

The antibacterial activity of green coffee and Arabica coffee extracts on cariogenic *Streptococcus mutans* isolated from dental caries: An in vitro study.

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Abstract:

Background: different plant derived substances are widely used in the prevention of dental caries through their activity on *Streptococcus mutans* which is considered to be major cariogenic bacteria.

Aim of the study: The aim of this study was to evaluate the antimicrobial activity of green coffee and Arabica coffee on *Streptococcus mutans*.

Materials and methods: Agar well diffusion method with measurement of inhibition zones of *S. mutans* were used to assess the antibacterial effect of different concentrations of green coffee and Arabica coffee with or without sugar. *Streptococcus mutans* bacteria were isolated from dental caries in patients consulted the central dental clinics in Basrah (Al Shaheed Qies center) for treatment.

Results: Without sugar Green coffee showed greater inhibitory effect on *S. mutans* than Arabica coffee. The minimum inhibition zone (0.5cm) was recorded at concentration of (1.5g/100ml) in green coffee compared to (0.5cm) in Arabica coffee that was obtained only at a higher concentration of (3.5g/100ml) .When sugar was added the antibacterial effect of both Green and Arabica coffee declined significantly leading to minimum inhibition zones of (0.7cm) and (0.2cm) at a concentration of (3.5g/100ml) for green coffee and Arabica coffee respectively. Boiling of both types of coffee showed no significant alteration in their antibacterial activity as compared to non-boiled preparations.

Conclusion: consumption of green coffee without adding sugar might be a promising adjunct in the prevention of dental caries owing to its antimicrobial activity against *S. mutans* bacteria.

Key word: Green coffee, Arabica coffee, *Streptococcus mutans*, Dental caries

التأثير ضد الجرثومي لخلصات القهوة الخضراء والقهوة العربية على جرثومة *Streptococcus mutans* كعامل رئيسي للتسوس المعزولة من حالات تسوس الأسنان: دراسة مختبرية .

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الخلاصة:

تهدف هذه الدراسة الى تقييم الفعالية ضد الجرثومية للقهوة الخضراء والقهوة العربية على جرثومة *Streptococcus mutans* كمسبب رئيسي لتسوس الأسنان .

المواد وطرائق العمل: استخدمت طريقة الانتشار بالأغار وقياس مناطق التثبيط على جرثومة *S. mutans* لتقييم الفعالية ضد الجرثومية بتركيز مختلفة للقهوة الخضراء والقهوة العربية بأضافة او عدم اضافة السكر .

النتائج: اظهرت هذه الدراسة ان عدم اضافة السكر للقهوة الخضراء له تأثير تثبيطي أعلى على جرثومة *S. mutans* المسببة من القهوة العربية. حيث كانت اقل منطقة تثبيط (0,5 سم) عند التركيز (1,5 غم/100مل) للقهوة الخضراء بينما كانت منطقة التثبيط (0,5 سم) عند تركيز اعلى (3,5 غم/100مل) للقهوة العربية. اما عند اضافة السكر فإن التأثير التثبيطي للقهوة الخضراء والعربية تتاقت بدرجة ملحوظة حيث كانت اقل منطقة التثبيط (0,7 سم) و(0,2 سم) عند التركيز (3,5 غم/100مل) للقهوة الخضراء والعربية على التوالي. وعند غلي كلا النوعين من القهوة سواء الخضراء او العربية لم يظهر اي تغيير واضح في الفعالية ضد الجرثومية عند مقارنتها مع حالة عدم الغلي لكلا النوعين من القهوة .

الاستنتاجات: نستنتج من الدراسة الحالية ان استهلاك القهوة الخضراء بدون اضافة السكر ربما يعزز او يساعد في تجنب حدوث تسوس الاسنان بسبب الفعالية ضد الجرثومية للقهوة الخضراء على جرثومة *S. mutans* المسببة.

Introduction

Dental caries is a worldwide health problem that affects people of different ethnicities and ages. It is considered as an infectious disease and results from interactions between different factors namely oral flora, the teeth and dietary habits. Carbohydrates in the diet, both mono- and disaccharides are absorbed into the dental biofilm, and because of the presence of the microorganisms in high concentrations there, they are broken down into organic acids. Acid production by the cariogenic bacteria through this carbohydrates metabolism will result in reduction of pH in the oral environment leading to demineralization of tooth surfaces leading to dental caries (Takahashi and Nyvad, 2008).

Although different types of bacteria are responsible for dental caries, the major cariogenic bacteria are the mutans Streptococcal group represented by *S. mutans* (Brandão *et al.* 2007; Liu *et al.*, 2011). Bacterial adherence to tooth surface is considered to be a key point in the development of dental caries and thus interference on this mechanism can play a great role in the prevention of the carious process (Oliveira *et al.*, 2007).

