The effect of an oral spray containing an aqueous extract of Triticum vulgare on dental plaque and gingival inflammation in schoolchildren: A randomized controlled trial

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Abstract

Aim To investigate whether the daily use of a spray containing an aqueous extract of Triticum vulgare (TV), belonging to the family of Graminaceae, associated with supervised toothbrushing may improve gingival health in schoolchildren with mixed dentition.

Materials and methods Study design: Randomised, controlled, single-centre, examiner blind, parallel-group study. The study population included 57 schoolchildren with plaque-induced gingivitis randomly allocated to test (n = 29) and control (n = 28) group. Both groups were enrolled in a mechanical plaque control programme for a period of 2 weeks. The test group was also instructed to use a gluten free spray formulation of TV spray twice daily after toothbrushing. Evaluations of plaque index, modified gingivitis index (GI), salivary pH and whole stimulated saliva quantity took place at baseline and after 1 and 2 weeks of study product use.

Results No side effects were observed. Plaque accumulation and GI statistically significantly improved compared with baseline in both groups (all P-values < 0.005), while salivary pH remained nearly unchanged. Between-group differences in index reduction were statistically significant only for GI favouring the test group (P = 0.013). Statistics: Repeated-measures ANOVA and the Friedman test were applied to evaluate the influence of time on quantitative variables within each treatment group. Differences between test and control groups were tested using the unpaired t test or the Mann-Whitney U-test with Bonferroni correction.

Conclusions This study found that TV in spray formulation is safe and effective in controlling gingival inflammation. Thus, it may be a potential adjuvant in the treatment of gingivitis in combination with mechanical plaque control in schoolchildren.

KEYWORDS Children; Dental plaque; Gingivitis; Herbal medicine; Oral hygiene.

Introduction

Plaque-induced gingivitis is the most common form of periodontal disease in childhood and adolescence [Albandar and Tinoco, 2002; Botero et al., 2015; Kolawole et al., 2011] and its prevalence and severity increase with age [Albandar and Tinoco, 2002]. A national survey in the United Kindom showed that about one third of 5-year-olds suffered from gingivitis, compared to two thirds of 8- and 12-year-old children and half of 15-year-olds [White et al., 2006]. In a recent cross-sectional study from Southern Italy the prevalence of this disease was about 55% among schoolchildren aged from 9 to 13 years [Paduano et al., 2018].

Based on the direct correlation between plaque accumulation and development of gingival inflammation, effective control of gingivitis could be achieved through primary and secondary prevention measures involving health education, oral hygiene instruction and motivation, supervised daily toothbrushing and professional plaque removal [Kwan et al., 2005]. This is even more relevant in children suffering from systemic diseases in which the impaired host's immune and inflammatory response may render an individual more susceptible to the effects of bacterial plaque [Meyle and Gonzales, 2001]. Poor oral health exerts a severe impact on general health and well being of an individual [Broder, 2007]. Despite the commitment of parents, children do not consistently perform home care procedures at an acceptable level [Kolawole et al., 2011]. Dental health interventions have demonstrated positive but temporary effects on plaque accumulation and transient improvement of gingival health [Stein et al., 2018]. Mixed dentition may further augment biofilm retention and contribute to gingival inflammation [Agarwal et al., 2009; Guimares et al., 2016]. In this context, the use of chemical adjuncts in addition to toothbrushing and flossing for routine home care may better control plaque accumulation and prevent gingival and oral diseases to initiate or progress [Cortelli et al., 2013].

Several antimicrobial agents, such as chlorhexidine digluconate (CHX), have been successfully used in prevention and treatment of gingivitis in mouthwash formulations [Cortelli et al., 2013; Shim et al., 2012]. However, unwanted side effects, such as unpleasant taste and tooth discolouration, limit their

long-term use and acceptability by patients [Brecx et al., 1993]. Moreover, the use of mouthwashes is not recommended in young children who are unable to spit effectively.

