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The Effect of Chlorhexidine and Tea Polyphenols Mouthwash in Adolescent Gingivitis

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Abstract: Periodontal Disease is one of the common diseases of the oral cavity. It is not only common in adults, but also in children and adolescents. As the public recognition of health concepts is growing fast, oral health has been focused. Mouthwash plays an important role in the lives of people with periodontal disease by effectively inhibiting plaque and reducing the risk of getting periodontal disease. Adolescent gingivitis is a common periodontal disease in the adolescent population. At present, it has been recognized that periodontal disease is a multi-factor disease, among which dental plaque biofilm is the most important pathogenic factor. The bacteria and their products of dental plaque are indispensable initiating factors for periodontal disease, which directly or indirectly participate in the process of periodontal disease. The ideal mouthwash should be able to reduce the number of bacteria in the oral cavity, eliminate or reduce the microorganisms on the surface of the teeth and the oral cavity, and inhibit the accumulation of plaque on the gums, tissue pathogenic bacteria re-colonized in the teeth and periodontal pockets, and prevent the recurrence of gingivitis. By introducing the prevalence and pathogenesis of gingivitis in adolescence, we analyzed the effect of two types of mouthwash of chlorhexidine and tea polyphenols on the control of gingivitis plaque in adolescence and the mechanism of bacterial inhibition, as well as the effect on the hard and soft tissues of the oral cavity, and evaluated the clinical efficacy of chlorhexidine and tea polyphenols in the control of gingivitis in adolescence.

Keywords: Tea Polyphenols, Chlorhexidine, Adolescent Gingivitis, Periodontal Disease, Gonadal Hormone

1. Overview of Adolescent Gingivitis

Periodontal disease is a widespread disease that affects 90.9% of the adult population in China [1]. Periodontal disease is not only prevalent in adults, but also children and adolescents. According to surveys, gingivitis affects more than 70% of children and adolescents over the age of 7 [2]. Gum disease is a group of diseases that occur in the gum tissue and includes inflammation of the gums and systemic pathology in the gums. It is the least symptomatic form of periodontal disease, does not affect the underlying supporting structures of the teeth and is reversible, usually caused by the accumulation of bacterial biofilm (plaque). The causative factors of gingivitis are related to genetic factors, environmental factors and smoking, in addition to the pathogenic bacteria of the plaque biofilm.

The new 1999 classification divides gum disease into plaque-induced gum and non-plaque gum disease, and classifies plaque-induced gum disease as "plaque-related only" and "systemic-influenced gum disease". Gingivitis in adolescence is classified in the new classification as one of the "gum diseases caused by plaque biofilm and influenced

by systemic factors"[3]. It occurs most often in adolescence and is slightly more common in women than in men. Gingiva is a target tissue for sex hormones and because of the endocrine changes that occur in individuals at a certain age, gingival tissue becomes more reactive to local irritants such as plaque, producing a more pronounced inflammatory response, or exacerbating existing chronic gingivitis. Changes in the level of sex hormones in adolescents are a systemic factor in the development of gingivitis during puberty. The disease is found on the gingival papillae and gingival margins of the labial side of the anterior teeth. The labial side of the gingiva is visibly swollen, and the gingival papillae are bulbous, dark red or blushing, shiny, soft and bleed easily on probing. Gingival sulci may form pseudo-periodontal pockets without loss of attachment level and resorption of alveolar bone. Adolescent gingivitis is usually diagnosed clinically by the patient's age at puberty and by seeing if the gingival inflammatory response exceeds that which can be induced by local irritants. During the individual's puberty phase, there is a temporary increase in the production of sex hormones and thus a temporary enhancement of gum inflammation, which can partially subside after puberty but can persist if local irritants are not removed. Puberty also has a significant impact on the composition of the periodontal flora. Studies have shown that the proportion of Gram-positive anaerobic colony-forming units gradually increases from pre-puberty to post-puberty [4]. In addition, the proportion of colony-forming units of anaerobic black bacteria increased from prepubertal to pubertal stages; the proportion of *P. intermedia*, *Capnocytophaga* and *E. corrodens* were also higher in adolescence than in pre-adolescence and post-adolescence [5, 6]. Gingivitis during puberty is associated with increased levels of sex hormones, which can also worsen the symptoms of gingivitis. According to the study, increased levels of oestrogen and progesterone can lead to increased blood vessels in the gums and increased inflammatory response in patients who are pregnant [7], pubescent or taking oral contraceptives [8].

