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Research Article

ANTIPLAQUE EFFICACY OF *GANODERMA LUCIDUM* TOOTHPASTE - AN *IN VITRO* STUDY

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ABSTRACT

Objective: The objective of the study was to evaluate the efficacy of *Ganoderma lucidum* toothpaste as an antiplaque agent and to compare its efficacy with herbal toothpaste and mouthwash.

Methods: Pooled saliva was collected in a sterile container from the volunteers after taking the consent. Tissue culture plate with 12 (3 × 4) wells was chosen. Pooled saliva of 20 mL was added to each well using the micropipette and was kept in the incubator at 37°C for 72 h. After 72 h, saliva was removed without touching the walls or the base of the wells. Each row was treated either with slurry prepared with Ganoderma/herbal/Colgate total toothpaste or herbal/chlorhexidine mouthwash/distilled water. One row of wells was kept as a control using erythrosine dye. After 30 s, all the wells were rinsed with distilled water. Erythrosine dye was added to all the wells, kept for 30 s, and rinsed with distilled water. The tissue culture plate was kept in the ELx800MS machine (ELISA reader) which was set at 540 nm, and the readings were obtained.

Results: The results showed that *G. lucidum* toothpaste slurry reduced plaque than herbal and chlorhexidine mouthwash. However, there was no significant difference in plaque reduction between herbal and *G. lucidum* toothpaste slurries.

Conclusion: The present study concluded that *G. lucidum* had better antiplaque efficacy than herbal toothpaste, herbal mouthwash, and chlorhexidine mouthwash.

Keywords: *Ganoderma lucidum*, Plaque, Mouthwash, Toothpaste.

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INTRODUCTION

Dental plaque is defined as a highly specific variable structural entity formed by sequential colonization of microorganisms on the tooth surface, epithelium, and restorations. It is also defined as soft deposits that form the biofilm adhering to the tooth surface or other hard surfaces in the oral cavity including removable and fixed restoration. Dental plaque is researched extensively and proved to be the paramount factor in the initiation and progression of gingival and periodontal disease.

Dental plaque accumulation is a prerequisite for the development of gingivitis [1]. Gingivitis may progress to periodontitis in susceptible individuals. If prevention of gingivitis is successful, progression to periodontitis can be delayed or prevented. Since both gingivitis and periodontitis are plaque-associated oral conditions, the removal of dental plaque will inhibit their occurrence and progression.

Potential removal of plaque by means of toothbrush remains the most widely accepted method of disease prevention. Continuation of effective personal oral hygiene regimens requires a well-motivated patient who does oral hygiene practices in a proper fashion for a sufficient duration of time and with adequate frequency. Chemical plaque control is considered as an adjunct to mechanical oral hygiene practices, and these agents are used in the form of mouth rinse.

Phytotherapy has been practiced in India since ages. There are many herbal agents used in toothpaste which serve as excellent antiplaque agents, namely triphala, meswak, tulsi, and aloe vera. Studies are also done using *Garcinia kola* stem wood extract in toothpaste which has shown very good antibacterial property and can be used for personal oral hygiene [2].

Spirulina, a cyanobacterium or blue-green algae which has a wide range of nutritional and health benefits, was assessed for its oxidative properties in the healing of oral submucous fibrosis and leukoplakia. Radhika *et al*. assessed its antiplaque efficacy when used in mouthwash. Their result showed that there was a significant reduction in dental plaque and gingivitis [3].

Ganoderma lucidum is a Basidiomycetes fungus belonging to the family Polyporaceae [4]. It has been used for more than thousand years for its medicinal properties in Traditional Chinese Medicine. It has many biologically active components such as triterpenes, polysaccharides, and ganoderic acids [3], and so on, giving it, its antimicrobial [5], antiviral [6], immunomodulatory [7], antioxidant [8], antitumor, anticancer, and antifungal [9] properties. Based on the above properties, the aim of the present study was to evaluate the anti-plaque efficacy of *G. lucidum* toothpaste and compare its efficacy with herbal toothpaste, herbal mouthwash, and chlorhexidine mouthwash.

