CLINICAL EFFICACY AND MECHANISM OF SWISS HERBAL TOOTHPASTES ANTIBACTERIAL AND ANTI-INFLAMMATORY EFFECTS ON PATIENTS WITH GINGIVITIS AND EARLY STAGES OF PERIODONTITIS

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Abstract

Introduction: Oral diseases are common diseases that occur frequently and are estimated to affect half of the global population. The occurrence and development of most oral diseases are closely associated with oral microbiome.

Methodology: Individuals with gingivitis and the early stages of periodontitis participated in a 2-month comparative clinical study comparing toothpaste with 1450 ppm sodium monofluorophosphate and xylitol (the control; 15 patients) and tooth paste containing further botanical extracts (the experiment; 35 patients). The Loe & Silness, CPITN, OHI-S, and PMA indices were used to measure the clinical indicators of gingivitis and periodontitis. In the gingival crevicular fluid and plaque, the pro-inflammatory and anti-inflammatory interleukins, nitrites/nitrates, total antioxidant activity, and bacterial distribution typical of gingivitis and periodontitis were measured.

Results: Clinically speaking, experimental toothpaste was more effective in reducing the bacterial load specifically associated with gingivitis/periodontitis. Herbal extracts had anti-inflammatory, anti-oxidant, direct, and indirect anti-bacterial activities through inhibition of bacterial defence versus phagocytes, whereas the control toothpaste had a modest direct anti-bacterial impact.

Conclusions: Due to their various modes of action, chemical and plant-derived anti-bacterials should be used in conjunction to treat gingivitis and periodontitis at their early stages. Due to their numerous beneficial impacts, plant-derived active ingredients for oral care could replace hazardous chemicals.

Keywords- Herbal Toothpastes**,** Anti-Inflammatory, Periodontitis, Antibacterial

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1. Introduction

It is believed that half of the world's population suffers from oral disorders, which are regular and prevalent diseases.[1] The oral microbiota is closely linked to the onset and progression of the majority of oral illnesses (Marsh, 2005).[2] According to Marsh and Zaura (2017), more than 700 species of oral bacteria, fungus, and other microorganisms are incorporated in extracellular matrix and operationally and physically adhere to the surface of teeth or the oral mucosa as biofilms.[3] Adversary As one of the original colonisers, Streptococcus sanguinis has the ability to create hydrogen peroxide, which mediates bacterial relationships and alters the spatial-temporal organisation of the species in dental plaque (Cheng et al., 2020).[4] Streptococcus mutans is thought to be the main cause of dental caries because of its acidogenic/aciduric properties and capacity to create extracellular matrix (Klein et al., 2015).5. Lipopolysaccharide (LPS), one of the virulence factors produced by Porphyromonas gingivalis, is significant in the development of gingivitis and periodontitis.[6]

These microbes can also drive the host to release cytokines like interleukin-1 (IL-1) and interleukin-6 (IL-6) within the oral biofilm, affecting the host's immunological homeostasis and leading to oral dysbiosis (Hajishengallis and Lamont, 2021).[7] Thus, it is crucial to prevent tooth plaque from forming in order to preserve oral health. Dental plaque can be reduced using a variety of mechanical and chemical techniques, such as flossing, mouthwash, and using a toothbrush and fluoride-containing toothpaste (Almas and Almas, 20148; Chapple et al., 20159). Studies have shown that such routine oral hygiene techniques are successful in lowering dental plaque, regulating dental caries, and controlling periodontal illnesses. Nevertheless, it is vital to apply antibacterial and anti-inflammatory medications in combination to the high-risk population.[6]

In the current clinical laboratory research, we assessed the clinical efficacy of toothpaste containing four medicinal plant extracts (Arnica montana, Salvia officinalis, Chamomilla recutita, and Echinacea purpurea) and chemical anti-bacterial substances (sodium monofluorophosphate (1450 ppm) and Xylitol) in a sample of patients with gingivitis and early stages of periodontitis. Herbal-free toothpaste was utilised as the control. The laboratory component of the clinical investigation was made to separate the antibacterial effects of the toothpaste's chemical and plant-derived ingredients and to clarify the methods by which these antibacterial benefits were accomplished. It was also done to compare the anti-inflammatory and redox balancing properties of the experimental and control toothpastes.

