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Oxalate-containing phytocomplexes as dentine desensitisers: An in vitro study

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Summary It is known that pulpal fluid movement through dentinal tubules causes dentinal hypersensitivity and that pain can be reduced by decreasing the fluid flow. The aim of this study was to evaluate dentinal permeability and morphology after a single exposure to experimental phytocomplex substances containing oxalates.

The treatments tested were experimental pastes, gels and solutions of phytocomplexes (extracted from rhubarb, spinach and mint), an experimental paste containing 5% potassium oxalate, and two commercial toothpastes recommended for dentinal hypersensitivity (Elmex and Sensodyne).

Dentine discs from human third molars were used in this study. Each sample was brushed for 3 min with each treatment in order to test reductions in dentinal permeability. Each treated sample was challenged with orthophosphoric acid for 90 s to determine changes in dentinal permeability and the sensitivity of treatments to acid challenge. Scanning electron microscopy was used to analyse the samples.

This study found that spinach and rhubarb phytocomplex treatments reduced dentinal permeability by occluding dentinal tubules through formation of calcium oxalate crystals. These results indicate that phytocomplexes extracted from rhubarb and spinach, used in different formulations, should be effective for topical treatment of dentinal hypersensitivity.

Introduction

Dentine is a permeable substrate due to the presence of dentinal tubules (diameter 1—2 μm) that connect the pulpal chamber with the oral cavity whenever enamel or cementum is lost. Dentinal

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tubules, partially filled with pulpal fluid which has a similar composition to plasma, allow communication between the pulp and the oral cavity.[1,2] The hydrodynamic theory suggests that pain and hypersensitivity are due to fluid movement within dentinal tubules. This theory explains why chemical (e.g. hyperosmotic solutions), thermal and mechanical stimuli cause pain due to fluid movement whenever dentinal tubules are open and exposed to oral fluids.[3–6] Reduction of the diameter of dentinal tubules should reduce fluid flow and may induce a clinically acceptable reduction in pain.[7–11] The fluid volume moving across the dentine and, consequently, the 'in vivo' sensitivity is proportional to the diameter and number of tubules.[12] Any substance that decreases dentinal conductance (i.e. dentinal permeability) by closing patent tubules is able to reduce pain.[13,14]

Previous studies concerning oxalate-based treatments on dentinal tissue showed significant reductions in dentinal permeability and hypersensitivity.[15–20] However, no information is currently available on the effect of oxalate-containing natural substances on dentinal permeability and hypersensitivity. It is well known that many vegetables, such as rhubarb, spinach and mint, contain oxalates either as soluble or insoluble salts or as oxalic acid.[21,22] Oxalic acid forms soluble salts with sodium, potassium or ammonium ions, and insoluble salts with calcium, magnesium and iron ions.[23–25] In neutral and alkaline environments, calcium and oxalate may bind together forming different shaped crystals of calcium oxalate.[17,25]

The purpose of this study was to evaluate the changes in dentinal permeability after application of several phytocomplexes containing oxalates. A scanning electron microscope (SEM) was used to analyse changes in dentine morphology induced by experimental treatments and commercial toothpastes. Finally, X-ray diffractometric analyses of tested extracts were undertaken to evaluate the presence of crystal phases in phytocomplex extracts.

Materials and methods

Phytocomplex extracts and experimental treatments

Cry-lyophilised extracts of spinach leaves (*Spinacia oleracia*), rhubarb stalks (*Rhubarb rhaponicum*) and mint leaves (*Mentha piperita*) were used in powdered form. The experimental solutions and mixtures, in the form of gels or pastes, were made on the weight percentage of all components. The extracts were provided by ABOCA Company (Sansepolcro, Arezzo, Italy).

Polyethylenglycol gel (PEG 400/4000 control gel) (100 g) was prepared as follows: 2 g of PEG 400/4000 gel 70:30 [1.4 g of PEG 400 (2.2%) (Merck) and 0.6 g of PEG 4000 (0.9%) powder (Merck)], 35 g of aluminium hydroxide (Fluka AG, Switzerland), 2 g of sodium dodecylsulphate [$CH_3(CH_2)_{11}OSO_3Na$, Sigma Aldrich Chemie, The Netherlands], 12.5 g of glycerol ($CH_2OHCHOHCH_2OH$, Carlo Erba, Italy), 12.5 g of sorbitol 70% ($C_6H_{14}O_6$, Carlo Erba, Italy), and bidistilled water (Carlo Erba, Italy) containing 0.2% methyl 4-hydroxybenzoate solution (Sigma Aldrich Chemie, The Netherlands).

