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Antibacterial Activity of *Camellia sinensis* Extracts Against Dental Caries

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Different bacteria were separated from saliva and teeth of cariogenic patients and identified by a variety of morphological and biochemical tests. Extracts of green tea strongly inhibited *Escherichia coli, Streptococcus salivarius* and *Streptococcus mutans*. The antibacterial effect of green and black tea extracts were compared with those of amoxicillin, cephradine and eugenol.

Key words : *Camellia sinensis*, Dental caries, Antibacterial activity

INTRODUCTION

Camellia sinensis (tea) belonging to the family Theaceae, is of three main types, oolong tea, black tea and green tea. Black tea is prepared from green tea by fermentation process, while oolong tea is intermediate between black and green tea (Varro *et al.*, 1988). Tea extracts have been found to be effective against many diseases. Several laboratory studies on crude extracts or constituents of black and green tea have shown antioxidative and antimutagenic effects. (Yeo *et al.*, 1995). Green tea extract is also effective in several types of cancer. Catechin and epigallocatechin gallate (a main constituent of green tea leaves), significantly inhibit the promotion of tumors and carcinogenesis in experimental animals (Fujiki *et al.*, 1992) Animal experiments have also shown the protective effects of flavonoids separated from tea extract against cardiovascular diseases which inhibit the oxidative modification of low density lipoprotein in macrophages (De Whalley *et al.*, 1990). Saponins extracted from green tea leaves have an anti-inflammatory, leukotriene antagonising activity and are also effective against peptic ulcer in experimental rats (Sagesaka *et al.*, 1996). Tea extracts inhibit the intestinal absorption of glucose and sodium in experimental rats due to reduced mucosal uptake of glucose (Kreydiyyeh *et al.*, 1994). All catechins separated from oolong tea exhibited a significant antiallergic activity except epicatechin (Ohmori *et al.*, 1995).

Green tea extracts effectively block oxidative DNA damage in the liver and has shown antihepatotoxicity in rats (Hamdaoui *et al.*, 1994). Thirty eight tea polyphenols were evaluated for their inhibitory effect against HIV replication in lymphocyte cells and demonstrated relatively potent anti HIV activity (Hashimoto *et al.*, 1996). Tea components are also used as a flavoring agent (Sugyama and Kenkichi, 1994), as a deodorant (Hayashi and Shinji, 1995), in skin cosmetics (Yamane *et al.*, 1995), in mouth wash and dentifrice (Enohara *et al.*, 1995). Polyphenols extracted from green tea significantly inhibit the growth of bacteria responsible for different types of dental caries (Otake *et al.*, 1991).

Dental caries is a bacterial disease in which the susceptible tissue is the tooth and the infectious agents are members of oral microflora. Occlusal dental caries is the most commonly occuring type among Pakistani population. Culture studies of specific sites on carious and non carious human teeth have shown that microorganisms responsible for caries differ from site to site, depending on the tooth surface involved (Roth and Calmes, 1981). Microorganisms responsible for dental caries production in human beings are *Streptococcus sanguis, Streptococcus mitis, Streptococcus mutans, Enterococci, Actinomycetes, Neisseria* and *Lactobacillus*. Gram positive bacteria and specifically lactobacilli predominates in the microbiodata of carious dentin. *Streptococcus mutans* is present in almost all types of carious lesions (Hillson, 1990).

The aim of the present study was to investigate the antibacterial activity of different extracts of green or black tea and to compare it with other drugs which are most commonly used in the treatment of dental caries.

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MATERIALS AND METHODS

Chemicals

Methanol (BDH), n-hexane (Merck), chloroform (BDH), ethylacetate (Merck), eugenol (Sigma), brain heart infusion agar (Oxoid), nutrient agar (Oxoid), amoxicillin (Oxoid), cephradine (Squibb), API 20S and API 20E Kit (Bio Merieux, France). All the chemicals and reagents used were of analytical grade.

Instruments

Incubator (Memmert), autoclave (Model. GI. Sc. General lab supplies corporation, Faisalabad Pakistan), microscope (Olympus BH2, Japan), new rotary vacuum evaporator (NE EYELA Tokyo Rokakai Co. Ltd) and refrigerator (Dawlance, Pakistan) were used.

Tea sampling

Dried green tea of sample1 (China), sample2 (Bangladesh) and black tea (Brook Bond Supreme) were used.

Solvent extraction

Green/black tea (200 g) each was weighed separately macerated in 2 liters of methanol, and kept at room temperature (16~25°C) for five days. Filtrate obtained was evaporated at 40°C to dryness with the help of a vacuum evaporator (New Rotary Vacuum Evaporator NE EYELA Tokyo Rikakai Co, Ltd). Dried extract (32.5 g) obtained was dissolved in 1 liter of the distilled water and filtered. Three separating funnels were taken and 200 ml of filtrate were added to each separating funnel. Chloroform, n-hexane and ethylacetate (50 ml each) were added into each of the three separating funnels which were then shaked vigorously for few minutes. Solvent layers were separated. Again 50 ml each of n-hexane, Chloroform and ethylacetate were added to separating funnels. All of the separating funnels were shaked vigorously and afterwards n-hexane, chloroform and ethylacetate layers were separted from each of the three separating funnels respectively.

