Evaluation of anti-microbial activity of spore powder of *Ganoderma lucidum* on clinical isolates of *Prevotella intermedia*: A pilot study

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Abstract

Aim: This study aimed at evaluating the anti-microbial activity of spore powder of *Ganoderma lucidum* on *Prevotella intermedia* isolated from subgingival plaque from chronic periodontitis patients. **Settings and Design:** Written informed consent was obtained from each subject enrolled in the study. The Institutional Ethics Committee granted the ethical clearance for the study. **Materials and Methods:** This study included 20 patients diagnosed with chronic periodontitis. Pooled subgingival plaque samples were collected using sterile curettes from the deepest sites of periodontal pockets. The collected samples were then transported in 1 mL of reduced transport fluid. The organisms were cultured and confirmed. These organisms were then used for minimum inhibitory concentration (MIC) procedure. **Statistical Analysis:** Mean of the MIC value obtained was calculated. **Results:** Thirteen out of the 20 clinical samples were tested that showed sensitivity at various concentrations. Five samples showed sensitivity at all concentrations. Twelve samples showed sensitivity at 8 mcg/ml. Eleven samples showed sensitivity at 4 mcg/ml, 8 samples showed sensitivity at 2 mcg/ml, and 5 samples showed sensitivity even at 1 mcg/ml. Mean MIC value of *G. lucidum* spore powder for *P. intermedia* obtained was 3.62 mcg/ml. **Conclusion:** *G. lucidum* with its multipotential bioactivity could be used as an anti-microbial, in conjunction with conventional therapy in periodontal disease.

Keywords: Anaerobic bacteria, anti-microbial agents, *Ganoderma lucidum*, Gram-negative bacteria, periodontitis, *Prevotella intermedia*

Introduction

Periodontitis is a disease that affects the tooth supporting tissues and is characterized by a loss of periodontal attachment including the alveolar bone.[1] The etiology of the disease is multifactorial and bacterial deposits play an essential role in the pathogenesis. The bacteria comprises predominantly of Gram-negative anaerobic rods.[1] Among them, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Bacteroides* spp., and *Selenomonas* spp. have been associated with chronic periodontitis. Black-pigmented anaerobes have been considered as the key pathogens in mixed anaerobic infections.[2] In the oral cavity, these bacteria, particularly *P. intermedia*, play an important role in the onset and subsequent development of the polymicrobial periodontal diseases.[3] Despite this, there are only a few published studies on this organism.

Ganoderma lucidum, a large, red mushroom, belonging to the class Basidiomycetes, is unique in its range of pharmacogenic components. Various parts of this mushroom, namely the mycelia, spores, and fruit body possess medicinal properties. The major physiologically active constituents in *G. lucidum* are polysaccharides, peptidoglycans, and triterpenes.[4,5]

Spore powder of *G. lucidum* is widely used in Traditional Chinese Medicine.[6] Scientific research has proved that spore powder of *G. lucidum* has demonstrated multiple functions such as blockade of release of histamine, inhibition of an overstimulated immune system, and a regulatory effect on cellular and humoral immunity.[7,8]

Most of the antibiotics and antivirals tend to exhibit undesired side effects and drug resistance. The mutation of the strains also complicates the issue. This makes the

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Access this article online	
Quick Response Code:	Website: www.contempclindent.org
	DOI: 10.4103/0976-237X.166834

How to cite this article: Nayak RN, Dixitraj PT, Nayak A, Bhat K. Evaluation of anti-microbial activity of spore powder of *Ganoderma lucidum* on clinical isolates of *Prevotella intermedia*: A pilot study. Contemp Clin Dent 2015;6:S248-52.

development of new agents an urgent necessity. Research today focuses at discovering agents that specifically inhibit viral and bacterial multiplication without affecting the normal cells. Researchers today are fast moving to tap the potential of medicinal plants and fungi with their antibacterial and antiviral activity.[9,10]

The objective of this study was to evaluate whether or not spore powder of *G. lucidum* has anti-microbial activity on *P. intermedia*.

Materials and Methods

This study included 20 patients diagnosed with chronic periodontitis. The criteria of the patients selected, included presence of bleeding on probing, probing depth ≥5 mm, and clinical attachment loss ≥3 mm. Patients on any antibiotic therapy and periodontal treatment up to 3 months prior to this study were excluded. Patients with any systemic diseases or conditions, pregnant, lactating women, and smokers were excluded from the study. Written informed consent was obtained from each subject enrolled in the study. The ethical clearance for the study was obtained from the Institutional Ethics Committee.