Carboxymethylcellulose paste (CMC control paste) (100 g) was prepared as follows: 2 g of CMC carboxymethylcellulose disodium salt (high density, Sigma Aldrich Chemie, The Netherlands), 35 g of aluminium hydroxide (Fluka AG, Switzerland), 2 g of sodium dodecyl sulphate [$CH_3(CH_2)_{11}OSO_3Na$, Sigma Aldrich Chemie, The Netherlands], 12.5 g of glycerol ($CH_2OHCHOHCH_2OH$, Carlo Erba, Italy), 12.5 g of sorbitol 70% ($C_6H_{14}O_6$, Carlo Erba, Italy), and bidistilled water (Carlo Erba, Italy) containing 0.2% methyl 4-hydroxybenzoate solution (Sigma Aldrich Chemie, The Netherlands).

Five percent potassium oxalate paste (100 g) was prepared as follows: 35 g of aluminium hydroxide [$Al(OH)_3$, Fluka AG, Switzerland)], 2 g of sodium dodecyl sulphate [$CH_3(CH_2)_{11}OSO_3Na$, Sigma Aldrich Chemie, The Netherlands], 2 g of CMC (high density, Sigma Aldrich Chemie, The Netherlands), 12.5 g of glycerol ($CH_2OHCHOHCH_2OH$, Carlo Erba, Italy), 12.5 g of sorbitol 70% ($C_6H_{14}O_6$, Carlo Erba, Italy), 20 ml of 28% potassium oxalate solution [potassium oxalate monohydrate $(COOK)_2$ H_2O Carlo Erba, Italy], and bidistilled water (Carlo Erba, Italy) containing 0.2% methyl 4-hydroxybenzoate solution (Sigma Aldrich Chemie, The Netherlands).

Dentine sample preparation

Erupted human third molars, extracted previously for periodontal reasons, were used in this study. All teeth were extracted after informed consent was obtained from each patient and according to a treatment protocol approved by the Internal Review Board, Department of Oral Sciences of Bologna University, Italy. The teeth were stored at 4 °C in physiological saline for no longer than 1 month.

Crown segments were obtained by sectioning the roots 1 mm beneath the cementum enamel junction (CEJ) using a low-speed water-cooled diamond saw (Remet, Bologna, Italy). The thickness of the crown segments was 2.5 ± 0.5 mm.

The occlusal enamel was cut off by a parallel section above the CEJ. The pulpal tissue was removed with small forceps, without altering the pre-dentine surface or the inner part of the pulpal chamber. The crown segments were cemented, using cyanocrylate (ROCKET^{TM} Heavy DVA, USA), to Plexiglas supports (2 cm $\times$ 2 cm $\times$ 0.5 cm) penetrated by an 18 gauge stainless steel tube.[9,13]

Each sample was finally connected with a hydraulic pressure device, working at 70 cmH_2O (6.9 kPa) pressure, in order to measure hydraulic conductance (Lp).

Apparatus and measurement of permeability

Evaluations of dentinal permeability were performed in accordance with standard procedures. This involves measurement of convective fluid flow through each crown segment under 70 cmH_2O (6.9 kPa) of pressure for three times every 3 min.[12,14]

The permeability apparatus included a 2.5-μl microcapillary tube (Microcaps, Fisher Scientific, Atlanta, GA, USA) positioned between the pressure reservoir and the crown segment, in which an air bubble indicated the fluid flow movement. This fluid flow movement permits measurement of dentinal permeability by calculation of Lp.[11]

Experimental design

A homogeneous smear layer was created on each dentine surface using abrasive paper for 30 s. Subsequently, the Lp was measured to evaluate the minimum permeability of each specimen and its acid resistance.[7,8,25] The smear layer was then removed, treating the dentine surface with a chelating agent (EDTA solution 0.5 M, pH 7.4) for 5 min.[9] Subsequently, the dentine surface was rinsed and the Lp was measured in order to obtain the highest permeability (Lp max = 100% was arbitrarily assigned).

Lp 100% permits evaluation of modifications in dentinal permeability following the test treatments. Moreover, each specimen was treated for 3 min with the experimental treatments in order to calculate the Lp of the specimen expressed as a percentage (Lp%) of the maximum Lp value (100%). Ultimately, each treated specimen was submitted to a final acid attack for 90 s using 37% orthophosphoric acid solution in order to evaluate the resistance of the treatments to acidic challenge. Table 1 shows the experimental design.

Treatment application

Eighteen different treatments were tested: experimental pastes, gels and solutions of phytocomplexes, an experimental potassium-oxalate-based paste, and Elmex and Sensodyne toothpastes as controls. Each treatment (approximately 0.45 g) was applied undiluted on the dentine surface for 3 min using a soft brush.[11] The tested treatments are listed in Table 2.

Table 1 Experimental design

Treatments	Lp values
Application of 0.5 M EDTA (5 min)	Lp maximum (arbitrary value of 100%)
Production of smear layer by abrasive paper (30 s)	Lp minimum
Application of treatment (3 min)	Lp modifications
Application of 37% H_3PO_4 (90 s)	Lp increase

Lp, hydraulic conductance.

