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ORIGINAL ARTICLE

Antimicrobial Effect of Honey on Streptococcus Mutans of Dental Plaque

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ABSTRACT

Purpose: Dental plaque consists of various pathogenic microorganisms like streptococcus mutans. Since ancient times it has been proved that honey has anti-inflammatory, antimicrobial, antiviral, antiparasitic, antimutagenic and antitumour effect. So, this study was conducted to evaluate the antimicrobial effect of honey on streptococcus mutans count before and after application of honey at a definite time interval.

Material and Method: The antimicrobial effect of Honey (Dabur honey, Dabur India) was tested on 20 volunteers from SGT Dental College who fulfilled the inclusion criteria. Plaque samples were collected from 6 teeth of all 20 individuals at baseline .Then, after 2 hours honey was applied with cotton applicator using paint on technique. After one hour of application, plaque samples were collected again from the same teeth. All the collected plaque samples were cultured on MSA agar plates and colonies were counted.

Results: Paired T test was applied to compare the mean bacterial count before and after application. After honey application, it was observed that the colony count of streptococcus mutans was reduced in significant amount. Conclusion - It was concluded from the study that honey has antimicrobial effect on streptococcus mutans.

Keywords: Antimicrobial, Honey, Dental plaque, Streptoccocus mutans

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INTRODUCTION

Dental caries and periodontal diseases are chronic diseases of human beings. Both these diseases are initiated by dental plaque. Dental plaque present at localized area on tooth surface can cause dental caries. After the ingestion of fermentable carbohydrates, the bacteria present in plaque produce acid which causes a rapid decrease in pH. As the pH level drops below 5.5 the demineralization process sets in and hence caries occur. Plaque bacteria enter the gingival connective tissue and cause ulceration in gingival epithelium.

After entering gingival connective tissue it can cause destruction to the alveolar bone and hence various periodontal diseases set in. So, plaque bacteria are responsible for both dental caries and periodontal diseases.

In the past four decades, microbial ecologists including microbiologists, taxonomists, molecular biologists, biochemists, epidemiologists, and dental scientists have accumulated information which has led to the identification of the presumed pathogens of human dental caries and periodontal diseases (1). So in order to prevent dental diseases it is important to decrease the bacterial load of plaque. Dental plaque consists of various pathogenic microorganisms of which streptococcus mutans is the most pathogenic.

Gargling solutions containing anti-

microbial agents was developed to prevent dental caries. Antibiotics are the representative antimicrobial agents but they are not good agents for use as a component in gargling solution due to potential induction of resistance to antibiotics (2). So, due to side effects the pathogenic microorganisms have develop a resistance against antibiotics. Recently much attention has been paid to extracts and biologically active compounds that are isolated from natural species and used in herbal medicine (3). Honey is the resinous material collected by honeybees from various plant sources. It is an important food for human in ancient times. Bees sip a little nectar when they make flowery rounds and they stash it in the hexagonal wax cells of the honeycomb to nourish young bees. The liquid nectar that is collected turns into honey when moisture evaporates to form a saturated and supersaturated solution of sugars, consisting typically of 17% water, 38% fructose, 31% glucose, 10% other sugars and a wide range of micronutrients (vitamins, amino acids and minerals) and some enzymes like Invertase, Glucose oxidase etc (4).

Honey flavor is an important quality for its application in food industry and also a selection criterion for consumer's choice. It has anti-inflammatory, antimicrobial, antiviral, antiparasitic, antimutagenic and antitumour effect (5). Honey contains various polyphenols like Caffeic acid, Chrysin, Galangin, Quercetin, Acacetin, Kampferol, Pinocembrin, Pinobanksin and Apigenin. These polyphenols have antitumour effect on liver and lung cancer cells (6).

Honey has antimicrobial effect on various microorganisms e.g. staphylococcus aureus, pseudomonas aeruginosa, Escherichia coli, Salmonella typhi (7). Studies have been conducted on antimicrobial effect of various forms of honey like Revamil source honey (8), raw and processed honey (3), manuka honey (Produced from Leptospermum Scoparium) (9), Propolis (Collected by Apis mellifera bees from various plant source) (10).

So, current study was conducted to investigate the antimicrobial effect of honey (Dabur honey, Dabur India) on Streptococcus mutans and to assess the reduction in Streptococcus mutans count before and after application of honey at a definite time interval.