Oral hygiene measures and mouth rinses are well known methods that fight dental caries. Chlorhexidine mouth rinse is considered the gold standard due to its antibacterial activity against cariogenic microorganisms, and thus decreasing the microorganisms count in the oral cavity that leads to reduction in the production of acids which destroy the tooth structure. However, chlorhexidine and other

chemical mouth rinses like fluoride are not without side effects (Sundas and Rao, 2011; Tehrani and *et al.*, 2011). In recent years, much attention was paid to alternative medicine preventing many human disorders remembering that most agents used by humans in the treatment of diseases are of plant origin although little is known about their mode of actions (Asokan *et al.*, 2009). This might be applied in the field of treatment of bacterial diseases with the problem that many microorganisms have developed resistance to many of the currently used chemotherapeutic agents due to indiscriminate use of antibacterial agents throughout world.

One of the promising solutions to combat these bacterial antimicrobial resistances is through the extraction and utilization of plant derived substances. The benefits of mouth washes derived from natural substances such as garlic , lime, green tea, alum have long been studied and used for their therapeutic properties to avoid complications of chemically derived mouth washes (Ankri and Mirelmon, 1999); (Dwhe-Ureghe *et al.*, 2010); (Hamilton-Miller, 2011); (Tehrani *et al.*, 2011); (Kukreja and Dodwad, 2012). The aim of this study was to in vitro evaluation of the antibacterial effect of green coffee and Arabica coffee on the growth of *Streptococcus mutans* bacteria isolated from patients with dental caries.

Materials and methods

Sources of coffee and bacteria

Green coffee powder was obtained from India markets whereas Arabica coffee was obtained from local markets in Basrah. *Streptococcus mutans* bacteria were isolated from dental caries in patients consulted the central dental clinics in Basrah (Al Shaheed Qies center) for treatment. The identification of these bacteria was carried on according to (Friedrich, J. 1981) in the laboratory of Microbiology department of Basrah Dentistry College.

Preparation of coffee solutions:

Different concentrations of Green coffee powder were prepared through mixing (0.5, 1.5, 2.5, 3.5, 4.5 and 5) grams of the powder in 100 ml of distilled water for each. The solutions were left at room temperature for about one hour after which, the fluid part from each concentration was used for the study. The same procedure was repeated for the Arabica coffee powder (Alade and Irobi, 1993).

A second set of solutions for both green coffee and Arabica coffee of exactly the same concentrations as used in the preparation of the previous solutions were arranged but with adding a tea spoonful amount of table sugar to each tube. After mixing with sugar, the solutions were left at room temperature for an hour after which the liquid part was used in the study.

A third set of solutions for both Green coffee and Arabica coffee of the same concentrations as used previously were prepared but with boiling for five minutes and the fluid part from each concentration was utilized.

Testing inhibitory effect of coffee solutions in different concentration on *S.mutans*:

Agar well diffusion method (Perez *et al.*, 1990) was utilized to assess the effect of Green coffee and Arabica coffee solutions on *S.mutans*. Bacterial suspension of *S.mutans* was prepared and made to 0.1 optical densities in spectrophotometer. Muller Hinton agar plates were inoculated with 0.1 ml of bacterial suspension after spreading the inoculums on the surface of the medium. Six holes were made on the medium surface and to each hole on separate plates ,0.1 ml of each concentration from the three sets (plain, sugar mixed and boiled solutions) of both Green coffee and Arabica coffee was added. The plates were then incubated for 24 hours at 37°C. The results of

antibacterial activity of the solutions were then recorded.

Results

Both green coffee and Arabica coffee solutions showed inhibitory action on the growth of *S. mutans* but at varying degrees depending mainly on concentration of the materials in the solutions and the method of preparation of the solution. In general green coffee resulted in greater antibacterial activity against *S. mutans* compared to Arabica coffee at comparable concentrations of the solution regardless the method of preparation used in the study.

For plain green coffee, the maximum inhibition zone on *S. mutans* growth was (2 cm) at concentration of (5.5 g/100 ml) whereas the minimum inhibition zone (0.5cm) was recorded at concentration of (1.5g/100 ml) .With addition of sugar the maximum inhibition zone obtained was (1.5 cm) at concentration of (5.5g/100ml) while the minimum inhibition zone (0.3cm) was at concentration of (2.5g/100ml) concentration as shown in table(1).

Table (1): The antibacterial activity of green coffee on *S. mutans* using different method of preparation.