Recently, a specific aqueous extract of *Triticum vulgare* (TV), belonging to the family of Graminaceae, has demonstrated antioxidant capacity and anti-inflammatory properties by decreasing the release of interleukin (IL)-6, tumor necrosis factor (TNF)-alpha and prostaglandin E2 (PGE2), and tissue repair modulation by enhancing chemotaxis and fibroblastic proliferation [Antonucci et al., 2018; Sanguigno et al., 2018]. TV is currently used in pharmaceutical formulations for the treatment of ulcers, burns, scarring delays, dystrophic diseases, and in conditions in which it is necessary to stimulate re-epithelialization or tissue regeneration processes and at the same time to control inflammation [Sanguigno et al., 2018; Serafini et al., 2012]. An in vivo study in rats demonstrated that TV can efficiently bind to sugar residues on the cell surface or to mucins in the salivary pellicle and can be still detected at similar levels after 2 hours [Smart et al., 2002].

To the best of our knowledge, whether TV could be effective in controlling gingival inflammation has not yet explored. Therefore, the aim of the present study was to investigate whether the daily use of a spray containing an aqueous extract of TV associated with supervised toothbrushing might improve gingival health in schoolchildren.

Materials and Methods

Study population

The study was carried out as randomised, controlled, single-centre, examiner blind, clinical trial in two parallel groups at the Section of Paediatric and Preventive Dentistry, Department of Surgical Sciences, University of Turin (Italy) from April 2016 to September 2018. It was approved by the Institutional Ethics Committee of the "AOU Città della Salute e della Scienza", Turin, Italy (protocol n° N-CS/689) and was conducted in accordance with the Declaration of Helsinki, as revised in 2012.

Parents or legal guardians of the minors were informed as to the objectives of the study and signed informed consent. The CONSORT guidelines were followed for clinical trials.

Schoolchildren were consecutively screened for enrolment according to the following criteria: age 6 to 14 years (inclusive); healthy conditions; normal cognitive and motor development; teeth in the mixed dentition phase; presence of permanent first molars and incisors; gingivitis score of ≥1.0, according to the Modified Gingivitis Index (GI) [Suomi and Barbano, 1968] at six index teeth and a full-mouth plaque score (FMPS) ≥50% at the screening visit. Exclusion criteria were as follows: antibiotic treatment within 1 month before the trial; use of any medications that could influence gingival tissue (e.g. phenytoin, cyclosporine); systemic diseases or conditions that could interfere with the study outcomes (immune deficiencies, uncontrolled diabetes, haematologic diseases, genetic conditions, disabilities); presence of orthodontic appliances or space maintainers; mucosal diseases; severe oro-pharyngeal infections; extensive caries; failure to comply with the spray regimen.

Sample size and randomisation

Assignment to the test group (supervised toothbrushing and daily application of TV spray) and the control group (supervised toothbrushing alone) was made using a computer-generated table by an independent operator who did not take part into the study and was provided to the dental hygienist in sealed and opaque envelopes. This study was designed to detect a potential mean difference between control and test groups of 0.3 with a power of 80% for detecting a statistically significant difference in GI scores at the 0.05 probability level. Assuming a standard deviation of 0.3 the estimated required sample size was 24 in each arm. The number was adjusted to 60 individuals (30 per group) to compensate for possible dropouts.

Clinical protocol

All patients received careful instructions on self-performed plaque control measures by an experienced dental hygienist according to the individual needs. The manual toothbrush and fluoride-containing toothpaste were provided for use through the study. Children were invited to replicate demonstrated movements in their mouth under the supervision of the hygienist. Afterwards they received professional plaque removal so that all patients could initiate testing under the same conditions. A separate hygienist provided the spray containing a gluten-free aqueous extract of TV (Jalma, Farmaceutici Damor SpA, Naples, Italy) to the test children and instructed them to use the spray two times a day for two weeks. A squirt was applied to the right/left side of the mouth on the buccal aspect and a squirt on the lingual aspect. Parents were asked to apply the spray and to supervise children during oral hygiene procedures.

