IN VITRO ANTIFUNGAL ACTIVITY OF POTASH ALUM AGAINST CANDIDA ALBICANS ON ACRYLIC RESIN

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ABSTRACT

Objective: To evaluate the in vitro antifungal activity of Potash Alum (Phitkary) solution on Candida Albicans growth on heat cured acrylic resin.

Study Design: Quasi-experimental study.

Place and Duration of Study: Department of Oral Pathology, Peshawar Dental College, Peshawar, from Nov 2019 to Jan 2020.

Methodology: Acrylic resin discs (n=120) of standard size were prepared following the manufacturer's instructions. The acrylic discs were contaminated with a suspension of 106cfus/ml of C. albicans in Sabouraud dextrose broth for 24 hours. After contamination, 30 acrylic discs were randomly selected, washed, sonicated, and plated for colony counts. The remaining 90 acrylic discs were randomly divided into 3 groups, 30 acrylic discs in each group. Group A discs were kept in 5.25% Sodium Hypochlrite solution, group B discs were kept in sterile Phosphate Buffered Saline and group C discs were kept in Potash-alum solution (10mg/ml). After 2 hours, all discs were washed, sonicated, and cultured on Sabouraud dextrose agar in serial dilutions for colony counts. Data was analysed using SPSS version-19.

Results: Both Potash Alum (10 mg/ml) and Sodium Hypochlorite were effective in completely removing the attached Candida cells from the acrylic resin. In contrast, the negative control was only able to reduce viable counts by 23.6%. Sodium Hypochlorite and Potash Alum both showed statistically significant activity (p<0.01) against C. albicans compared to the negative control.

Conclusion: Potash Alum has a significant in vitro antifungal activity against C. albicans on acrylic resin. Its antifungal effect is comparable to the effects of Sodium Hypochlorite and therefore, it may offer a cost-effective and safe alternative to commercial denture cleaning agents.

Keywords: Acrylic resin, Candida albicans, Denture cleaning agent, Potash Alum.

INTRODUCTION

Growth of microbial biofilm on the denture surfaces is one of the major problems denture wearers face. In particular, fungal growth on denture surfaces predisposedenture wearers to a variety of oral health issues. These may include denture stomatitis, chronic candidiasis, and papillary hyperplasia[1]. Since majority of denture wearers are elderly people with co-morbid systemic conditions, microbial growth on the denture surface may affect their systemic health[2]. Plaque accumulation on dentures is facilitated by irregularities and imperfections on the unpolished denture surfaces. The problem is compounded by poor oral hygiene due to reduced manual dexterity of the elderly denture wearers. Candida Albicans is an opportunistic pathogen that can be found in the oral cavities of up to 60% of healthy individuals[3]. It is also one of the most common microorganisms growing on the denture surfaces.

Maintenance of good oral and denture hygiene is one of the best ways to discourage microbial accumulation on denture surfaces[4]. Good oral hygiene should be complemented by regular mechanical and chemical cleaning of the denture[5]. Ultrasonic cleaning is another method to remove denture biofilm, however its usefulness is limited by its high cost and user-unfriendly nature. Use of mechanical and chemical denture cleaning agents becomes more relevant in cases of geriatric and handicapped population[6]. While

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mechanical cleaning can be very effective in eliminating the biofilm growth on the denture surfaces, it can abrade the denture surface over a period and is difficult for people with reduced manual dexterity. Thus, chemical cleaning agents are more practical and easier to use for the geriatric and handicapped denture wearers. Chemical agents used for denture cleaning should be effective in reducing the biofilm, biocompatible and affordable.

Sodium hypochlorite, hydrogen peroxide, mouth washes containing chlorhexidine and household solutions such as vinegar and sodium chloride are some of the most common chemical denture cleaning solutions[7]. Some of these chemical solutions are expensive while others damage the denture surface. Some chemical solutions have a strong odour which can be unpleasant for the denture wearers.

Potash Alum ($KAl(SO_4)2 \cdot 12H_2O$) is a naturally occurring compound with antibacterial and antifungal properties. Potash Alumhas been in use of Egyptian, Indian and Chinese civilizations since antiquity[8]. It is known by the name of Phitkary in the subcontinent and is a household item. Potash Alum is odourless, cheap, and nontoxic in small quantities. Due to its lack of toxicity, the United States, Food and Drug Administration (FDA) has approved it as a food additive[9].