2. Methodology

Sodium monofluorophosphate (1450 ppm), xylitol, and Swiss medicinal herbs were the active constituents in the toothpaste under investigation (experimental toothpaste (ETP), Trisa Revital Sensitive, manufacturer TRISA AG, Triengen. The medicinal plants used as the active ingredients were aqueous-ethanol extracts of Chamomilla recutita leaves (containing no less than 0.1% alpha-bisabolol), Salvia officinalis leaves (containing no less than 10% total phenols), Arnica montana flowers (containing no less than 0.04% sesquiterpene lacton), and Echinacea purpurea flowers (containing no less than 1% echinacoside). The control toothpaste (CTP) included xylitol and sodium monofluorophosphate (1450 ppm) in addition to the identical excipients as the experimental toothpaste (ETP). These two toothpastes were used for clinical and laboratory evaluation in order to distinguish clinical and biological effects of herbal constituents from those of fluoride and xylitol. Individual herbal extracts of Chamomilla recutita leaves, Salvia officinalis leaves, Arnica montana flowers, and Echinacea purpurea flowers (all purchased from Biologica AG) were incorporated into the bacteria in the in vitro experiments to demonstrate their specific anti-bacterial effects. These extracts may have also been combined in the toothpaste's proportions. To assess the impact on bacterial catalase and intracellular bacterial death, they were also given to bacteria before phagocytosis by human granulocytes. At Department of the Public Health Dentistry at Rama Dental College in Kanpur, 50 patients of both sexes (aged 35 to 55) with gingivitis or early periodontitis participated in the investigation. The Institutional Ethical Committee carefully reviewed and approved the research protocol. The patients were split into experimental and control groups at randomly. Table 1 displays the demographic breakdown of those with periodontitis in the various groups.

Group Patients	Age,	Years	M	F		Gingivitis	Initial PD
Experimental (conventional treatment + ETP twice a	35	35–55	12	23	5	8	27
day for 60 days)							

Control (conventional treatment + CTP twice a day for 60 days)	15	36–55	7	8	3	3	12

If necessary, each of them received treatment using conventional therapeutic and hygienic procedures. The traditional course of therapy includes instruction in good oral hygiene, plaque removal, polishing of the teeth's enamel, and, if necessary, tartar eradication. Prior to the clinical investigation, all patients who were enrolled in it agreed to a "wash-out period" of 72 hours without using any toothpaste. Participants in the control (n = 15) and experimental (n = 35) groups were instructed to brush their teeth twice daily for 60 days with CTP or ETP, respectively. The participants received the free toothpaste samples from the distributor. The patients received dental hygiene instruction. Patients were not informed if they were using placebo or experimental toothpaste, and the toothpaste tubes were given numbers. The usage of CTP or ETP was not disclosed to the laboratory staff or medical professionals who were performing the measurements and clinical evaluation procedures. These reasons lead to the classification of this pilot clinical investigation as a double-blind, placebo-controlled research. Healthy donors matched by sex and age (n = 25) were recruited from the Medical Department staff and trainees, who donated gingival crevicular fluid (GCF). The normal ranges of different markers in GCF derived from the measurements performed on this biological material. Individuals with virus hepatitis and subjects with severe chronic and/or infectious disorders in the acute phase have been removed from the research. No subjects or controls had used any medications or nutritional supplements known to affect redox status or inflammation for at least six weeks before to enrollment in the trial. None of the three cohorts under study had any alcohol or drug abusers. Three smokers made up the control group while there were five smokers in the experimental group. All participants gave their agreement for biological material sample and the gathering of individual and anamnestic information. The subjective opinions of doctors and patients as well as objective clinical indicators of gingivitis and chronic mild periodontitis were used to evaluate the clinical efficacy of ETP and CTP. These indices included the Löe and Silness method's gingival and plaque indexes, Parma's papillae-gum margin-alveolar (PMA) index measuring gingival inflammation, the International CPITN test, and the OHI-S index. The clinical research's indicators were all determined twice, on days 0 and 60. The Periodontal World Health Organisation index defines the need of therapy against periodontal pathologies of any type. With the help of a special graduated periodontal probe, the clinical state of gingival sulcus and periodontal tissue in the vicinity of six teeth was registered and expressed as a score: 0—absence of pathology; 1—bleeding after the probe introduction; top of the gum is slightly inflamed; 2—pathological gingival pocket of 4–5 mm in depth; 3—pathological gingival pocket of 6 mm and more in depth. The final result was calculated from the ratio of the score sum divided by 6. The clinical significance of the CPITN score was as follows: 0—no therapy needed; 1—instructions on individual oral hygiene are needed; 2–3—professional oral/teeth hygiene is needed plus instruction on vidual oral hygiene; 4—complex therapy of periodontal tissues is necessary. The PMA index allows semi-quantitative assessment of the gum state and diagnosis of gingivitis. Gums were stained by a special non-toxic dye and the PMA score was determined by analysis of the dye penetration into gingiva: 0—no penetration, no inflammation; 1—moderate inflammation of gingival papilla (P); 2—inflammation of marginal gum (M); 3—inflammation of alveolar gum (A). The PMA index was expressed in % and calculated by the formula:

