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Effect of Benzylisothiocyanate on acid production of salivary sediment

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Abstract
Benzylisothiocyanate (BIT) is an aromatic compound extracted from the root of Salvadora Persica L. It has been found to have antibacterial effect against certain microorganisms. Salivary supernatant has buffering capacity that helps to reduce acid production and hence enamel demineralization. The effect of BIT on acid production by salivary sediment was investigated in the presence and absence of salivary supernatant. Six experiments were prepared as the following : (1) suspended salivary sediment (SSS) system (16.7%)(negative control) ,(2) glucose (positive control)(5%) ,(3) glucose and 100 µg/ml BIT ,(4) salivary supernatant (33.3%) ,(5) salivary supernatant with glucose ,(6) salivary supernatant , glucose and 100 µg/ml BIT . Incubation was done in a water bath at 37°C and pH was monitored at 15-minute intervals for a period of one hour. BIT at a concentration of 100 µg/ml had a slight inhibitory effect on acid production by SSS system. When the supernatant was added to the incubation mixture, a dramatic inhibitory effect on acid production was found. An additive effect on acid production inhibition was shown when both BIT and supernatant were added to the incubation mixture. The inhibitory action of BIT on acid production was almost the same in the absence and presence of supernatant. In conclusion, BIT like hypothiocyanate inhibits acid production by oral flora. In addition, BIT and salivary supernatant have an additive effect in preventing pH fall which helps to prevent enamel demineralization.
Keywords: Salivary sediment, benzylisothiocyanate, acid production.

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Introduction

Saliva is a unique biological fluid that is very dilute because it is composed of more than 99% water. It is a complex mixture of Salivary glands secretions, crevicular fluid, transudate from the oral mucosa, bacteria, remains of food and desquamated epithelial cells [1].Two of the most important functions of saliva are : the antibacterial activity and buffering actions [2] . Saliva contains a collection of components that interact to serve those two interrelated salivary functions. Saliva performs the buffering action to protect the mouth as follows: 1) it prevents colonization of potentially pathogenic microorganisms by denying them optimization of environmental conditions. 2) Saliva neutralizes the acids produced by acidogenic microorganisms thus preventing enamel demineralization [1,3].

The main buffer systems known to contribute to the total buffer capacity of saliva are the bicarbonate, phosphate systems and those based on proteins. These systems have different pH ranges of maximal buffer capacity, the phosphate and bicarbonate systems having pK values of 6·8 - 7·0 and 6·1 - 6·3, respectively, whereas the proteins contribute to the salivary buffer capacity at very low pH values only [4,5,6]. The buffering action of saliva works more efficiently during stimulated high flow rates but is almost ineffective during periods of low flow with unstimulated saliva [2]. The bicarbonate buffer system is the major buffer in stimulated saliva and is responsible for 90% of the buffering capacity [3].

Benzylisothiocyanate (BIT) is a steam distillable volatile oil and one of the naturally occurring isothiocyanates compounds which is obtained from the root of Salvadora persica L [7,8]. This plant has medicinal properties and is termed the "Arak" in ancient Arabic literature. Its root has been used for centuries as a substitute for a toothbrush in many countries. In addition, BIT is found in the tissues of Indian cress ,garden cress and in relatively large amounts in cruciferous vegetables such as cabbage , Brussels , cauliflower and broccoli [7] . Many isothiocyanates like BIT have important biological effects as they demonstrate anticarcinogenic and antimicrobial activities [8,9]. Benzylisothiocyanate displayed rapid and strong bactericidal effect against oral pathogens involved in periodontal diseases and against other Gram-negative bacteria , while it mainly showed inhibitory effect to the growth of Gram-positive bacteria [10,11]. It was also shown to be effective against a spectrum of microorganisms such as Streptococcus mutans , Candida albicans ,and Herpes simplex type I and capable of preventing acid production by oral flora [7,12,13]. The purpose of this study is to investigate the effect of BIT on the

salivary sediment pH profile in the absence and presence of salivary supernatant.

