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Citrus Volkameriana: A Potential Adjunct Herbal Extract in Management of Chronic Gingivitis

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ABSTRACT

Using plant extracts as adjunct natural sources in treating various oral diseases mainly dental caries, periodontal diseases and oral malodour could provide an innovative strategy in controlling bacterial infection. Bacterial dental plaque biofilm-dependent oral diseases such as dental caries, periodontal and endodontic diseases are considered as major worldwide medical, economic and public health problems. Presence of pathogens in plaque biofilm leads to microbiological affront which disturbs the immunological balance leading to an atypical host tissues destructive reaction resulting in oral diseases and other serious systemic conditions. Commercially existing medications have been tried and examined against bacterial plaque pathogens. Unluckily, diverse antimicrobial drugs cannot be utilized safely due to the presence of some accompanied side effects in addition to the possibility of the development of antibacterial opposing strains of microorganism associated with the wide-scale misuse of antibiotics. The utilization of herbal commodities for therapeutic purposes has been established in numerous ancient and resident cultures and its therapeutic merits are recognized. The importance of plants having antibacterial and anti-inflammatory activity has been well recognized in the field of alternative medicine. Citrus species are previously discussed as an important adjunct in curing several diseases in traditional medicine. In this article we will try to provide a hypothesis discussing the possibility of using Citrus Volkameriana (one of the citrus species) as a potential adjunct herbal extract in management of chronic gingivitis.

Key words: Citrus Volkameriana, chronic gingivitis, herbal extract.

Introduction

Gingivitis is an inflammation restricted to the gingiva without losing attachment or alveolar bone. It is one of the commonest oral diseases that involve more than 90% of the populace apart from age, sex or race (Seneviratne *et al*., 2011). The initial clinical indication of gingivitis is the bleeding which is caused by a vascodilatory effect accompanying the inflammatory process. Other signs and symptoms include presence of swollen gums which appear bright red or purple. Tissues feel tender or painful on touch. Bleeding after brushing and/or flossing with bad breath (halitosis) is also common. In addition to disappearance of stippling that is usually present in the gum tissue of several persons thus the gums may show a shiny appearance when the gum tissue becomes engorged and tense over the inflamed underlying connective tissue. To prevent gingivitis every day and efficient supragingival plaque control using tooth brushing and dental floss is mandatory to detain the probable succession of gingivitis to periodontitis (Marsh, 2010).

Even though mechanical plaque control methods have the prospective to preserve sufficient levels of oral hygiene, clinical practice and populace based studies have proven that such methods are not being working as correctly and efficiently as they should be in many individuals. Consequently, numerous chemotherapeutic agents such as triclosan, essential oils and chlorhexidine have been used to control bacterial plaque, aiming at recuperating the efficiency of daily control methods. The recognition of the importance of plants with antibacterial and anti-inflammatory activity has enlarged

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as a result of existing problems accompanying the wide-scale abuse of antibiotics that provoked multiple side effects in addition of development of microbial drug resistance (Miri *et al*., 2013).

Citrus is an important fruit crop of tropical and subtropical areas with several years' history of cultivation. Consumption of citrus has long been known to prevent many human diseases, from scurvy to several types of cancers (Balestrieri *et al*., 2011). Citrus is mainly treasured for the fruits, which is either eaten as fresh fruit (sweet orange, tangerine, grapefruit, etc.), processed into juice, jams or extra to plates and beverages e.g. lemons. Citrus species are important in traditional medicine. The use of citron and lemon antidote for poison and venom is recorded in earliest research. In Samoa, a mix made with sweet orange incorporated is utilized against oral sores in infants. Citron leaves are used jointly with other plant parts to make mixtures to treat postpartum sickness, serious flu, and internal injuries (Benavente-Garcia *et al*., 2008). In USA, citrus is recommended as an ingredient of a healthy diet because it highly contains vitamin C, lycopene and flavonoids, which are well recognized in reduction of prostate and breast cancer risks, reduction of viral infectivity and inflammation. Recovery of capillary activity and control of cholesterol levels are also among the beneficial effects (Elevitch, 2006, Lopes *et al*., 2011). Citrus species have well documented pharmacological activities. Results have shown that citrus extract exerted many bioactivities including anticancer (Visalli *et al*., 2014), antioxidant, analgesic, anti-inflammatory (Sood *et al*., 2009), antiulcer (Bitra *et al*., 2012), hepatoprotective (Balestrieri *et al*., 2011), cardioprotective (Lopes *et al*., 2011), anxiolytic (Carvalho-Freitas *et al*., 2002), antiviral (Cardile *et al*., 2015), antimicrobial, antiobesity and antidiabetic activities (Pamar and Kar, 2007, Sood *et al*., 2009, Wendakoon *et al*., 2012,). These actions were correlated to the wide variety of secondary metabolites produced by citrus including polyphenolic compounds, alkaloids, limonoids, pectins and dietary vitamin C (Benavente-Garcia and Castillo, 2008, Marti *et al*., 2009, Tundis *et al*., 2014, Visalliet *et al*., 2014).

