

Can Coffee Prevent Caries? - An In-Vitro Study

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Abstract

Streptococcus mutans has been implicated as a primary causative agent of dental caries in humans. The micro-organisms produce 3 types of glucosyltransferase, and synthesize an adherent and water-insoluble glucan from sucrose by their cooperative action, which causes the organisms to adhere firmly to the tooth surface. The adherent glucan also contributes to the formation of dental plaque, in which the accumulation of acids leads to localized decalcification of the enamel surface. Roasted coffee extract possesses antibacterial activity against a wide range of microorganisms, including *Staphylococcus aureus* and *Streptococcus mutans*. The in-vitro study conducted at Oral Maxillofacial Pathology and Microbiology laboratory at KLE Society's Institute of Dental Sciences showed that coffee has antibacterial action to *Streptococcus mutans* and hence has an anticariogenic action but no antiadhesive action. This was studied by counting the colony forming units of the *Strep. Mutans* and the zone of inhibition around the different combinations of coffee.

INTRODUCTION

Caries is considered as one of the main problems of public health, so, many researchers in the world, have been searching for alternatives to prevent the occurrence of this process. The adherence of bacterial cells to teeth surface is of great importance to the

development of caries lesions and the interference on some of these mechanisms can prevent the formation of carious lesions. Antimicrobial and anticariogenic properties of tea have been extensively studied. ¹ Few studies on the antimicrobial activity of coffee-based solutions are found in the literature. Coffee contains several compounds which are known to affect human body chemistry. It helps in reducing risk of Alzheimer's disease, of Parkinson's disease, increases the effectiveness of pain killers, as an antidiabetic, prevents liver disease and cancer and is an antioxidant. ² Coffee is classified in the Rubiaceae family, *Coffea* genus, and the species cultivated in Brazil are *Coffea arabica* and *Coffea canephora*. Coffee grain is composed by water, mineral substances, glucides, lipids, organic acids, alkaloids, tannic acid ³ theobromine, caffeine and several vitamins. ⁴

Toda et al. related the effects of coffee on microbial species such as *Staphylococcus aureus*, *Salmonella thypi*, *Shigella dysenteriae*, *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Yersinia enterocolitica* and attributed this bactericidal effect

to the

tannic acid. Daglia et al. studying dark roasted coffee described that a compound originated during the roasting process and it had very strong antibacterial activity. Caffeine in the coffee is responsible for its antibacterial effect but not for anti adhesive properties. A six-ounce cup of coffee contains 100-150 mg of caffeine. This compound was effective against Gram-positive and Gram-negative reference strains, such as *Streptococcus Mutans*, the organism causing dental caries. The roasted coffee also has anti adhesive properties. Trigonelline one of the components of coffee is responsible for its anti adhesive properties. The study data suggest that trigonelline, a water-soluble compound in coffee that contributes to the aroma and flavour of the beverage, "may have the major responsibility for coffee's anti-adhesive activity." "Nevertheless," researchers conclude, "we can hypothesise that due to antibacterial and anti-adhesive activity, and coffee might reduce *S. mutans* colonisation of tooth surface and might be effective in preventing *S. mutans*-induced tooth decay." ⁵ The aim of this study was to evaluate the in vitro antimicrobial activity of coffee-based solutions from different origins, obtained by three distinct methods, on *S. mutans* adherence to human dental enamel and dentine. ¹

