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Antibacterial Activity of Green Tea and Black Tea on *Streptococcus mutans*: An in Vitro Study

Abstract

Background: *Camellia sinensis (*green tea & black tea) has been shown to possess antimicrobial properties and also, its consumption is associated to lower incidences of various pathological conditions, including cardiovascular disease, strokes, obesity, diabetes, inflammatory conditions and aging process. However there is paucity of literature regarding its inhibitory effect on *Streptococcus mutans.* **Materials and methods:** An in-vitro experimental study was conducted using ethanolic extract of Green and Black tea. The extract of each was then diluted with an inert solvent, Dimethyl Formamide, to obtain 4 different concentrations (100mg/ml, 200mg/ml, 300mg/ml, and 400mg/ml) of each at 10,20,30,50 & 75 microlitres. 0.2% chlorhexidine mouthwash was used as a positive control and dimethyl formamide was used as negative control. The different extracts, along with controls, were then subjected to microbiological investigation. **Results:** Green and Black tea extracts presented the largest zone of inhibition 14.4 and 20.4mm respectively at the concentration of 400mg/ml. **Conclusion**: The comparison between Green tea and Black tea extracts with Chlorhexidine showed significant results while the mean zone of inhibition of Black tea extract was found to be highly significant than that for Green tea suggesting higher anti microbial activity of Black tea ($p = 0.008$) than Green tea ($p = 0.017$).

Key Words

Green tea; black tea; in vitro, zone of inhibition

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INTRODUCTION

Tea is an infusion of flavorful leaves that has been consumed for centuries as a beverage and is valued for its medicinal properties.[1] Being rich in natural antioxidants, tea is reported to be used in the management of various systemic conditions like colon, esophageal, and lung cancers.[2] Tea contains elements, such as polyphenols, which showed promising antibacterial properties against cariogenic bacteria, especially S. *mutans.* Many *in vitro* and *in vivo* effects of tea polyphenols have been reported including antioxidant, anticarcinogenic and hypolipidemic properties.[2] *Streptococcus mutans* is considered as the major etiological pathogen of dental caries. It possesses a variety of mechanisms to colonize tooth surfaces. Its one of the bacteria in dental plaque that produces organic acids which causes enamel demineralization.[3] Tea is of three different types; namely, non fermented (green tea), semi-fermented (Oolong tea) and fermented type (black tea). The anti *Streptococcus mutans* effect of nonfermented and semi-fermented types of tea have been reported.[3] Tannic acid, found in tea phenols, is an important inhibitor of bacterial growth and glucosiltransferase activity.[4] Moreover, tea components have been shown to increase acid resistance to tooth enamel.[5] Thorough exploration of the available literature revealed that not many studies have been conducted on fermented and non-fermented tea as an antibacterial agent. Hence the present study was conducted to evaluate the antibacterial effect of green tea and black tea on *Streptococcus mutans.*

Table 1(a): Zones of Inhibition of different concentration of Green tea extracts

Concentration	Zone of inhibition (mm)					Mean ±SD
	10 µl	20 µl	30 µl	50 µl	75 µl	
100mg/ml	R	R	R	R	11mm	2.2 ± 4.9193
200mg/ml	R	R	R	R	13mm	2.6 ± 5.8137
300mg/ml	R	R	14mm	14mm	16mm	8.8 ± 8.0746
400mg/ml	R	16mm	16mm	18mm	22mm	14.4 ± 8.4142

Table 1(b): Zones of Inhibition of different concentration of Black tea extracts

Concentration	Zone of inhibition (mm)					Mean ±SD
	10 µl	20 µl	30 µl	50 µl	75 µl	
100mg/ml	R	R	R	13mm	14mm	5.4 ± 7.4027
200mg/ml	R	R	R	16mm	16mm	6.4 ± 8.7635
300mg/ml	R	17mm	17mm	19mm	21mm	14.8 ± 8.4380
400mg/ml	18mm	18mm	20mm	22mm	24mm	20.4 ± 2.6076

Table 2: Zones of Inhibition of Controls

CONTROLS	Zone of inhibition (mm)					Mean ±SD
	10 µl	20 µl	30 µl	50 µl	75 µl	
0.2% Chlorhexidine Mouthwash	18mm	20mm	20mm	22mm	24mm	20.8 ± 2.2803
Dimethylformamide	R	R	R	R	R	-

MATERIALS AND METHODS

Procurement of Tea Leaves

Tea leaves manufactured in Darjeeling were obtained from the local market.

