

Antibacterial Activity of Iranian Green and Black Tea on *Streptococcus Mutans* : An In Vitro Study

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Abstract:

Objective: Dental caries is a common infectious disease. *Streptococcus mutans* is the prevalent decay microorganism. The anti *Streptococcus mutans* activity of non fermented and semi-fermented tea has been shown. The aim of this study was to determine the anti *Streptococcus mutans* activity of Iranian green and black tea (non fermented and fermented type).

Materials and Methods: The study was experimental. The aerial parts of wild-growing *Camellia sinensis* were collected from Lahijan province. The methanolic extract of green and black tea were examined on *Streptococcus mutans* (ATCC3566). Five different concentrations (50mg/ml, 100mg/ml, 200mg/ml, 300mg/ml and 400 mg/ml) of tea extracts were tested using the well assay method. The agar dilution method recommended by the NCCLS standards (National Committee for Clinical Laboratory Standards) was used. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of extract inhibiting visible growth of the organism on the agar media plate. Minimum bactericidal concentration (MBC) was detected from MIC.

Results: The Iranian green and black tea had an antibacterial effect on 100 to 400 mg/ml concentrations. The minimum inhibitory concentration of green and black tea was 150 and 50 mg/ml, respectively. The mean diameter of inhibition zone were 9.5 mm and 10.9 mm for methanolic extract of green and black tea, respectively.

Conclusion: Both Iranian non fermented (green tea) and fermented (black tea) have anti *Streptococcus mutans* activity in vitro. The anti *Streptococcus mutans* activity of black tea appears on a lower concentration than green tea.

Key Words: Green Tea; Black Tea; Antibacterial Effect; *Streptococcus Mutans*

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INTRODUCTION

Dental caries is a common infectious disease. Some different types of *Streptococcus* are known as caries producing microorganisms. *Streptococcus mutans* is the most important

tooth decay causing microorganism [1]. It has been shown that tea has antibacterial effects [2-4]. For the first time, in 1989, it was demonstrated that Japanese green tea has an inhibition effect on *Streptococcus mutans* [5]. The

inhibitory effect of Japanese and Chinese green tea on caries has been confirmed in other reports [6-7].

Tea has three different types; namely, non fermented (green tea), semi-fermented (Oolong tea) and fermented type (black tea) [2]. The anti *Streptococcus mutans* effects of non-fermented and semi-fermented types of tea have been reported [8-11]. In spite of several different studies on anti *Streptococcus mutans* effects of non-fermented and semi-fermented teas, little is known about the fermented type. Although black tea is a common daily beverage in Iran, our information about its anti *Streptococcus mutans* activity is not sufficient. The aim of this study was to determine the anti *Streptococcus mutans* effects of Iranian green and black tea.

MATERIALS AND METHODS

The aerial parts of wild-growing *Camellia sinensis* were collected from Lahijan province, north of Iran at an altitude of 220 m. Samples were pure without any aromatic or additive materials. Freshly dried leaves were ground into fine powder with an electric grinder. Obtained powder was stored in desiccators. 100 gm powdered plant was stored in a round bottom flask (no.72) and refluxed with 95% methyl alcohol (Merck, Germany). Watman No.1 paper with 150µm diameter was used for liquor filtration. The methanol soluble fraction was evaporated under reduced pressure to obtain methanol extract. Dried plant extract was stored in 4°C. The bacteria were obtained from the bacterial stock, Department of Microbiology, Medical Faculty, Shahed University.

Table 1. Diameter of Inhibition Zones Induced by Methanolic Extract of Iranian Green and Black Tea in Three Examinations

Tea		Concentraons					
		50mg/ml	100 mg/ml	150 mg/ml	200 mg/ml	300 mg/ml	400 mg/ml
First Examination	Methanolic extract of green tea	-	-	10mm	13 mm	15 mm	18 mm
	Methanolic extract of black tea	-	9 mm	10 mm	13 mm	16 mm	18 mm
Second Examination	Methanolic extract of green tea	-	-	10 mm	12 mm	15 mm	18 mm
	Methanolic extract of black tea	-	9 mm	10 mm	13 mm	16 mm	21 mm
Third Examination	Methanolic extract of green tea	-	-	10 mm	15 mm	16 mm	19 mm
	Methanolic extract of black tea	-	-	10 mm	14 mm	17 mm	21 mm

Extracts were screened against *Streptococcus mutans* (ATCC35668) at different concentrations of 50 mg/ml, 100 mg/ml, 200 mg/ml, 300 mg/ml to 400mg/ml using well diffusion method. Blood agar plates (Merck, Germany) were inoculated using a sterile swab dipped into culture in columns adjusted to 1.5×10^8 bacteria/ml (0.5 Mc Farland turbidity standards). Camellia sinensis extracts were placed on the blood agar plate's media surface. Plates were kept at room temperature for 30 minutes and then microorganisms were incubated under aerobic conditions at 37°C. After 24 h incubation time, the zones of inhibition were measured using an antibiotic zone reader. The MIC (Minimum Inhibitory Concentration) was determined as the lowest concentration of extract inhibiting visible growth of organism

on the agar plates. MIC was determined by inhibition of bacterial

growth in the plates containing test extract . It was measured by comparison with growth in last plate. MBC (Minimum Bactericidal Concentration) was detected from MIC range.