Concentration gram/100ml	Inhibition zone of <i>S.mutans</i> (cm)		
	Plain solution	Sugar added solution	Boiled plain solution
0.5	0	0	0
1.5	0.5	0	0.5
2.5	1	0.3	1
3.5	1.3	0.7	1.3
4.5	1.8	1.1	1.8
5.5	2	1.5	2

For Arabica coffee, it was found that the maximum inhibition zone on bacterial growth using plain solution was (1.2 cm) at concentration of (5.5 g/ 100 ml) while minimum inhibition zone of 0.5 cm was reported at concentration of (3.5g/100ml).

When sugar was added to Arabica coffee, the maximum inhibition zone was (0.9cm) at a concentration of (5.5g/100ml) and the minimum inhibition zone was (0.2cm) at a concentration of (3.5g/100ml) as shown in table (2).

Boiling of green coffee and Arabica coffee solutions showed no change in their antibacterial activities on *S. mutans*. The results are the same as for non boiled solutions for both substances.

Table (2): The antibacterial activity of Arabica coffee on *S.mutans* using different methods of preparation.

Concentration gram/100ml	Inhibition zone of <i>S.mutans</i> (cm)		
	Plain solution	Sugar added solution	Boiled plain solution
0.5	0	0	0
1.5	0	0	0
2.5	0	0	0
3.5	0.5	0.2	0.5
4.5	0.8	0.5	0.8
5.5	1.2	0.9	1.2

Discussion

The use of herbal derived remedies in the treatment of dental caries is an ongoing issue. More and more research is needed in order to obtain such a substance that possesses a wide range of pharmaceutical properties and at the same time lacking adverse side effects that are frequently encountered in systemic medications (Walker, 1996).

Green coffee bean extract, in particular, has received special attention from researchers since it shows, due to presence of some substances in it, important antimicrobial activity against many gram positive and negative bacteria (Pane *et al.*, 2012).

Volatile and non volatile organic acids (Chlorogenic acid-CGA-and caffeic acid) together with phenols and aromatic compounds are the components of green coffee that were shown to have antibacterial effects against pathogenic bacteria (Fardiaz, 1995).

The proposed antibacterial activity of caffeine in green coffee is attributed to its ability to pass easily through bacterial cell wall and then starts to inhibit bacterial DNA synthesis leading to inhibition of bacterial enzyme protein synthesis and thus slowing the activity of all bacterial cells (Nonthakaew, A. *et al.*;2015).

The current study showed that both green coffee and Arabica coffee solutions showed inhibitory action on *S.mutans* growth .The extent of inhibition increased as the concentration of the material in the solution was increased. The mode of preparation of the coffee in the study also showed difference in the magnitude of its antibacterial activity. Boiling showed no any significant change in the antibacterial activity for both Green coffee and Arabica coffee while adding sugar during preparation resulted in a recognizable decrease in the antibacterial activity for both substances.

Several studies are in agreement with current study reporting the inhibitory action of green coffee on the growth of different bacterial species. Fardiaz S. (1995)

reported that roasted green coffee beans inhibited the growth of gram positive bacteria like *Staphylococcus aureus* ,*Bacillus cereus*, *Lactobacillus bulgaricus* , *Streptococcus lactis* and *Streptococcus faecalis* and Gram-negative bacteria like *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* .Toda *et al.*(1989) attributed the antimicrobial bactericidal effect of coffee on the tested microorganisms in their study to the tannic acid present in coffee . They reported in their study that caffeine isolated from coffee didn't have antimicrobial activity against *Streptococcus mutans*, and there was no difference for coffee with or without caffeine. A study by Daglia *et al.*(2002) showed that green and roasted coffee solutions interfere with *Streptococcus mutans* adherence to saliva coated hydroxyapatite beads. This antiadhesive properties together with the bactericidal effect on *S. mutans* can explain the anticariogenic role of green coffee. The *S. mutans* anti-adhesive property of green coffee was not investigated in the current study

The reduction of inhibition zone observed in the current study following the addition of sugar to coffee (whether green or Arabica coffee) was also observed in one study in which adding sugar to coffee resulted in total prevention of inhibition of *S. mutans* indicating that changing the component of the food greatly affects oral environment and thus the growth of cariogenic bacteria in mouth. According to (Kashket *et al.* 1985) presence of specific components of the consumed food together with the way of food consumption may modify the effects of sugar especially sucrose on dental caries as this will interfere with ability of cariogenic bacteria to form extracellular polysaccharides.

In conclusion green and Arabica coffee have in vitro activity against the cariogenic bacteria *Streptococcus mutans* and might be used in the recent era of alternative medicine as an example of plant remedies with medicinal properties. However more extended research is needed for better validation of the specific compounds present in green coffee to which the antibacterial activity is attributed. This might enable the purification of these compounds in order to start their clinical use on a sound medical background.

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