A clinical trial by Olmez *et al* 1998, demonstrated that a mouth rinse containing Potash Alum was significantly more effective in reducing plaque and salivary levels of *oral streptococci* compared to baseline[10]. However, to the best of our knowledge its use as a denture cleaning agent has not been investigated earlier. We hypothesize that Potash Alum can be a useful chemical cleaning agent for dentures. Therefore, the aim of our study was to compare the antimicrobial effectiveness of Potash Alum solution with commercially available denture cleaning agents.

METHODOLOGY

This quasi-experimental comparative study was carried out at the department of Oral Pathology, Peshawar Dental College, Peshawar, Pakistanbetween November 2019 to January 2020. The study was approved from the institutional ethics committee (EC Ref. No. RCD-19-09-22). Sample size was calculated using G power software version 3.1 (Faul, Erdfelder, Lang, & Buchner, 2007) at an effect size of 0.4 and alpha of 0.05.

Heat cured acrylic resindiscs (n=120, Lucitone 199, standard powder, original shade, Densply, Pennsylvania, USA) of standard size (40 × 12 × 3mm) were prepared following manufacturer's instructions using a stainless-steel mould. All samples were cooled to room temperature after deflasking and excess acrylic was removed with low speed carbide burs. Finally, the acrylic discs were polished with fine wet pumice powder and kept in distilled water at room temperature for 72 hours to remove residual monomer[11].

C. albicans previously isolated from clinical samples of denture wearing patients and cultured on Sabouraud dextrose agar. After incubation at 37°C for 48 hours, C.albicans was Gram stained observed under light microscope. Observation of fungal hyphae confirmed presence of C.albicans. A suspension containing 10 6cfu/ml in sabouraud dextrose broth was prepared. Acrylic discs were disinfected with 70% alcohol for 15 minutes and washed with sterile distilled water. Each sample was then transferred to 50 ml sterile test tubes containing 10 ml of C. albicans suspension (3×10^6 cfu/ml). Samples were incubated at 37°C for 24 hours. C. albicans suspension was inoculated on sabouraud dextrose agar plates in serial dilutions to verify the cfu/ml.

After 24 hours acrylic samples were washed with sterile distilled water. Thirty acrylic samples were randomly selected, washed three times with sterile PBS and transferred to separate 50 ml test tubes containing 10 ml of PBS. The tubes were sonicated for 5 minutes to detach viable adherent C. albicans cells and inoculated in serial dilutions on Sabouraud agar plates.

Cleaning protocol for contaminated acrylic samples

The 90 remaining samples were randomly divided into three groups (30 samples each). Each group of acrylic samples were separately immersed in 5.25% NaOCl (Canal Pro NaOCl, Coltene-Endo) as positive control, sterile PBS as negative control and Potash Alum solution (10mg/ml) in sterile test tubes. This concentration of Potash Alum solution (10gm/ml) was found in our pilot study to be effective against C. albicans. All test tubes were incubated at 37°C for two hours. Acrylic samples were washed three times with sterile PBS and transferred into separate test tubes containing 5 ml PBS. These tubes were agitated on a sonicator for 5 minutes and inoculated on Sabouraud dextrose agar plates for *C. albicans* colony count.

The mean colony counts of *c. albicans* were calculated and compared using paired sample t-test. The *p*-value of ≤0.05 was considered significant. All statistical tests were done using IBM SPPSS version 19.

RESULTS

A total of 120 acrylic resin samples were prepared and contaminated with *C. albicans*. These samples were subdivided into 4 groups of 30 samples each. Groups were labelled as baseline samples (untreated), treated either with sodium hypochlorite (positive control), phosphate buffered saline (PBS, negative control) or Potash alum.