Pooled subgingival plaque samples were collected using sterile curettes from the deepest sites of the periodontal pockets. The samples were transported in 1 mL of reduced transport fluid. Culture procedures were carried out in the Laboratory of Molecular Biology and Immunology at our institute.

Methodology

Isolation of the organism

The plaque sample was vortexed to break down the plaque and then the sample was inoculated in blood agar with incorporated kanamycin. Then, the plates were kept in anaerobic jar for 48–72 h at 37°C. *P. intermedia* showed black-reddish minute colony morphology and so the plates incubated were then removed and examined for black-reddish minute colonies. Gram staining and sugar fermentation tests were performed to confirm the organism. Confirmed colonies were subcultured in thioglycollate broth to grow the organisms.[11] These organisms were then used for minimum inhibitory concentration (MIC) procedure.

Preparation of the stock solution

The stock solution was prepared by taking 1 ml of sterile saline in a sterile vial in which 10 mg of the *G. lucidum* spore powder was added. From this, 100 µl stock solution was used.

Minimum inhibitory concentration procedure

Ten tubes, each having 100 µl thioglycollate broth were used for the MIC procedure. One hundred µl stock solution was added in the first MIC tube containing 100 µl broth. After mixing well, 100 µl solution from this tube was transferred to the second MIC tube. This process was continued till the 10th tube. From the 10th tube which was the last tube, 100 µl final solution was discarded. The concentrations of the aqueous extract achieved by this serial dilution method were as following - 500, 250, 125, 62.5, 31.25, 16, 8, 4, 2, and 1 mcg/ml.[12] One hundred µl isolated strain of *P. intermedia* was added to each of the 10 such prepared MIC tubes with varying concentrations such that the final volume per tube was 200 µl. The tubes were then incubated at 37°C for 48–72 h. After the incubation period, by visual inspection of the tubes, the MIC values were determined. With the test group, positive and negative controls were put up. The positive control showing turbidity constituted broth and the bacterial strain [Figure 1]. The negative control containing broth and extract appeared clear [Figure 2]. The MIC value was obtained by visualizing each series of tubes, and the last tube with clear supernatant was taken as the MIC value. The clear supernatant was considered to be without any growth. Turbidity in the MIC tube indicated growth of the bacteria implying that the bacteria were resistant to the aqueous extract of the powdered spores of *G. lucidum*.

Results

Table 1 shows that 13 out of the 20 clinical samples were tested that showed sensitivity at various concentrations ranging from 500 to 1 mcg/ml.

In our study, aqueous extract of the powdered spores of *G. lucidum* demonstrated anti-microbial activity on *P. intermedia.* Thirteen out of the 20 clinical samples were tested that showed sensitivity at various concentrations. Sensitivity was tested from 500 to 1 mcg/ml. Thirteen samples were sensitive at 16 mcg/ml. Twelve samples demonstrated sensitivity at 8 mcg/ml, 11 samples at 4 mcg/ml, and 8 samples at 2 mcg/ml. Five samples exhibited sensitivity even at 1 mcg/ml. The mean MIC value of an aqueous extract of the powdered spores of *G. lucidum* for *P. intermedia* obtained was 3.62 mcg/ml.

Figure 1: Turbidity implying the presence of bacterial growth

Discussion

P. intermedia has been implicated in extra-oral and intra-oral infections. Extra-oral infections caused by *P. intermedia* are mainly nasopharyngeal infection and intra-abdominal infection.[13] *P. intermedia* belonging to the orange complex of secondary colonizers is an important pathogen involved in the pathogenesis of periodontal disease and the organism contributes for the disease progression along with other organisms. In our study, we tested the anti-microbial activity of an aqueous extract of the spores of *G. lucidum* against the clinical isolates of *P. intermedia*. Out of the 20 clinical samples tested, 13 exhibited sensitivity at various concentrations. Mean MIC value of the aqueous extract of the powdered spores of *G. lucidum* for *P. intermedia* obtained was 3.62 mcg/ml [Graph 1]. Activity of the aqueous extract of the spores of *G. lucidum* at 500 mcg/ml showed that 65% of organisms were sensitive and 35% of organisms were resistant [Graph 2]. Activity of the aqueous extract of the spores of *G. lucidum* at 16 mcg/ml showed that 65% of organisms were sensitive and 35% of organisms were resistant [Graph 3].

