

Flavonoids as a Possible Preventive of Dental Plaque

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Abstract □ To test flavonoids for antibacterial activity against oral microorganisms, flavonoids, quercetrin and naringenin, were incorporated into two pharmaceutical preparations in the form of tooth paste. Samples of dental plaque, the most accused dental deposit which initiates the gingival and periodontal diseases, were collected from the teeth surface of ten dental students at one week interval before and after using placebo, followed by two formulae of tooth paste containing 0.1% of quercetrin and naringenin (formulas I and II, respectively). The amount of dental plaque was assessed by the Quigley and Hens index. Then plaque samples were subjected to bacteriological examination of Gram stain and plate counts of microorganisms. The results revealed that most of Gram negative cocci and bacilli were highly affected by the two formulae: the number of actinomycetes were decreased after using formula I and disappeared completely by the use of formula II, while the number of Gram positive streptococci was highly decreased after the treatment with the two formulae. These results indicate a possible use of flavonoids to inhibit dental plaque formation.

Keywords □ Flavonoid, inhibition of dental formation.

Dental plaque is considered to be a primary etiological factor which initiates the periodontal and gingival diseases. It is the mucous mass attached to the tooth. It consists of salivary proteins, food debris, microorganisms and varying quantities of polysaccharides of bacterial origin. Sucrose which is the most frequent dietary sugar in man serves as a substrate for the synthesis of intra and extracellular polysaccharides by coccal organisms. Plaque growth is related to the number of microorganisms and the volume of extracellular polysaccharides synthesis by these organisms¹). Some flavonoids inhibited the dextran sucrose which catalyzes formation of α -1,6-linkage rich (soluble) glucan²). The effects of flavonoids on adhesive glucan formation by *Streptococcus mutans* on human dental plaque were also examined *in vitro*. This result obtained from *in vitro* tests indicates possible use of some flavonoids to prevent dental plaque formation and led our attention to clinical efficacy of these natural compounds on plaque inhibition.

EXPERIMENTAL METHODS

Preparation of flavonoids

Two flavonoids were prepared from plant materials known to contain flavonoids, *Bauhinia purpurea* L. (Family Leguminosae) and *Citrus paradisi* Macf., (Family Rutaceae). Air-dried plant materials were pulverized and extracted with 70% methanol in Soxhlet apparatus. The extracts were evaporated under reduced pressure at 40°C. The concentrated extracts were fractionated by using preparative cellulose tlc. The separation of the bands was effected by using BuOH: HOAc: H₂O (4:1:5), followed by H₂O: HOAc (9.5:0.5) as solvent systems. Acid hydrolysis was carried out with 1 N HCl³). The structure of the pure compounds obtained was determined by standard spectral and chromatographic data and comparison with authentic compounds.

Preparation of tooth pastes

Tooth pastes were prepared according to the method in Romington's Pharmaceutical Sciences⁴).

Estimation of the efficacy of two types of tooth paste

Ten volunteers of dental students with no sign of gingivitis participated in this study. Dental plaques were collected from the tooth surface by using sterile sickle scalere and deposited in sterile test tubes, each of which containing 10 ml normal saline solution. These samples were considered as control. Every participant used three samples of tooth paste, i, e., a placebo, and two formulae of tooth paste containing 0.1% quercetrin (Formula I) and naringening (Formula II) respectively. The students were asked to use every sample for one week only. At the end of every week, they stopped the use of tooth paste and tooth brush to permit accumulation of dental plaque. The next day morning dental plaque was collected from one surface of the last molars only. Then the amount of dental plaque was assessed by the Quigley and Heins index⁵⁾, after staining with disclosing tabletes for plaque scoring. The previous procedure was repeated at the end of every week during the whole experimental period of 3 weeks.

Antimicrobial study

All plaque samples were enumerated by the spread plate technique in triplicate.⁶⁾ Serial dilutions of the samples under test were prepared (10^{-1} to 10^{-4}) and agar plate (Difco Lab, Detroit, USA) was used to determine counts of bacterial population. The incubation was carried out at 35°C for 48 hours. From all samples, the well grown colonies were selected and Gram strain.