Removal of local irritants remains the key to the treatment of adolescent gingivitis. Plaque is still the main cause of adolescent gingivitis. Scaling or gingivectomy is currently prescribed treatment for the disease. Effective prevention of the disease and slowing its progression have also become particularly important. Good oral hygiene, proper brushing, rational use of mouthwash and effective flossing are the most important means of preventing and slowing down the progression of gingivitis in adolescence. In this paper, we focus on the control of plaque and the effect on the hard and soft tissues of the oral cavity with chlorhexidine and tea polyphenol rinses to evaluate the effect of these two drugs on the management of adolescent gingivitis.

2. Tea Polyphenols

Tea polyphenols (TP) are a polyphenolic compound isolated and purified from natural tea-leaves, which have a strong inhibitory effect on a wide range of bacteria in nature [9], and have the advantages of regulating the balance of flora [10], reducing or eliminating the odor produced by flora [11], and not generating resistance to repeated use [12]. Tea polyphenols have been shown to have antibacterial activity for over 100 years [13]. Researchers at home and abroad are still working on the antibacterial properties of tea polyphenols for a long time. At present, domestic research on the antibacterial properties of tea polyphenols is still limited to the types of tea polyphenols, their antibacterial effects and influencing factors, while foreign research is relatively more in-depth. Therefore, it still has a long way to go in China.

According to their chemical structure tea polyphenols can be divided into four categories, namely catechins, anthocyanins, flavanols and phenolic acids. Among them, catechins are the most abundant and are the main functional active substances of tea polyphenols. There are four main types of catechins, including epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC). EGCG is the main catechin substance (50%-80% of catechins) and the most important functional substance [14].

Tea polyphenols have a broad-spectrum antibacterial effect, inhibiting both G+ and G- bacteria. The antibacterial effect of tea polyphenols is not only reflected in their antibacterial activity but also in their ability to act synergistically with a variety of known antibiotics, such as tea polyphenols and tetracycline against Staphylococcus aureus [15]. In recent years, an increasing number of studies have shown that tea polyphenols have a good inactivating effect on bacterial toxins. Staphylococcal enterotoxin B (SEB), an exotoxin of Staphylococcus aureus, can cause acute gastroenteritis. It has been shown that intraperitoneal injection of green tea extract or EGCG in a mouse model of acute gastroenteritis, was found to have some inhibitory effect on SEB [16]. It was found that EGCG has a significant inhibitory effect on the virulence factor glucosyltransferase of Streptococcus mutans, which can play a good role in preventing dental caries [17].

At present, the antibacterial mechanism of tea polyphenols is still unclear and is mostly believed to be the result of the joint action of a variety of factors in China and other countries. At present, the research on the antibacterial mechanism of tea polyphenols at home and abroad is mainly reflected in the following three aspects. 1. Tea polyphenols disrupt the function of cell membranes - EGCG binds very easily to the peptidoglycan layer exposed on the cell surface of G+ bacteria, which may eventually cause damage to the cell membrane, thus weakening the ability of the bacteria to bind to the host cell [18, 19, 20]; 2. Inhibitory effect of tea polyphenols on specific target proteins of bacteria - it was found that catechin substances could inhibit the activity of purified DNA rotase by binding to the ATP conjugation site of the β-subunit of DNA rotase, which eventually led to the disruption of DNA synthesis [21]; 3. oxidative bacterial inhibition of tea polyphenols - there is increasing evidence that tea polyphenols can generate hydrogen peroxide through autotrophy in neutral to weakly alkaline media, thereby

inhibiting the effect on bacteria [22, 23].