Materials and Methods

Preparation of Salivary Sediment:

Salivary sediment was used for this study as it represents a very convenient method of obtaining mixed oral flora. Wax stimulated whole saliva was collected into a test tube from subjects who had avoided all forms of oral hygiene for 24 hours and had not eaten for 8-10 hours prior to collection [14]. The samples were pooled and centrifuged at 10,000 g for 15 minutes at 4°C. The supernatant was decanted and stored at 4°C until required which was usually within 1 hour. The salivary sediment was washed 2 times by re-suspension in distilled water and re-centrifugation at 1,740 g for 15 minutes each. A final stock concentration of 50% (v/v) of the sediment was prepared by adding equal volume of distilled water to the measured volume of the sediment. Fresh salivary sediment was prepared on the day of each experiment [15].

Benzylisothiocyanate:

Benzylisothiocyanate (MW 194.22) was obtained from Eastman Kodak Co. A stock solution of BIT was prepared by dissolving 0.36 ml in 4 ml methanol. Distilled and sterilized water was used for subsequent dilutions in order to prepare the required concentration of 100 µg/ml [7].

Effect of BIT on Salivary Sediment pH profile:

Six experiments were carried out to determine the effect of BIT on Saliva pH profile in the absence and presence of salivary supernatant. In each of these experiments, the final incubation concentration of various components of suspended salivary sediment (SSS) system were; (1) suspended salivary sediment (16.7%)(negative control) ,(2) glucose (5%) (positive control),(3) glucose and 100 µg/ml BIT ,(4) salivary supernatant (33.3%) ,(5) salivary supernatant with glucose ,(6) salivary supernatant , glucose and 100 µg/ml BIT . The initial pH was determined for each experiment and then incubated in a water bath at 37°C. The pH values ware monitored at 15 minutes interval for a period of one hour using a Cole Parmer pH meter.

Results

All six experiments were carried out in duplicate and the means of pH values were reported in **Table 1** and illustrated in **Figure 1**. The supernatant with the suspended salivary sediment (SSS) mixture had maintained the pH value of 7.3 which is higher than the pH value of 7.0 of the suspended salivary sediment mixture alone (experiments 1 and 4).

It had been found that Benzylisothiocyanate (BIT) at a concentration of 100 µg/ml had a slight inhibitory effect on acid production by SSS system (experiments 2 and 3). When the supernatant was added to the incubation mixture, a dramatic inhibitory effect on acid production was found (experiments 2 and 5). An additive effect on acid production inhibition was

Table 1: pH values of suspended salivary sediment system in the presence of 100 µg/ml BIT and salivary supernatant

SSS Mixture/pH values	0 min	15 min	30 min	45 min	60 min	0 min
1-Suspended Salivary Sediment (SSS) alone	7.3	7.3	7.2	7.0	7.0	7.3
2-SSS +5% Glucose	7.3	5.2	4.6	4.5	4.3	7.3
3-SSS +5% Glucose +100 µg/ml BIT	7.3	5.8	5.1	4.8	4.7	7.3
4-SSS +Salivary Supernatant	7.4	7.4	7.4	7.3	7.3	7.4
5-SSS +Salivary Supernatant +5% Glucose	7.4	7.1	6.6	6.0	5.5	7.4
6-SSS +Salivary Supernatant +5% Glucose+100 µg/ml BIT	7.4	7.3	7.0	6.3	5.8	7.4

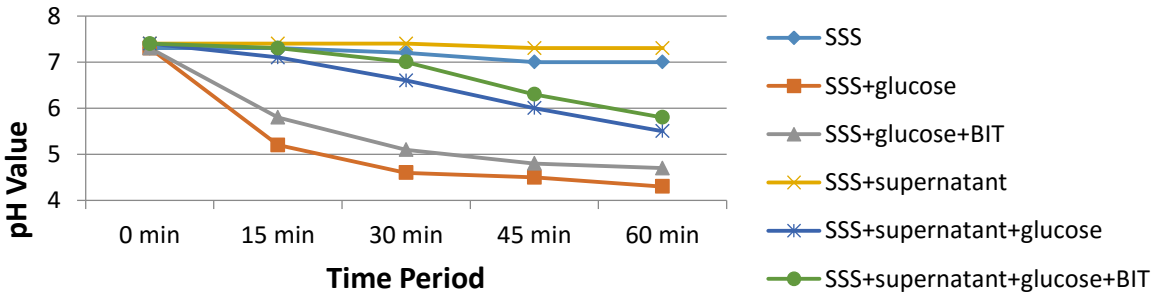


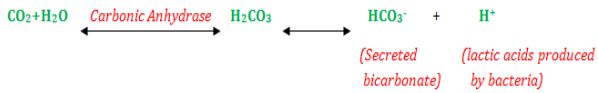
Figure 1: pH profile of suspended salivary sediment system in the presence of 100 µg/ml BIT and salivary supernatant

shown when both 100 µg/ml of BIT and supernatant were added to the incubation mixture (experiments 3, 5 and 6). It was noticed that the acid production inhibition activity of 100 µg/ml of BIT was almost the same in the absence and presence of supernatant (experiments 2, 3, 5 and 6).