MATERIALS AND METHODS

The present work was an *in vitro* study. Pooled saliva was collected in a sterile container from the volunteers after obtaining their written informed consent. Materials used for the *in vitro* study were two tissue culture plates with 12 (3 × 4) wells, micropipette (10 mL), Ganoderma toothpaste, Himalaya Herbal Complete Care toothpaste, chlorhexidine mouthwash (0.2%), Himalaya Hiora Herbal Mouthwash, distilled water, erythrosine dye, and ELISA reader were used. 20 mL of pooled saliva was added in each well of tissue culture plate using micropipette which was set at 10 mL (Fig. 1).

The culture plates were kept in the incubator which was set at 37°C, for 72 h. The temperature set was equivalent to that of the oral cavity. After

72 h, the saliva in each well was removed using a pipette leaving behind the plaque on the walls and base of the wells (Fig. 2).

G. lucidum and Himalaya Herbal Complete Care toothpaste were mixed in distilled water separately to make a smooth slurry, and chlorhexidine 0.2% mouthwash, Himalaya Hiora mouthwash, and distilled water were used for analysis.

For the first row of the tissue culture plate (4 wells), two drops of disclosing agent (erythrosine) were added in each well. After 30 s of adding the disclosing agent, it was pipetted out and rinsed with 20 mL of distilled water using micropipette. This well was taken as a control. The second and third row, Ganoderma toothpaste slurry and Himalaya Herbal Complete Care toothpaste slurry, were added and kept for 30 s and rinsed with 20 mL of distilled water using micropipette.

In fourth, fifth, and sixth row, chlorhexidine mouthwash, herbal mouthwash, and distilled water were added and were kept for 30 s and rinsed with distilled water. Two drops of disclosing agent were added in all the wells from second to sixth row and were kept for 30 s and rinsed with 20 mL of distilled water using micropipette.

After the analysis, using various agents, 20 mL of distilled water was added in all the wells of tissue culture plates and kept in the ELx800MS machine (ELISA Reader) (Fig. 3) for the analysis.

The ELx800MS was set at 540 nm as the absorbency range of disclosing agent (erythrosine) is 525–530 nm. The readings were obtained by the printer which was connected to ELx800MS machine. The results were analyzed using SPSS 20 software and tabulated using one-way analysis of variance (ANOVA) followed by *post hoc* Tukey's test.

RESULTS

Comparison of multiple agents was performed using ANOVA followed by *post hoc* Tukey's test, and p value was kept at 0.005. Overall, there was a significant difference in the mean score between the control (Group 1), Group 6, and other Groups (p=0.000). *Post hoc* analysis showed that Group 1 had a higher mean score than Groups 2–5. Similarly, Group 6 had higher mean score than Groups 2–5. This implies that there was less amount of plaque in Groups 2–5. Group 2 showed better results than Groups 4 and 5 (p=0.001 and 0.005). Similarly, Group 3 showed better results than Group 4 (p=0.003). There was no significant difference between Groups 2 and 5. There was no significant difference between Groups 2 and 3 (p=0.965) and between Groups 4 and 5 (p=0.916). (Table 1)

DISCUSSION

Plaque is characteristically observed on the gingival third of the tooth surface [10]. A common method for detecting plaque is by the use of a disclosing agent. They are available as tablets, lozenges, or wafers, which contain dye or other coloring agents. The various available disclosing agents are erythrosine (PLAKSEE), two tone dye (alpha plaque), PLAKLITE, skinners iodine, mercurochrome solution (0.5%), Bismark brown (Easlick disclosing solution), and malachite green [11].

In the present study, erythrosine was used as a disclosing agent. Erythrosine has a single wavelength, and this can be easily measured using ELISA reader or any colorimetric analysis. Erythrosine is an extremely colored molecule which absorbs light nearly 500nm and emits longer wavelength. The Lamda max (λ max) of erythrosine was 525 nm, as UV spectrum of erythrosine showed its maximum absorbance at 529 nm by Ramakrishna SP 2007 [12]. Other studies by Tinsley and Chadwich in 1997 said that λ max of erythrosine to be 530 nm [13]. Based on the previous studies, wavelength used in the present study was 540 nm.