Compound Tea Polyphenol Rinse is a newly developed natural plant oral rinse, its main ingredients are tea polyphenol, licorice sweetener, DP300, glycerin and vitamin E. It can inhibit and kill pathogenic bacteria in the mouth, kill oral deformation streptococcus and viruses, and inhibit the growth of fungi. It can freshen the mouth without irritation. In 2004, 180 juvenile patients with juvenile gingivitis were selected in an elementary school, and 60 cases in the test group were randomly selected for a trial of mouthwash with compound tea polyphenol rinse [24], There was a significant difference between the same group before and after seven days of treatment ($t=27.89$, $P<0.01$), which proved that the compound tea polyphenol mouthwash could effectively relieve the symptoms of gingivitis. In 2007, 78 patients with chronic periodontitis were selected to demonstrate through clinical trials [25]. Periodontal basic treatment with compound tea polyphenol mouth rinse group was a significant comparison group with periodontal basic with distilled water mouth rinse group. In periodontitis, although traditional methods such as scaling and scraping can remove subgingival microorganisms, they have limitations on deep periodontal pockets and deep soft tissues, so the application of antibacterial drugs is an important part of treatment [26]. Some research has shown that tea polyphenols are effective against Streptococcus mutans, and inhibit plaque by inhibiting the growth of Streptococcus mutans, thus producing the effect of preventing gingivitis and dental caries [26]. Triclosan (DP300), is a non-ionic, highly effective and broad-spectrum anti-microbial agent. It has been shown that DP300 has a minimum inhibitory concentration of 5.26×10-2mol/L against the main oral pathogenic bacteria *Streptococcus albicans*, *Rhodococcus intermedius*, *Rhodococcus melanogaster*, *Streptococcus pyogenes*, *Rhodococcus oralis*, *Actinobacillus eusocialis*, *Rhodococcus non-dissociated*, *Lactobacillus* and *Rhodococcus maculatus*. The minimum inhibitory concentration of 2.63 × 10-2mol/L for *Aeromonas gingivalis*, *Bacillus nucleatum*, *Bacillus mycoides*, *Bacillus discoideus* and *Streptococcus anaerobicus* is highly effective in killing and inhibiting CO2-loving and specific anaerobic bacteria causing gingivitis and periodontitis [27]. Gingivitis can develop into periodontal disease under certain conditions. Therefore, the treatment and prevention of gingivitis are crucial. Current research has developed mouth rinses containing antibacterial drugs that have been shown to have therapeutic and preventive effects on gingivitis. It has been confirmed that the compound tea polyphenol-containing rinse has positive efficacy on gingivitis, non-toxic side effects, good taste, especially suitable for adolescents and children in adolescence, and can be clinically promoted as a medicine for the treatment and prevention of gingivitis in adolescence [24].

3. Application of Chlorhexidine in Periodontal Antimicrobial Therapy

Chlorhexidine (hibitane), a broad-spectrum bactericide, is effective against both gram-positive and negative bacteria. Due to its good antibacterial properties, it has been widely used in treatment of dental clinic. Chlorhexidine Gluconate (CHX) is biguanide chlorobenzene that has been used clinically since the 1950s to increase cell membrane permeability by causing loss of intracellular components (including nucleotides) due to cell lysis [29, 30]. Currently, it has been utilized clinically for cleaning non-biological clinical surfaces and disinfection of medical catheters, as well as in dentistry as a disinfectant rinse for preventing the growth and accumulation of plaque biofilms because of its good biocompatibility [31]. Also as an adjunct to oral hygiene and non-surgical treatment [32]. The effects of chlorhexidine gluconate on systemic health have not been adequately studied and therefore only topical administration is recommended. Chlorhexidine gluconate binds to the skin and mucous membranes as a cationic oxidant and is also difficult to be absorbed, with 30% remaining in saliva for up to 5 hours and mucosal tissue for up to 12 hours after a single flush, and is not detectable in plasma [33, 34].

Chlorhexidine rinses antimicrobial plaque action mechanism is mainly manifested in the following aspects. 1. Reduce the number of bacteria that can be adsorbed to the dental surface in saliva: Chlorhexidine adsorbs to the bacterial surface and interacts with the anion of the bacterial cell wall, increasing the permeability of the cell wall, thus making it easy for chlorhexidine to enter the cell and kill the bacteria by precipitating the cytoplasm, so the number of bacteria adsorbed to the dental surface is reduced; 2. The acidic group of salivary glycoprotein is closed by binding with the acidic group of salivary glycoprotein so that the adsorption ability of salivary glycoprotein to the tooth surface is weakened and the formation of acquired film and plaque is inhibited; 3. Chlorhexidine binds to the enamel of the tooth surface and covers the tooth surface, thus hindering the adsorption of salivary bacteria to the tooth surface; 4. Chlorhexidine competes with calcium ions while replacing the acidic agglutinating network of calcium ions that interact with agglutinating bacteria in saliva and precipitation, thus changing the cohesion of plaque bacteria and inhibiting the accumulation of bacteria and the adsorption of bacteria on the tooth surface.