After 7 days (T1) and 14 days (T2) the oral hygiene standards of children of both groups were reviewed and oral hygiene procedures were reinforced. Compliance with the use of the spray was evaluated at each visit.

Clinical measurements

Clinical parameters were assessed at baseline, 7 and 14 days by a single calibrated operator masked to the group assignment using a periodontal probe (PCPUNC 15, Hu-Friedy, Chicago, IL, USA) along the gingival margin. Intraexaminer calibration was performed on a group of 10 children before the study and the kappa coefficients for plaque and gingivitis indices were 0.83 and 0.89.

The presence or absence of supragingival plaque at four sites of each tooth (distobuccal, centrobuccal, mesiobuccal, oral) was assessed by means of plaque control record (0 = absence; 1= any presence of plaque) using a disclosing solution and expressed as percentage of tooth surfaces harbouring plaque (FMPS) [O'Leary et al., 1972].

Gingival inflammation was recorded using GI according to Suomi and Barbano [1968] on the facial and lingual surfaces of six index teeth (16, 11, 26, 36, 31 and 46) [Guimaraes Nobre et al., 2016] and scored on a scale from zero to three based on visual inflammatory changes and bleeding of the gingiva (0= no inflammation; 1= inflammation; 2= severe inflammation).

Wax-stimulated whole saliva samples were collected at least 2 hours after meals and at least 1 hour after toothbrushing to measure the quantity of stimulated saliva and pH using a chair side kit (Saliva-Check Buffer System GC America, Inc., Alsip, IL). Children were instructed to chew on a piece of wax for 30 seconds and to spit saliva into the measuring cup, then continue chewing for 5 minutes and expectorating every 15 to 20 seconds. The volume of saliva was measured and scored as very low (<3.5 ml), low (3.5 to 5.0 ml) and normal (>5.0 ml). Immediately after collection pH measurement was done dipping the pH strip into the stimulated saliva for 10 seconds and then using the Saliva-Check Buffer Mat for comparison with a measuring range from 5.0 to 7.8. The investigator also took notes in case of adverse effects.

Data analysis

Data were recorded in a Microsoft Excel file and analysed with statistical software (SPSS for Mac, SPSS version 24.0, IBM Corporation, Armonk, NY, USA). The primary efficacy analysis was conducted on the intention-to-treat analysis set including all randomised subjects who had at least one treatment assessment of the efficacy parameters. Data were first examined for normality by the Kolmogorov-Smirnov test, and if they did not achieve normality, analyses were performed using non-parametric methods. Repeated-measures ANOVA (FMPS, GI) and the Friedman test (pH) were applied to evaluate the influence of time on each variable within each treatment group, followed by post-hoc tests. Changes in the stimulatory salivary quantity were analysed by means of the chi-square test. Differences between test and control groups were tested using the chi-square test for categorical variables and the unpaired t test or the Mann-Whitney U-test, as appropriate, for quantitative variables. The Bonferroni correction was applied for multiple comparisons. Differences were considered statistically significant when P was < 0.05.

Results

As reported in Figure 1, 142 children were screened for inclusion: 82 were excluded because they did not meet the inclusion criteria or parents withdrew their consent.

The enrolment process resulted in a study population of 60 subjects, with 30 subjects in the test group and 30 subjects in the control group. Three children dropped out from the study because they did not attend the baseline visit. Finally, a total of 57 patients were treated: 29 in the test group (mean age: 10.8 ± 2.2 years) and 28 in the control group (mean age: 9.9 ± 2.3 years). Three patients did not attend the 1-week examination for personal reasons.

The baseline characteristics of the participants, including mean age, and sex, did not significantly differ between the two groups (Table 1). None of the patients experienced adverse effects, such as irritation on the oral mucosa or tooth staining.

