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Antifungal activity of a toothpaste containing Ganoderma lucidum against Candida albicans - an in vitro study

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Abstract

Introduction : Candida albicans is the most common oral fungus associated with oral candidial infections. Various antifungal agents are in use and the search is on for more agents showing anti candidial properties. Ganoderma lucidum has been in use in Traditional Chinese Medicine for years. Literature supports the use of this Ganoderma lucidum as a medicinal mushroom for its antimicrobial, antiviral properties. *Objectives:* Varying concentrations of a toothpaste containing Ganoderma lucidum was tested in vitro for its antifungal properties against Candida albicans. *Method:* The activity of a Ganoderma containing toothpaste against Candida albicans was tested by serial broth dilution method and was expressed by minimum inhibitory concentration (MIC). *Results:* The toothpaste exhibited antifungal properties against the tested organism. The MIC value of Candida albicans was found to be less than 02 mgm /ml.

Key words: Ganoderma lucidum, toothpaste, Candida albicans, serial broth dilution, MIC.

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Introduction

In a healthy host, opportunistic fungal pathogens are commensal fungi commonly colonizing human mucosal surfaces. Candida albicans is one such opportunistic human pathogen that colonizes at several anatomically distinct sites such as oral cavity, skin, gastrointestinal tract and the vagina. Candida albicans has been found in all ages especially in the oral cavity of babies and geriatric age groups. Over-proliferation of Candida albicans and its infiltration into the mucosal or cutaneous surface results in a pathogenic infection. This infection is known as Candidiasis. Oral Candidiasis can occur in patients with long term use of antibiotics in immune-compromised hosts and in patients undergoing cytotoxic chemotherapy and radiotherapy. This could also predispose the patient to systemic candidial infection.

Many of the currently available antifungal drugs have undesirable side effects and lead to the rapid development of drug resistance, causing profound effects on human health (1).This has necessitated the need for discovering new effective antifungal drugs. Traditional medicine has a huge treasury of herbs and remedies that can be tapped as a source for obtaining antifungal agents from the plant, animal and fungal kingdom after scientific research.

Ganoderma lucidum is a Basidiomycetes fungus belonging to the family Polyporaceae. (2) It has been in use for thousands of years for its medicinal properties in Traditional Chinese Medicine. (3). It is known to have many biologically active components like triterpenes (4,5), polysaccharides (6,7), ganoderic acids (5), and so on, giving it, its antimicrobial (8,9,10,11,12,13), antiviral (10,14,15,16,17), immunomodulatory (15), antioxidant (18), antitumour (19) and anticancer (20) properties. As the antimicrobial and antiviral activity of Ganoderma has already been documented scientifically, with just a few mentions in scientific papers regarding its antifungal activity on human pathogens, it was decided that a preliminary study to observe its antifungal effects on Candida albicans would be in order.

Toothpastes are the most common vehicles for delivery of drugs to the oral cavity. This preliminary study was aimed at determining the Minimum Inhibitory Concentration (MIC) of one such toothpaste containing Ganoderma lucidum * on Candida albicans in vitro by serial broth dilution method. This toothpaste does not contain any other constituents that could demonstrate anticandidial properties.

Materials and Methods

A standard procedure for performing the MIC test was followed (21). The standard strain of Candida albicans used in this study was ATCC 2091.Sabouraud dextrose broth, Ganoderma lucidum containing paste, sterile MIC tubes and micropipettes were the other armamentarium used.

Procedure: Revival of the organism – The Candida albicans strain from the stock was revived by plating on blood agar medium. After overnight incubation at 37^0 C, isolated colonies were selected and the identity of the organism was confirmed. Isolated colonies were transferred to sterile Sabouraud dextrose broth and once again incubated overnight. The growth concentration was adjusted to 10^5 organisms / ml by using 0.5 McFarland's turbidity standard.

To prepare stock of the toothpaste, one gram of the toothpaste was added to one ml. of sterile saline in a sterile vial.

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*A branded proprietary product (Ganozhi toothpaste manufactured by DXN Industries (M) SDN, BHD. Malaysia) containing Ganoderma lucidum in a food gel base with menthol is available worldwide since 1993.

Two hundred µl of the Sabouraud dextrose broth was added in ten MIC tubes. In the first MIC tube containing 200 µl broth, 200 µl of stock was added. After mixing well, 200 µl was transferred to the second MIC tube. This was continued till the last (10^{th}) tube. From the last tube 200 µl final solution was discarded. By following this serial dilution, the concentration of the paste was achieved as the following – 500, 250,125, 62.5, 31.25, 16,8,4,2 mgm. /ml. respectively.

To each of the ten such prepared MIC tubes with varying concentrations, 200 µl of the earlier prepared strain of Candida albicans was added, such that the final volume per tube was 400 µl. The tubes were then incubated for 48 hours at 35^0 C.

After the incubation, the MIC values were determined by visual inspection of the tubes. With the MIC tubes, positive and negative controls were put up. Positive control containing broth plus candidial strain showed turbidity and negative control containing broth only appeared clear. In the tubes tested, the last tube with clear supernatant was considered to be without any growth and taken as MIC value.

Turbidity in the MIC tube indicated growth of the fungus implying that it was resistant to the toothpaste.

Results

Results, as shown in Table 1, indicated that the MIC value for Candida albicans was less than 02 mgm. /ml.