RESULTS AND DISCUSSION

The results shown in Table I which represent the mean plaque index scores in different groups revealed that a mechanical cleaning with the dentifrice decreased the amount of dental plaque but a significant decrease of 32% and 34% was observed after using formula I and II, respectively. The total numbers of the different types of microorganisms compared with that of the control were shown in Table II and Fig. 1. The results revealed that the total number of microorganisms in the dental plaque samples obtained from the students using placebo only for seven days decreased significantly, whereas a remarkable significant decrease of 99.2% and 99.6% in the total viable count was observed after using formula I and formula II, respectively. On the other hand the results of the bacterial plaque ratio were shown in Fig. 2. The analysis of the control dental plaque revealed that most of these groups were Gram positive streptococci,

Table I. Mean plaque indices in different groups

No. of students	Control	Placebo	Formula I	Formula II
1	189.0	154.8	106.6	103.3
2	181.6	141.0	132.6	128.6
3	169.3	113.3	101.8	103.7
4	191.3	156.4	139.6	121.0
5	194.6	162.1	121.8	130.7
6	188.3	142.1	106.2	103.6
7	198.3	161.6	155.1	150.3
8	168.2	151.6	123.4	127.4
9	165.1	143.8	101.7	106.1
10	178.1	153.0	136.6	128.7
Mean plaque index	182.3	148.0*	122.5*	120.3*
SD	11.789	14.314	18.369	15.789
SE	± 3.728	± 4.527	± 5.809	± 4.993
% of Reduction		18	32	34

Value significantly differed from those of the control
* $p < 0.001$

Table II. Comparative effectiveness of two types of tooth paste on the total number of microorganisms

	Controls	Placebo	Formula I	Formula II
\bar{X}	1456667	233330*	11222*	5667*
SD	1245389	1319	12765	7483
SE	± 41513	± 4640	± 4255	± 2494
% of Reduction		83.98	99.2	99.6

Values significantly differed from those of the control
* $p < 0.001$

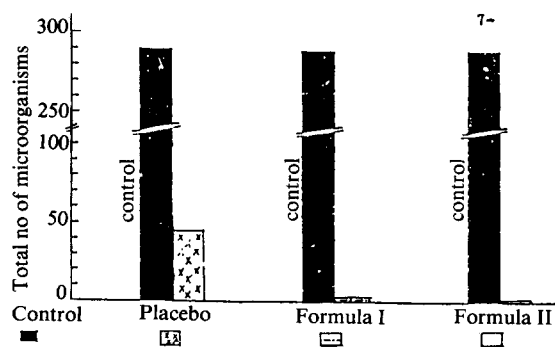


Fig. 1 Comparative effectiveness of different types of tooth paste on the total no of microorganisms.

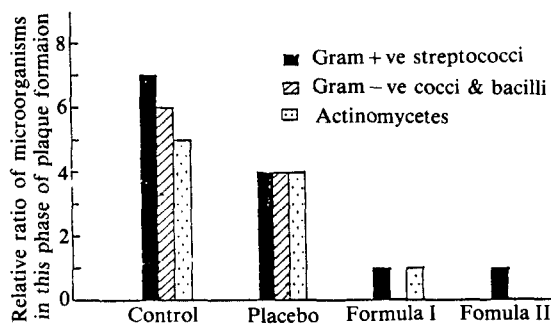


Fig. 2. Ratio of microorganisms in collected plaque samples.

whereas number of Gram negative cocci and bacilli and actinomycetes was less in this stage of plaque formation and the ratio of these groups was 7:6:5 respectively. By comparing with that of the control group, the number of the different types of organisms in the plaque samples obtained from the students after using placebo for seven days decreased approximately in the same ratio, while by using formula I which contained 0.1% quercetrin, Gram positive streptococci and actinomycetes were significantly decreased in the ratio of 7:1 and 5:1, and Gram negative cocci and bacilli completely disappeared. By using formula II which contained 0.1% naringenin there was a remarkable significant inhibition of the number of Gram positive cocci in the ratio of 7:1, while Gram negative cocci and bacilli disappeared completely.

These results showed that quercetrin (quercetin 3-L-rhamnoside) and naringenin (4,5,7-trihydroxy flavanone) in 0.1% concentration significantly inhibited the dental plaque formation by a significant decrease in the accumulation of plaque microorganisms and an inhibitory effect on bacterial adhesion to the tooth surface, and that flavonoids are a very interesting class of natural compounds which play an important role in the defense mechanisms.

Besides their role in plants against plant pathogens when they are working as phytoalexins, they also possess remarkable antimicrobial activity against plaque microorganisms and can inhibit the dental plaque formation.

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LITERATURE CITED

1. Berman, K.S. and Gibbons, R.J., *Arch. Oral Biol.* **12**, 849. (1966)
2. Lio, Massayoshi *et al.*, *Agric. Biol. Chem.* **48**, 2143 (1984).
3. Mabry, T.J., "The Systemic Identification of Flavonoids" Springer Verlag, Inc. New York, (1970).
4. Remington's Pharmaceutical Sciences, Macke Publishing Company, Easton, Pennsylvania, USA (1980).
5. Quigley, G.A. and Hein, J.W., *J. Amer. Dent. Ass.* **65**, 26 (1962).
6. American Public Health Association, "Standard Methods for the Examination of Water and Waste Water". 16 ed., American Public Health Association, Washington, D.C., (1985).