Statistical analysis

The means and standard deviations of each group were calculated from Lp% obtained from the treatments. Statistically significant differences were identified among the groups by ANOVA. Fisher's least

Table 2 List of the phytocomplex treatments tested in this study

Treatments	pH
Solution containing 25% spinach extract	7.1
Paste containing polyethylene glycol 400/4000 (70:30) + 15% rhubarb extract	6.5
Paste containing polyethylene glycol 400/4000 (70:30) + 20% rhubarb extract	6.4
Paste containing 15% rhubarb extract	6.3
Paste containing 20% rhubarb extract	6.3
Solution containing 25% rhubarb extract	6.5
Solution containing 25% mint extract	6.1
Solution containing 25% rhubarb + 12.5% mint extracts	6.4
Paste containing 20% rhubarb + 10% mint extracts	6.5
Gel containing 20% rhubarb + 10% mint extracts	6.2
Elmex	Neutral
Sensodyne	Neutral
Paste containing 5% potassium oxalate	7.5
Solution containing 25% rhubarb + 25% spinach extracts	6.8
Paste containing 25% rhubarb + 25% spinach extracts	6.7
Paste containing 25% spinach extract	6.9
Paste containing carboxymethylcellulose	6.5
Gel containing polyethylene glycol 400/4000 (70:30)	6.6

significant difference (LSD) test and Bonferroni's test were used to isolate and compare the significant differences ($P < 0.01$) between the groups. SPSS Version 8.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

SEM analysis

Dentine specimens were rinsed with de-ionised water and then fixed in 2% cacodylate-buffered glutaraldehyde water solution (pH 7.3) for 24 h at room temperature. Each fixed specimen was slowly dehydrated with ascending alcohols to 100%. After being coated with gold, the specimens were examined with an SEM (Jeol Model 5400, Jeol Co., Tokyo, Japan) at 5—10 kV.

X-ray diffractometric analysis

X-ray diffractometric analyses were performed on the tested treatments with a Philips 1050 diffractometer, utilizing Cu α radiation, a nickel filter and a scanning speed of 0.5°/min of 2θ.

Results

The results of permeability analysis after treatment with the different test products and subsequent acid attack with orthophosphoric acid (37% H_3PO_4 solution) are listed in Table 3.

Dentinal permeability (Lp) results are expressed as mean ± standard deviation. Table 3 also reports the percentage reduction in dentinal permeability (Lp *R*%) for each treatment with respect to Lp max%, and the percentage increase in dentinal permeability (Lp *I*%) subsequent to the acid attack (37% H_3PO_4).

The application of different substances had various effects on dentinal permeability and produced several morphological alterations in dentine surface morphology.

Permeability test

EDTA treatment

Dentine samples treated with 0.5 M EDTA were considered as the benchmark for maximum permeabil-

Table 3 Dentinal permeability (Lp%) after treatment application and acid attack

Treatments	(Lp% max) EDTA	Lp% dentinal permeability after treatment application (Lp *R*)[a,b]	Lp% dentinal permeability after acid attack (Lp *R*)[c,d]
Smear layer	100	30 ± 5.2 (−70%)[e,f]	103 ± 9.5 (+63%)[f]
25% Spinach solution	100	56.1 ± 11.0 (−43.9%) A	67.6 ± 15.4 (+11.5%) a
15% Rhubarb gel	100	79.7 ± 7.1 (−20.3%) DE	88.8 ± 12.8 (+8.1%) cd
20% Rhubarb gel	100	78.7 ± 8.1 (−21. 3%) DE	82.2 ± 10.0 (+3.5%) bc
15% Rhubarb paste	100	77.9 ± 8.8 (−22.1%) DE	98.3 ± 14.0 (+20.4%) de
20% Rhubarb paste	100	74.1 ± 7.4 (−25.9%) CD	93.2 ± 13.1 (+19.1%) de
7 25% Rhubarb solution	100	72.1 ± 4.7 (−27.9%) BC	87.8 ± 12.8 (+15.7%) cd
25% Mint solution	100	96.2 ± 9.8 (−3.8%) F	98.5 ± 11.6 (+2.3%) de
25% Rhubarb + 12.5% mint solution	100	72.7 ± 3.9 (−27.3%) BC	85.3 ± 4.8 (+12.6%) bc
20% Rhubarb + 10% mint paste	100	74.8 ± 4.7 (−25.2%) CD	88.9 ± 7.0 (+14.1%) cd
20% Rhubarb + 10% mint gel	100	77.4 ± 4.4 (−22.6%) DE	88.5 ± 6.5 (+11.1%) cd
Elmex	100	96.0 ± 4.1 (−4.0%) F	94.7 ± 6.1 (−1.3%) de
Sensodyne	100	75.5 ± 4.0 (−24.5%) CD	86.5 ± 11.6 (+11.0%) cd
5% Potassium oxalate paste	100	68.7 ± 4.4 (−31.3%) BC	72.0 ± 3.3 (+3.3%) a
25% Rhubarb + 25% spinach solution	100	57.7 ± 6.6 (−42.3%) A	74.8 ± 10.0 (+17.1%) a
25% Rhubarb + 25% spinach paste	100	52.7 ± 8.6 (−47.3%) A	79.1 ± 6.2 (+26.4%) a
25% Spinach paste	100	72.9 ± 3.3 (−27.1%) BC	85.1 ± 10.0 (+12.2%) bc
Carboxymethylcellulose control paste	100	82.8 ± 8.4 (−17.2%) DF	84.7 ± 12.4 (+1.9%) bc
Polyethylene glycol 400/4000 (70:30) gel	100	86.0 ± 8.5 (−14%) DF	88.0 ± 9.6 (+2.1%) cd