For gingivitis and periodontitis, the main microorganisms involved are gram-negative anaerobic bacteria such as *Porphyromonas gingivalis*, *Clostridium perfringens*, *Proteus spp*. and *dentate dense spirochetes* [35, 36]. In vitro studies have shown that chlorhexidine gluconate can affect the growth and reproduction of oral disease-associated flora, such as *Porphyromonas gingivalis* [37]. In a clinical trial, the diversity and abundance of bacteria on the tongues of healthy individuals were reduced with the use of 0.12% chlorhexidine gluconate gargle twice daily for 7 days [38, 39]. Also under the effect of 0.20% chlorhexidine gluconate gargle, the species and abundance of bacteria in the saliva of healthy people were reduced for the same treatment [40]. Therefore, chlorhexidine gluconate causes changes in the oral microflora and whether these changes affect healthy oral microbiota has not been elucidated. However, some studies

have shown a significant decrease in salivary pH and buffering capacity after 7 days of using chlorhexidine gluconate rinse, possibly due to increased salivary lactate and glucose levels [40]. Also in vitro experiments have found a similar situation [41]. For non-bacterial microorganisms, chlorhexidine gluconate also acts on fungi and viruses, such as *Candida albicans* associated with oral candidiasis [42]. It has been shown that some *Candida albicans* form resistant colonies after the use of chlorhexidine gluconate rinse, which can reduce the effectiveness of chlorhexidine gluconate rinse over time [43]. Chlorhexidine gluconate is used as a mouthwash will reduce the abundance of anaerobic bacteria such as *Veronella*, which may cause fungi such as Candida albicans to dominate and cause disease [44, 45]. Therefore, some researchers believe that the use of chlorhexidine gluconate gargle should be avoided in immunocompromised patients to avoid morbidity and mortality due to fungal infections [46].

The effective concentration of chlorhexidine gluconate rinse can also affect the growth and reproduction of oral microflora. The concentrations of chlorhexidine gluconate rinse currently on the market are roughly 0.20%, 0.12%, and 0.06%. In Europe, a 0.2% CHX solution was developed as the international standard concentration [47], and several studies have tested lower concentrations of CHX (0.12%) and have also shown clinical effects [48, 49]. The optimal dose of chlorhexidine gluconate is usually considered to be 20 mg twice daily [50, 51, 52]. This optimal dose balances efficacy, local side effects and user acceptance [53]. The study showed that 0.20% chlorhexidine gluconate rinse was significantly better than 0.12% and 0.06% chlorhexidine gluconate rinse in preventing supragingival plaque [54]; While 0.12% and 0.06%, chlorhexidine gluconate with rinse showed no statistical difference in the inhibition of bacterial plaque [55, 56]. However, some diseases have been reported to be associated with contaminated chlorhexidine gluconate rinse [57], it indicates that bacteria also can adapt to chlorhexidine gluconate [58]. Therefore, future studies are obliged to explore the development of bacterial resistance against chlorhexidine gluconate.

4. Comparison of Studies on Chlorhexidine and Tea Polyphenols

There is a lack of unified standards for the detection of antibacterial effects of tea polyphenols, and it is difficult to compare and analyze many data from different literature; so much so that the effect of the remaining ingredients in mouthwash on the antibacterial effect of tea polyphenols needs further study; and the synergistic effect of tea polyphenols and other sources (such as plants, chemical synthesis) of antibacterial substances is not sufficient. The research and development of substances with synergistic activity on tea polyphenols may also be an important development direction in the future; the drug resistance of periodontal pathogenic bacteria is becoming more and more serious, especially those originating from food, and there is not much research on the inhibitory effect of tea polyphenols on food-derived drug-resistant bacteria; tea polyphenols are widely used because of the recognition of their safety, however, the research on the interaction between tea polyphenols and bacteria is still very insufficient of tea polyphenols, especially on whether tea polyphenols induce bacteria to develop tolerance to other antimicrobial agents and other unfavorable conditions, lack of risk assessment. At present, the antibacterial mechanism of tea polyphenols is not very clear, and the application of tea polyphenols still lacks scientific basis and guidance, which largely limits the further promotion of tea polyphenols in the field of medicine (especially dentistry, oral prophylaxis, etc.). To better develop the application of tea polyphenol-related mouthwash, the research on the antibacterial properties and mechanism of tea polyphenol-containing mouthwash must be more extensive and in-depth [28].

