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Original Article

Dentistry Section

An In vitro Study to Compare the Effect of Different Types of Tea with Chlorhexidine on *Streptococcus mutans*

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ABSTRACT

Introduction: Tea is the second most commonly consumed beverage in the world after water. The leaf and bud of the plant *Camellia sinensis* produces tea. The different forms of tea are 'non-fermented' green tea, 'semi-fermented' oolong tea and 'fermented' black tea according to the manufacturing process. *Streptococcus mutans* is the main causative organism in dental caries and plaque formation.

Aim: The present study was undertaken to determine the antibacterial effectiveness of aqueous and ethanol extracts of green tea, black tea and oolong tea against *S. mutans* in comparison with 0.2% chlorhexidine.

Materials and Methods: An in vitro study was conducted to compare the effectiveness of aqueous and ethanol extracts of green tea, black tea and oolong tea with 0.2% chlorhexidine against *S. mutans*. Chlorhexidine 0.2% commercially available as mouthwash was used as such for comparison. The antimicrobial activity was determined using agar well diffusion method. About 50 µl of the aqueous and ethanol extracts of tea and 0.2% chlorhexidine were inoculated into the wells prepared on blood agar plates smeared with *S. mutans*. The agar plates were incubated for 24 hours after which the diameter of the zone of inhibition was measured. Analysis of Variance (ANOVA) followed by Tukey's post hoc test were used for statistical analysis.

Results: The mean zone of inhibition of the aqueous extracts of green tea, black tea, oolong tea and chlorhexidine was found to be 16.33 mm, 10.33 mm, 19.66 mm and 22 mm respectively. The mean zone of inhibition of the ethanol extracts of green tea, black tea, oolong tea and chlorhexidine was found to be 14 mm, 9 mm, 20.66 mm and 22 mm respectively. The study result state that the inhibitory effect of chlorhexidine is almost similar to that of oolong tea followed by green tea and black tea.

Conclusion: From the present study, it can be concluded that the aqueous and ethanol extracts of oolong tea showed highest antimicrobial activity compared to green tea and black tea.

Keywords: *Camellia sinensis*, Dental caries, Mouthwashes, Oolong tea

INTRODUCTION

Man is turning towards the nature as natural herbal products are being increasingly used in prophylaxis and treatment of different diseases [1]. Because of its low incidence of serious adverse effects, low cost and their perceived efficacy, herbal medicine is gaining more importance [2].

Water is the most commonly consumed beverage in the world followed by tea. Consumption of tea has become part of a daily routine in many parts of the world. For more than 2000 years, tea has been known to lead the beverage list [3]. Tea, which found its origin in China, has been used since centuries for medicinal purpose [2]. *Camellia sinensis* is the plant from which tea is obtained. Tea can be found in non-fermented form as green tea, semi-fermented as oolong tea and fermented form of black tea [3].

Tea contains significantly high amounts of flavonoids. These flavonoids are well known for their antioxidant capacity as well as in impeding the free radical activity, thereby reducing the risk of coronary heart disease and decreasing Low-Density Lipoprotein (LDL) cholesterol [4]. Antioxidants are known to protect DNA and cell membranes from oxidation. Therefore more emphasis is being laid on antioxidant-rich foods in public health nutrition [4].

Dental caries is a ubiquitous multifactorial infectious disease [5]. *Streptococcus mutans* is the main causative organism in dental caries and plaque formation. In the presence of sucrose, *S. mutans* sticks onto the surfaces of the teeth, ferments the dietary sugars and results in the release of acids [1]. It also degrades the dietary carbohydrates producing lactic acid leading to localized demineralization eventually resulting in dental caries. Inspite of mechanical plaque control methods, the removal of plaque from inaccessible areas of the oral cavity remains a challenge, making the use of chemical agents mandatory.

Among the chemical plaque control agents, chlorhexidine is considered as the gold standard. Its major advantage is longer period of action, as it binds to the hard and soft tissues of the oral cavity [5].

Among Asians, green tea is more preferred while Western countries largely consume black tea. During the processing of tea leaves, the characteristic flavor components of black tea are produced by fermentation. Oolong tea undergoes less fermentation than black tea. Chinese restaurants are known for oolong tea [6]. The antioxidant, anticarcinogenic, anti-inflammatory, thermogenic, probiotic, and antimicrobial properties of green tea has been well established [1,3]. Black tea has been proven to reduce the risk of coronary heart disease and cancer owing to its antioxidant capacity [2,4]. Oolong tea too has been proved to have effects on cardiovascular disease, cancer and obesity [6]. Oolong tea has not been researched much even though its beneficial effects have been reported [3].