Evidence and possible mechanisms

The Volkamer lemon (Citrus Volkameriana) has been known for centuries (Lopes *et al*., 2011). Citrus Volkameriana (CV) herbal extract may be a possible adjunctive method in management of chronic gingivitis. The health enhancing effects of citrus consumption is attributed to the wide assortment of bioactive compounds that make up citrus phytochemicals. The major classes of citrus phytochemicals include flavonoids, carotinoids, limonoids, folic acid, pectin, coumarins, vitamin C, high quality dietary soluble fibers and mineral potassium. Dietetic fibers, including pectin, have revealed a positive effect on a wide spectrum of pathological conditions. Their positive control on human health status is based on their anti-oxidative, hypocholesterolemic, anti-inflammatory and anti-cancer properties. Modest evidence has been reported about their activity on the immunological system (Lopes *et al*., 2011).

A possible method mediating the protective outcome is linked to bioactive compounds that act mainly by interfering with reactions of detrimental oxidizing agents and free radicals. CV contains various types of antioxidants such as phenolic compounds, flavonoids, carotenoids, ascorbic acid and others. Amid them are carotinoids which have been confirmed to have a vital function in preventing oxidative damage resulting from singlet oxygen and free radicals presence (Lina *et al*., 2003). Folic acid is used by all mammalian cells as coenzyme for amino acid interconversions and for the production of pyrimidine purine required for deoxyribonucleic acid (DNA) creation. The maturation of the junctional epithelium, which has a quick turnover rate, is of major import in the deterrence and direction of the destruction accompanying periodontal disease. Folic acid deficit has been allied with abnormalities in speedily proliferating epithelial cells, including buccal squamous cells. It is accordingly likely that the junctional epithelium would also be affected. This in actuality has been experienced in experimentally induced folic acid deficiency. The basal cell layer showed increased nuclear staining with various levels of degeneration in the spinous layer including widening of the intercellular spaces. This histological depiction was coupled with clinical evidence of severe widespread gingival inflammation (Lina *et al*., 2003, Galati and O'Brien 2004, Williams *et al*., 2004).

Vitamin C has been previously recognized as a contender for modulating periodontal diseases. Contemplates of scorbutic gingivitis which is characterized by ulcerative gingivitis and quick periodontal pocket development with tooth loss and the effects of vitamin C on extracellular matrix and host immunologic and inflammatory responses offer a justification for hypothesizing that vitamin

C is a risk factor for periodontal disease. Vitamin C deficit has been shown histologically to cause a lack of collagen formation by disturbing hydroxylation of proline and increased permeability of endotoxins from the oral mucosal tissues. Vitamin C can influence the retort of the periodontium by influencing collagen turnover, epithelial barrier and leukocyte functions. It is also implicated in the synthesis of the matrix of calcified tissues such as alveolar bone and teeth. Thus it is likely that inadequate vitamin C supply could exacerbate established periodontitis. Vitamin C is also recognized as scavenger of hydroxyl radicals. Hypochlorous acid, a physically powerful oxidative agent, can activate mutually neutrophil-derived and gingival crevicular fluid collagenase and this oxidative commencement can be prohibited by vitamin C (Lina *et al*., 2003, Galati and O'Brien 2004, Williams *et al*., 2004). Flavonoids are well known for their antioxidant activities. Antioxidants are substances responsible for cells protection against the oxidative effect of reactive oxygen sorts, like singlet oxygen, peroxyl radical, hydroxyl radical, superoxide radical, nitric oxide and peroxynitrite. The impaired balance between these reactive oxygen species and antioxidants result in a condition commonly referred to as oxidative stress. This oxidative stress may lead to cellular damage which is linked to various systemic problems such as diabetes, cancer, cardiovascular disorders, neurodegenerative diseases and aging. Furthermore, oxidative stress can harm numerous biological molecules. Proteins and DNA are important targets of cellular damage. Antioxidants interfere with radical producing systems and increase the function of endogenous antioxidants, protecting the cells from damage by these free radicals. Naturally occurring flavonoids have been also recognized for their antimicrobial activity. This makes them significantly important in the field of medical microbiology. Many researchers have secluded and recognized the structures of flavonoids having antifungal, antiviral and antibacterial effects. These properties of flavonoids enable them to be used extensively in the fields of nutrition, food safety, and health (Lina *et al*., 2003, Galati and O'Brien 2004, Williams *et al*., 2004).