At present, foreign countries have made relatively clear comparisons between the clinical applications of tea polyphenols and chlorhexidine, but there is a relative lack of corresponding research comparisons in China, probably because the domestic research on the antimicrobial properties of tea polyphenols is still limited to the types of tea polyphenols, antibacterial effects and influencing factors. Despite this, there is still some research progress in the comparison of the two in China. By comparing the effects of compound tea polyphenol rinse and compound chlorhexidine rinse on subgingival flora and gingival sulcus fluid endotoxin levels in patients with periodontitis [25], it was found that the compound chlorhexidine rinse group and the compound tea polyphenol rinse group had the same effect and were slightly better than the chlorhexidine group in terms of the change of G-bacteria count before and after the experiment. The side effects of chlorhexidine, such as poor taste, ease to stain the surface of the teeth, mucosal irritation and long-term use can lead to the normal flora of the oral cavity, so tea polyphenols in clinical use may be more due to chlorhexidine. Domestic experimental research. In the study of compound tea polyphenol rinse for the treatment of juvenile gingivitis made a comparison of the efficacy of tea polyphenol and chlorhexidine, the data showed no significant difference in the efficacy of the two groups ($X2=0.683$, $P>0.05$) [24]. However, the relevant domestic studies showed that the authors of the experimental conclusions are slightly subjective, and lack objective evaluation of the comparison of tea polyphenols and chlorhexidine two. However, in foreign countries, scholars have made a relatively more objective view, and the results of an experiment in 2021 through Meta-analysis proved that tea polyphenols do have a more positive effect on PI, GI, GBI, BOP and other clinical parameters. Based on these results, that is, there is enough evidence to support the use of tea polyphenols for the prevention and treatment of periodontal disease, but there is not enough evidence to prove or recommend the use of chlorhexidine for the treatment or prevention of dental caries. However, the authors also stated that there is still insufficient

evidence to suggest that tea polyphenols can completely replace chlorhexidine. Therefore, chlorhexidine remains the recommended regimen for the treatment of gingivitis and periodontitis. It is very valuable in managing the treatment of gingivitis in adolescence. Finally, it is worth mentioning that the removal of local irritants remains the key to the treatment of adolescent gingivitis. The plaque remains the main etiology of adolescent gingivitis. The effect of sex hormones on periodontal health should also be considered. Traditional chlorhexidine gluconate rinses may be resistant to periodontal microorganisms, so the next research should focus on whether tea polyphenols have an inhibitory effect on drug-resistant bacteria. Further research should also be considered to see if tea polyphenols can mitigate the aggravating effects of sex hormones on gingivitis.

5. Conclusion

The key to the prevention and treatment of adolescent gingivitis is to remove local factors and control plaque, and chlorhexidine and tea polyphenol mouthwash can effectively inhibit plaque and reduce inflammation. There are few studies on the comparative efficacy of chlorhexidine and tea polyphenol rinses in China, and chlorhexidine rinses may cause periodontal microorganisms to develop drug resistance. For adolescent gingivitis, the therapeutic effect of each active ingredient of the two types of mouthwash through different mechanisms should be differentiated to help clinicians treat periodontal problems and prevent the occurrence of periodontal disease according to individual patient conditions.

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References

[1] Feng Xiping. Oral health status of the Chinese population - Report of the fourth Chinese oral health epidemiological survey. Paper presented at: The 18th Annual Conference of the Chinese Society of Stomatology on Preventive Stomatology 2018; Xi'an, Shaanxi, China.

[2] Page RC, Schroeder HE. Pathogenesis of inflammatory periodontal disease. A summary of current work. Lab Invest. Mar 1976; 34 (3): 235-249.

[3] Chapple ILC, Mealey BL, Van Dyke TE, et al. Periodontal health and gingival diseases and conditions on an intact and a reduced periodontium: Consensus report of workgroup 1 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Clin Periodontol. Jun 2018; 45 Suppl 20: S68-s77.