At Sterile condition, 1 ml of incubated solution with determined MIC was cultured and saved at 37°C for 24 hours. The first concentration which had no bacterial growth was considered as MBC.

The agar dilution method recommended by the NCCLS standards (National Committee for Clinical Laboratory Standards) was used [12]. Each examination was tested three times.

Penicillin disc was used as the positive control. The inhibition zone diameter of penicillin disc was 28 mm.

Table 2. MIC Induced by Methanolic Extract of Iranian Green and Black Tea in Three Examinations

Tea		Cconcentrations					
		50mg/ml	100 mg/ml	150 mg/ml	200 mg/ml	300 mg/ml	400 mg/ml
First Examination	Methanolic extract of green tea	+	+	+	-	-	-
	Methanolic extract of black tea	+	-	-	-	-	-
Second Examination	Methanolic extract of green tea	+	+	-	-	-	-
	Methanolic extract of black tea	+	-	-	-	-	-
Third Examination	Methanolic extract of green tea	+	+	+	-	-	-
	Methanolic extract of black tea	+	-	-	-	-	-

The Iranian green and black tea had an antibacterial effect on *Streptococcus mutans* growth in 100 to 400 mg/ml concentration.

The mean diameter of inhibition zone was 9.5 mm and 10.9 mm for methanolic extract of green and black teas, respectively. The inhibition zone diameter in three examinations is summarized in Table 1. The minimum inhibitory concentrations of Iranian green and black tea were 150 mg/ml and 50 mg/ml, respectively. The minimum bactericidal concentration of Iranian green and black teas were achieved as 400 mg/ml and 200 mg/ml, respectively.

The MIC of methanolic extracts of green and black teas are summarized in Table 2.

DISCUSSION

The results of the present study show that Iranian green tea in 150, 200, 300 and 400 mg/ml concentrations and Iranian black tea in 100, 150, 200, 300 and 400 mg/ml concentrations have antibacterial activity on *Streptococcus mutans*. The inhibitory effect of black tea on *Streptococcus mutans* was in lower concentrations than green tea. Tea is a herbal substance with anti caries effect; the inhibitory effect of green tea on decay has been reported [4-7-11-13]. The result of this study shows that Iranian green tea has an inhibitory effect on *Streptococcus mutans*. This finding is in agreement with the previous studies about Chinese and Japanese green tea [6-9-10].

In spite of different in vivo and in vitro studies on the antibacterial effects of green tea, our knowledge about black tea is not sufficient. Linke and Legeros suggest that even with sugar consumption in the diet, black tea can decrease caries formation [14]. Touyz and Amsel have demonstrated that two weeks use of black tea has an anticariogenic effect on 18 day mice [15]. Based on our findings, Iranian black tea has an anti *Streptococcus mutans* activity. This finding is in agreement with Hamdi et al's study. They found that black tea has more preventive effects on biofilm formation than

green tea. In this study, the anti *Streptococcus mutans* activity of black tea was more than green tea [16]. On the contrary, Rasheed and Haider 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 [17].

It has been suggested that the volatile components of semi fermented tea are the cause of higher anticaries effect of this tea compared with the non fermented type [18].

The polyphenols, catechins, gallic acid and the aflavins are introduced as antibacterial agents of tea [8].

Although black and green tea both contain similar amount of flavonoids, their chemical structure is different. Green tea contains more catechins (simple flavonoids). Leaf oxidation during black tea processing converts these simple flavonoids to the aflavins and the arubigins [2].

The catechin content depends on the leaf process before tea drying, the geographic location of the farm, soil, climate and the type of tea such as blended or decaffeinated [2]. It seems that the anti *Streptococcus mutans* activity depends on the type of tea.

We used pure green and black tea leaves without any aromatic or additive materials. Similar to Hamdi et al's study, the tea leaves were selected from Lahijan province.

Considering the same material that was used in both studies, it seems that the antibacterial effect of Iranian black tea is more than green tea. Since the chemical content of tea depends on factors such as the processing method, the geographic location of the farm, the soil and the climate, we contributed this difference to geographical variation.

This study was performed on standard microorganisms. Further studies on green and black tea effects on mouth derived *Streptococcus mutans* are necessary.

CONCLUSION

Iranian green and black tea have anti *Streptococcus mutans* effects in vitro. The anti *Streptococcus mutans* activity of Iranian black tea is more than green tea.

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