Therefore, this study was undertaken to determine the antimicrobial effectiveness of aqueous and ethanol extracts of green tea, black tea and oolong tea on *S. mutans* in comparison with 0.2% chlorhexidine.

MATERIALS AND METHODS

An in vitro study was conducted at the Department of Microbiology, AJ Institute of Medical Sciences, Mangalore, India, to check the antibacterial effectiveness of five samples each of the three types of tea: green tea, black tea and oolong tea against *S. mutans*.

Preparation of Culture Media

Once the freeze-dried strains of *S. mutans* (MTCC 497) was obtained from IMTECH (Institute of Microbial Technology, Chandigarh, India), it was transferred onto Brain Heart Infusion (BHI) broth. After 24 hours of incubation, *S. mutans* was smeared on blood agar plate. *S. mutans* strains were transferred into 10 ml of BHI broth for the antibacterial test after incubation at 37°C for 24 hours.

Preparation of Tea Extracts

Three different types of tea leaves of the brand, Kanan Devan Hill Plantations, Munnar, India, were used for the study, including Green tea, Black tea and Oolong tea.

Aqueous Tea Extracts

Ten grams of each tea leaf sample was weighed and added to 100 ml of boiling distilled water and further boiled for 30 minutes. The extract obtained was reduced to 10 ml over a water bath to obtain 100% concentration.

Ethanol Tea Extracts

About 5 g of each tea sample was weighed and added to 50 ml of ethanol. A mortar and pestle was used to grind the tea leaves with ethanol and the mixture was filtered using Whatman filter paper to obtain extract. The extract obtained was reduced to 5 ml over a water bath to obtain a 100 % concentrated ethanol extract of each tea.

Prior to antibacterial testing, freshly prepared tea extracts were allowed to cool down to room temperature.

Chlorhexidine gluconate 0.2 % commercially available as mouthwash was used as such for comparison and it served as a positive control.

Antibacterial Test

The antimicrobial activity was determined using agar well diffusion method. *S. mutans* strains obtained was spread evenly on blood agar plate using a sterile swab. Wells of diameter 6 mm were punched on the blood agar plate using the tip of a 1 ml micropipette. About 50 µl of the aqueous extracts of tea and 0.2 % chlorhexidine were inoculated into the wells prepared on blood agar plate smeared with *S. mutans*. The ethanol extracts of tea and 0.2 % chlorhexidine were inoculated in the same manner in a separate plate. A total of 10 plates were prepared for all the three types of tea and chlorhexidine. The agar plates were incubated for 24 hours after which the diameter of the zone of inhibition was measured in mm using a vernier calliper.

STATISTICAL ANALYSIS

The data was analyzed using SPSS software (version 17.0). Analysis of variance (ANOVA) followed by Tukey's post hoc test were used for statistical analysis. The level of significance was set at 0.05.

RESULTS

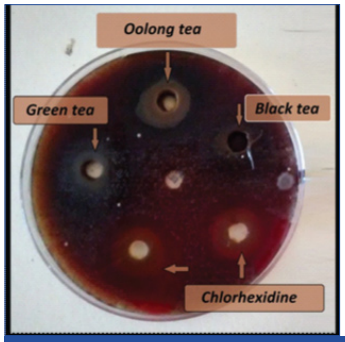
The mean zone of inhibition of the aqueous extracts of green tea, black tea and oolong tea was found to be 16.33 mm, 10.33 mm and 19.66 mm respectively. The mean zone of inhibition of the ethanol extracts of green tea, black tea and oolong tea was found to be 14 mm, 9 mm and 20.66 mm respectively. Chlorhexidine 0.2% which served as the positive control showed mean zone of inhibition of 22 mm. The data shows a statistically significant difference between aqueous extracts of tea and chlorhexidine. Similar results were shown between ethanol extracts of tea and chlorhexidine. The study results states that the inhibitory effect of chlorhexidine is almost similar to that of oolong tea followed by green tea and black tea [Table/Fig-1].