G. lucidum showed better results than chlorhexidine and Hiora mouthwash. However, there was no statistical difference between the two toothpastes. Supragingival plaque mainly consists of Gram-positive bacteria. *G. lucidum* being a mushroom extract acts mainly on the Gram-positive organisms. Kosanić and Ranković, in 2011, said that Gram-positive bacteria are more susceptible to various mushroom extracts than Gram-negative bacteria, due to the absence of lipoproteins in the cell wall [14]. Turkoglu *et al*. demonstrated that phenols present in mushroom extracts are the major carriers of antibacterial activity [15]. Karaman *et al*. reported the inhibitory activity of *Ganoderma applanatum* and *G. lucidum* and chloroform extracts against *Staphylococcus aureus*

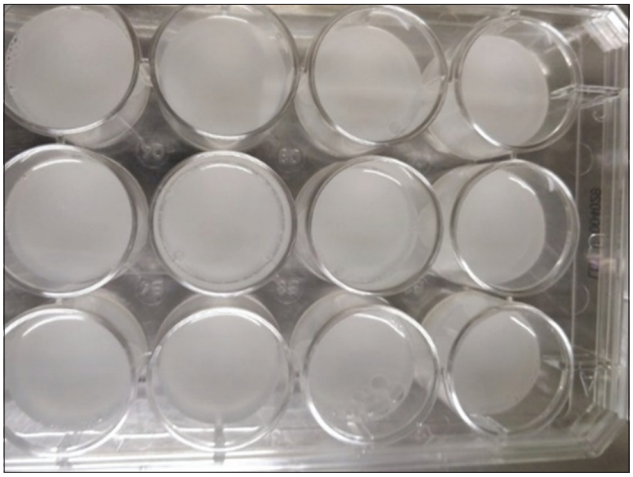

Fig. 1: 20 mL of pooled saliva in tissue culture plate (3 × 4)

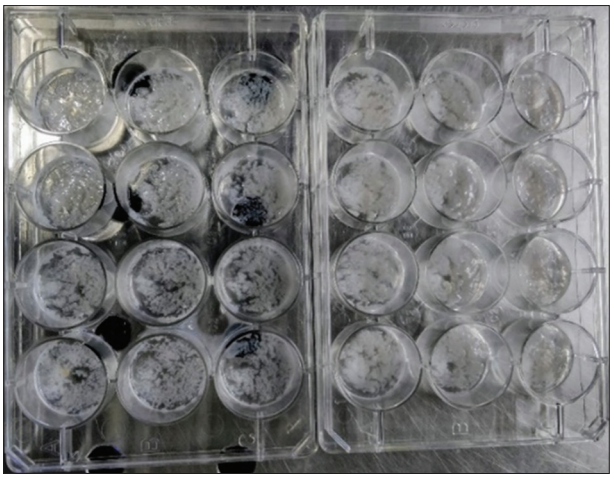

Figure 2: Plaque formed on the walls and base of the tissue culture plates after 72 h of incubation at 37°C

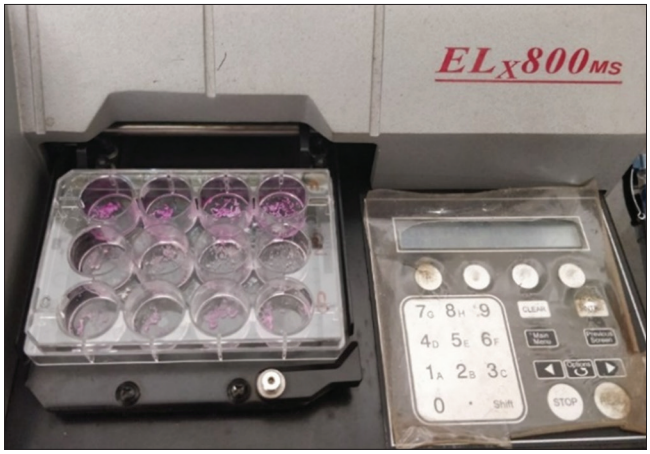

Figure 3: Culture plate after treatment kept for analysis in ELISA reader

Table 1: Comparative evaluation of antiplaque efficacy of *G. lucidium,* Himalaya complete herbal care, chlorhexidine mouthwash, Hiora mouthwash, and distilled water