Preparation of Green Tea and Black Tea Extract

The tea leaves were dried and powdered with an electric grinder. Obtained powder was stored in a desiccator. 100 gm of tea powder each of green and black tea were stored in a round bottom flask (no.72) and refluxed with 100% ethyl alcohol. It was then subjected to filtration with whatman filter paper to obtain a clear filtrate. The filtrate so obtained was reduced in a borosilicate glass beaker at a low temperature of less than 40 degree celcius with the help of a Soxhlet Extraction Unit (heating mantle) MSW- 436 of MAC (Macro Scientific Works Limited), to obtain semi solid residue of tea extract. From 100gm of green tea and black tea powder dissolved in 500ml of ethanol, 5gm residual of tea extract was obtained of concentration of 20% w/w.[3] 4gm of residual extract was dissolved in 10ml of Dimethylformamide to counteract the effect of ethanol on *Streptococcus mutans* and to obtain a concentration of 400mg/ml (40%). 1ml of this extract was taken in a sterilized test tube and labeled as 40%. The remaining 9ml of extract was further dissolved by Dimethylformamide to obtain different concentration of 100mg/ml, 200mg/ml and 300mg/ml respectively.[6]

Controls

0.2% of chlorhexidine mouthwash which is considered as gold standard was used a positive control.

Procurement of Microorganisms

MTCC strain No 497 was obtained from Microbial Type Culture Collection (MTCC) and Gene Bank, Chandigarh. The strain belonged to genus Streptococcus while the species was mutans. i.e., *Streptococcus mutans*, was used for the study purpose.

Preparation of Culture Media

The Brain Heart Infusion agar powder (Special Infusion Agar) for in vitro diagnostics, M211, was obtained from HiMedia Laboratories Limited, Mumbai. Fifty grams of this powder was suspended in 1000ml of distilled water. It was then boiled to dissolve the medium completely and then sterilized by autoclaving at 15 lbs pressure and 121 degree Celsius for 15 minutes. The pH of the agar was maintained at 7.4 at 25 degree Celcius. The media was then mixed well and poured into petri-dishes. The process of making culture media was carried out as per the instructions provided by the manufacturer. *Streptococcus mutans* MTCC was then added to nutrient broth which was incubated at 37^0 C for 24 hours. It was sub-cultured onto nutrient agar plate and incubated at 37^0 C for 24 hours. The inoculum for antimicrobial activity was prepared by adjusting the density of organism to approximately

Table 3: Comparison of mean of Zones of Inhibition of different concentration of Green and Black tea extracts with 0.2% Chlorhexidine mouthwash (positive control)

Statistical Procedure	Mean of Zone of Inhibition of Green Tea extract 0.2% Chlorhexidine Mouthwash (positive control)	Mean of Zone of Inhibition of Black Tea extract with 0.2% Chlorhexidine Mouthwash (positive control)
Mean ± standard deviation	-13.80000 ± 5.78504	-9.05000 ± 7.14306
t-value	-4.771	-2.534
p-value	.017	0.008
Statistical significance	Significant	Highly Significant

10^8 colony forming units/ml with the help of 0.5 Mcfurland opacity standards. Then it was inoculated on agar plate by lawn culture method. The growth conditions were aerobic as specified by Gene bank, Chandigarh.[3]

RESULTS

Zone of inhibitions were measured from the edge of the punched hole (ditch) to outer border of bacterial inhibition (translucent area) at four different randomly selected perpendicular places. The zones of inhibition were measured with the help of HiAntibiotic Zone scale from HiMedia Laboratories Limited, Mumbai. These zones of inhibitions were measured after 24 and 48 hours but no difference was observed. Table 1(a) shows zone of inhibition of various concentrations of ethanolic extracts of Green tea. The zones observed for Green tea extract at 100mg/ml, 200mg/ml, 300mg/ml and 400mg/ml were 2.2 ± 4.9193, 2.6 ± 5.8137, 8.8 ± 8.0746 and 14.4 ± 8.4142 respectively. Green tea at lower concentration ie 100mg/ml exhibited a minimum zone of inhibition of 11 mm which was achieved at a high volume of 75 µl. At lower volumes, *Streptococcus mutans* was resistant to the action of Green tea extract. A maximum zone of inhibition of 22 mm was observed at a concentration of 400mg/ml when subjected to a volume of 75 µl. Table 1(b) shows zone of inhibition of various concentrations of ethanolic extracts of Black tea.

The zones observed for Black tea extract at 100mg/ml, 2000mg/ml, 300mg/ml and 400mg/ml were 5.4 ± 7.4027, 6.4 ± 8.7635, 14.8 ± 8.4380 and 20.4 ± 2.6076 respectively. Black tea at lower concentration ie 100mg/ml exhibited a minimum zone of inhibition of 13 mm which was achieved at a volume of 50 µl. At lower volumes, *Streptococcus mutans* was resistant to the action of Black tea extract. A maximum zone of inhibition of 24 mm was observed at a concentration of 400mg/ml when subjected to a volume of 75 µl. Table 2 shows zone of inhibition of the positive and negative controls. Zone of inhibition observed for 0.2% Chlorhexidine mouthwash (positive control) 20.8 ± 2.2803 but was absent in case of Dimethylformamide suggesting that *Streptococcus mutans* were resistant to its action (negative control). Table 3 shows comparison of mean of zones of inhibition of different concentrations of green and black tea with 0.2% chlorhexidine mouthwash (positive control). The mean zone of inhibition for green tea with 0.2% chlorhexidine mouthwash was at -13.80000 ± 5.78504 while the mean zone of inhibition for black tea with 0.2% chorhexidine was -9.05000 ± 7.14306. The comparison of mean zone of inhibition for green and black tea with 0.2% chlorhexidine mouthwash were found to be significant (p=0.017) and (p=0.008) respectively.