MATERIALS AND METHODS

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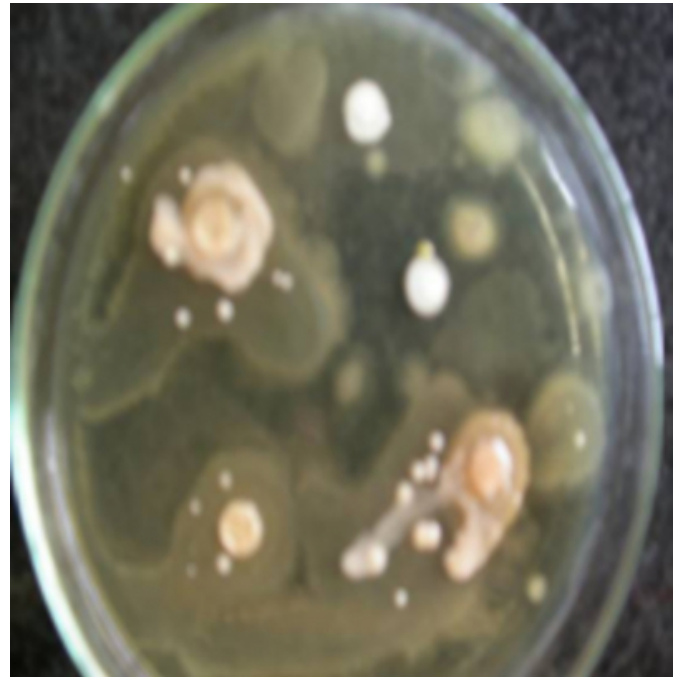
Thirty volunteers, both males and females about the ages of 18-25 years, from KLE Society's Institute of Dental Sciences, Bangalore, randomly picked, with no significant past or present medical history were selected for the study. Volunteers below 18 and above 25 years were excluded from the study. Their in-formed consent was obtained. This study was approved by Local Ethical Committee (KLE Societys Institute of Dental Sciences, Belgaum)

Coffee solution was obtained by boiling for 2 minutes, either alone, or in combination with sugar and milk. One milliliter(ml) of saliva of these volunteers, was diluted in 100 ml of distilled water and 1ml of this 100 ml, which contained saliva and distilled water, was added to BHI(Brain Heart Infusion) broth and incubated for 48 hrs at 37°C. One µl of this turbid broth was plated on a Mitis Salivarius Bacitracin(MSB) agar plate and again incubated anerobically at 37 °C for 48 hours. Greenish yellow colonies were identified and counted. Confirmation for strep mutans was done using Grams stain and a negative catalase testing

Colony forming units per $\times 10^3$ ml of saliva were counted. Wells containing 1) coffee 2)coffee+milk-sugar 3)coffee+milk+sugar were made on a S.mutans streaked BHI agar plate. The colonies formed around these beverages were counted and was called as the colony forming unit(CFU). (figure 1)

Figure 1

Figure 1: showing the CFU(colony forming unit) around 1)(left down),2)(above left) and 3) (below right) in a streaked BHI agar plate



Filter paper disks 5 millimeter in size soaked in 1) distilled water, 2)boiled coffee without sugar and milk and 3) boiled coffee with sugar and milk and boiled coffee with milk and no sugar were placed on BHI agar plate streaked with streptococcus mutans from the the MSB plate(figure 2)

Figure 2

Figure 2: Filter paper disks soaked in 1) coffee 2)coffee+milk-sugar, 3)coffee+milk+sugar, showed zone of inhibition(ZOI) around coffee(left up and down) but reduced ZOI in coffee+milk-sugar(right up), but no zone of inhibition around coffee+milk+sugar(right down), on a S.mutans streaked BHI agar plate.



. The different combinations of milk and sugar with coffee were taken as this is the method by which the coffee is consumed in the world. Zone of inhibition around the filter paper disks were measured in millimeters.

Glass beads about 0.5 mm diameter and soaked in saliva of the 30 volunteers were placed on the streptococcus mutans streaked MSB agar plate. A well dug in the media around the glass beads was filled with 1)coffee, 2)coffee+sugar+milk and 3) coffee+milk-sugar(figure 3)

Figure 3

Figure3: Turbidity was noted in the test tubes with coffee+milk-sugar(left) and coffee+milk+sugar(extreme left). But no turbidity in coffee-sugar-milk(right).