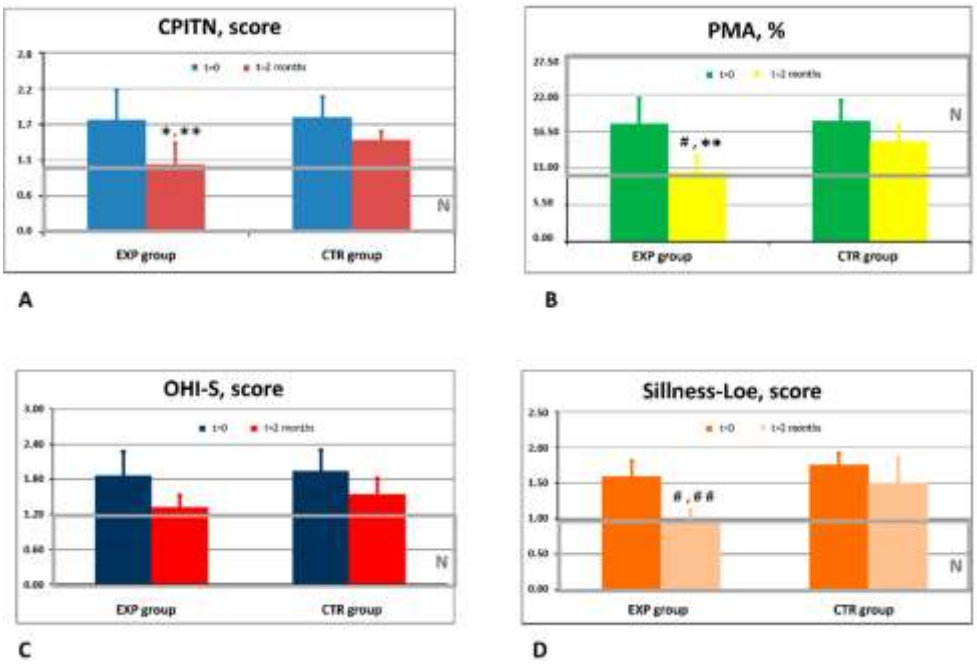
$$PMA = \frac{\text{scores}}{3 \times \text{number of teeth} \times 100\%}.$$

The clinical significance of PMA defines mild gingivitis at 30%, moderate gingivitis at 31%–60%, and severe gingivitis at 61% and above. A streamlined version of the Green-Vermillion index called Index OHI-S was launched in 1964. OHI-S = (TP/n) + (TT/n), where n is the number of teeth, where TP and TT are the amounts of plaque and tartar on each tooth, is an index that measures the level of oral hygiene. The Silness-Loe Index assigns a score between 0 and 3 depending on how thick the plaque is next to the gums. Sigma Chemical Co. provided almost all of the chemical reagents and solvents, the H2O2 standard, and the media for growing human and bacterial cells. Cayman Chem. Co. provided the enzyme activity and nitrite/nitrate assay kits, and Bio-Rad Laboratories provided the monoclonal antibodies for the ELISA interleukin kits. The in vitro investigation included ten distinct Staphylococcus aureus strains that were obtained from the nasal and oral cavities. Table 1 contains a collection of the strains. As previously stated, S. aureus was cultured in tryptic soy broth at 37 °C with constant shaking.