Water extraction

Green/black tea (5 g each) was taken in a steel pan, 500 ml of water were added and heated it to boiling for 15 minutes.

Preparation of discs

Discs (4 mm in diameter) were prepared by using whatmann filter paper No.1.

1-Water extract (1 ml) was separated for the preparation of 20 discs (0.5 mg/disc).

2-With 100 ml of water extract 2 g of sugar were mixed for the preparation of discs Twenty discs were dipped in 1 ml of extract for three days.

3-With another 100 ml of the portion of water extract 2 g of sugar and 50 ml of milk were mixed for the preparation of 20 discs.

Patients selection criteria

Fourty patients suffering from caries were selected after thorough screening from a private clinic. Among the fourty patients, 28 were male and 12 were female and their ages ranged from 18 to 45 years.

Separation and identification of bacteria

Source of bacteria: Bacteria were taken from the saliva and dental plaque of carious teeth of cariogenic patients.

Preparation of bacterial culture

Brain heart infusion agar was prepared by dissolving 3.7 g in 100 ml of distilled water; by sterilizing in the autoclave for 15 minutes at 15 pound pressure (121°C); and by pouring 5~8 ml of the prepared media in to each petridish and then storing in refrigerator.

Sample of the saliva of a cryogenic patient was taken and spread over brain heart infusion agar with the help of a sterilized cotton swab. The petridishes were incubated at 37°C for 48 hours.

Samples of dental plaque of carious teeth were taken from the cariogenic patient, homogenized by grinding with a pestle and mortar containing 4 ml of 0.05 M phosphate buffer (pH 7.3). The homogenates were centrifuged at 1,000 rpm for 5 minutes to remove debris. Supernatant fluid was spread over brain heart infusion agar with the help of a sterilized wire loop. The plates were incubated at 37°C for 48 hours. (Hunt *et al.*, 1969).

Caries samples were taken with the help of wooden tooth pick inserted into the space of the cariogenic tooth of a patient. The tooth picks were then pressed in petri dishes containing brain heart infusion agar just after having taken the sample and incubated at 37°C for 48 hours. (Kristofferson and Bratthall, 1982).

Purification of bacterial culture

Colonies grown on the brain heart infusion agar plates were further isolated and purified by streak plate technique (Pelczar and Reid, 1983). Colonies of streptococcus were separated and their slants were prepared. Pure culture of *Escherichia coli* was supplied by microbiology laboratory of Combined Mililtary Hospital (CMH); purity of the culture was further tested by different biochemical tests and by a API 20E kit.

Identification of bacteria

Bacteria were identified by different biochemical and morphological tests. Shape, size and color of the colonies of bacteria were studied by naked eye. Then, other tests like Gram, spore and capsule staining, size determination and biochemical tests were performed according to the "Bergey's Manual" to confirm the strains and types of bacteria (Holt and Murray, 1984).

Use of API kits

Bacterial identification was finally confirmed by using the standardized identification system of API 20E kit for Enterobacteriaceae and API 20S kit for gram positive streptococci.

Culture sensitivity test

Streptococcus mutans, Streptococcus salivarius and *Escherichia coli* were separated from the pure culture of individual bacteria.

Bacterial cells were taken from a 24 hours old pure bacterial culture with the help of a sterilized wire loop and streaked over media in petri dishes. Discs of different tea extracts, velocef (cephradine), amoxicillin and eugenol were placed over media with the help of a sterilized needle. Petri dishes were incubated at 37°C for 48 hours in an incubator and zone of inhibition appeared were measured with a vernier calliper.

RESULTS AND DISCUSSION

Sensitivity of bacteria

Bacterial sensitivity was checked against tea extracts, eugenol, velosef and amoxicillin (Table I, II, III).

Streptococcus mutans showed sensitivity against tea extracts of ethylacetate (Table III). Zone of inhibition was observed and measured by a vernier calliper. For green tea extract of sample#1 zone of inhibition was 0.95 cm and for that of sample#2 0.92 cm. Zone of inhibition for eugenol was 0.73 cm, for amoxicillin 1.5 cm, and for velosef 1.1 cm. No sensitivity was observed against chloroform, n-hexane extracts or water extracts of tea. Black tea extracts did not showed any response against any type of bacteria.

Streptococcus mutans showed sensitivity against ethylacetate extract of green tea (Table III). Zone of inhibition for ethylacetate extract of green tea sample#1 was 0.71 cm and for green tea sample#2 0.73 cm. *Streptococcus salivarius* also showed sensitivity against eugenol, amoxicillin or velosef. Zone of inhibition of eugenol was 0.96 cm, of amoxicillin 1.27 cm and of velosef 8.5 cm. No sensitivity was observed when discs of chloroform extracts, n-hexane extracts and water extracts were used.