. The adhesion of the organism to the saliva coated glass beads was studied by inserting the glass beads from this plate to a BHI broth which was again incubated for 48 hrs at 37° C. The glass beads were agitated using a vortex mixer. The turbidity in the broth would indicate that organism adhered to glass beads and clear solution would indicate no adhesion.

Confirmation of this study was done using Streptococcus Mutans ATCC strain no 25175(figure 4)

Figure 4

Figure 4: showing the ATCC strain 25175 stick



RESULTS

The results showed that there was colony growth, colony forming unit (CFU) in the MSB agar plate and it was about 1.02×10^5 CFU in case of coffee added to milk without sugar and it reduced to 0.06×10^5 CFU in case of coffee without milk and sugar and to 1.47×10^5 CFU in case of coffee with milk and with sugar, in case of water it was 1.83×10^5 CFU (figure 1). The zone of inhibition around the disks was maximum i.e. 3.43 m.m. in the only coffee disk, reduced in case of coffee+milk and no sugar which was 2.67 m.m. and no inhibition with coffee+milk+sugar and in case of water inhibition of [0.77mm] was seen (figure 2). This indicated the anticaries or antibacterial action of coffee reduced when consumed with milk and no sugar and further reduced if taken without both sugar and milk. There was no anticaries action when coffee was consumed with sugar and milk The streptococcus mutans did not show any adherence to the glass beads as shown by the positive turbidity in the BHI broth(figure 3).

Figure 5

Table :Mean, standard deviation and median values of CFU/mL obtained for the experimental groups, zone of inhibition and the adherence to glass beads are shown in table.

	ZONE OF INHIBITION	COLONY FORMING UNITS	ADHERENCE TO GLASS BEADS- Y=1
c only	3.43±1.71	0.06±0.09	1
c+m+s	0.43±0.58	1.47±0.45	1
c+m-s	2.67±0.65	1.02±0.35	1

Where c =coffee, m=milk, s=sugar, Zone of inhibition in millimeters, CFU= () $\times 10^5$, adherence to glass beads--

[Y=Yes=1]; [N=no adherence =2]

No statistically significant difference was observed among log CFU/mL values obtained for experimental groups and control (p=0.05).

Zone of inhibition :=> Mean=1.5, Median=1, SD=1.914, Variance=3.666

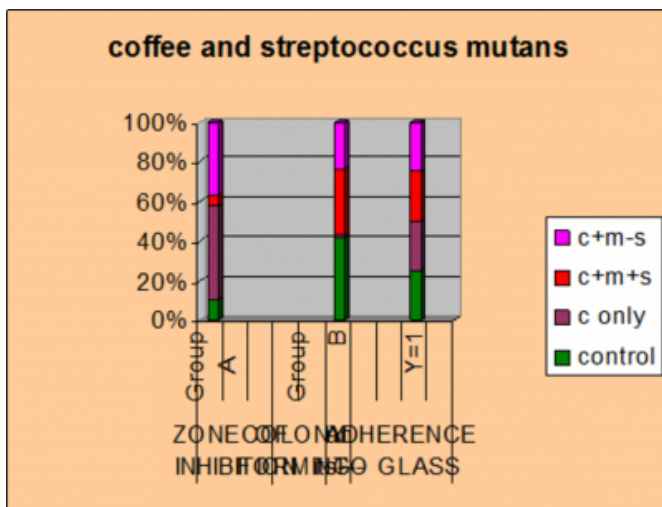
CFU =>Mean=1.075, Median =1.285, SD= 0.72968 and Variance was 0.53243

A student 't' test calculated by Maths Calculator showed a value of :

t = 0.4150; df = 6; standard error of difference = 1.024; sdev= 1.45; degrees of freedom = 6 and a confidence interval of 95% was obtained.