Control treatments are not statistically efficient to reduce dentinal permeability.
[a] Reduction in dentinal permeability (Lp *R*%) after treatment application.
[b] For dentinal permeability after acid attack, treatments with the same letter are not statistically significant (Fisher's least significant difference test and Bonferroni's test; $P > 0.01$).
[c] Increase in dentinal permeability (Lp *I*%) after acid attack.
[d] For dentinal permeability after treatment application, treatments with the same letter are not statistically significant (Fisher's least significant difference test and Bonferroni's test; $P > 0.01$).
[e] The smear layer is not acid resistant and is not statistically efficient to reduce dentinal permeability.
[f] Means and standard deviations of Lp% values obtained after treatment application and acid attack with 37% orthophosphoric acid solution.

ity, in accordance with previous studies. Maximum permeability was calculated as $100 \pm 0.1\%$.[11,12,14]

Smear layer

The production of a new smear layer, obtained using wet abrasive paper for 30 s, lowered dentinal permeability by almost 70%.

Treatment applications

All treatments that were applied to exposed occlusal dentine modified Lp. Table 3 shows the changes in Lp after the application of each treatment.

Treatment with phytocomplexes such as 25% spinach extract solution, 25% rhubarb + 25% spinach extract paste and 25% rhubarb + 25% spinach extract solution was most effective in reducing dentinal permeability. Their application decreased dentinal permeability by 43.9%, 47.3% and 42.3%, respectively. The reduced permeability showed a modest increase after acid attack: +11.5% for 25% spinach extract solution, +26.4 for 25% rhubarb + 25% spinach extract paste, and +18.1% for 25% rhubarb + 25% spinach extract solution. This suggests that the application of such phytocomplexes may generate acid-resistant crystals on the dentine surface and inside the dentinal tubules. This was confirmed by subsequent SEM investigations. As a result, the three cited treatments were the most effective in reducing dentinal permeability.

Treatment with 25% spinach extract paste provided favourable results, with a reduction in Lp of 27.1% that was followed by a modest increase in Lp (+12.2%) after successive application of orthophosphoric acid solution.

Treatment with 25% rhubarb + 12.5% mint extract solution produced similar results to 25% spinach extract paste; that is, a reduction in Lp of 27.3% after treatment with phytocomplex that was followed by a successive increase (+12.6%) after acid challenge.

Treatment with 25% rhubarb extract solution decreased Lp by 27.3%. When the treated surface was challenged with phosphoric acid, the Lp increased by 15.7%.

In this regard, the application of 15% and 20% rhubarb extract pastes resulted in reductions in Lp after treatment of 22.1% and 25.9%, respectively, but this effect disappeared after acid attack, especially after treatment with 15% rhubarb extract paste.

Treatments with 15% and 20% rhubarb extract gel reduced Lp by 20.3% and 21.3%, respectively; this reduction was maintained after acid treatment, especially after application of rhubarb extract gel.

In summary, the treatments containing rhubarb and mint extracts as solutions, pastes or gels reduced Lp by 27.3%, 25.2% and 22.6%, respectively, while acid treatment halved the effectiveness of treatment.

Treatment with 25% mint extract solution only reduced the Lp of dentine by 3.8%, and the effect was almost completely reversed following acid attack.

The experimental potassium oxalate paste reduced Lp by 31.3%, and this only increased by 3.3% following acid challenge.

Commercial products did not markedly reduce dentinal permeability. In particular, the limited reduction in Lp caused by Sensodyne (24.5%) or Elmex (4.0%) treatment did not show any resistance to acid attack. Lp values of the different treatments are summarised in Table 3.

Statistical analyses

Fisher's LSD test and Bonferroni's test found significant differences ($P = 0.01$) between the treatments. Control treatments with CMC paste and PEG 400/4000 gel were not statistically effective in reducing dentinal permeability. The most effective treatments were 25% spinach extract solution, 25% rhubarb + 25% spinach extract paste and 25% rhubarb + 25% spinach extract solution. In contrast, Elmex and 25% mint extract solution were the least effective treatments.

Multiple significant differences were obtained from various treatment groups; both Fisher's LSD test and Bonferroni's test showed similar significant differences between the most effective treatments: 25% spinach extract solution, 25% rhubarb + 25% spinach extract paste, and 25% rhubarb + 25% spinach extract solution. Both statistical tests found Elmex and 25% mint extract solution to be the least effective treatments.