Materials and methods

The short term antimicrobial effect of Honey was tested on 20 Interns both males and females from SGT Dental College. The materials used in the study were:

- Honey (commercially available)
- Disclosing solution
- Sterilized throat swab stick
- MSA agar plates
- Saline
- Before starting the study ethical clearance was obtained from ethical committee of SGT Dental College and consent form was obtained from each participant.

Inclusion criteria

- Subjects with DMFT < 3
- Subjects who gave informed consent
- Subjects whose oral hygiene is fair.

Exclusion criteria

- Subjects having systemic diseases
- Subjects on medication
- Subjects having an adverse habit
- Subjects undergoing orthodontic treatment.
- Subjects who have received any complex therapy within 6 months.

Out of 100 interns a total of 58 interns voluntarily agreed to participate in the study. Out of these 58 interns 20 fulfilled the inclusion criteria. These 20 subjects were included in the study. In order to bring uniformity amongst volunteers, oral prophylaxis was performed one week before and carious lesions were restored. The study was conducted over a period of fourteen days. All the participants were asked to report to department of microbiology SGT Dental College in the morning. They were allowed to have breakfast on the day of sample collection two hours before coming to microbiology department. First participants were asked to rinse with disclosing solution for 30 seconds in order to make the plaque visible which was earlier not visible by naked eye. Baseline samples were collected from six surfaces of six teeth.

- Buccal surface of upper right first molar (16)
- Buccal surface of upper left first molar (26)
- Lingual surface of lower left first molar (36)
- Lingual surface of lower right first molar (46)
- Labial surface of upper right central incisor (11)
- Labial surface of lower left central incisor (31)

Samples were collected on sterile cotton swab stick after soaking the swab with sterile water. These samples are directly transferred to MSA agar plates and streaking was performed. This entire process was done in an aseptic condition near spirit lamp. These plates were then placed in an anaerobic jar, which was closed after collecting all samples and placed in the incubator for 48 hours at 37°C. After 2hours, honey was applied to the subjects on the same 6 teeth with cotton applicator (paint on technique) and the participants were asked to abstained from drinking and eating for an hour.

After one hour of application plaque samples were collected again from the same surfaces of 6 teeth after applying disclosing solution and soaking the swab in sterile water. All the collected swab samples were directly transferred on MSA agar plates. Streaking was performed and plates were kept in an anaerobic jar. This jar was then placed in incubator for 48 hours at 37°C. Streptococcus mutans colonies were identified after performing smear-

ing procedure. Then colonies were counted and recorded.

STATISTICAL ANALYSIS

Mean and standard deviation values were calculated. SPSS version 17 was used. Paired T test was used to compare the mean colony count before and after application of honey. $P < 0.05$ was significant level.

RESULTS

The study was conducted to assess the antimicrobial effect of honey. Streptococcus colony counts were recorded before and after honey application. The mean bacterial colony count of each individual has decreased in significant amount.

The mean bacterial colony count of all the individuals before honey application was 48.32 and after honey application it has reduced to 27.93. t-value was calculated and it was found that there was significant reduction in streptococcus mutans colony count after honey application in all the volunteers with the p value 0.001. (Table 1)

DISCUSSION

The present study is an experimental study done on 20 volunteers from SGT Dental College. Streptococcus mutans is the organism chosen for the study as it is present in the oral cavity in majority and it has an important role in the dental diseases. In the results of present study it has been proved that honey has antimicrobial activity against Streptococcus mutans. Streptococcus mutans is gram positive cocci which are present in dental plaque.

Honey consists of polyphenols and these polyphenols have beneficial effects on dental caries, oral cancer and periodontal diseases (11). Role of antimicrobial effect of polyphenols on oral streptococci (12):

- A direct effect against S.mutans
- An interaction with microbial membrane proteins inhibiting the adherence of bacterial cells to the tooth surface
- The inhibition of glucosyltransferase and amylase.

Some authors found honey as cariogenic and it was advised that use of honey in nursing bottle should be discouraged (13). But, according to electron microscope studies the ingestion of honey causes no erosion of tooth enamel as observed after drinking fruit juice. Ten minutes after consumption of fruit juice tooth erosion was observed, while 30 minutes after honey ingestion the erosion was only very weak. This effect can be explained only partially by the calcium, phosphorus and fluoride levels of honey and other colloidal honey components might also play a role. So, honey is not as cariogenic as other sugars (5).