The probability of this result, assuming the null hypothesis, is 0.69

Figure 6
GRAPH



DISCUSSION

Dental plaque has been implicated as the principal etiological factor in both dental caries and periodontal diseases. Mutans streptococci, which have their prime habitat in plaque, can colonize tooth surfaces and initiate plaque formation. Sucrose-dependent adherence and agglutination processes (Hamada and Slade, 1980) seem to be of prime importance. Recently, isolation of natural anti-plaque and anti-caries substances from plants has been reported (Palenik et al., 1979; Rosen et al., 1984; Wolinsky and Sote, 1984; Southard et al., 1984;-Gravenmade and Jenkins, 1986). For example, tannins from tea and beverages

were found to inhibit growth and glucosyltransferase (GTF) activity of S. mutans (Paolino et al). 6

It is well known that, free radical induced, oxidative stress is an important factor in the etiology of many pathological processes. Much attention has been focused on the

antioxidants in food that may have beneficial physiological effects. Antioxidants help support the immune system and help fight the spread of cancer.

Roasting markedly affects the composition of the coffee polyphenols through the Maillard reaction and confers to coffee its pleasant taste and aroma (Richelle et al, 2001). However, although natural antioxidants 7 are lost during heating, the overall antioxidant properties of coffee brews can be maintained or enhanced by the formation of new antioxidants such as the Maillard reaction products (Nicoli et al, 1997a). 8 Roasted coffee extract possesses antibacterial activity against a wide range of microorganisms, including Staphylococcus aureus and Streptococcus mutans, whereas green coffee extract exhibits no such activity. 9 Arabica coffee (from C. arabica) is considered more suitable for drinking than robusta coffee (from C. canephora); robusta tends to be bitter and have fewer flavors than Arabica. 10 Consuming coffee up until 20 minutes after brewing will deliver 300 phytochemicals that are antioxidants 11 and will stay in the human system up to one month. The ingestion of coffee also provides the equivalent amount of antioxidants as three glasses of orange juice. 12

Caffeine 13 which is one of the ingredients of coffee, acts as a natural pesticide that paralyzes and kills certain insects feeding on the plants. Studies have found that the addition of caffeine, which has weak intrinsic antibacterial activity, to a mixture of ̢-dicarbonyl compounds at the concentrations found in coffee, demonstrated that caffeine synergistically enhances the antibacterial activity of ̢-dicarbonyl compounds and that glyoxal, methylglyoxal, and diacetyl in the presence of caffeine account for the whole antibacterial activity of roasted coffee. 3 This observation has been confirmed by our study.

Antibacterial action of coffee was proved by this study both by the large zone of inhibition and by the reduced CFU. But the combination of both milk and sugar reversed the antibacterial action of coffee, with sugar and milk ,more than milk without sugar.

Considering that coffee is constituted by several substances

such as water, mineral substances, glucides, lipids, organic acids, alkaloids, tannic acids, theobromine, caffeine and several vitamins, the isolated evaluation of each compound may clarify the specific agent related to its anti-adhesive effect [1]. It was suggested that trigonelline, an alkaloid and a water-soluble compound in coffee that contributed to the aroma and flavour of the beverage, may be responsible for coffee's anti-adhesive activity. In the absence of animal model data, caution is advised in the interpretation of the in vivo significance of these results. Melanoidin was another one of the reasons for this antiadhesive action reported Science News.

But roasting reduces the trigonelline to niacin, and hence antiadhesive action of coffee on streptococcus mutans is reduced. Since the branded coffee available in the market is roasted, the trigonellin has no role to play and hence the antiadhesive action of coffee is absent in our study, as proved by the turbidity of the BHI broth.

SUMMARY

Coffee anti-caries potential is related to its capacity of altering the biosynthesis of extra cellular polysaccharides (mainly mutans), avoiding the adhesion of streptococci.

“Black coffee must be strong and very hot; if strong coffee does not agree with you, do not drink black coffee. And if you do not drink black coffee, do not drink any coffee at all.” ~ Andre Simon

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