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ORIGINAL ARTICLE

A randomised crossover trial to compare the potential of stannous fluoride and essential oil mouth rinses to induce tooth and tongue staining

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Abstract This study compared the staining potential of two experimental amine fluoride/stannous fluoride mouth rinses (A and B), a phenolic/essential oil rinse (C) and a negative control, water, rinse (D). The study was a single centre, randomized, single-blind, four treatment crossover study design among healthy participants. Prior to each study period, participants received a dental prophylaxis. On the Monday of each period, subjects suspended oral hygiene, and under supervision, rinsed with the allocated mouth rinse immediately followed by a warm black tea solution at hourly intervals eight times a day for 4 days. On Friday, the area and intensity of staining on the teeth, the primary outcome measure and dorsum of tongue were assessed. This regimen was repeated for all the three subsequent treatment periods. Rinse B produced less stain than rinse A, but the difference was not significant ($p=0.20$). Rinse B produced significantly more stain than rinse C ($p<0.05$) and D ($p<0.001$). For tongue staining, rinse B produced significantly more staining than D ($p<0.01$) but not A or C. Overall, all test rinses produced more staining than placebo with an overall pattern for more staining with stannous formulations. Individuals using stannous or phenolic/essential oil mouth rinse formulations should be advised of the possible staining side effect and that this can be easily removed by a professional dental cleaning.

Keywords Discolouration · Mouth rinse · Forced stain model · Stannous fluoride · Phenolic/essential oils

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Introduction

It is 40 years since the pivotal publication, on chlorhexidine mouth rinse [1], which demonstrated that plaque control and the prevention of gingivitis could be achieved using a chemical agent. Since then, numerous studies using many chemicals, in a variety of vehicles, have been published, although, the number of agents fulfilling the European Federation of Periodontology "anti-plaque" definition [2, 3] has been proportionately small (for review see [4]). Arguably, opinion has moved away from the idea of chemical plaque control replacing mechanical tooth cleaning to one of adjunctive benefit. One reason for this has been local side effects of some anti-plaque chemicals. Thus, it is widely appreciated that cationic antiseptics, notably chlorhexidine, phenolic/essential oil formulations and polyvalent metal salts, such as stannous fluoride, cause extrinsic discolouration of the teeth [5–9].

The mechanism of staining, previously widely debated, has been proven as caused through an interaction of such actives with dietary chromogens, essentially precipitating the chromogens onto oral surfaces [10, 11] (for reviews see [4, 12, 13]). Both laboratory and clinical studies have shown that cationic and polyvalent metal salts alone do not cause staining, the data which disproved the hypothesis of denaturation of pellicle proteins by chlorhexidine and formation of metal sulphides [10, 11].

Alterations, in formulations by the addition of other ingredients, have been attempted particularly for chlorhexidine mouth rinses, to reduce staining. Thus far, any benefit to reduce staining has been balanced by loss of anti-plaque efficacy. A recent example is a chlorhexidine rinse with an anti-discolouration system, where the claimed reduction in staining in vivo [14, 15], challenged by a study in vitro [16], was at the expense of significant loss of plaque inhibition [17].

The initial laboratory studies, employed to investigate the aetiology and mechanism of chlorhexidine staining, involved repeatedly exposing a substrate to chlorhexidine followed by black tea or coffee [18]. Randomised, controlled, clinical trials followed in which subjects, in a crossover design, rinsed with chlorhexidine and drank large amounts of tea, coffee or water [19]. Similar laboratory and clinical models were used to study staining by other cationic antiseptics and polyvalent metal salts and investigate stain inhibition and removal. Later and based on laboratory studies [20], the so-called forced staining clinical model was developed which, essentially involved, eight times per day, reciprocal rinsing with the test agent and tea [21]. In addition, the model used warm tea prepared at three times the normal tea leaf-to-water ratio. Thus, the propensity of a mouth rinse formulation, to cause oral and dental staining, can be evaluated and compared with controls, over a few days under standardised conditions.