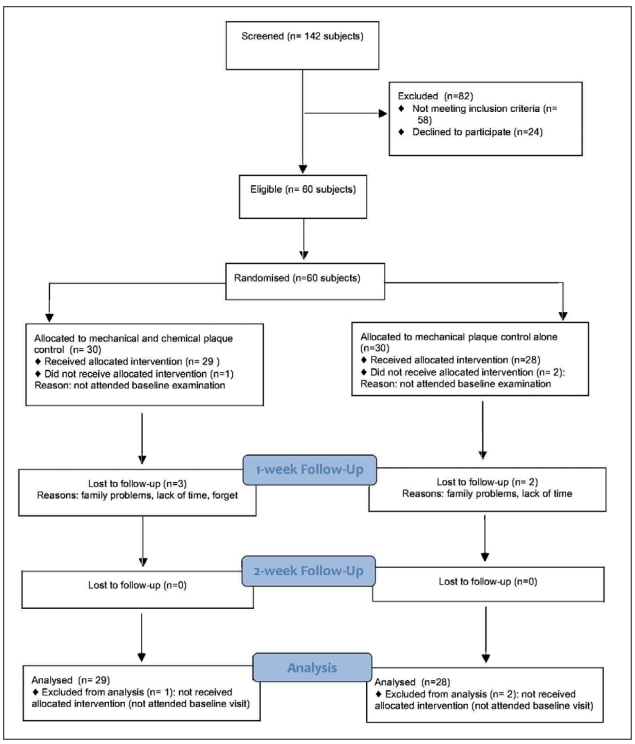

FIG. 1 Flow chart of the study.

All parents reported that they observed no difficulty in using the spray and children offered no resistance to the treatment.

The comparative analysis between test and control groups over the experimental period in relation to periodontal clinical parameters is shown in Table 2. Both treatments were associated with a statistically significant reduction in the percentage of sites with plaque and in the severity of gingival inflammation with a decrease from baseline to 2-week follow-up (all P < 0.005). The reduction in GI from baseline was significantly greater in the test group than in the control group at week 2

Variables	Test Group (N = 29)	Control Group (N = 28)	p value test vs control
Age (years)	10.8 ± 2.2	9.9 ± 2.3	0.107†
Range	6 - 14	6 - 14	
Females	16 (55.2)	19 (67.9)	0.325‡

†Unpaired t-test ‡Chi-square test

TABLE 1 Basic demographic characteristics of the study population (mean ± standard deviation or N [%]).

Variables	Group	Baseline	1 week	Δ0-1 week	2 weeks	Δ0-2 week
FMPS (%)	Test	68.8 ± 14.3*	43.9 ± 18.1**	24.9 ± 21.9	34.7 ± 15.4**	34.1 ± 22.7
	Control	70.3 ± 14.4*	44.3 ± 19.0 **	26.0 ± 19.3	38.8 ± 16.6 **	31.5 ± 15.6
Difference between groups		NS†	NS‡		NS‡	
GI	Test	1.7 ± 0.4*	1.3 ± 0.5**	0.4 ± 0.5	0.6 ± 0.4**	1.1 ± 0.5
	Control	1.4 ± 0.5*	1.1 ± 0.6 ***	0.3 ± 0.6	1.0 ± 0.7**	0.4 ± 0.6
Difference between groups		NS†	NS‡		0.013‡	

FMPS, Full-Mouth Plaque Score; GI modified gingivitis index; NS, difference between groups not statistically significant (P > 0.05)
* P < 0.005, p values represent changes among the three time points (ANOVA test).
**P < 0.005, p values represent longitudinal changes from baseline (Tukey test).
***P < 0.05, p values represent changes from baseline (Tukey test).
†Unpaired t test
‡Bonferroni-corrected t test

TABLE 2 Changes in clinical variables (mean ± standard deviation) over the experimental period in both treatment groups.