The distribution of permeability values (permeability scatterplot) showed a non-homogeneous distribution for 25% spinach extract solution, whereas such treatments produced the higher reductions in Lp.

SEM evaluation

EDTA application

After application of this chelating solution, dentine surfaces appeared completely free of smear layers. All dentinal tubules were open and smear plugs were absent. Peritubular dentine was dense and homogeneous, and intertubular dentine was characterised by exposed collagen. No crystals were present (Fig. 1) on EDTA-treated dentine surfaces.

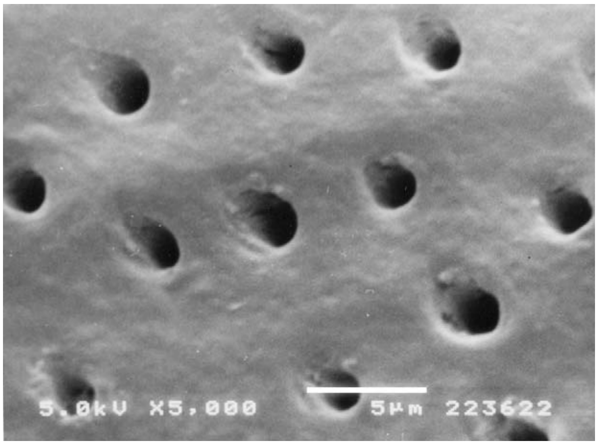

Figure 1 Micrograph of dentine surface. EDTA treatment removes the smear layer and the dentinal tubules appear completely open. This treatment was used to simulate the tubular condition in the presence of severe dentinal hypersensitivity (original magnification 5000×).

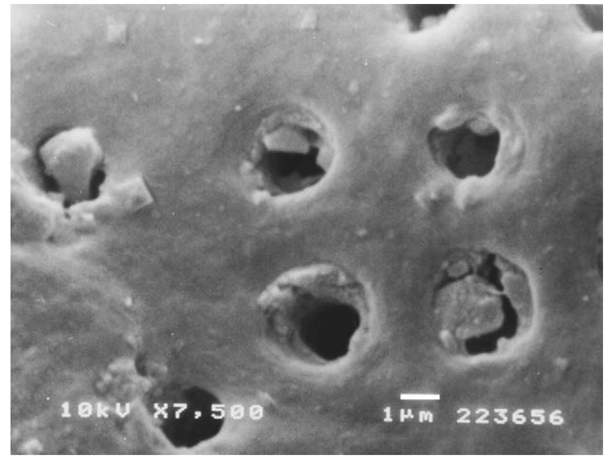

Figure 3 Dentine surface treated with 25% rhubarb + 25% spinach extract solution. Note the presence of clusters of microcrystals in dentinal tubules (original magnification 7500×).

Smear layer

After the surface treatment with abrasive paper, the smear layer appeared uniform and homogeneous.

Treatment applications

Following application of preparations of spinach (Fig. 2), spinach + rhubarb (Fig. 3) or rhubarb (Fig. 4), numerous clusters and microcrystals occluded the dentinal tubules. These crystals had the shape of an eight-faced bipyramid, approximately 1–2 μm across.

Orthophosphoric acid challenge

The morphology of dentine surfaces treated with 25% spinach extract solution and then submitted to acid attack showed acid-resistant microcrystals occluding the dentinal tubules (Fig. 5). The same observations were made following treatment with 25% rhubarb + 25% spinach extract solution (Figs. 6 and 7), 25% spinach extract paste (Fig. 8) and 25% rhubarb extract solution (Fig. 9).

X-ray diffraction analysis

Figs. 10 and 11 show the X-ray diffractograms of lyophilised concentrates of rhubarb and spinach. The lyophilised rhubarb roots presented patterns without well-defined reflections, indicating low-crystallinity. On the contrary, lyophilised spinach presented patterns with characteristics of a crystalline substance with defined reflections of soluble oxalates, such as potassium and sodium oxalate.

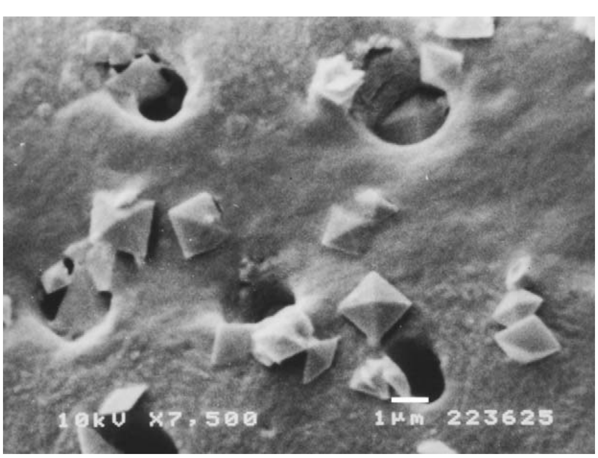

Figure 2 Dentine surface treated with 25% spinach extract solution. Note the crystal shapes and position in the dentinal tubules (original magnification 7500×).