Aim The aim of the present clinical study, employing the forced staining methodology, was to compare tea staining of the teeth and tongue induced by two experimental amine fluoride/stannous fluoride test rinses (A and B), compared to a placebo control rinse (D), water, and a commercially available, phenolic/essential oil benchmark control rinse (C). The phenolic/essential oil mouth rinse was chosen as the comparator because of data reported from a related forced staining investigation [9].

Objectives The primary objective was to compare whole mouth, tooth staining area/intensity product derived from the gingival crescent and body of the assessed teeth following use of the four rinses. The secondary objective was to compare the area/intensity product staining on the dorsum of the tongue following the use of the four rinses.

Materials and method

The investigation was a single centre, randomized single-blind, four treatment crossover study performed to compare the staining potential of two experimental amine fluoride/stannous fluoride mouth rinses (A and B), a commercial phenolic/essential oil rinse (C) and a placebo control, water, rinse (D). Randomisation allocated participants equally to different sequences of the four treatments according to a Latin square design incorporating balance for possible carryover from the immediately preceding period. The simple randomisation schedule allocating randomisation numbers to screening numbers was provided by the study statistician. Screening numbers were allocated to subjects by study staff, determined by the time of their arrival at the screening session and were unknown to the statistician. Prior to the study, approval from the University of Bristol Faculty of Medicine and Dentistry Ethics Committee was sought and given. The study adhered to the guidelines for good clinical practice and was monitored regularly by an individual from the sponsor company. After an ethical approval, potential participants derived from the staff and students of Bristol Dental School and Hospital were invited to a screening visit where they were asked to read and sign a Participant Information Sheet and Consent form, if they agreed to take part in the study and prior to any study procedures being performed. Both clinical visits and data collection took place at Bristol Dental School. Screening took place within 2 weeks prior to day 1 of the first treatment period. A dentally qualified clinician recorded the participant's demographics, medical history, current/concomitant medications, performed an oral soft tissue examination and ensured the participant fulfilled the eligibility criteria for the study. Twenty healthy participants, of either gender, aged 18 and over, not using any tobacco products and with no medical or pharmacotherapy history, which could compromise the conduct of the study, were recruited. Participants had to have at least 12 scorable teeth and good oral hygiene and oral and gingival health. Subjects who successfully fulfilled all the necessary entrance criteria were provided with a standard commercially available toothpaste and toothbrush to use from the screening visit through until morning 1 of their first treatment. No oral hygiene other than the use of the test rinse was performed during treatment weeks, but was resumed during the washout periods. Subjects were asked not to drink red wine and other high potential staining food products during the study.

During the week before the study, the subjects received a prophylaxis to remove all staining, plaque and calculus deposits. On the Monday of the following week, subjects returned to the clinic to receive their rinses and to check if their dentition was stain free. A further prophylaxis was provided to remove any residual stain still present at the

start of each study period. Following this prophylaxis, and under direct supervision, each subject rinsed for 30 s with 10 ml of their allocated mouth rinse (30 s with 20 ml of the phenolic/essential oil rinse), immediately followed by a 60 s rinse of 10 ml of a warm (50°C) black tea solution[1]. This rinsing regimen was repeated hourly, eight times throughout the day and on the following 3 days up to end of Thursday. Throughout this period, volunteers omitted all forms of oral hygiene except the use of the mouth rinses.

On Friday, subjects were questioned as to adverse events, and an oral soft tissue examination was performed. Then, using the method described by Lobene [22], the primary outcome, intensity of stain and stain area on the gingival crescent and body of the tooth on the buccal surfaces of each assessable incisor, canine and premolar teeth were observationally scored using a three-point scale. As a secondary outcome, the dorsum of the tongue was also scored for stain area and intensity using similar scales. For both scoring of tongue and teeth, a standardised light source for stain assessment was used (Color-i-dent II®, Waldmann GMBH, Germany) and was performed by a researcher blinded to the treatment. At the end of each study period, each subject received a thorough prophylaxis to remove all plaque, calculus and staining before starting subsequent periods of the study. This regimen was repeated for all the three subsequent treatment periods. An assessment of repeatability of scoring was made by arbitrarily selecting ten subjects for re-scoring of the teeth and tongue staining, at least 30 min after the first scoring. The assessor was blinded to the treatment group. A washout period of the intervening weekend was allowed between test periods, when subjects returned to their usual oral hygiene regimens. A prophylaxis was performed at the end of the final test period.