(P = 0.013), while the individual oral hygiene status did not significantly differ between the groups at any assessment time points (P > 0.05). In terms of changes in salivary parameters (Table 3), not statistically significant results were obtained in both test and control groups for pH. Although stimulated saliva quantity increased more in the test group at week 2 compared to baseline, no statistically significant difference was observed as compared to the control group.

Discussion

Due to the high prevalence of plaque-induced gingivitis in schoolchildren, the aim of the present study was to assess the activity of an oral spray containing an aqueous extract of TV on plaque and inflammatory parameters. All study participants presented a high degree of plaque and gingivitis and were therefore a target population in need to improve oral hygiene. As failure to brush effectively limits mechanical plaque control, chemical means could represent an effective adjunct.

The aqueous extract of TV obtained from the whole germinated plant is an active component of some pharmaceutical products already marketed in Italy and abroad under the brand name Fitostimoline. They promote tissue repair and wound healing by stimulating fibroblast chemotaxis and maturation, and by increasing the fibroblastic index, which are crucial points in the repairing processes [Antonucci et al., 2018; Serafini et al., 2012]. It has been suggested that these activities are due to the accelerated protein synthesis and to the enhanced ability of caption and incorporation of proline from tissues. TV mitogenic effect has been demonstrated both in plants and in the mouse model [Favit et al., 1992]. Recently, Sanguigno et al. [2018] demonstrated anti-inflammatory properties of TV, using the lipopolysaccharide-stimulated microglia model of neuro-inflammation. The aqueous extract of TV reduced the release of pro-inflammatory mediators such as IL-1ß, IL-6, PGE2, and TNF-a. This inhibitory activity on inflammation was also confirmed in primary rat microglia cultures [Sanguigno et al., 2018]. Notably, such regenerative and anti-inflammatory properties are strictly connected as the same mediators and cellular components are involved in the regulation of both inflammatory and repairing processes [Ariel and Timor, 2013]. In particular, cytokines such as IL-1, TNF-α, and IL-6, that are activators of inflammation, usually decrease to favour the transition from the inflammatory to the regenerative phase during wound healing [Ariel and Timor, 2013].

The 2-week use of the TV spray obtained greater statistically and clinically significant reduction in GI scores compared with the control group, while similar decrease in mean FMPS values was observed for both groups. This improvement in plaque index was most likely due to the professional oral hygiene session together with the repeated personalised home oral hygiene instructions received by children, which enhanced knowledge in adopting and sustaining appropriate brushing technique and frequency, contributing to the difference from the baseline. Consistent with previous studies, parents were actively involved in the education programme and had the direct supervision of the biofilm control [Guimaraes Nobre et al., 2016; Santos et al., 2007]. This is an important aspect because without a tailored oral hygiene education, most parents tend to give to the children the total responsabilities of their own oral health without any supervision [Kolawole et al., 2011].

We used a spray formulation to deliver TV. Oral spray has been recommended for physically and mentally challenged patients and institutionalised elders but it may also represent the most suitable delivery system for systemically healthy children who may be unable to follow instructions for mouthrinsing, and has less side effects than conventional mouthwashes [Chibinski et al., 2011; Viana et al., 2014]. In the present study TV oral spray was well accepted by patients and easily delivered by parents. In addition, no side effects were observed supporting that TV is safe in use.

A recent systematic review reported that oral sprays are an acceptable delivery method for chemotherapeutic agents and can be effective against dental biofilm and gingival inflammation, for managing gingivitis and for prevention of dental caries, when used in addition to daily oral hygiene measures [Zhang et al., 2019]. However, the heterogeneity of the included studies was high, and no definitive conclusions could be drawn.