Discussion:

The incidence of Candida albicans isolated from the oral cavity has been reported to be 45% in neonates, (22) 45%–65% of healthy children,(23) 30%–45% of healthy adults (24,25), 50%–65% of people who wear removable dentures, (25) 65%–88% in those residing in acute and long term care facilities, (25,26,27) 90% of patients with acute leukemia undergoing chemotherapy, (28) and 95% of patients with HIV(29). Candida species now rank as the fourth most common cause of nosocomial bloodstream infections in the United States and the attributable mortality rate is 30% (30).

Advances in medical technology, chemotherapeutics, cancer therapy, and organ transplantation have greatly reduced the morbidity and mortality of life-threatening diseases. Patients who are critically ill and in medical and surgical ICUs have been the prime targets for opportunistic nosocomial fungal infections, primarily due to *Candida* species. On a daily basis, virtually all physicians are confronted with a positive *Candida* isolate obtained from one or more anatomical sites. High-risk areas for *Candida* infection include neonatal, paediatric, and adult ICUs, both medical and surgical (31).

Table 1 – MIC of the tested organism

Organism	Concentration of the toothpaste containing Ganoderma lucidum in mg / ml.								
	500	250	125	62.5	31.25	16	08	04	02
Candida albicans	S	S	S	S	S	S	S	S	S

S = Sensitive R = Resistant

Oropharyngeal candidiasis can manifest as pseudomembranous candidiasis, acute atrophic candidiasis, chronic hyperplastic candidiasis, chronic atrophic candidiasis, median rhomboid glossitis and angular cheilitis (32). Dentures have always been found to be a breeding ground for Candida albicans. Astrid Vanden Abbeele et al (33) in their study observed that screening of upper prostheses demonstrated Candida colonisation of upper prosthesis in 75.9% of individuals. The most frequent species isolated were Candida albicans 77.9% of the positive cultures, Candida glabrata (44.1%) and Candida tropicalis (19.1%). Hirokowi Nikawa et al (34) concluded that denture plaque (plaque on denture) containing candida could cause not only oral candidiasis, like oral thrush or denture-induced stomatitis, but also caries, root caries and periodontitis of abutment teeth.

In HIV disease, up to 90% of HIV+ persons have a symptomatic episode of oropharyngeal candidiasis. Both innate resistance and acquired immunity play some role in maintaining C. albicans in the commensal state and protecting the systemic circulation (35)

Abate screened about 60 different basidiomycetes cultures for antimicrobial secondary metabolites. Amongst these the culture filtrate extract of the polypore, G. lucidum produced the most effective antifungal compounds. The minimal inhibitory concentration (MIC) of 201A against Candida albicans was less than 1 mcg/ml (36). The Ganoderma containing toothpaste that was tested by us demonstrated a MIC of 2 mgm/ml against Candida albicans. This is reasonable considering that the toothpaste contains Ganoderma in a food gel base with no other ingredients with antifungal activity.

Hexaiang Wang (37) isolated a 15-kDa antifungal protein, designated Ganodermin, from Ganoderma lucidum. Ganodermin inhibited the mycelial growth in the phytopathogenic fungi B. cinerea, F. oxysporum and P. piricola. It will have to be ascertained in future studies, whether Ganodermin has a similar antifungal activity against Candida albicans.

Very few bioactive proteins, such as a lectin (38) and a ribonuclease (39) have been isolated from G. lucidum. There is scant literature on the antifungal action of Ganoderma lucidum on other fungi.

Both innate resistance and acquired immunity play some role in maintaining C. albicans in the commensal state and protecting the systemic circulation. Polymorphonuclear leucocytes are critical for protection against systemic infections, whereas cell-mediated immunity (CMI) by Th1-type $CD4^+$ T-cells is important for protection against mucosal infections (35). Fidel (35) recently found that epithelial cells from saliva and vaginal lavages of healthy individuals inhibit the growth of Candida in vitro. This epithelial cell anti-Candida activity requires cell contact by viable cells with no role for soluble factors, including saliva. Interestingly, oral epithelial cells from HIV positive persons with OPC had significantly reduced activity, indicating some protective role for the epithelial cells. TNF-α was also implicated as an important mediator in the recovery from oropharyngeal candidiasis (40). Candida albicans triggers interleukin-6 and interleukin-8 responses by oral fibroblasts in vitro. The secretion of proinflammatory cytokines - interleukin-6 and interleukin-8 by oral mucosal fibroblasts in response to C. albicans suggests that these cells have the potential to enhance the host defense against this organism in vivo. This may have important implications in controlling fungal overgrowth in the oral cavity (41).

Virulence factors in Candida albicans have been attributed to the utilization of several genes whose functions in adhesion, proteinases secretion, hyphal formation and phenotypic switching are required for their virulence (1). Taking into consideration all these local factors, we thought it would be prudent to use a Ganoderma containing toothpaste as it could prove convenient and useful for application on mucosa and dentures and toothbrushing

Hossain et al (42) concluded that carious teeth may constitute an ecologic niche for C. albicans potentially responsible for recurrent oral and non-oral candidiasis.

Fagade & Oyelade in their study (43) on testing ethanolic extracts of Ganoderma lucidum on various microorganisms found that the MIC of Candida albicans was 750 mgm/ml. In our study, we tested a toothpaste containing Ganoderma

lucidum against Candida albicans. It was highly sensitive with an MIC of less than 2 mgm. / ml. This is because the toothpaste contains spore powder, whereas Fagade used ethanolic extracts of the fruiting body. Therefore this toothpaste could be highly effective in patients with HIV infections, patients undergoing radiotherapy or chemotherapy, immunocompromised individuals, in high risk areas such as medical and surgical ICU's, as a local application on dentures and oropharyngeal candidiasis. However a clinical study to corroborate these laboratory findings is suggested.

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