3. Results

Every single person in the experimental group reported enjoying the flavour and scent of the ETP, as well as the toothpaste's mild but pleasant foaming, decreased gum bleeding (32 patients), and tooth whitening (17 patients). Although none of the control group participants noticed any clinical effects, they did notice a pleasant flavour and minor foaming of CTP. Doctor-investigators noted that ETP had a high clinical efficacy, and their findings were in perfect agreement with the findings of impartial instrumental investigations (Figure 1).

Figure 1. Effects of experimental (ETP) and control toothpaste (CTP) on clinical markers of gingivitis and periodontitis.

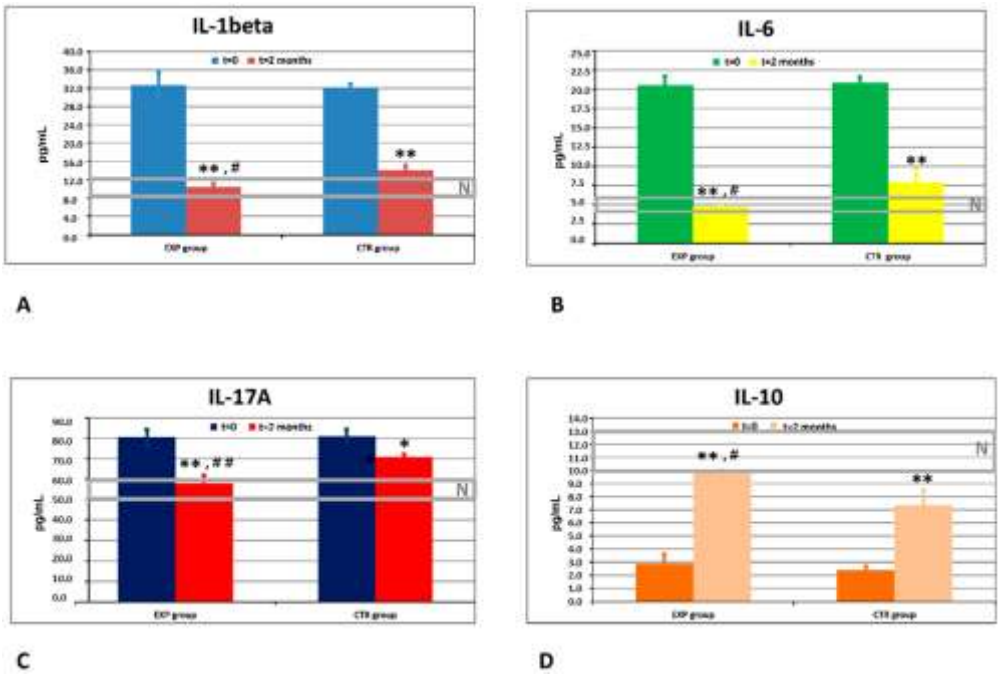


(A) Dynamics of the CPITN score in the experimental (EXP) and control (CTR) groups of patients. *N*-normal range of values obtained in healthy people. * $p < 0.05$ vs. baseline values; ** $p < 0.05$ vs. CTR. (B) Dynamics of the PMA index in the experimental (EXP) and control (CTR) groups of patients. *N*-normal range of values obtained in healthy people. # $p < 0.01$ vs. baseline values; ** $p < 0.05$ vs. CTR. (C) Dynamics of t hOHI-S score in the experimental (EXP) and control (CTR) groups of patients. N-normal range of values obtained in healthy people. (D) Dynamics of the gingival and plaque Sillness–Loe score in the experimental (EXP) and control (CTR) groups of patients. N-normal range of values obtained in healthy people. # $p < 0.01$ vs. baseline values; ## $p < 0.01$ vs. CTR. The evaluation with the traditional objective indicators of gingival and periodontal states revealed that the recruited patients assigned to both the control and experimental groups had clinical signs of gingivitis and the early stage of periodontitis (Figure 1). The experimental and control groups shared the same initial values for the four clinical indices. During a 60-day use of both ETP and CTP, the indicators of gingivitis, general oral hygiene, plaque and tartar presence, and mild periodontitis decreased. In the EPT group, as opposed to the CTP group, the effects measured objectively by the four indices (CPITN, PMA, OHI-S, and Loe & Sillness) were statistically substantially greater (p 0.05). By the end of the research, these indices returned to normal levels in the EPT group, while indexes in the CTP group still exceeded normal values.