We are suggesting the following approaches to test the hypothesis:

Patients with an age range (18-55 years) with chronic gingivitis based on clinical signs and symptoms should be selected. All patients should have at least 24 natural teeth. Patients having a probing depth of ≥ 4mm in any tooth, narration of antibiotic treatment in the last 6 months before starting the study or long-term treatment with anti-inflammatory drugs should not be included. History of periodontal therapy together with oral prophylaxis in the last 6 months should not be added in the contemplate. Smokers, pregnant females, and those who were tested allergic to the Citrus Volkameriana fruit should be excluded from the study. The patients should be divided into four equal groups:

1st group: supra and sub-gingival scaling should be performed together with application of the test material (Citrus Volkameriana extract).
2nd group supra and sub-gingival scaling should be performed together with application of a placebo.
3rd group: application of the test material alone.
4th group: application of a placebo alone.

Oral hygiene instructions should be educated to all patients in all the study groups including the use of daily tooth brushing. The test material should be applied either in the form of a topical gel or used as mouth rinse or incorporated in a tooth paste. 21 days experimental study period is suggested.

1. During the 21- day experimental period, that is on baseline, 7th day, after 14 days, and after 21 days, the plaque index (PI) (Löe and Silness, 1963) gingival index (GI) (Löe, 1967) and papillary bleeding index (PBI) (Ainamo and Bay, 1975) and salivary pH will be recorded.
2. Plaque samples should be collected using sterile curettes from just above the gingival margins from one particular surface for each patient to make a reliable quantitative evaluation of different types of bacteria including Streptococci, Porphyromonas gingivalis, Prevotella intermedia, Aggregatibacter (Actinobacillus) actinomycetemcomitans, Treponema denticola, Tannerella forsynthia, Fusobacterium nucleatum, Fusobacterium necrophorum, Fusobacterium species, Spirochetes, Peptostreptococcus micros, Campylobacter rectus, Bacteroides species, Eubacterium species and Enterobacteria using real-time polymerase chain reaction (RT-PCR) (Busin *et al*., 2009).
3. Gingival biopsies should be obtained for chronic inflammatory cells distribution detection with histopathological analysis using different stains (whenever it is indicated).

4. Testing the level of various inflammatory mediators in gingival crevicular fluid (GCF), saliva and plasma such as interleukin 1 (IL-1), prostaglandin E2 (PGE2), matrix metalloproteinases (MMPs), leukotriens B4, IL-2 IL- 4, IL-5, IL-6, IL-8, IL-10, IL-12 (p70), IL-13, Interferon- γ (IFN-γ) and tumour necrosis factor- α (TNF-α) before and after application of the CV extract using enzyme-linked immunosorbent assay (ELISA) (Lequin, 2005).
5. Survey of the extracellular and intracellular environments and detection of microbe linked molecular patterns (MAMPS) present on or released from microbial cells and bacterial interactions should be assessed using carbohydrate-based microarrays (Flannery *et al*., 2015).

The patients should be instructed to brush his teeth twice daily for the 21 days experimental study period with the provided tooth paste.

Instruction to the patients should be given to massage the provided gel onto their gums twice daily, at morning and evening, for 3-5 minutes for 21 days. Specially made thermoplastic mouth guard trays can be constructed to allow the accuracy that the gel should make contact with the gingival margin of the experimental teeth. The patients should be instructed to use the provided mouth wash three times daily for 21 days.

Conclusion

Accordingly, we can hypothesize that Citrus Volkameriana extract could be used as adjunct herbal extract in management of chronic gingivitis based on its previously discussed beneficial effect in managing of such inflammatory condition.

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