Study products evaluated

A Experimental AmF/SnF_2—mouthrinse 250 ppm; F—430 ppm Sn (GABA International AG, Therwil, Switzerland)
B Experimental AmF/SnF_2—mouthrinse 250 ppm; F—430 ppm Sn (GABA International AG, Therwil, Switzerland)
C Listerine Cool Mint Mouthrinse (Johnson & Johnson, New Brunswick, NJ, USA)
D Water control (Volvic still water, Danone Company, Paris, France)

NB A differed from B in having less antibacterial actives other than SnF_2 and a reduced concentration of complexing agents.

Rationale for sample size

The proposed sample size of 20 volunteers was chosen to enable detection of a mean difference of 0.25 units in the primary outcome with power of 80% using a test at the conventional 5% two-sided alpha level. For the secondary outcome, the detectable difference is 0.22 units. These figures were based on the results from a study [23], in which the degree of variation between changes from baseline in different subjects was represented by standard deviations of 0.39 units for intensity and 0.36 units for area. Relative to a mean intensity and area of 1.91 and 2.32 units for these measures on a water control, this amounts to 13% and 10% differences, respectively.

Statistical analyses

The product of stain area and intensity scores was calculated for two sites, gingival crescent and body of each scoreable tooth. The primary outcome measure was the whole mouth mean of these product scores across all scoreable gingival crescent and body of tooth sites. The secondary outcome measure was the product of stain area and intensity scores for the dorsum of the tongue. As distributional form for tooth and tongue staining was acceptably close to Gaussian, analysis of variance (ANOVA) was performed to model the effects of three factors, subject, period and treatment. Point estimates were calculated and 95% confidence intervals constructed to characterise differences between selected pairs of treatments. To avoid over-interpreting multiple comparisons, the best practice is to specify a restricted set of contrasts between treatments as of prior interest. Here, the protocol identified the contrasts of prior interest as those involving product B, viz. B vs. A, B vs. C and B vs. D. Nevertheless, an important observation in this study was a very clearly greater degree of tooth staining on the phenolic/essential oil preparation C compared to the water control D. Previous evidence on the staining potential of this agent is conflicting, with the systematic review of Stoeken [24] which was unable to produce a meta-analysis due to differences in methodology between the eight studies considered. In view of this lack of clear evidence, it is important for the present study to report this comparison also.

[1] Tea solution: the standard tea solution was prepared by immersing 3 g of tea leaves (Marks & Spencers' Extra Strong Tea, Marks & Spencers, Reading, UK) in 100 ml of freshly boiled water for 5 min. Following stirring and passage through double layer gauze, the solution was stored for distribution at 50°C in thermos flasks.

Results

Twenty-three healthy subjects were screened by the study site so that 20, who fulfilled all the entry criteria, were accepted into the study and randomized to a treatment schedule. All the 20 subjects who were enrolled completed the study. Of the 20, 17 returned complete data. Three subjects (one in period 1, one in period 2 and one in period 4) did not provide complete data. Intention to treat (ITT) analyses using all available stain data, therefore were regarded as primary. Per protocol (PP) analyses, excluding data from the three subjects with incomplete data, were qualitatively similar to ITT analyses. Tables 1 and 2 summarise tooth and tongue stain data for the ITT population. In mean terms, the order of greatest to least tooth area/intensity product scores was A, B, C and D (Table 1). ANOVA indicated that the difference between the four treatments was highly significant ($p<0.001$), as were the differences between subjects and periods ($p<0.001$ and $p<0.01$, respectively). For tongue area/intensity product scores, the descending order was B, A, C and D (Table 2).

Tables 3 and 4 show the pre-identified contrasts (bold) and also the additional C vs. D contrast in normal typeface for the ITT and PP populations. These comparisons show that for tooth staining, products B and C both led to highly significantly greater staining than water ($p<0.001$) in both ITT and PP populations. The degree of staining was rather greater on B than on C, at a borderline level of significance, and was apparently slightly lower than when product A, the less modified amine/fluoride stannous fluoride product, was used.