Escherichia coli also showed sensitivity against ethylacetate extracts of green tea (Table III). Zone of inhibition of ethylacetate extract of green tea sample#1 was 0.91 cm and for sample#2 0.92 cm. Zone of inhibition of eugenol was 0.76 cm and of velosef 1 cm. No sensitivity was observed against chloroform extract, n-hexane extract, water extract, or amoxicillin. *Strep-*

Table I. Effect of water extract of different tea samples on the zone of inhibition of test organisms

Solvent for Extract	Sample tea	Incubation time (hr)	Zone of inhibition (cm) of test organisms		
			S. salivarius	*S. mutans*	*E. coli*
Water	G.T.1	48	Nil	Nil	Nil
Water	G.T.2	48	Nil	Nil	Nil
Water	Black T	48	Nil	Nil	Nil
Water+S	G.T.1	48	Nil	Nil	Nil
Water+S	G.T.2	48	Nil	Nil	Nil
Water+S	Black T	48	Nil	Nil	Nil
Water+S+M	G.T.1	48	Nil	Nil	Nil
Water+S+M	G.T.2	48	Nil	Nil	Nil
Water+S+M	Black T	48	Nil	Nil	Nil

G.T.1=Green tea of sample 1, Black T=Black tea, M=Milk
G.T.2=Green tea of sample 2, S=Sugar

Table II. Effect of different drugs on the zone of inhibition of test organisms

Solvent for extract	Concentration	Incubation time (hr)	Zone of inhibition (cm) of test organisms		
			S. salivarius	*S. mutans*	*E. coli*
Eugenol	40 µg	48	0.96±0.05*	0.73±0.02*	0.76±0.01*
Amoxicillin	25 µg	48	1.27±0.12***	1.5±0.04***	Nil
Velosef (Cephradine)	30 µg	48	0.85±0.03*	1.1±0.10**	1±0.06**

(M±SEM) *P<0.01, **P<0.05, ***P<0.001

Table III. Effect of tea extracts on the zone of inhibition of test organisms

Solvent for extract	Sample tea	Incubation time (hr)	Zone of inhibition (cm) of test organisms		
			S. salivarius	*S. mutans*	*E. coli*
Ethylacetate	G.T.1	48	0.71±0.02*	0.95±0.06**	0.91±0.05*
Ethylacetate	G.T.2	48	0.73±0.10*	0.92±0.03**	0.92±0.14**
Ethylacetate	Black T	48	Nil	Nil	Nil
Chloroform	G.T.1	48	Nil	Nil	Nil
Chloroform	G.T.2	48	Nil	Nil	Nil
Chloroform	Black T	48	Nil	Nil	Nil
n-Hexane	G.T.1	48	Nil	Nil	Nil
n-Hexane	G.T.2	48	Nil	Nil	Nil
n-Hexane	Black T	48	Nil	Nil	Nil

M±SEM, *P<0.01, **P<0.05
G.T.1=Green tea of sample 1
G.T.2=Green tea of sample 2
Black.T=Black tea

tococcus mutans and *Streptococcus salivarius* were maintained on brain heart infusion agar while *Escherichia coli* on nutrient agar. After incubation at 37°C for 24 hours, cultures were freeze dried.

The present study showed the antibacterial activity of tea extract against cariogenic bacteria such as *Streptococcus mutans* and *Strepococcus salivarius,* isolated from saliva and teeth of different cariogenic patients. Different methods were used to culture bacteria present in saliva and teeth.

The water extract of green tea sample#1, green tea sample#2 and black tea did not show any antibacterial activity. Ethylacetate extracts of green tea sample #1 and sample#2 gave the maximum zones (Table III). The antibacterial activity may be due to tannins, gallotannic acid, volatile oil or polyphenols such as catechin, epigallocatechin and epigallocatechin gallate. The results of our study correlate with the observation of You (1993) who showed that *Streptococcus mutans* will be inhibited completely with 0.1% chinese green tea polyphenols.

Our study also correlated with the study of Yu (1992) who observed the effect of green tea extract on caries inhibition of hamsters and on acid resistance of tooth enamel. Both *in vitro* and *in vivo* experiments showed that green tea extract had significant inhibitory effects on the microorganisms causing dental caries.

Water extract of all types of green and black tea did not show any effect probably due to the reason that, during their preparation, the extracts were not dried under vacuum and the active constituents responsible for antibacterial activity might have been destroyed.

Chloroform and n-hexane extracts of green and black tea did not show any antibacterial activity because the constituents in green tea responsible for antibacterial activity may not be present in chloroform and n-hexane extract.

All extracts of black tea also did not show any anitbacterial activity because black tea was prepared from green tea leaves by fermentation process and during fermentation the constituents responsible for antibacterial activity might have been distroyed.

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