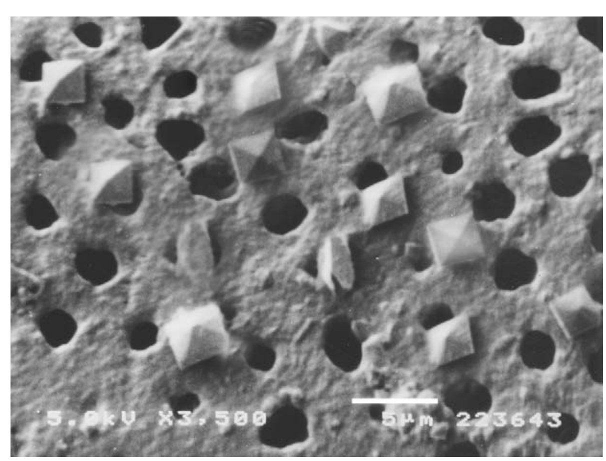

Figure 4 Dentine surface treated with 25% rhubarb extract solution and rinsed with bidistilled water. Crystals partially covering or occluding dentinal tubules can be observed (original magnification 3500×).

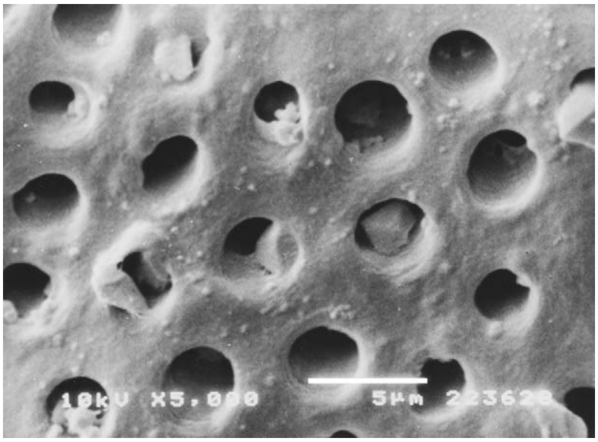

Figure 5 Dentine surface treated with 25% spinach extract solution and following acid challenge with orthophosphoric acid solution (37%). In spite of acid treatment, microcrystals occluding dentinal tubules remain in place, but their shape is sometimes modified (original magnification 3500×).

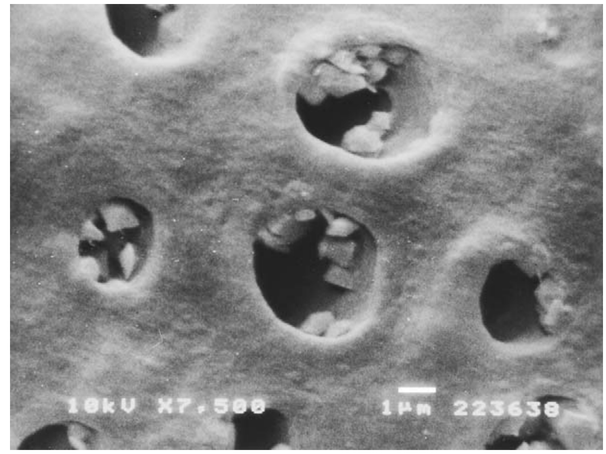

Figure 7 Higher magnification of Fig. 6 showing partial tubule occlusion following acid treatment. Note the presence of crystals adhering to the tubular walls (original magnification 7500×).

Discussion

The functional and anatomical occlusion of tubules reduces the flow of dentinal fluid. Any substance that leads to a decrease in dentinal conductance (i.e. dentinal permeability) by reducing the diameter or closing the tubules and diminishing their number is able to reduce dentinal hypersensitivity and pain.[13]

Several in vivo[16,20,26,27] and in vitro[15,17–19] studies have shown that pastes or aqueous solutions based on potassium oxalate occlude dentinal tubules by creating acid-resistant calcium oxalate crystals on the dentine surface and inside dentinal tubules.

In the present study, the SEM results show that treatment with oxalate-containing phytocomplexes induces microcrystal deposition on dentine and inside dentinal tubules. These treatments reduce the tubular diameters by forming crystals or crystal-like structures, as confirmed by permeability evaluations. All tested phytocomplex-based treatments contained oxalate salts.