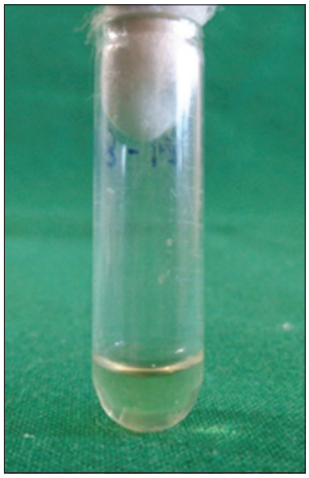


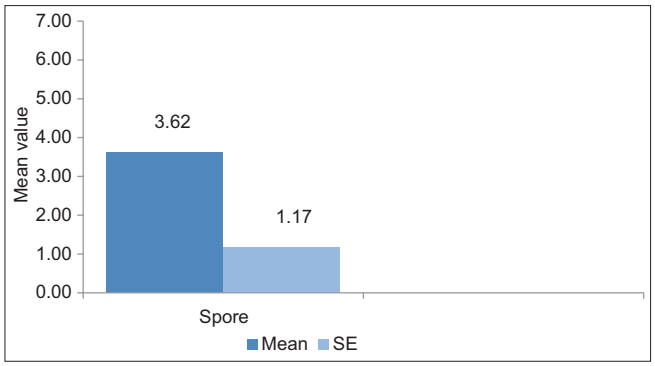
Figure 2: Clear solution implying the absence of any growth of bacteria

Yoon *et al*.[14] studied the antibacterial activity of the spores of *G. lucidum* against Gram-positive and Gram-negative bacteria by serial broth dilution method. Against standard strains and among five species of Gram-positive bacteria tested, the most prominent anti-microbial activity of *G. lucidum* was shown in *Micrococcus luteus* at a MIC of 0.75 mg/ml. Anti-microbial activity of *G. lucidum* was tested against the 10 species of Gram-negative bacteria and the strongest antibacterial activity was shown against *Proteus vulgaris* and *Escherichia coli* at MIC values of 1.25 mg/ml and 1.75 mg/ml, respectively. In this study, the anti-microbial activity was tested against the standard strains of various Gram-positive and Gram-negative organisms, but in our

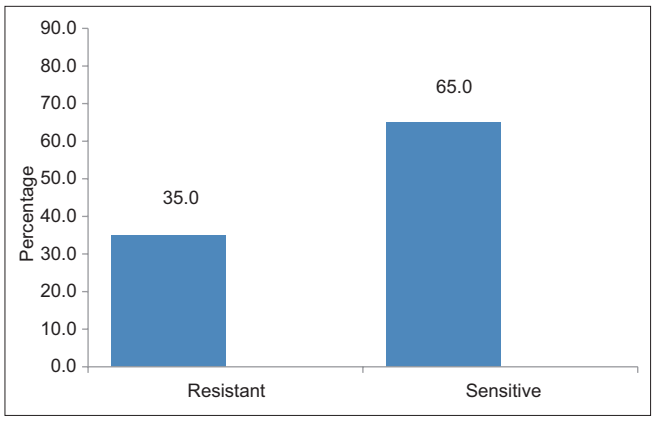
Table 1: MIC of aqueous extract of the powdered spores of *Ganoderma lucidum* for *Prevotella intermedia*

Samples	Concentration of aqueous extract of the powdered spores of *Ganoderma lucidum* in mcg/ml									
	500	250	125	62.5	31.25	16	08	04	02	01
1	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Resistant
2	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
3	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
4	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Resistant
5	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
6	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
7	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
8	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
9	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
10	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
11	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
12	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
13	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
14	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Resistant
15	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Resistant	Resistant
16	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant
17	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant
18	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
19	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Resistant	Resistant	Resistant
20	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant

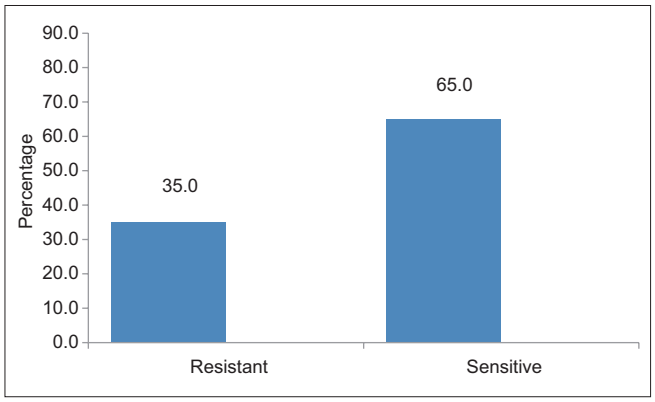
MIC: Minimum inhibitory concentration



Graph 1: The mean minimum inhibitory concentration value of aqueous extract of the powdered spores of *Ganoderma lucidum* for *Prevotella intermedia* is 3.62 mcg/ml