The antiseptic most frequently delivered via spray is CHX. Data from the literature indicated that the 0.2% CHX spray has a lower antiplaque and anti-inflammatory efficacy than 0.2% CHX mouthrinse. The systematic review reported an overall reduction of 0.74 for the Silness and Löe Plaque Index and 0.20 for the Gingival Index compared with the reduction

Variables	Baseline		1 week		2 weeks	
	Test	Control	Test	Control	Test	Control
pH (median [interquartile range])	6.7 (0.7)*	7 (1.0)*	6.8 (0.5)	6.8 (0.6)	6.8 (0.4)	7.0 (0.6)
Difference between groups (Mann-Whitney U-test)	NS		NS		NS	
Saliva quantity (N [%])						
Very low	7 (24.1)	9 (32.1)	3 (10.4) **	4 (14.2) **	4 (13.8) **	8 (28.6) ***
Low	6 (20.7)	5 (17.9)	8 (27.6)	12 (42.9)	6 (20.7)	5 (17.9)
Normal	16 (55.2)	14 (50)	18 (62.0)	12 (42.9)	19 (65.5)	15 (53.5)
Difference between groups (Chi-square test)	NS		NS		NS	

NS, difference between groups not statistically significant (P > 0.05) *P > 0.05, p values represent changes among the three time points (Friedman test). **P ≤ 0.01, p values represent changes from baseline (Chi-square test). ***P > 0.05, p values represent changes from baseline (Chi-square test).

TABLE 3 Changes in salivary variables over the experimental period in both treatment groups.

of 0.9 to 1.2 achieved with the corresponding mouthrinse formulation [Zhang et al., 2019]. In addition, CHX application is often associated with unwanted local side effects such as tooth staining, burning sensation, and alteration of taste limiting its long-term use [Brecx et al., 1993].

Data on other antiseptic agents like hexetidine, stannous fluoride and triclosan are few and inconclusive. This highlights the need for further studies on the potential benefits of alternative non-CHX chemotherapeutic agents applied via spray. In the last decade the beneficial effects of plant products on gingival health have been widely investigated in the adult population, whereas limited information has been provided for paediatric patients. The available evidence demonstrated that, beside being without any major side effect, herbal mouthwashes were equally effective than CHX in decreasing plaque scores, gingival inflammation and Streptococcus mutants salivary counts in the oral cavity of children [Haffajee et al. 2008; Kamath et al., 2019; Padiyar et al., 2018]. However, data on the potential efficacy of oral sprays containing plant-derived products are still lacking.

The present study acknowledges some limitations. Firstly, only children aged 6–14 years with mixed dentition were considered in the study, so that the results cannot be generalised to other age groups. Pseudo-pocketing around partially erupted teeth may favour plaque accumulation and gingival inflammation. It has been also demonstrated that at the same level of oral hygiene, the degree of gingival inflammation is lower in preschoolers than in older children [Agarwal et al., 2006]. Secondly, although the study showed positive effects, clinical changes observed for short duration cannot be predicted in the long run. However, since no adverse events occurred, it can be envisaged to extend the spray application beyond the time set by this study, until clinically healthy gingival conditions have been recovered. Further long-term prospective studies with larger sample size are necessary to confirm the findings of this short-term clinical trial.

Conclusions

This 2-week home use study on the efficacy on TV oral spray clearly confirmed the primary role of mechanical plaque control, as bacterial biofilm is the main aetiological factor of gingivitis. This product, therefore, proved to be an effective adjunct to the home oral care measures. Finally, the TV in spray formulation, together with the absence of side effects and the fact of being gluten free, is suitable for children and potentially useful to improve their oral health conditions. Considering that severe periodontitis is the six most prevalent human inflammatory disorder worldwide [Richards, 2014], early treatment and prevention of this initial inflammatory pathology is of critical importance for public health, in order to reduce the risk of periodontitis in adulthood.

Based on these promising results, further studies are recommended to explore the use of TV spray in medically compromised children and childhood cancer survivors who are at increased risk of oral health late effects.

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