Discussion

Saliva serves as a defense mechanism in human oral cavity through its buffering capacity and non-immunological antimicrobial contents such as lysozymes, lactoferrin and lactoperoxidase system. These two salivary defense mechanisms are among the most important functions of saliva [2,16,17].

Buffering capacity of saliva is carried out through the following components: bicarbonate, phosphate, urea, and amphoteric proteins and enzymes. Carbonic acid/bicarbonate (H2CO3/HCO3) buffer is the most important buffering system in stimulated saliva [2]. The concentration of bicarbonate in the saliva is greatly increased at increased flow rates. Therefore, bicarbonate is responsible for most of the salivary buffering capacity operative during food intake and mastication [5,18]. Carbonic anhydrase VI helps to maintain a high bicarbonate level in saliva. Thus , it catalyses the following reversible reaction [3,4,16]:



Another essential feature of this buffer system is "Phase Buffering" in which an increased carbonic acid concentration will cause carbon dioxide to be converted from the dissolved state into the gas phase allowing more bicarbonate to bind hydrogen ions which will increase the efficacy of the acid neutralization reaction [3,5,19].

Earlier studies showed that sediment mixtures without supernatant were more acidic than those mixtures with supernatant which clearly indicates that salivary supernatant inhibits the fall in pH [14,15,19]. In the present study, it had been found that the addition of supernatant to the incubation mixture decreased acid production. This finding is in agreement with a study done by Al Bagieh and Salako in 1993 which confirms earlier observations that saliva is capable of neutralizing acids being produced during glycolysis as well as raising the overall pH of the system. The neutralization of acids is achieved by bicarbonate buffer in such system of stimulated saliva. However, the raising of the overall pH of saliva is due to ph-rise factors present in saliva such as sialin, urea and amino acids notably arginine. Sialin is a peptide that stimulate base production by oral bacteria while urea and arginine are converted by urease and arginine deiminase respectively to ammonia [6,8,20].

Peroxidase or Lactoperoxidase is an essential salivary enzyme that offers antimicrobial activity because it serves as a catalyst for the oxidation of the salivary thiocyanate (SCN-) ion by hydrogen peroxide (H2O2) into hypothiocyanate (OSCN-), a potent antibacterial substance, according to the following reaction [3,17,21]:

When saliva samples are supplemented with H2O2 and SCN-, the mean concentration of OSCN- increased. The peroxide generated by the bacteria reacts with SCN- and lactoperoxidase both present in saliva and dental plaque, yielding OSCN- and possibly other short-lived oxidation products [17,21]. The oxidation products cause inhibition of bacterial metabolism by inhibiting sugar transport and by inactivating key glycolytic enzymes [17]. In the present study, it had been found that BIT has a slight inhibition of acid production which agrees with the findings of studies of Al Bagieh in 1998 and Al Bagieh and Weinberg in 1989 .[7,12] The finding of the present study that BIT and supernatant had an additive effect on acid production was concluded from the pH values of experiments 2,3,5 and 6 which indicate that pH values in experiment 6 were almost the same as the sum of pH values of experiments 3 and 5 .

The mechanism of action of BIT is either through the peroxidase system where BIT is oxidized by H2O2 in the presence of lactoperoxidase into benzyl-isohypothiocyanate or independently by its ability to oxidize the sulfhydryl (SH-) groups on enzymes involved in microbial growth and acid production [8,9,12].

Conclusion

In conclusion, Benzylisothiocyanate like hypothiocyanate was found to inhibit acid production by oral flora. In addition, BIT and salivary supernatant have an additive effect in preventing pH

fall which will help to prevent demineralization of the enamel and hence dental caries.

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