Graph 2: The activity of aqueous extract of the spores of *Ganoderma lucidum* at 500 mcg/ml showed that 65% of organisms were sensitive and 35% of organisms were resistant



Graph 3: The activity of aqueous extract of the spores of *Ganoderma lucidum* at 16 mcg/ml showed 65% of organisms were sensitive and 35% of organisms were resistant

current study, clinical isolates of *P. intermedia* were tested. In another study by Nayak *et al*.[15] against the standard strains of *Staphylococcus aureus*, *E. coli*, *Enterococcus faecalis* and *Klebsiella pneumoniae*, and antibacterial effect of the aqueous extract of the spore powder of *G. lucidum* were studied. The results suggested that the above-mentioned microorganisms were sensitive and the MIC value for *S. aureus* was 125 mcg/ml, *E. coli* was 125 mcg/ml, *E. faecalis* was <2 mcg/ml, and for *K. pneumoniae* the MIC was 62.5 mcg/ml. In our current study, mean MIC value obtained was 3.62 mcg/ml indicating that *P. intermedia* was sensitive even at this low concentration. Studies demonstrated that antibacterial components present in *G. lucidum* such as peptidoglycans were able to inhibit Gram-positive and Gram-negative bacteria.[16-18] Some other investigators through their research suggested that tissue and cellular damage following infections may be decreased by the immunosuppressive activity of *G. lucidum*.[16-18]

According to Gao *et al*. *G. lucidum* and other *Ganoderma* species often in combination with chemotherapeutic agents have been used to treat various bacterial diseases and found that polysaccharide components were the principal bioactive components which play an important role in the antibacterial activity.[16] Smania *et al*. observed maximum antibacterial activity of methyl australate, a derivative from *G. lucidum* against *E. coli* and *Pseudomonas aeruginosa* followed by *S. aureus* and the least zone of inhibition was recorded for *Bacillus* species.[19] In our current study, property of individual components of *G. lucidum* were not studied, but the anti-microbial property of spore powder as a whole was assessed. Klaus and Miomir have studied the influence of various extracts isolated from *G. lucidum* on *E. coli*, *Bacillus* species, *S. aureus*, and *Salmonella* species. The aqueous fruiting body extract showed the maximum zone of inhibition against *Bacillus* species while least zone of inhibition was reported for *E. coli* and *Salmonella* species.[20] In our study, the anti-microbial activity of only the spore powder was tested against *P. intermedia*. Cowan reported that the most active components present in the mushroom are generally water insoluble, expecting that low polarity organic solvents would yield a more active extract,[21] In our study, an aqueous extract was used. Yet without an organic solvent, excellent MIC activity up to 1 mcg/ml against *P. intermedia* was demonstrated. This opens up avenues for more studies on the anti-microbial effect of organic solvent extract of the spore powder of *G. lucidum* on *P. intermedia* which could possibly show even more enhanced MIC activity.

Our study demonstrates anti-microbial activity *in vitro* only. However, since *G. lucidum* is known to have immune modulating activity,[4,5] its effectiveness clinically could be much better and *in vivo* studies would probably demonstrate better control of infections due to synergistic actions. Many active substances are present in mushrooms and these individually contribute to the bioactivity observed *in vitro* and *in vivo*. Some roles of individual constituents are known whereas some still unknown. In summary, *G. lucidum* with its multipotential constituents may play an important role as an adjunct in the management of infectious and inflammatory diseases, periodontal disease being one. To the best of our knowledge, this is probably the first study to assess the

anti-microbial activity of *G. lucidum* against clinical isolates of *P. intermedia* isolated from chronic periodontitis patients.

Conclusion

G. lucidum, with its multipotential bioactivity, can be used as an anti-microbial, as an adjunct to conventional therapy in periodontal disease. Our study is probably the first of its kind to demonstrate antibacterial activity of the spore powder of *G. lucidum* against *P. intermedia*. Therefore, topical application of spore powder or aqueous or organic solvent extract directly on the infected tissues could be an efficient drug delivery system for the control of *P. intermedia* associated periodontitis. Systemic administration of the spore powder of *G. lucidum* with its proven immunomodulatory[4,5] activity could possibly enhance the response to local drug delivery.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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