To Evaluate The Antimicrobial Efficacy Of Green Coffee Bean Extract On Periopathogens- A Clinico-microbiological Study

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Abstracts: Aim - To evaluate the Antibacterial efficacy of three different dilution of green coffee bean extract on periopathogens. Objective- To achieve a dilution of green coffee bean extract which has the maximum inhibitory effect on P. gingivalis and A. actinomycetocomitans. Methods- The sterilised blood agar culture plate was prepared on which colonies of P. gingivalis and A. actinomycetocomitans were cultured by subgingival sample taken from Chronic Generalized Periodontitis cases. Three dilution of green coffee bean extract were prepared i.e 10^{-8}, 10^{-9} and 10^{-10} with the serial dilution method using Distilled water. Then streaking of colonies was done on three different areas of culture plate on which different dilution was added. Minimum inhibitory concentration (MIC) on culture plates was observed to see the inhibitory effect of Green Coffee Bean extract on periopathogens by Agar Diffusion Method.Result- 10^{-9} was found have maximum inhibitory effect on periopathogens especially P.Gingivalis Conclusion- P.Gingivalis is more susceptible to 10^{-9} concentration of Green Coffee Bean Extract [Sachin M NJIRM 2016; 7(5):56-59]

Key Words: Chlorogenic acid, Green coffee bean extract, Minimum inhibitory concentration, Periopathogens, thioglycolate broth.

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Introduction: Widespread use of synthetic chemicals and drugs has resulted in the emergence of side effects, uncommon infections and antimicrobial resistance. Natural products appear to be suitable alternatives as they are available and accessible at affordable prices Coffee has demonstrated significant antibacterial properties against cariogenic bacteria. Among all the natural products with antibacterial properties, coffee is the most popular owing to its safety and pleasant odour and taste. Coffea arabica and Coffea canephora are the two most commercialized coffee species and a recent study indicated higher efficacy of the latter.[1,2]

Human oral cavity is inhabited by more than 500 species of bacteria at 10^8-10^9 bacteria per milligram of dental plaque. A distinct difference exists between composition of supragingival and subgingival plaque.[3]

Chlorogenic acid (CGA) is a polyphenolic natural compound. Structurally, it is an ester of caffeic acid with the 3-hydroxyl group of a quinic acid. It has been reported to possess many health benefits including antibacterial, antifungal, antiviral, antiphlogistic, antioxidant, chemopreventive, and other biological activities.[5] Green (or raw) coffee is a major source of CGA in nature (5–12 g/100 g).[6] However, 30–50% of CGA decomposes during roasting.[4]

Recent studies demonstrated that the consumption of green coffee extracts produced antihypertensive effect in rats and humans, improvement in human vasoreactivity, inhibitory effect on fat accumulation and body weight in mice and humans, and modulation of glucose metabolism in humans. Such biological effects have been attributed to CGA present in green coffee.[6] Hence, the present study was undertake into assess the inhibitory effect of green coffee bean extract on P. gingivalis and A. actinomycetemcomitans, to assess the time-kill curve of on P. gingivalis and A. actinomycetemcomitans, and to determine the antibacterial activity of green coffee bean extract on P. gingivalis. The study was carried out according to the Ethical Standards of the 1975 Helsinki Declaration and was approved by institutional review board, MCDRC, Durg

Methods:Preparation Of Coffee Bean Extract: Stock solution of the antimicrobial agent was prepared by adding 10 mg of green coffee bean extract to 10 ml of Distilled water. For MIC, ten dilutions of the drug were prepared with distilled water using Serial dilution method by means of standard protocols given by Schwalbe et al [7]Further, 1 ml of drug from the stock solution was added into the initial tube which contains 9 ml of distilled water. For dilutions, 1ml of stock solution was added into the next nine tubes separately. Then from the initial tube, was considered as 10^{-1} dilution. From 10^{-1} diluted tube, 1ml was transferred to the second tube to make 10^{-2} dilution. The serial dilution was repeated up to 10^{-10} dilution for each drug.

Bacteria and growth condition: Stock culture of periodontal pathogens (P. gingivalis and A. actinomycetemcomitans) used in this study were obtained Kanamycin blood agar was used to isolate P. gingivalis. Major ingredients included trypticase blood agar base with 5% sheep blood, supplemented with yeast extract, hemin, vitamin k1, L‑cysteine, and in addition, contained 100 mg/L of kanamycin.

Dentaid agar was used to isolate A. actinomycetemcomitans. Dentaid was prepared using Brain heart infusion (BHI) agar to which 5 g of yeast extract, 1.5 g of sodium fumarate (Sigma Chemical Co., St. Louis, Mo.), and 1 g of sodium formate per liter were added.

The medium was autoclaved for 15 min at 121°C. The final pH was estimated to be 7.2 ± 0.2. Once the medium was cooled to 50°C, vancomycin was added to a final concentration of 9 µg/ml. Subculturing of P. gingivalis and A. actinomycetemcomitans was done by incubating then at 35‑37 ^{0}C for 48‑72 h. The agar plateswere inoculated, placed in the anaerobic jars, and incubated for 48 h, and re‑incubated for another 2‑4 days, so as to allow those slow‑growing organisms to form colonies. P. gingivalis was formed as black and mucoid colonies. A. actinomycetemcomitans was formed as tiny, translucent colonies.

