

drinking water reduced caries occurrence in an in vitro and in vivo study.² According to Sakanaka in 1992, the total number of fissure caries lesions was significantly reduced by the addition to the diet or to drinking water of tea polyphenols extracted from green tea. It was also observed that polyphenols inhibit the growth, glucan synthesis, and cellular adherence of cariogenic streptococci *in vitro*.³ Plaque formation inhibition by a polyphenol gargling solution was reported in 1997.⁴

Although green tea extracts have proven to have anticariogenic effects, there is no evidence for caries prevention with common drinking or food patterns. This study investigated the pH levels, plaque quality, and the amount of *Streptococcus mutans* after consuming polyphenol-containing milk.

MATERIALS AND METHODS

Thirty volunteers between 20 and 40 years old were evaluated in this study. The inclusion criteria were that a volunteer must be a non-smoker, have no systemic disease, and have over 1×10^5 *Streptococcus mutans* in 1 mL of saliva.

Two weeks prior to the study observation, volunteers received full-mouth scaling. Before the study, all volunteers were instructed in oral hygiene instructions and to use the same toothpaste. A mouth rinse was not allowed. During the study period, all volunteers were asked to follow the oral hygiene instructions and to use the same toothpaste. A mouth rinse was not allowed. During the study period, all volunteers were asked to maintain their normal dietary habits, and to consume 250 mL of polyphenol-containing milk 3 times a day. The test milk was formulated with 0.02% polyphenol (Sun Chemical Co., Tokyo, Japan) in 250 mL of milk (Kwan-Chun Co., Taoyuan, Taiwan). The Volunteers were not allowed to consume food related to this study, such as tea.

Forty-eight hours before the second and third examinations, volunteers were not allowed to brush their teeth or use dental floss until the examination was completed. At the second examination, the pH level,

weight of the dental plaque, and amount of *S. mutans* in the dental plaque were measured.

The third examination occurred 2 weeks after the second examination, and both were conducted in the morning. Volunteers were allowed to drink water but could not eat anything. Six hours before the examination, volunteers were not allowed to drink or eat.

pH Value Change

The pH value of the dental plaque was measured using a micro pH meter (Model 701, Orion Research, Cambridge, MA, USA) by the same dentist. The detecting area was located on the canine and the first premolar of each quadrant. pH values were determined at the second and third examinations. The data obtained at the second examination served as the baseline.

Plaque Weight

Data obtained at the second examination served as the baseline. Dental plaque was harvested with a Gracey curet supragingivally over the canine and the first premolar of each quadrant. The weight of the dental plaque was measured with a micro-weight measurement scale (Hitachi, Tokyo, Japan). The weight of the dental plaque was measured at the second and third examinations.

Amount of *Streptococcus mutans*

Diluted plaque solution was smeared on brain heart infusion agar and cultured in a 5% CO₂ and 95% N₂ atmosphere at 37 °C for 5 days. Then the total number of bacteria colonies was calculated. The obtained dental plaque was dissolved in 1 mL of 0.5 M phosphate buffer (pH 7.3) with 30 s of mixing. The diluted plaque solution was then smeared onto the Mitis Salivarius Bacitracin Agar (MSB agar). The bacterial colony-forming units (CFU/mL) were determined after developing in a 5% CO₂ and 95% N₂ at 37 °C atmosphere for 3 days. The amount of *S. mutans* was defined as follows:

$$\text{Streptococcus mutans in dental plaque} = \frac{\text{colonies of } S. \text{ mutans in dental plaque}}{\text{total bacteria colonies}} \times 100\%$$