Group	Mean±SD	Groups	p value 0.005	*Post hoc* test
Group 1	0.71±0.06	2	0.000	1>2,3,4,5
		3	0.000	
		4	0.000	
		5	0.000	
		6	0.03	
Group 2	0.06±0.006	1	0.000	2<4,5
		3	0.965	
		4	0.001	
		5	0.005	
		6	0.000	
Group 3	0.08±0.010	1	0.000	3<4
		2	0.965	
		4	0.003	
		5	0.027	
		6	0.000	
Group 4	0.18±0.008	1	0.000	4>2,3
		2	0.001	
		3	0.003	
		5	0.916	
		6	0.000	
Group 5	0.16±0.017	1	0.000	5>2
		2	0.005	
		3	0.027	
		4	0.916	
		6	0.000	
Group 6	0.30±0.039	1	0.003	6>2,3,4,5
		2	0.000	
		3	0.000	
		4	0.000	
		5	0.000	

G. lucidium: Ganoderma lucidium

and *Bacillus* species, and they checked for the presence of their total phenolic contents [16].

Madhumitha *et al*., in 2013 [17], studied the efficacy of Complete Care Herbal Toothpaste for its antiplaque anti gingivitis and for periodontal disease. They concluded that Complete Care Herbal Toothpaste was effective and safe to use in the prevention and management of dental plaque, and this could be attributed to antimicrobial properties of *Punica granatum*, *Zanthoxylum alatum*, *Acacia arabica*, Triphala, *Vitex negundo*, *Salvadora persica*, *Mimusops elengi*, *Trachyspermum ammi*, and *Azadirachta indica* which were present in the toothpaste.

Herbs and plant extracts have been used in oral hygiene products for many years. Hiora mouthwash is a herbal preparation, made from natural herbs with their beneficial properties such as anticariogenic and antiplaque as it contains trimethylamine, salvadorian, chlorides, high amounts of fluoride and silica, sulfur, Vitamin C; small amounts of tannins, saponins, flavonoids, and sterols. Its antibiotic property is due to the presence of piper betle and Elettaria cardamomum; and anti-inflammatory and immunity booster, due to the presence of Terminalia bellerica [18].

Chemical agents have antiseptic or antimicrobial action to inhibit supragingival plaque formation and development of gingivitis. Commonly used chemical agents include phenolic compounds, Bis-biguanides, pyrimidines, quaternary ammonium compounds, oxygenating agents, halogens and heavy metal salts [19]. Among these agents, chlorhexidine was the most effective antiseptic for plaque inhibition and prevention of gingivitis when used twice daily as mouth rinse [20]. Chlorhexidine is considered to be the gold standard as an anti-plaque agent than other antiplaque agents available in the market. Jenkins *et al*. suggested that chlorhexidine has plaque inhibitory action as it gets adsorbed on the tooth surface rather than its oral retention or initial bactericidal effect. Chlorhexidine molecule attaches to pellicle by one cation, leaving the other free to interact with bacteria attempting to colonize the tooth surface [21]. It is dicationic at pH levels above 3.5. It prevents plaque accumulation, and hence, it is an antiplaque and anti gingivitis agent [22] and reduces the adherence of *Porphyromonas gingivalis* to epithelial cells [23]. It can be bacteriostatic or bactericidal depending on the dose. The process of plaque prevention would, therefore, occur at the tooth surface itself by tooth bound chlorhexidine [24,25].

CONCLUSION

G. lucidum toothpaste has shown good results and can be considered as an effective antiplaque agent. Mechanical plaque control always remains the primary method to maintain oral health. The use of chemical plaque control adjunct to mechanical plaque control aids in improving the oral health of the individual.

AUTHORS' CONTRIBUTION

Dr. Prabhjeet Singh: Protocol preparation, ethical submissions, conducting the study, and review of the final manuscript. Dr. Meena Anand Kukkamalla: Selection of the topic, protocol preparation, ethical submissions, conducting the study, data analysis, drafting the manuscript, and review of the final manuscript. Dr. Jesil Mathew: Providing resource material and review manuscript.

CONFLICTS OF INTEREST

There is no conflict of interest in the present study. The study was self-funded.

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