Evaluating the Effect of Fulvic Acid on Oral Bacteria and Cancerous Oral Cells

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Shilajit is a homeopathic treatment used by local inhabitants of India and Pakistan. It may have specific components that inhibit the formation of cavities and the growth of cancer cells. This experiment analyzed the effects of fulvic acid, an active component of shilajit, on the growth of oral bacteria and squamous cell carcinoma. The effect of fulvic acid was evaluated on early *Streptococcus mutans* (*S. mutans*) biofilm formation and established *S. mutans* biofilm by treating each group with different concentrations of fulvic acid for 24 hours in sterile 96-well flat-bottom microtiter plates. *S. mutans* was used because it is a common cause of dental caries. The optical density (OD) of the *S. mutans* biofilm was measured after crystal violet staining using a SpectraMax190; greater growth correlated to greater OD. It was determined that fulvic acid inhibits the growth of newly forming *S. mutans* biofilm at fulvic acid concentrations greater than 1.25% (vol. %) and established *S. mutans* biofilm at fulvic acid concentrations greater than 5% (vol. %). To evaluate the effect of fulvic acid on squamous cell carcinoma (SCC-25) cells, six-well plates seeded with SCC-25 cells ($1*10^5$ cells/well) were exposed to different concentrations of fulvic acid (buffered to a pH of 7.5) for 72 hours. The cytotoxicity and cell proliferation were measured using a cytotoxicity detection kit and a water soluble tetrazolium kit (Roche Applied Science), respectively. It was determined that fulvic acid inhibits the growth of SCC-25 cells at concentrations of fulvic acid above 2% (volume %). The effects of fulvic acid (0.5%) on matrix metalloproteinase expression and collagen degradation ability of SCC-25 cells is being analyzed. The suppressive mechanisms observed by fulvic acid on both *S. mutans* and SCC-25 cells could improve overall oral health.

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