The morphology of calcium oxalate crystals is an eight-faced bipyramid shape (tetragonal system) which corresponds to weddellite or dihydrated calcium oxalate $CaC_2O_4{\cdot}2H_2O$. Weddellite is the

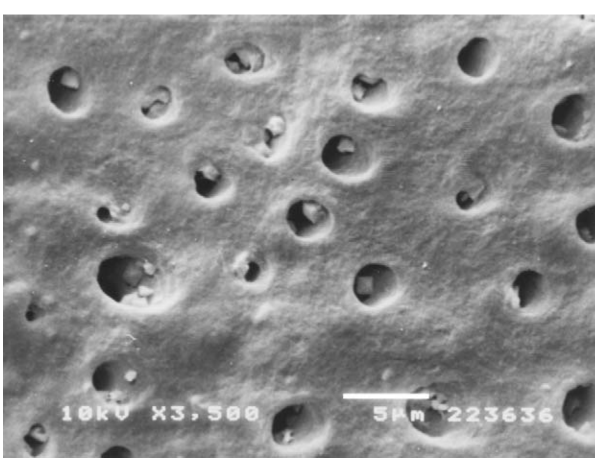

Figure 6 Treatment with 25% rhubarb + 25% spinach extract solution followed by treatment with orthophosphoric acid solution (37%) for 90 s. Dentinal tubules are partially occluded by acid-resistant microcrystals (original magnification 3500×).

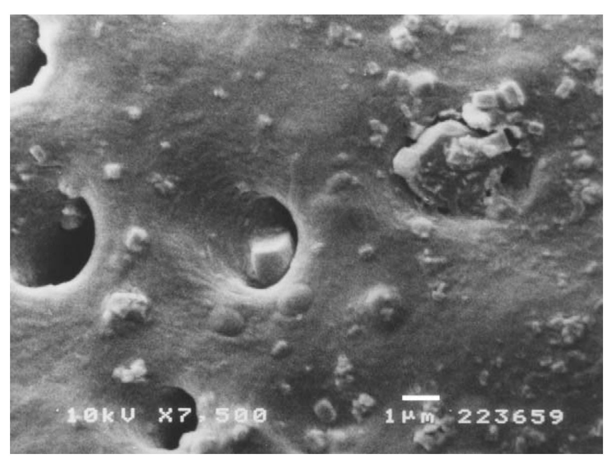

Figure 8 Dentine surface treated with 25% spinach extract paste, followed by treatment with orthophosphoric acid solution (37%) for 90 s. Note the presence of clusters and single crystals occluding dentinal tubules, consistent with reductions in permeability (original magnification 7500×).

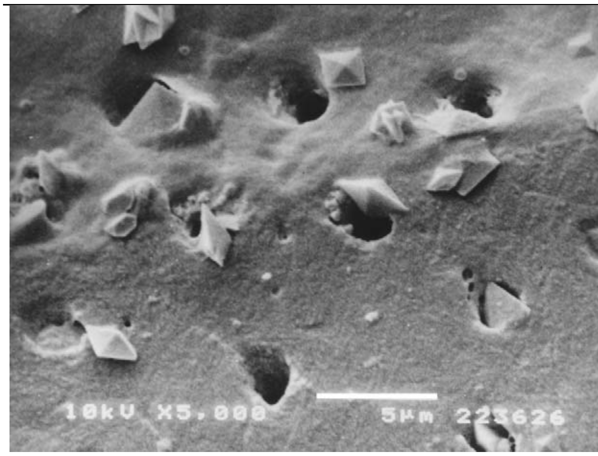

Figure 9 Partial occlusion of tubules following acid treatment after treatment with 25% rhubarb extract solution. Note the presence of several crystals in tubule orifices and their angular morphology (original magnification 5000×).

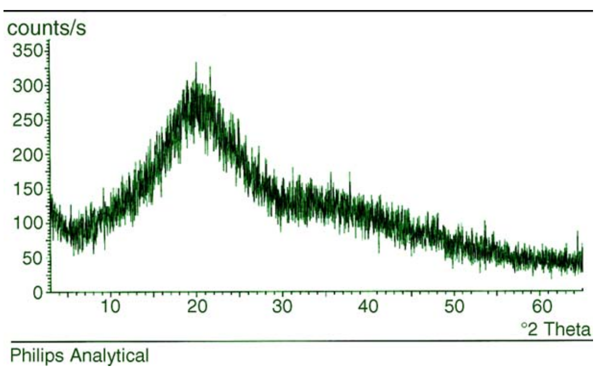

Figure 10 X-ray diffractogram of lyophilised extract of rhubarb stalks. The amorphous pattern is evident.

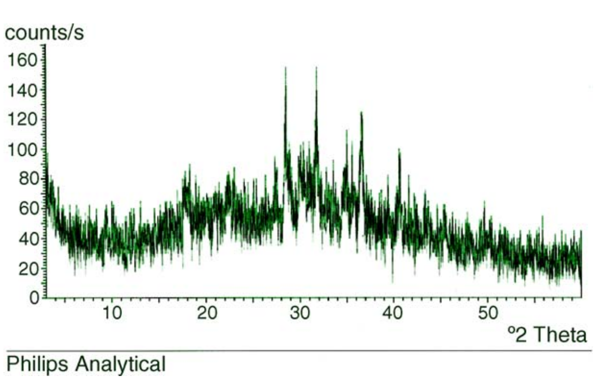

Figure 11 X-ray diffractogram of lyophilised extract of spinach leaf showing oxalate crystals reflexes.

metastable form of calcium oxalate. No whewellite crystals were found.[21]

It is known that crystals have been classified as weddellite or whewellite only on the basis of their shape. The crystal polymorphism, defined as the formation of crystals with different shapes, seems to be associated with different environmental parameters during crystalogenesis.