[4] Wojcicki CJ, Harper DS, Robinson PJ. Differences in periodontal disease-associated microorganisms of subgingival plaque in prepubertal, pubertal and postpubertal children. J Periodontol. Apr 1987; 58 (4): 219-223.

[5] Delaney JE, Ratzan SK, Kornman KS. Subgingival microbiota associated with puberty: studies of pre-, circum-, and postpubertal human females. Pediatr Dent. Dec 1986; 8 (4): 268-275.

[6] Nakagawa S, Machida Y, Nakagawa T, et al. Infection by Porphyromonas gingivalis and Actinobacillus actinomycetemcomitans, and antibody responses at different ages in humans. J Periodontal Res. Jan 1994; 29 (1): 9-16.

[7] Löe H. Periodontal changes in pregnancy. The Journal of periodontology. 1965; 36 (3): 209-217.

[8] Kalkwarf KL. Effect of oral contraceptive therapy on gingival inflammation in humans. Journal of periodontology. 1978; 49 (11): 560-563.

[9] Wang Yefei. Inhibitory effect of tea polyphenols (TP) on bacteria. Tea. 1994 (03): 37-41.

[10] Ishihara N, Chu D-C, Akachi S, Juneja L. Improvement of intestinal microflora balance and prevention of digestive and respiratory organ diseases in calves by green tea extracts. Livestock Production Science. 2001; 68 (2-3): 217-229.

[11] Tang, Yu-Fang, Gong, Zheng-Li. A preliminary study on the deodorization mechanism of tea extracts. Tea Letters. 2000 (03): 35-36.

[12] You Shiqi, Hong Fangyao, Li Zheguang, Huang Ketai, Xu Yuan, Yang Xianqiang. Study on the prevention of caries by green tea polyphenols in China. Chinese Journal of Stomatology. 1993 (04): 197-199+254.

[13] McNaught J. On the action of cold or lukewarm tea on Bacillus typhosus. BMJ Military Health. 1906; 7 (4): 372-373.

[14] Shen T, Khor SC, Zhou F, et al. Chemoprevention by lipid-soluble tea polyphenols in diethylnitrosamine/phenobarbital-induced hepatic pre-cancerous lesions. Anticancer research. 2014; 34 (2): 683-693.

[15] Sudano Roccaro A, Blanco AR, Giuliano F, Rusciano D, Enea V. Epigallocatechin-gallate enhances the activity of tetracycline in staphylococci by inhibiting its efflux from bacterial cells. Antimicrob Agents Chemother. Jun 2004; 48 (6): 1968-1973.

[16] Hisano M, Yamaguchi K, Inoue Y, et al. Inhibitory effect of catechin against the superantigen staphylococcal enterotoxin B (SEB). Arch Dermatol Res. Sep 2003; 295 (5): 183-189.

[17] Xu X, Zhou XD, Wu CD. The tea catechin epigallocatechin gallate suppresses cariogenic virulence factors of Streptococcus mutans. Antimicrob Agents Chemother. Mar 2011; 55 (3): 1229-1236.

[18] Sirk TW, Brown EF, Friedman M, Sum AK. Molecular binding of catechins to biomembranes: relationship to biological activity. J Agric Food Chem. Aug 12 2009; 57 (15): 6720-6728.

[19] Sirk TW, Brown EF, Sum AK, Friedman M. Molecular dynamics study on the biophysical interactions of seven green tea catechins with lipid bilayers of cell membranes. J Agric Food Chem. Sep 10 2008; 56 (17): 7750-7758.

[20] Sharma A, Gupta S, Sarethy IP, Dang S, Gabrani R. Green tea extract: possible mechanism and antibacterial activity on skin pathogens. Food Chem. Nov 15 2012; 135 (2): 672-675.

[21] Gradisar H, Pristovsek P, Plaper A, Jerala R. Green tea catechins inhibit bacterial DNA gyrase by interaction with its ATP binding site. J Med Chem. Jan 25 2007; 50 (2): 264-271.

[22] Akagawa M, Shigemitsu T, Suyama K. Production of hydrogen peroxide by polyphenols and polyphenol-rich beverages under quasi-physiological conditions. Biosci Biotechnol Biochem. Dec 2003; 67 (12): 2632-2640.

