Effect of custom-made probiotic chocolates on *Streptococcus mutans*, plaque pH, salivary pH and buffering capacity in children- A randomized controlled trial

Aim:

The aim of the study was to compare the plaque pH, salivary pH and buffering capacity of custom-made probiotic chocolates with plain chocolates in children and to evaluate the antimicrobial efficacy of the custom-made probiotic chocolates against *Streptococcus mutans*

Method:

Chocolate with probiotics was prepared according to FDA regulations. Finety children with DMFT/dmft ≤ 3 were included for the study. For phase 1 trial they were randomly divided into 3 groups Milk chocolate (MC), White chocolate (WC) and Dark chocolate (DC) with 30 children in each group. Randomization was done by a single blinded examiner using list of random numbers and allocated into different groups by the same examiner. The effect of the probiotic Vs plain chocolates was tested on plaque pH, salivary pH and buffering capacity in children. Baseline, 10 minutes, 30 minutes and 60 minutes samples were recorded. The plaque, salivary pH were measured immediately by two blinded examiners using Hannah pH meter. Forsdicks (1957) method modified by Rugg-Gunn (1975) was used for estimation of plaque pH. Buffering capacity of saliva was determined by the classical Ericson’s test (1959) by the same examiners. For phase 2 trial, children were allocated to their respective probiotic groups. Similar procedure was repeated for plaque pH, salivary pH and buffering capacity in children.

Second part of the trial was to determine the *S. mutans* colony count in children. They were randomly divided into 3 groups (n=20) as: Group I - Probiotic milk chocolate, Group II - Probiotic white chocolate, Group III - Probiotic dark chocolate.
They were given probiotic chocolates for 5 consecutive days in a week. Baseline, post intervention, 15 days and 30 days colony count were measured. Thioglycolate sucrose blood bacitarcin agar (TSBB) was used for culturing *S. mutans*. Saliva samples of about 1 ml were serially diluted up to $10^3$ dilution. 1ml of diluted saliva was added in the center of sterile Petri dish using a sterile pipette. Diluted saliva of about 1 ml was plated by using a bent glass rod on the agar media and the plates were inverted and incubated at 37°C for 48 to 72 hours. The colonies were identified by morphology and confirmed using gram staining and catalase test. Single blinded investigator performed the colony counting.

Results:

- Probiotic milky bar followed by probiotic dark chocolate was found to have the least drop in salivary ph
- Among the normal chocolates, Dark chocolate had the least drop in ph
- All the 3 chocolate groups significantly reduced the colony count from baseline to 30 days. Among which dark chocolate group showed the maximum reduction

Conclusion:

Chocolates can serve as a vehicle for delivering probiotics in children making it beneficial for health. Probiotic chocolates have the advantage of reducing the chocolates acidogenicity and hence making them tooth friendly.

Keywords:

Chocolate, probiotics, *S mutans*