For tongue staining, preparation B led to a degree of staining that was highly significantly greater than on preparation D and rather greater than on preparations A and C with borderline significance in the PP analyses. However, tongue staining was not significantly greater on preparation C than on D.

Repeatability

There was a very high correlation between original and repeat scores for the ten subjects for teeth ($r^2=0.862$, $p=0.001$) and tongue ($r^2=0.96$, $p<0.001$). The data suggested, however that the repeat scores tended to be higher than the original scores. The latter findings would not be expected to distort the comparisons between the four treatments.

Table 1 Whole mouth tooth mean area/intensity product score

Treatment	*N*	Mean	SD	Median	Minimum	Maximum
A	19	5.75	1.19	6.03	2.90	7.15
B	20	5.50	1.24	5.28	2.78	7.50
C	18	4.89	1.16	5.11	2.28	6.65
D	20	3.75	1.08	3.64	2.00	5.81

Table 2 Tongue stain area/intensity product score

Treatment	*N*	Mean	SD	Median	Minimum	Maximum
A	19	3.21	2.12	2.00	0	6
B	20	3.90	2.49	4.00	0	9
C	18	2.94	2.44	2.00	0	9
D	20	2.55	1.67	2.00	0	6

Adverse events

One adverse event was reported in one individual which resulted in withdrawal of that subject from that leg of the study. This was described as oral intolerance to the rinse (C), producing burning and discomfort on rinsing. Other adverse events reported included one individual with a headache (no action taken with regard to the study) and one person had a head cold. The latter completed only two treatment days and was discontinued for the rest of that particular study period but recommenced for the next study period.

Protocol violation/deviation

None were reported in this study.

Discussion

In this forced stain model, with eight times per day reciprocal rinsing with the test solutions and warm black tea, some staining with all rinses, including the water

Table 3 Estimated differences in whole mouth mean stain product score and tongue stain product score between pairs of treatments specified for the ITT population ($n=20$)

Assessment area	Treatment comparisons	Estimated difference	95% confidence interval	*P* value
Whole mouth mean stain product score	B vs. A	−0.31	−0.77 to +0.16	0.20
	B vs. C	+0.51	+0.03 to +0.99	0.04
	B vs. D	+1.75	+1.29 to +2.21	<0.001
	C vs. D	+1.24	+0.76 to +1.71	<0.001
Tongue stain product score	B vs. A	+0.71	−0.29 to +1.72	0.16
	B vs. C	+0.86	−0.16 to +1.89	0.10
	B vs. D	+1.35	+0.37 to +2.33	0.008
	C vs. D	+0.49	−0.54 to +1.51	0.51

Table 4 Estimated differences in whole mouth mean stain product score and tongue stain product score between pairs of treatments specified for the PP population (n=17)

Assessment area	Treatment comparisons	Estimated difference	95% confidence interval	P value
Whole mouth mean stain product score	B vs. A	−0.33	−0.86 to +0.20	0.22
	B vs. C	+0.46	−0.07 to +0.99	0.09
	B vs. D	+1.75	+1.22 to +2.28	<0.001
	C vs. D	+1.29	+0.77 to +1.82	<0.001
Tongue stain product score	B vs. A	+1.02	−0.03 to +2.07	0.06
	B vs. C	+1.09	+0.05 to +2.14	0.04
	B vs. D	+1.56	+0.51 to +2.60	0.004
	C vs. D	+0.46	−0.57 to +1.50	0.37

control, was to be expected. Extrinsic staining of teeth is mainly through the incorporation of chromogens into the pellicle layer, indeed from studies in vitro, "naked" enamel takes up very little stain, even when exposed continuously to tea solutions for 24 h or more [25]. Stain area/intensity product scores were used as the outcome measure, since area scores alone are less discriminatory. Thus, in this model, where there is no tooth cleaning during test periods, stain scores by area, particularly on the teeth, tend to be high, because the whole pellicle-coated buccal surfaces are exposed to the chromogen, irrespective of the test rinse, and remain relatively undisturbed. Of course any agent, which actively interacts with the chromogen, will indirectly create an apparent increase in stain area, as the increased stain intensity will improve detection of stain.