Minimum bactericidal concentration: The minimum concentration of the drug in the tube which does not show any turbidity is considered as the MIC of the drug.

After the MIC procedure, four dilution tubes which were showing sensitivity to antibacterial agent at lower concentrations were taken and inoculated into respective culture medium to check the growth of microorganisms. Formerly plates were incubated in anaerobic jar/chamber for ≥48 h and then colonies.

Result: Antibacterial activity of Green Coffee Bean Extract on P. gingivalis and A. actinomycetemcomitans by Disc diffusion methodOne way ANOVA test was used to compare the time-kill assay of Green Coffee Extract for both the microorganisms. 10^{-9} dilution of green coffee bean extract was more effecting against P.Gingivalis than A. Actinomycetocomitans (Table 1,2,3)

Table:1 The Antibacterial Activity Of Green coffee Bean Extract On A. Actinomycetocomitans

Green Coffee Bean Extract	Zone ofInhibition(mm)
10^{-8}	8
10^{-9}	11
10^{-10}	9

Table:2 : The Antibacterial Activity Of Green Coffee Bean Extract On P.Gingivalis

Green coffee Bean Extract	Zone of Inhibition (mm)
10^{-8}	16
10^{-9}	25
10^{-10}	20

Table:3 :Comparison of different Concentration

MO	T	Mean±SD	P value
Pg	0	140.0±26.4	0.02*
	2	176.6±5.7	
	4	199.6±30.9	
	6	176.0±27.4	
	24	500.0±0	
Aa	0	215.3±138.8	0.04*
	2	58.3±23	
	4	60.3±11.9	
	6	55.6±47.2	
	24	470±0	

MO-Microrganisms, T- Time,SD- Standard deviation

Discussion: Synthetic antimicrobial agents have resulted in considerable side effects, antimicrobial resistance and the emergence of previously uncommon infections owing to their improper usage.[8] Instead, plant extracts may prove to be better and safer alternatives if they are supported by scientific-based evidence. Several common natural products like lemon peel, neem stick and tulsi have been tested for their antibacterial properties.[9,10,11] Most of these plants that are used for traditional medicines can be cultivated locally and their extracts can be obtained locally by public for therapeutic use. Also, resistance by pathogenic bacteria to currently used antibiotics and chemotherapeutics has increased the global requirement for alternative safe, efficacious and cost-effective treatment options for such infections, particularly in developing countries.[6]

Coffee is the beverage of choice for many people, who love its rich taste and aroma and its stimulating effect. Until recently, coffee wasn't considered to be a health

food unlike tea and other leading hot beverages. Now researchers are finding that coffee seems to have a range of health benefits, including reducing the risk of type 2 diabetes, gallstones, liver cancer, Parkinson's disease and Alzheimer's disease. In addition, green coffee beans have been found to have antibacterial effects against pathogenic microorganisms.[6]

Green coffee bean extract is a natural supplement whose primary ingredients help people be well and healthy.[13] CGA, as the active ingredient present in the unroasted green coffee beans, is responsible for its antimicrobial property. Polyphenol is an organic compound found in this extract is responsible for its antioxidants property. Leptin is another compound found in this extract is responsible for regulating the energy intake and expenditure.[14]

The growth of P.gingivalis was inhibited showing 16mm, 20mm and 25 mm zone of inhibition and the growth of A.actinomycetocomitans was inhibited showing 8mm, 11mm and 9mm zone of inhibition.
Analysis of this data revealed that 10^{-9} dilution of Green Coffee Bean extract showed the maximum zone of inhibition of periopathogens and P.Gingivalis was more susceptible to Green coffee bean extract

According to Fardiaz[4] CGA and caffeic acids, are nonvolatile organic acids found in coffee, inhibit the growth of some Gram-positive bacteria like (S. aureus, B. cereus, L. bulgaricus, S. lactis and S. faecal) and Gram-negative bacteria viz. (E. coli, S. typhi and P. aeruginosa), but not molds and yeast. Pruthviraj et al.[15] demonstrated that the caffeine extracted from the leaves and leaf buds of Camellia sinensis (green tea), and beans of Coffea arabica (coffee) inhibits the growth of gram-negative bacteria like E. coli, Proteus mirabilis,Klebsiella pneumonia, P. aeruginosa. Brandao et al.[10] explored that coffee solutions of different origin that is,Coffea arabica and Coffea canephora, known as “arabic coffee” and “robust coffee”, respectively cultivated in Brazil had no antimicrobial activity against Strep mutans, but the tested coffee solutions reduced significantly the adherence of Strep mutans to glass surface which suggest potential anticariogenic activity of coffee solutions. Todaet al.[16] related the effects of coffee on microbial species such as S. aureus, S. thiphi, Shigella dysenteriae,Vibrio cholerae, Vibrio parahaemolyticus and Yersinia enterocolitica and attributed this bactericide effect to the tanic acid. The present in vitro antibacterial assessment study helps us to focus on an intervention approach to design and conduct a clinical trial to detect the beneficial effect of pure green coffee bean extract on patients at risk for periodontitis.

Conclusion: 10^{-9} concentration of Green Coffee Bean Extract was effective against periopathogens especially P.Gingivalis and furthur studies are required to validate our results and to achieve a concentration which is bacteriocidal to other periopathogens.

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