Effects of ETP and CTP on Pro- and Anti-Inflammatory Cytokines in GCF

Figure 2A–D compares the measurement and dynamics of three pro-inflammatory cytokines (IL-1, IL-6, and IL17A) and one anti-inflammatory cytokine (IL-10) in GCF to the comparable levels in healthy individuals. Basal levels of IL-1, IL-6, and IL17A, all pro-inflammatory cytokines, were significantly and equally raised in the GCF, although IL-10 values were lowered in both the experimental and control groups when weighed against healthy control subjects. The GCF cytokine concentrations in the patients in the ETP group attained normal levels by day 60 of the clinical investigation, whereas normal GCF cytokine levels were never attained in the control group. Differences between the groups were statistically significant ($p < 0.05$).

Figure 2. Effects of ETP and CTP on inflammatory cytokines in gingival crevicular fluid (GCF).



(A) Dynamics of cytokine IL-1beta (pg/mL) in the experimental (EXP) and control (CTR) groups of patients. *N*: normal range of values obtained in healthy people. ** $p < 0.01$ vs. baseline values; # $p < 0.05$ vs. CTR. (B) Dynamics of cytokine IL-6 (pg/mL) in the experimental (EXP) and control (CTR) groups of patients. *N*: normal range of values obtained in healthy people. ** $p < 0.01$ vs. baseline values; # $p < 0.05$ vs. CTR. (C) Dynamics of cytokine IL-17A (pg/mL) in the experimental (EXP) and control (CTR) groups of patients. *N*: normal range of values obtained in healthy people. * $p < 0.05$ vs. baseline values; ** $p < 0.01$ vs. background values; ## $p < 0.01$ vs. CTR. (D) Dynamics of cytokine IL-10 (pg/mL) in the experimental (EXP) and control (CTR) groups of patients. *N*: normal range of values obtained in healthy people. ** $p < 0.01$ vs. baseline values; # $p < 0.05$ vs. CTR. Data from healthy donors who were matched for age and sex (n = 25) and recruited patients were contrasted. In comparison to normal values, the NO2 and NO3 levels in GCF were significantly higher in the experimental and control groups. The experimental and control groups had the same initial NO2/NO3 concentrations. By the 60th day, levels of this pro-inflammatory marker had significantly decreased in the ETP group and had returned to normal levels, while they had remained elevated in the control group.

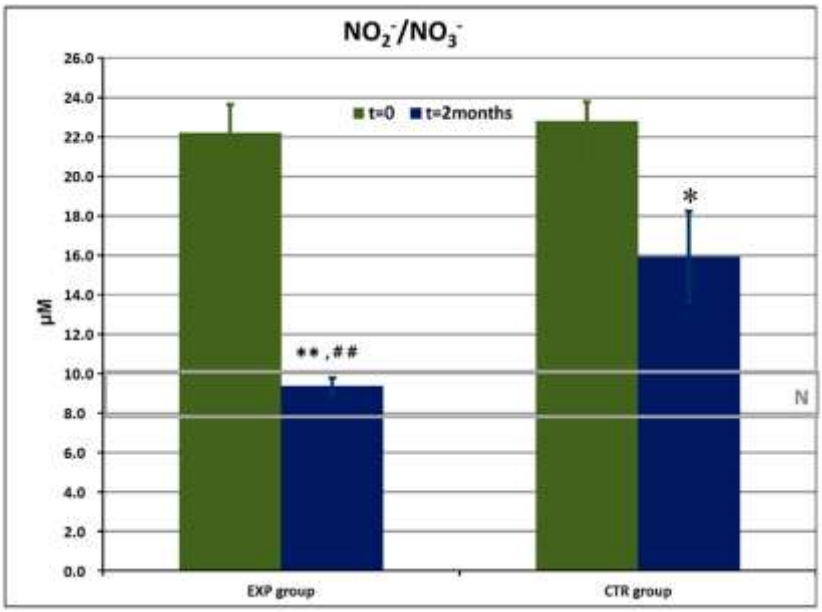


Figure 3. Nitrite and nitrate (NO_2^-/NO_3^-) levels (µM) in gingival crevicular fluid in the experimental(EXP) and control (CTR)

groups of patients before and after the trial. N: normal range of values obtained in healthy people. * $p <$ 0.05 vs. baseline values; ** $p < 0.01$ vs. baseline values; ## $p < 0.01$ vs. CTR.