The choice of control(s) in randomised controlled clinical trials of oral care or hygiene formulations, whether parallel or crossover in design, is always problematic and influenced by many factors, including ethics, aim(s), nature of the study (exploratory, explanatory, pilot for power size calculations, etc.), basic clinical science and logistics (for review see [4, 26]. When possible or even available, a placebo or negative control is ideal, particularly, where test formulations have not been evaluated before for their effects or within a specific clinical model, both of which apply here. When mouth rinses are under test, virtually in any investigative scenario, water, as the placebo is an ideal choice, perhaps not necessarily the case if toothpastes are under study. A positive control would be a second choice, but in many areas of dental research, such controls are not available or accepted as such. This was not the case here and chlorhexidine could have been used; this would have positioned the stannous fluoride rinses between the placebo and positive control. Chlorhexidine, however was not chosen for several reasons. First and foremost, it is established that chlorhexidine causes staining more frequently and to a greater extent than stannous fluoride; the typical colour being a darker brown than the light golden brown of stannous fluoride staining. Secondly, most if not all reviews have concluded that the anti-plaque properties of stannous fluoride are less than chlorhexidine, but local side effects are less in number, less common and less severe. Thirdly, the recent change in status of chlorhexidine rinses as a potential therapeutic chemical has made an agreement for its routine use in clinical trials as a positive control more difficult to obtain. Finally, a comparison of stannous fluoride with chlorhexidine was not the purpose of this investigation and to include it just for interests sake merely would have added another treatment cell to the crossover design.

The phenolic/essential oil rinse was chosen as a benchmark control (for review see [4]) because it is a commonly used mouth rinse product in many countries and arguably has similar anti-plaque activity to an existing amine fluoride/stannous fluoride rinse product (for review see [4]). Also, staining by the phenolic/essential oil rinse has been both reported and investigated in a clinical staining model [9, 24, 27]. It could be argued that the rinses should have been used, followed by a water rinse to determine their staining potential alone, however a great deal of already cited evidence supports the theory that staining by cationic antiseptics and polyvalent metal salt results from an interaction with dietary chromogens and alone these agents do not cause staining. This stated the choice of chromogen could influence the outcome and certainly the appearance of the stain. Thus, coffee is known to produce a less intense stain than tea [28] and although never tested in a controlled clinical study, red wine would be expected to produce a darker and more intense staining than tea.

The differences albeit not significant between the two SnF_2 are worthy of some discussion. Rinse B as stated contained more complexing agents and a higher amount of antimicrobial agents, other than SnF_2, than rinse A. The science behind this was that the complexing agents might shield the stannous ion and thereby reduce staining, but the probable loss of antimicrobial properties of the stannous component would be compensated by other antimicrobials present, which do not have the propensity to stain. For tooth staining, the trend was for less staining with B compared to A, however this was reversed for tongue staining. These equivocal findings suggest that even if rinse B had less available stannous ions, it was insufficient to significantly influence staining in this model.

The findings, therefore, of this placebo- and benchmark-controlled study suggest that the experimental stannous fluoride formulations have the potential to cause slightly more staining than the phenolic/essential oil rinse, which in turn would be expected to stain considerably more than water. On a positive note, one can probably safely infer from the stain data that these experimental stannous fluoride rinses would be expected to have significant plaque inhibitory action in

keeping with the existing commercial rinse product [7], although this would require testing within an appropriate clinical model. As this study used an in situ model allowing normal salivary pellicle formation on samples in healthy volunteers, the results for the staining of dietary chromogens can be generalised to the population.

In conclusion, both experimental rinses A and B induced extrinsic staining by an interaction with tea. The experimental rinse B would not appear to offer any appreciable advantages in reduced staining when compared to experimental rinse A. The phenolic/essential oil mouth rinse product clearly has the potential to cause extrinsic staining of the teeth and tongue albeit to a lesser degree than the tested experimental stannous fluoride rinses. Given that this rinse is a combination of phenol and essential oils it would be of interest to determine which specific agent(s) induced the tea staining, a question that could almost certainly be addressed using laboratory staining methodologies.

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Conflicts of interest None of the authors are aware of any conflicts of interest arising from conducting and reporting the findings of this study.

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