Table-I shows viable counts (mean ± SD) of *C. albicans* (CFU/mL ± SD) attached to acrylic samples before and after the disinfection protocols. Both Potash Alum (10 mg/ml) and Sodium Hypochlorite were effective in comple-tely (100%) removing the attached Candida cells from the acrylic resin. In contrast, the negative control (PBS) was only able to reduce viable counts by 23.6%. Both Sodium Hypochlorite and Potash Alum showed statistically significant activity (p<0.0001) against C. albicans compared to the negative control (PBS).

Table-II presents the susceptibility profile of C. albicansagainst Potash Alum solution. Concentrations of 18.25 mg/ml of Potash Alum was found to be the minimum fungicidal concentration for C. albicans. Concentrations of 4.5 mg/ml (MIC50) and 10 mg/ml (MIC90) were found to be the minimum inhibitory concentrations of Potash Alum, inhibiting the growth of 50% and 90% of C. albicans, respectively.

Table-I: Effectiveness of Potash Alum solution and percentage removal of C. albicans from thirty experimentally contaminated acrylic resin discs.

Disinfectants	Initial Culture (mean ± SD)	After Disinfection (mean ± SD)	Removing Ability %	*p*-value
Sodium hypochlorite (positive control)	830 ± 75	-	100	<0.001
Phosphate Buffered Saline (negative control)	830 ± 75	634 ± 48	23.6	0.083
Potash Alum (10mg/ml)	830 ± 75	-	100	<0.001

Table-II: Susceptibility profile of candida albicans to potash alum solution.

Variable	Potash Alum (mg/ml)
Minimum Fungicidal Concentration (MFC)	18.25
Minimum Inhibitory Concentration 90 (MIC 90)	10
Minimum Inhibitory Concentration 50 (MIC 50)	4.5

DISCUSSION

The current study investigated the antifungal effects of Potash Alumto find a cheap alternative for routine chemical cleaning of acrylic dentures. We found that Potash Alumis as effective as Sodium Hypochlorite in inhibiting fungal growth on acrylic dentures.

Potash Alum is a part of Ayurvedic and Chinese medicine and has traditionally been in use as a topical antiseptic and aftershave solution[12,13]. The safety profile of Potash Alum is well established which is apparent from its use in food

processing and preservation[14,15]. In dentistry, it has been found effective against oral bacteria and plaque control[16]. Its safety for oral use and gingival health has also been documented[17] and ina study by Thomas *et al* 2015, Potash Alum was found to be effective in control of oral bacteria when routinely used as a mouth rinse[18].

Studies by Kolaei *et al* 2013 and Flayeh 2010, have described the antifungal properties of Potash Alum previously[19,20], however, to the best of our knowledge, its use as a denture cleaning agent has never been reported. Antifungal activity of Sodium hypochlorite, peroxides and chlorhexidine have been investigated by Nakamoto *et al* 1991 and Buergers *et al* 2008[21,22]. These chemical agents have been found effective as denture cleaning agents, however, most of them are either costly or have unpleasant side effects such as foul odour and bad taste. As a denture cleaning agent, Potash Alum is an odourless, cheap, and safe alternative to these chemicals. In the current study, we found that 10 mg/ml of Potash Alum is effective in inhibiting the growth of C. albicans. One previous study[23] has reported 12.5 mg/ml as an effective concentration of Potash Alum which inhibits the growth of C. albicans. This concentration of Potash Alumis very similar to our findings (10mg/ml). Another study investigated inclusion of Potash Alumparticles into denture soft liners 24 and found it effective in discouraging C. albicans adherence.

A combination of mechanical and chemical cleaning has proven to be the best method of denture cleaning[24]. However, in patients who are dependent on others and those wary of damage to the denture due to mechanical cleaning, chemical cleaning methods can be as effective if used properly. In this study we evaluated the antifungal effects of Potash Alumin laboratory settings. Although, it has a clear in vitroinhibitory effect on the growth of C. albicans growth, long-term prospective clinical studies are required to further evaluate the role of Potash Alumas a denture cleaning agent. In addition, its long-term effects on denture acrylic resin also needs to be investigated.

CONCLUSION

Potash Alum has a significant in vitro antifungal activity against C. albicans on acrylic resin. Its antifungal effect is comparable to the effects of sodium hypochlorite and therefore, it may offer a cost-effective and safe alternative to commercial denture cleaning agents.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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