Similar data were obtained with total AOA in GCF (Figure 4).

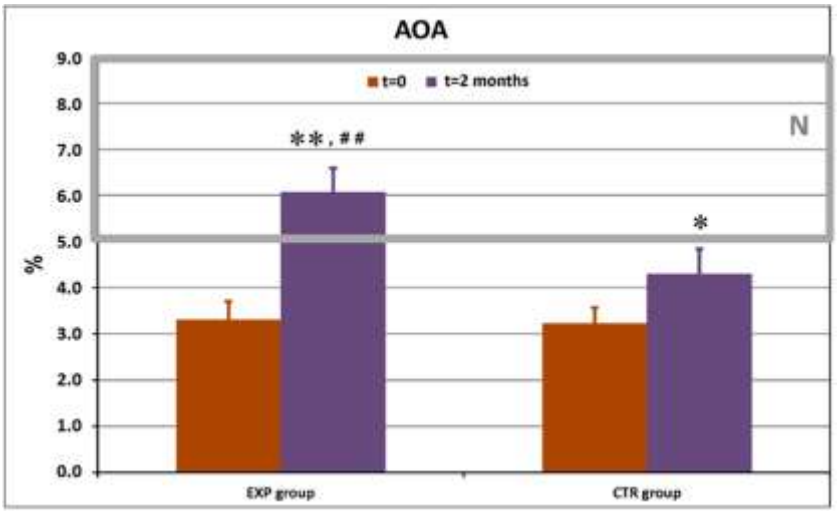


Figure 4. Total antioxidant activity (AOA, %) in gingival crevicular fluid in the experimental (EXP) and control (CTR)

groups of patients before and after the trial. *N*: normal range of values obtained in healthy people. * $p < 0.05$ vs. baseline values; ** $p < 0.01$ vs. baseline values; ## $p < 0.01$ vs. CTR. Both groups' initial total AOA readings were below average. The traditional oxidative stress measure was brought back to normal following the application of ETP. The mean total AOA value was somewhat raised by CTP, although it remained below average (p 0.05).Prior to the start of the trial and following its completion, quantitative real-time PCR research was done on a subset of patients from the experimental (n = 15) and control (n = 5) groups. The levels of seven periodontal pathogens that contribute to the development of gingivitis and periodontitis, chronic inflammatory diseases of the periodontal tissues, were all identified. This data showed that both toothpastes eliminated several bacterial periodontal pathogens, diminishing their local gingivalconcentrations below a pathologically significant threshold. However, ETP was more

efficient than CTP against three pathogens: *P.g.*, *F.n.*, and *P.i.*

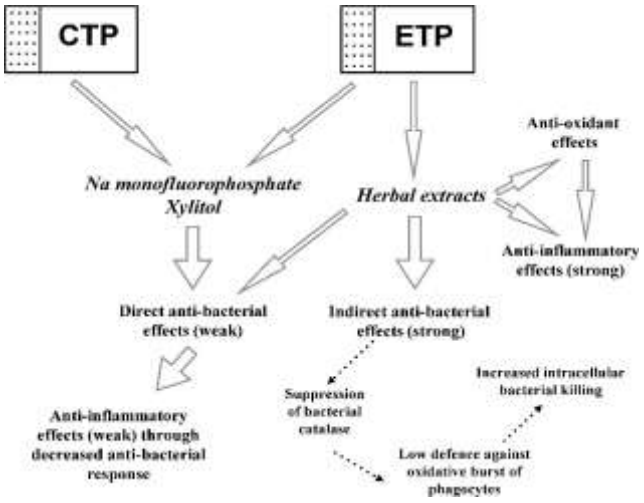
Table 2. The sources and characteristics of S. aureus strains used in experiments on phagocytosis, intracellular killing, catalase-inhibiting, and anti-microbial activity of ETP and CTP.