DISCUSSION

Dental caries is one of the most common diseases in humans. It is an infectious multifactorial microbiologic disease of the teeth that results in localized dissolution and destruction of the calcified tissue. A wide group of microorganisms are identified from carious lesions of which *streptococcus mutans* and *lactobacillus* are the main pathogenic species involved in the initiation and development of dental caries. Many studies suggested that *mutans streptococci, Lactobacillus* and salivary buffering capacity are important risk factors for dental caries.[4] *Streptococcus mutans* is considered as the major etiological pathogen of dental caries. It was classified into 3 serotypes C, E and F due to the different compositions of the serotype-specific polysaccharides. These bacteria in presence of surface-adsorbed salivary amylase, sucrose and starch can produce bacterial enzymes such as glucosyltransferases, and fructosyltransferase that synthesize water-insoluble and soluble linked glucans from sucrose. They adhere on the tooth surface with other oral bacteria. Consequently, the adhesion of glucan brings about the formation of dental plaque. Furthermore, these bacteria in dental plaque produce organic acids which cause the enamel demineralization.[4] In the last few years, an increased attention has been focused on the natural plant extracts, especially those containing phenolic compounds with antimicrobial and antioxidant properties.[9] Tea,

which originated in China, has conquered the world's taste over the last 2000 years. It is obtained from the leaf and bud of the plant Camellia sinensis.[10,11] Depending on the manufacturing process, tea can be 'non-fermented' green tea, 'semifermented' oolong tea, or 'fermented' black tea. Consumption of tea is a daily routine in many parts of the world.[12] The per capita mean consumption of tea in the world has been reported to be 120 ml/day.[13] In India, the per capita consumption of tea annually is 706 gm.[14] The chemical composition of tea is complex; tea contains polyphenols, alkaloids (caffeine, theophylline, and theobromine), amino acids, carbohydrates, proteins, chlorophyll, volatile compounds, minerals, trace elements, and other unidentified compounds. Among these, polyphenols constitute the most interesting group and are the main bioactive molecules in tea and, in consequence, tea can be considered an important dietary source of polyphenols, particularly flavonoids.[12] Subramaniam P *et al*., clearly indicates that the conformational changes due to polymerization of catechins can critically affect the inhibitory action of the glucosyltransferases on *Streptococcus mutans*. It indicated that the both aqueous and organic extracts of oolong tea showed greatest zones of inhibition, followed by green tea and black tea.[12] Several authors reported that the green tea contains catechin and polyphenols. These compounds have been found to possess antibacterial and antiviral action as well as anticarcinogenic and antimutagenic properties.[1] Green tea flavonoids have well-established in vitro antibacterial, antimicrobial, antiviral, antifungal activities against a variety of infectious agents, e.g., *Staphylococcus aureus*, *Vibrio cholerae*, *Escherichia coli*, *Helicobacter pylori*, *Legionella pneumophila*, influenza virus, HIV type 1, *Herpes simplex virus*.[15,16] Chung *et al*., and Peter *et al*., demonstrated *in vitro* microbial studies that high consumption of tea (3-4 cups/day) exhibited caries resistances properties. This is due to their high contents of fluoride and polyphenolic catechin components.[1] The results of the present study shows that green tea and black tea at 100mg/ml, 200mg/ml, 300mg/ml and 400 mg/ml concentrations have antibacterial activity on *Streptococcus mutans*. The inhibitory effect of black tea on Streptococcus mutans was higher than that of green tea. This finding is in agreement with the previous studies about Chinese, Iranian and Japanese green and black tea. On the contrary, Rasheed A, Haider M (1998), concluded that the antibacterial activity of black tea is not extensive. They suggested that since black tea is prepared from green tea leaves, as a result of fermentation, the antibacterial activity of black tea changes. The results of the present study strongly suggests that certain components of tea exert a significant anticariogenic effect by virtue of their inhibitory activity against *S. mutans*.

RECOMMENDATIONS

1. Further studies can be conducted using different concentrations of green tea and black tea in vivo.
2. Further studies can be conducted using different tea types on various transient bacteria.
3. Tea extracts can possibly be incorporated into chewing gums, toothpastes, mouthwashes, and dental floss for its preventive actions.

CONCLUSION

The comparison between Green tea and Black tea extracts with 0.2% chlorhexidine mouthwash showed significant results while the mean zone of inhibition of Black tea extract was found to be highly significant than that for Green tea suggesting higher anti microbial activity of Black tea than Green tea .

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