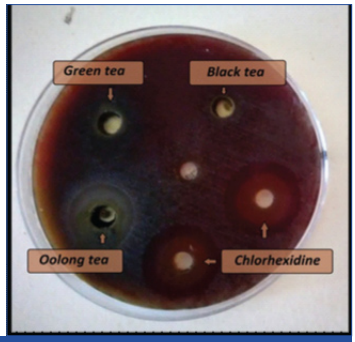
Group	Mean zone of inhibition (mm)	
	Aqueous extract	Ethanol extract
Green tea	16.33	14
Black tea	10.33	9
Oolong tea	19.66	20.66
p-value	0.001	0.02

[Table/Fig-1]: Mean zone of inhibition of *Streptococcus mutans* on different types of tea.
Results of ANOVA test

Comparison group	Aqueous extracts (p-value)	Ethanol extract (p-value)
Green tea v/s chlorhexidine	0.17	0.000
Black tea v/s chlorhexidine	0.000	0.000
Oolong tea v/s chlorhexidine	0.958	0.999
Green tea v/s black tea	0.148	0.777
Black tea v/s oolong tea	0.000	0.000
Green tea v/s oolong tea	0.187	0.003

[Table/Fig-2]: Comparison of aqueous and ethanol extracts of different types of tea.
Results of Turkey's post hoc analysis





[Table/Fig-3]: Zone of inhibition obtained with aqueous extracts of tea and 0.2 % chlorhexidine. **[Table/Fig-4]:** Zone of inhibition obtained with ethanol extracts of tea and 0.2 % chlorhexidine.

On comparison within groups, chlorhexidine was significantly better than the aqueous extract of black tea whereas there was no statistically significant difference between the mean zones of inhibition of the aqueous extracts of green tea, oolong tea and chlorhexidine. Chlorhexidine was found to be significantly better than the ethanol extracts of black tea and green tea, whereas there was no statistically significant difference between the ethanol extract of oolong tea and chlorhexidine [Table/Fig-2-4].

DISCUSSION

The present study was conducted to compare the effectiveness of aqueous and ethanol extracts of green tea, black tea and oolong tea with 0.2 % chlorhexidine on *S. mutans.* Recently, plant-derived stimulant beverages like chocolate, coffee and tea have demonstrated anticariogenic activity both in vitro and in vivo [3].

In this study, there was no statistically significant difference between inhibitory effect of chlorhexidine and aqueous extracts of oolong tea. This is in contrast to a study done by Subramaniam P et al., [3] in which it was found that compared to 0.2 % chlorhexidine, aqueous extract of oolong tea exhibited greater inhibition against *S. mutans*.

In the present study, ethanol extract of green tea and black tea showed lesser inhibition compared to the ethanol extract of oolong tea. The aqueous extracts of green and oolong tea showed

significantly greater zones of inhibition than the aqueous extract of black tea. Similar results were obtained in a study conducted by Subramaniam P et al., [3].

All the three types of tea showed an inhibitory effect on the growth of *S. mutans* in the present study. Both the extracts of oolong tea showed greater inhibitory effect compared to green tea and black tea. Similar results were shown in the study conducted by Subramaniam P et al., [3] who suggests that this may be due to the presence of greater amounts of phytochemicals in oolong tea compared to green and black tea. This is in accordance with the findings of Sasaki H et al., [7] who reported that the synergistic effect of monomeric polyphenols makes oolong tea extract bactericidal. Chatterjee P et al.,[2] states that the higher flavonoid contents of green tea is responsible for its better activity than black tea. According to Subramaniam P et al., more catechins is present in green tea, whereas black tea contains mostly polymerized catechins as it undergoes fermentation leading to its less inhibitory action against *S. mutans* [3].

The green tea catechin mouthwash and chlorhexidine mouthwash showed comparable results in plaque reduction in a single-blinded cross-over study by Arab H et al., [8].

The newer mouth rinses are studied against chlorhexidine considering it as the gold standard mouth rinse [9]. It maintains the high level due to its superior substantivity and antiplaque effect [10].It acts against *Streptococcus mutans* making it anticariogenic in nature [9]. Inspite of all its merits, chlorhexidine is known to provoke an immediate hypersensitivity reaction and exhibit cytotoxic activity against human periodontal ligament cells [11]. Chlorhexidine also causes alteration in taste sensation, erosion of the oral mucosa as well as brownish discoloration of teeth and other restorative materials [9].

LIMITATION

There were a few limitations associated with this study as a high concentration of tea extract was used, even though it is not consumed at such high concentration; efforts could have been made to check the effectiveness of tea extracts at varying lower concentrations.

Limited number of studies have investigated the antimicrobial effect of oolong tea extracts against *S. mutans* in aqueous and ethanol extracts [3,5], thus further in vivo studies are recommended to compare the effectiveness of green tea, black tea and oolong tea extracts against *S. mutans*.

CONCLUSION

It can be concluded that the aqueous and ethanol extracts of oolong tea showed higher antimicrobial activity compared to green tea and black tea. Since people all over the world drink tea mostly on a daily basis, promotion of oolong tea might bring down the prevalence of caries and also it might be used in the form of mouthwashes.

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