[23] Arakawa H, Maeda M, Okubo S, Shimamura T. Role of hydrogen peroxide in bactericidal action of catechin. Biological and Pharmaceutical Bulletin. 2004; 27 (3): 277-281.

[24] Wu DH, Guan ZEM, Li YAD. Study on the treatment of juvenile gingivitis with compound tea polyphenol rinse. Paper presented at: The First Annual Meeting of the General Practice Stomatology Committee of the Chinese Society of Stomatology 2009; Tianjin, China.

[25] Wang P, Ji P, Luo QW, Zhang W, Tang M. The effect of a compound tea polyphenol rinse on subgingival flora and gingival sulcus endotoxin levels in periodontitis patients. Effect of compound tea polyphenol rinse on subgingival flora and gingival sulcus fluid endotoxin levels in patients with periodontitis. Journal of Chongqing Medical University. 2007 (01): 72-75.

[26] Gillette W. Re: Antibiotics and periodontal disease. J Periodontol. Jul 1996; 67 (7): 726.

[27] Xiao Yue, Liu Tianjia, Huang Zhengwei, Zhou Xuedong, Zhan Ling, Li Jiyao. Experimental study on the effect of tea polyphenols on the caries-causing power of oral bacteria. Guangdong Dental Disease Control. 2002 (01): 4-6.

[28] Yang Hailun, Liu Xiaoxiang, Zhu Junli, Li Jianrong. Research progress on the antibacterial properties of tea polyphenols. Food Industry Science and Technology. 2015; 36 (21): 385-389.

[29] Gilbert P, Moore LE. Cationic antiseptics: diversity of action under a common epithet. J Appl Microbiol. 2005; 99 (4): 703-715.

[30] Cieplik F, Jakubovics NS, Buchalla W, Maisch T, Hellwig E, Al-Ahmad A. Resistance Toward Chlorhexidine in Oral Bacteria - Is There Cause for Concern? Front Microbiol. 2019; 10: 587.

[31] Janakiram C, Venkitachalam R, Fontelo P, Iafolla TJ, Dye BA. Effectiveness of herbal oral care products in reducing dental plaque & gingivitis - a systematic review and meta-analysis. BMC Complement Med Ther. Feb 11 2020; 20 (1): 43.

[32] Wade WG. The oral microbiome in health and disease. Pharmacol Res. Mar 2013; 69 (1): 137-143.

[33] Hoffmann T, Bruhn G, Richter S, Netuschil L, Brecx M. Clinical controlled study on plaque and gingivitis reduction under long-term use of low-dose chlorhexidine solutions in a population exhibiting good oral hygiene. Clin Oral Investig. Jun 2001; 5 (2): 89-95.

[34] Roberts WR, Addy M. Comparison of the in vivo and in vitro antibacterial properties of antiseptic mouthrinses containing chlorhexidine, alexidine, cetyl pyridinium chloride and hexetidine. Relevance to mode of action. J Clin Periodontol. Aug 1981; 8 (4): 295-310.

[35] Hajishengallis G, Liang S, Payne MA, et al. Low-abundance biofilm species orchestrates inflammatory periodontal disease through the commensal microbiota and complement. Cell Host Microbe. Nov 17 2011; 10 (5): 497-506.

[36] Bartold PM, Van Dyke TE. An appraisal of the role of specific bacteria in the initial pathogenesis of periodontitis. J Clin Periodontol. Jan 2019; 46 (1): 6-11.

[37] James P, Worthington HV, Parnell C, et al. Chlorhexidine mouthrinse as an adjunctive treatment for gingival health. Cochrane Database Syst Rev. Mar 31 2017; 3 (3): Cd008676.

[38] Tribble GD, Angelov N, Weltman R, et al. Frequency of Tongue Cleaning Impacts the Human Tongue Microbiome Composition and Enterosalivary Circulation of Nitrate. Front Cell Infect Microbiol. 2019; 9: 39.

[39] Hyde ER, Luk B, Cron S, et al. Characterization of the rat oral microbiome and the effects of dietary nitrate. Free Radic Biol Med. Dec 2014; 77: 249-257.

[40] Bescos R, Ashworth A, Cutler C, et al. Effects of Chlorhexidine mouthwash on the oral microbiome. Sci Rep. Mar 24 2020; 10 (1): 5254.