Strain Number	Source of Isolation	Resistance to 5–10	Catalase Activity (Units/2 × 10^7 Bacteria)
1523	Throat, tonsils (chronic	+++	**5.1**
1546	Oral epithelia	–	**2.1**
1549	Throat, tonsils (chronic	++	**3.7**
1555	Oral epithelia	+	**2.3**
1561	Nasal sinuses (sinusitis)	−	**2.2**
1612	Oral epithelia	–	**2.2**
1620	Throat, tonsils (chronic	+	**2.6**
1643	Throat, tonsils (chronic	–	**2.2**
1670	Oral epithelia	–	**2.1**
1780	Throat, tonsils (chronic	–	**2.0**

4. Discussion

The experimental and control groups were randomly assigned to patients who joined this clinical laboratory study on the basis of the inclusion and exclusion criteria. By sex, age, and baseline clinical ratings for gingivitis and early-stage periodontitis, individuals in the two groups were matched (Table 1 and Figure 1). In the experimental group of patients, as compared to the start of the trial and as contrasted to the control group, clinical indicators of gingivitis and periodontitis, such as PMA, Sillness-Loe, and CPITN indexes, statistically improved significantly. Nevertheless, the clinical impact of CTP did not reach statistical significance. For the control group, the indicators of gum health and plaque, including the presence of plaque at the gingival border, the number of teeth with thick plaque and tartar, inflammation, bleeding, and bleeding on the probe, showed a tendency of enhancement. In light of these factors, we draw the conclusion that ETP significantly enhanced periodontal tissues' clinical state, reducing gum inflammation (bleeding and redness), plaque accumulation, and early signs of periodontitis. After these optimistic clinical results and evaluations made by physicians and patients, we moved on to in-depth laboratory analyses to clarify the processes underlying the ETP's exceptional clinical performance. Although it is widely acknowledged that poly-microbial infection plays a significant role in the development of gingivitis and periodontitis, mechanical clearance of microbial biofilms as well as systemic and topical antibiotics are the main therapeutic options. An extensive search for alternative non-toxic, clinically- and cost-effective treatments, such as toothpastes, mouthwashes, and gingival gels, to avoid and reduce local bacterial overload was prompted by the need for frequent painful cleaning procedures and the developed resistance of dental bacteria to antibiotics. In recent times, it has been proposed that molecules that limit the growth of biofilm (plaque) could both prevent and treat gingivitis and periodontitis. Flavonoids, 2-aminoimidazole alkaloids, and halogenated furanones of plant origin have all been found to be among the most powerful disrupters of microbial biofilms.[10] The herbal ingredients that make up ETP are well known for their many positive health benefits. All four of the medicinal plants utilised in the ETP formulations have acceptable safety profiles for topical usage. They are lauded for their ability to exert in vitro and in vivo antibacterial, anti-inflammatory, and anti-oxidant actions at low non-toxic doses.[11,12] First, S. aureus bacterial cultures were used to investigate the direct anti-bacterial activity of ETP, CTP, and herbal extract-constituents of ETP. ETP was quite effective in preventing or killing bacterial development, it should be mentioned. The efficiency of ETP was