Honey has three enzymes like diastase (amylase), decomposing starch or glycogen into smaller sugar units, Invertase (sucrose, □-glucosidase), decomposing sucrose into fructose and glucose, as well as glucose oxidase, producing hydrogen peroxide and gluconic acid from glucose (5). This H2O2 produced is responsible for the antibacterial activity of honey. In the present study also, there is reduction in bacterial colony count which suggest the presence of glucose oxidase enzyme as it causes formation of H2O2 and gluconic acid.

Similarly, in a study done by Paulus *et al* it has been proved that hydrogen peroxide is produced by Apis mellifera (honeybee) glucose oxidase enzyme on dilution of honey. In the same study RS honey when diluted to 40-20% accumulated high levels of H2O2 24h after dilution (14).

Cases of honey poisoning are also reported in the literature and have concerned individuals from the following region: Caucasus, Turkey, New Zealand, Australia, Japan, Nepal, and South Africa also some countries in North and South America. Observed symptoms of such honey poisoning are vomiting, headache, stomach ache, unconsciousness, delirium, nausea and sight weakness. In general the poisonous plants are known to local bee keepers and honey which can possibly contain poisonous substances are not marketed. To minimise the risk of honey born poisoning in countries where plants with poisonous nector are growing, tourist are advised to buy honey from shops only and not on road and from individual beekeepers (5). In the present study also, commercially available honey was taken for evaluating the antimicrobial effect on Streptococcus mutans in twenty volunteers.

Presence of active Glucosyltransferase within dental plaque facilitates the formation of glucan in situ, thereby providing distinct binding sites for oral microorganisms. A study was done to explore the effect of compounds found in Propolis on streptococcus mutans growth and on glucosyltransferase activity. Results have shown that several

Table 1: Bacterial colony count before and after honey application in all participants

	Mean bacterial colony count i.e CFU (Before)	Mean bacterial colony count i.e CFU (After)	SD (Before)	SD (After)	N	t-value	p-value
Total n = 20	48.32	27.93	18.68	14.61	120	8.638	0.001***

*CFU = Colony forming units, SD = Standard deviation, N = Total no. of tooth surfaces, n = number of participants, *** = very highly significant*

compounds were identified in propolis which inhibited the GTF activity and bacterial growth. Apigenin is a novel and potent inhibitor of GTF activity and tt-farnesol was found to be an effective antibacterial agent present in propolis (10). So, propolis inhibit Glucosyltransferase enzyme of plaque and hence important in the pathogenesis of dental caries. Propolis contains flavinoids and these flavinoids are responsible for inhibition of Glucosyltransferase activity.

In a study when antibacterial efficacy of methanol, ethanol and ethyl acetate extracts of raw and processed honey was tested against gram positive bacteria (Staphylococcus aureus, Bacillus Subtilis, Bacillus cereus, Enterococcus faecalis and Micrococcus luteus) and gram negative bacteria (Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi), both showed antibacterial activity against tested organisms. Honey samples used contain biocomponents whose antibacterial activities are highly comparable with those of the two regular antibiotics (tetracycline and ciplofloxacin) (3).

In general also honey has various benefits due to which it is used since long in herbal medicine. Honey is used as burn wound healers. So, it can be safely used on burns and inserted into cavities, including sinus, to clear the infection (15).

Kim *et al* did a study on Koreans to determine the optimal concentration of Korean propolis against clinical isolates of mutans streptococci. The results had shown that MIC value for propolis were 35ug/l and propolis had a bacteriostatic effect on Streptococcus mutans and bactericidal effect on Streptococcus sobrinus at > 2 x MIC concentration (2).

The present study concluded that honey has antimicrobial effect on streptococcus mutans after a definite time interval. Results can be due to production of hydrogen peroxide, inhibition of glucosyltransferase activity or presence of polyphenols in honey. All these factors are responsible for antimicrobial effect of honey on streptoccocus mutans.

Study finding are important for public health intervention. Results suggest that honey can be used to develop an oral hygiene product such as a toothpastes and mouthwashes to prevent dental caries. It can also be used in chewing gum and candies which are most frequently used by children.

Further studies are required to assess the long term effect of honey on plaque microorganisms. Another limitation of the present study is that commercially available honey was used for testing the effect on streptoccocus mutans. Artifical honey contains various sugars and other constituent which can alter the results of the study. so, further studies are required to be conducted using natural honey.

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