[41] Chatzigiannidou I, Teughels W, Van de Wiele T, Boon N. Oral biofilms exposure to chlorhexidine results in altered microbial composition and metabolic profile. NPJ Biofilms Microbiomes. Mar 20 2020; 6 (1): 13.

[42] Mayer FL, Wilson D, Hube B. Candida albicans pathogenicity mechanisms. Virulence. Feb 15 2013; 4 (2): 119-128.

[43] LaFleur MD, Kumamoto CA, Lewis K. Candida albicans biofilms produce antifungal-tolerant persister cells. Antimicrob Agents Chemother. Nov 2006; 50 (11): 3839-3846.

[44] Samonis G, Gikas A, Anaissie EJ, et al. Prospective evaluation of effects of broad-spectrum antibiotics on gastrointestinal yeast colonization of humans. Antimicrob Agents Chemother. Jan 1993; 37 (1): 51-53.

[45] Sam QH, Chang MW, Chai LY. The Fungal Mycobiome and Its Interaction with Gut Bacteria in the Host. Int J Mol Sci. Feb 4 2017; 18 (2).

[46] Barnes RA. Early diagnosis of fungal infection in immunocompromised patients. J Antimicrob Chemother. Jan 2008; 61 Suppl 1: i3-6.

[47] Franco Neto CA, Parolo CC, Rösing CK, Maltz M. Comparative analysis of the effect of two chlorhexidine mouthrinses on plaque accumulation and gingival bleeding. Braz Oral Res. Apr-Jun 2008; 22 (2): 139-144.

[48] Keijser JA, Verkade H, Timmerman MF, Van der Weijden FA. Comparison of 2 commercially available chlorhexidine mouthrinses. J Periodontol. Feb 2003; 74 (2): 214-218.

[49] Van Strydonck DA, Timmerman MF, van der Velden U, van der Weijden GA. Plaque inhibition of two commercially available chlorhexidine mouthrinses. J Clin Periodontol. Mar 2005; 32 (3): 305-309.

[50] Cumming BR, Löe H. Optimal dosage and method of delivering chlorhexidine solutions for the inhibition of dental plaque. J Periodontal Res. 1973; 8 (2): 57-62.

[51] Agerbaek N, Melsen B, Rölla G. Application of chlorhexidine by oral irrigation systems. Scand J Dent Res. Sep 1975; 83 (5): 284-287.

[52] Jenkins S, Addy M, Newcombe RG. Dose response of chlorhexidine against plaque and comparison with triclosan. J Clin Periodontol. Apr 1994; 21 (4): 250-255.

[53] Flötra L, Gjermo P, Rölla G, Waerhaug J. Side effects of chlorhexidine mouth washes. Scand J Dent Res. 1971; 79 (2): 119-125.

[54] Haydari M, Bardakci AG, Koldsland OC, Aass AM, Sandvik L, Preus HR. Comparing the effect of 0.06% -, 0.12% and 0.2% Chlorhexidine on plaque, bleeding and side effects in an experimental gingivitis model: a parallel group, double masked randomized clinical trial. BMC Oral Health. Aug 18 2017; 17 (1): 118.

[55] Turesky S, Gilmore ND, Glickman I. Reduced plaque formation by the chloromethyl analogue of victamine C. J Periodontol. Jan 1970; 41 (1): 41-43.

[56] Löe H. The Gingival Index, the Plaque Index and the Retention Index Systems. J Periodontol. Nov-Dec 1967; 38 (6): Suppl: 610-616.

[57] Rupp ME. Do chlorhexidine patient baths prevent catheter-associated urinary tract infections? Lancet Infect Dis. Jan 2016; 16 (1): 8-9.

[58] Saleem HG, Seers CA, Sabri AN, Reynolds EC. Dental plaque bacteria with reduced susceptibility to chlorhexidine are multidrug resistant. BMC Microbiol. Sep 15 2016; 16: 214.

[59] Mazur M, Ndokaj A, Jedlinski M, Ardan R, Bietolini S, Ottolenghi L. Impact of Green Tea (Camellia Sinensis) on periodontitis and caries. Systematic review and meta-analysis. Jpn Dent Sci Rev. 2021 Nov; 57: 1-11.