around two orders of magnitude more than that of CTP, although the direct anti-bacterial effects of the herbal extracts were quite marginal. Our findings were consistent with evidence that had been published on the rather mild anti-bacterial effects of the two chemical components of CTP, disodium fluoride monophosphate and xylitol.[13,14,15,16] The herbal actives of ETP suppressed a microbial defence against an oxidative burst thus, bacteria were effectively killed intracellularly. Striking a resemblance to the catalase-suppressing effects observed for ETP, plant extracts used in ETP and standardised fermented papaya gel17 suggests that polyphenols, secondary metabolites in abundance in all these plant products, could be excellent anti-bacterials, acting at the level of granulocyte-bacteria interaction. Reactive oxygen and reactive nitrogen species (ROS and RNS, respectively) represent the first line of the anti-microbial host defence. They are produced in great excess during primary oxidative responses of phagocytes to fight bacteria, viruses, parasites, etc. Local and generalized oxidative stress have, for a long time, been considered a molecular hallmark of gingivitis and periodontitis. Here, we found that all recruited patients with clinically proven gingivitis and moderate periodontitis had background levels of nitrite and nitrates (RNS) that were significantly elevated compared to values within the normal range. When compared to day 0 and the control group, the administration of ETP completely normalised the nitrite/nitrate radio, whereas the use of CTP caused a less pronounced drop (p 0.05 vs. day 0). The total antioxidant activity in GCF, on the other hand, was lower than normal in both groups at day 0 and returned to normal levels in the ETP group by the end of the research (p 0.01 vs. day 0 and vs. CTP group). Despite a small increase, the total AOA continued to be below the lower border of normalcy (p 0.05). We suggested that the normal balance of pro- and anti-oxidants in GCF achieved after a 2-month-long use of ETP could be attributed to herbal constituents known for their antioxidant properties.[18,19] Pro-inflammatory and anti-inflammatory cytokines, proteins made by immune cells in reaction to biotic and abiotic stimuli, operate as regulators of cell-cell interactions during inflammatory reactions while ROS and RNS are low molecular weight mediators of inflammation. Periodontal bacteria are among the adaptive stress responses that are brought on by any change in producing cytokines.[20,21] Since the inflammatory (IL-1, IL-6, and IL-17A) and anti-inflammatory (IL-10) cytokine patterns in GCF were fully normalised, the current investigation demonstrated the impressive anti-inflammatory impact of ETP. Reduced bacterial excess brought on by chemical anti-bacterials and herbal components may help to explain the apparent reduction of inflammation in both groups. Our findings are consistent with studies on the therapeutic effects of dental care products containing essential oils, which showed superior results in reducing plaque and gingival inflammation when contrasted with placebo or control toothpastes and mouthwashes. Based on the findings, we draw the conclusion that the experimental toothpaste, which contained sodium mono-fluoride phosphate, xylitol, and therapeutic Swiss herbs, was more clinically effective than the control toothpaste, which contained only sodium mono-fluoride phosphate and xylitol. We further assume that more indirect anti-bacterial action through suppression of bacterial defence against oxidative bursts of host phagocytes, as well as direct anti-inflammatory and anti-oxidant effects, may be molecular events underlying greater-than-control clinical impacts on patients with gingivitis and early stages of periodontitis. These incidents are shown graphically as a whole.



Sodium monofluorophosphate and Xylitol are active components in both CTP and ETP that have negligible antibacterial effects. The subsequent inflammatory response from the host immune cells was reduced (mild anti-inflammatory effects) as a result of the decreased bacterial burden generated by the two drugs. ETP also includes Swiss medicinal plant extracts that have poor direct and high indirect antibacterial actions. These effects are based on the suppression of adaptive bacterial catalase activity in response to oxidative bursts in host phagocytes. Enhanced intracellular death of bacteria ingested during phagocytosis results from bacterial catalase suppression. Additionally, the medicinal plants from Switzerland have powerful direct anti-oxidant and anti-inflammatory capabilities.

5. Conclusion

There is still a critical need for safer therapy with great efficacy, despite several attempts to establish clinically and economically viable regimens to prevent and treat gingivitis and the early stages of periodontitis. Plant-derived extracts offer lower toxicity profiles towards human organisms when compared to the standard chemical antiseptics utilised in dental care products. While the individual anti-septic effects of the herbs used in the experimental toothpaste are quite mild, when combined with other herbs and chemical anti-septics, they have stronger anti-septic effects because of several bacterio-killing and bacterio-static mechanisms. Moreover, herbal actives, such as secondary metabolites, could interact with chemical anti-septics, thus invigorating their potential to fight against bacteria. Swiss medicinal plant-derived actives for oral care exert multiple positive effects, such as anti-inflammatory, anti-oxidant, direct anti-septic, and indirect anti-bacterial actions through the inhibition of bacterial defence against host phagocytes. On the grounds of the results obtained, it could be concluded that safe and efficient oral care products to prevent/treat gingivitis and early stage periodontitis should contain a combination of chemical and plant-derived anti-bacterials amid their different mechanisms of action.

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