Weddellite is the metastable form of calcium oxalate, and whewellite or monohydrated calcium oxalate $CaC_2O_4{\cdot}H_2O$ is the stable form of the same component that crystallises in a monoclinic shape (it is a rare form of calcium oxalate). A certain number of crystallisation behaviours have been described for both the hydratation states: raphids, prisms, styloids, druses and crystal sand.[22,28]

It is possible to affirm that soluble oxalates and oxalic acid present in the tested phytocomplexes form calcium oxalate crystals by reacting with dentinal calcium.[25] The calcium oxalate crystals already present in the lyophilised phytocomplexes may penetrate inside dentinal tubules if their dimensions are less than 1–2 μm. Calcium oxalate crystals are insoluble and they may bind to anionic macromolecules, such as dentinal proteins. The binding of crystals to anionic macromolecules is a surface-related process; the greatest adhesion and adsorption (specific binding) occur on to specific crystal faces.[28] The different effectiveness of each phytocomplex may be explained by different amounts of total oxalate and the diverse presence of soluble oxalates inside phytocomplexes.[29]

Spinach leaves contain both soluble and insoluble oxalates; the soluble oxalate content comprises approximately 80% of the total oxalates (970 mg/100 g of spinach leaves). In rhubarb stalks, both soluble and insoluble oxalates are present. The soluble oxalate content in rhubarb stalks comprises approximately 30% of the total oxalates (805 mg/100 g of rhubarb stalks). The soluble oxalate content in mint comprises approximately 10% of the total oxalates (170 mg/100 g of mint leaves).[29]

The ratios of different elements, particularly calcium and oxalate/calcium, determine the effect of phytocomplexes on dentinal tubule occlusion.[20] Low amounts of calcium and excess oxalate (oxalate/calcium ratio > 1) induce binding of oxalate to calcium, producing calcium oxalate directly inside dentinal tubules. Crystal precipitation in the orifices of the dentinal tubules leads to occlusion of the tubules.

The oxalate/calcium ratio of spinach leaves was lower than that of rhubarb stalks (Table 4). The low effectiveness of mint extracts was probably due to the low oxalate content in the phytocomplex.

The permeability results and SEM morphological analysis demonstrated an interaction between some of the test products and the dentine surface.

Table 4 Oxalate content, oxalate/calcium ratio and pH of spinach, mint and rhubarb

Foodstuffs	Oxalate (mg/100 g) range (mean)	Calcium (mg/100 g) range (mean)	Oxalate/ calcium (mEq)
Spinach (*Spinacia oleracia*)	320—1260 (970)	80—122 (101)	4.27
Rhubarb (*Rhubarb rhaponicum*)	275—1336 (805)	40—50 (45)	7.95
Mint (*Mentha piperita*)	140—200 (170)	180—290 (235)	0.32

X-ray diffraction analysis, which showed the absence of crystals in rhubarb extracts, confirmed in situ formation of insoluble calcium oxalate inside dentinal tubules and on the dentine surface when treated with other phytocomplex extracts.

The present in vitro study of natural product extracts that induced a series of structural and physiological changes in dentine may be correlated in vivo with the relief of pain and dentinal hypersensitivity.

It is well known that oxalates are able to create crystals, most likely calcium crystals, when applied to dentinal tissue. They produce a layer of crystals that reduces dentinal permeability. However, only a few foods are high in oxalates. In nature, for example, some common vegetables such as spinach, rhubarb and mint contain phytocomplexes that may be easily prepared and used for pastes or gels for dental hygiene. These preparations are able to create oxalate crystals that were detectable inside tubule orifices. As SEM analysis showed, these crystals are enclosed within the tubules. Oxalate crystals are small enough to penetrate the tubules and occlude tubular orifices. Other tested agents, such as toothpastes (Elmex and Sensodyne), CMC and PEG 400/4000, do not contain oxalates, and were unable to induce crystal formation on the dentine surface or reduce dentinal permeability. Treatment with 5% potassium oxalate paste proved that oxalates are able to reduce dentinal permeability and form crystals on dentine surfaces.

It is important to note that paste and gel formulations, unlike most commercial products, do not contain any abrasives or other agents that may cause alterations in dentine morphology.

In conclusion, these results show that phytocomplexes extracted from rhubarb and spinach, used in different formulations, may be suitable for topical treatment of dentinal hypersensitivity. The decrease in dentinal permeability, combined with increased resistance to acid attack and the formation of microcrystals produced by phytocomplexes from rhubarb and spinach, indicates that they may be useful products for dentinal hypersensitivity therapy. Further evaluations are in progress to define and create more suitable clinical formulations for commercial products and their application in vivo.

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