# Little Ironweed and Java Tea in Herbal Toothpaste Reduced Dentine Permeability: An *In Vitro* Study

La-ongthong Vajrabhaya<sup>1</sup>, Suwanna Korsuwannawong<sup>2</sup>, Cholthacha Harnirattisai<sup>3</sup>, Chayada Teanchai<sup>2</sup>, Weena Salee<sup>2</sup>

<sup>1</sup>Endodontics Section, College of Dental Medicine, Rangsit University, Pathumthani, Thailand, <sup>2</sup>Research office, Faculty of Dentistry, Mahidol University, Bangkok, Thailand, <sup>3</sup>Department of Operative Dentistry and Endodontics, Faculty of Dentistry, Mahidol University, Bangkok, Thailand Aim: To determine the effect of an herbal toothpaste containing Little Ironweed and Java Tea, on reducing dentine permeability in vitro. Materials and Methods: Dentine discs from human mandibular third molars were divided into three groups and brushed with herbal toothpaste, nonherbal toothpaste, or deionized water. Each group was immersed in artificial saliva (AS) or 6% citric acid. The permeability of each dentine disc was evaluated before and after saliva or acid challenge using a fluid filtration system. The morphology of dentine discs after treatment was observed using scanning electron microscopy (SEM). The mean permeabilities were statistically analyzed using analysis of variance and Tukey's test. Results: The nonherbal and herbal toothpaste groups demonstrated reduced dentine permeability. AS immersion decreased dentine permeability in both toothpaste groups with values lower than the control group. Dentine permeability values increased after acid immersion in the toothpaste groups and were similar to each other. SEM revealed small granular crystal-like and round particles on the dentine surface and opening of dentinal tubules of both toothpaste groups. More dentinal tubules were opened after brushing with deionized water. Conclusions: The reduction of dentine permeability caused by the herbal toothpaste was similar to that of the nonherbal toothpaste after brushing and the simulated oral conditions. Both herbal plants have the possibility to alleviate clinical hypersensitivity by reducing dentine permeability. Little Ironweed and Java Tea in the toothpaste composition is a potential choice for treating hypersensitive dentine.



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## INTRODUCTION

 $\mathcal{H}$  ypersensitivity of dentine is a sharp, shooting pain of short duration in a tooth. The dentine and dentinal tubules are exposed and patent to the pulp. The hydrodynamic theory has been proposed to explain the dentine hypersensitivity mechanism.<sup>[1]</sup>

Loss of enamel or cementum in the cervical area due to tooth brushing, gingival recession from periodontitis, erosion, or abrasion causes hypersensitivity. Placing a restorative material is recommended to occlude the exposed dentinal tubules.<sup>[2]</sup> However, desensitizing toothpaste is an alternative substance to use to relieve dentine hypersensitivity pain.

The treatment and prevention of dentine hypersensitivity is to eliminate the introduction of external stimuli in the dentinal tubules. These treatments include occluding the dentinal tubules, depolarizing electrical nerve conduction, direct or indirect restorations, and periodontal surgery to cover the exposed dentine with gingival tissue. Pro-Argin<sup>™</sup> and NovaMin<sup>®</sup> are kinds of toothpaste containing arginine and novamin,<sup>[3,4]</sup> respectively, that can occlude the dentinal tubules.

Most of the occluding substances in tubes of toothpaste are synthetic chemical agents. Currently, many people are concerned about the adverse effects of the chemical components in tubes of toothpaste and prefer to use a natural product. Vernonia cinerea or Little Ironweed was found to have potassium nitrate in the whole plant after chemical constituent extraction.<sup>[5]</sup> A chemical component evaluation demonstrated that the samples from the aerial part of the plant and leaves from Orthosiphon aristatus Blume, Java Tea, also contained potassium nitrate  $\leq 0.7$ , 2% w/w pH value ≤5.0.<sup>[6]</sup> Dok Bua Ku Sensitive Herbal Toothpaste (Twin Lotus Co. Ltd., Bangkok, Thailand) is the only Thai herbal toothpaste available in Thailand, that is, claimed to reduce tooth hypersensitivity. Potassium nitrate was extracted from both plants, which is the same as the active ingredient in Sensodyne Fresh Mint® (nonherbal toothpaste, GlaxoSmith Kline, Middlesex, UK), that is, recommended for reducing tooth hypersensitivity. However, the effect of these two herbal plants on occluding dentinal tubules to reduce dentine permeability has not been reported.

This study aimed to determine the consequence of an herbal toothpaste containing Little Ironweed and Java Tea, on reducing dentine permeability *in vitro*. The

null hypothesis is that there is no difference between Sensitive Herbal Toothpaste and Sensodyne Fresh Mint<sup>®</sup> in reducing dentine permeability.

## **MATERIALS AND METHODS**

## **EXPERIMENTAL DESIGN**

The design of this study was randomized controlled trials type. Only noncarious and nonoccluded extracted mandibular molars teeth were used. Dentine permeability after brushing with nonherbal and herbal toothpastes and also after immersion in AS and acid were evaluated by comparing with water; both hydraulic conductance (LP value) and scanning electron microscopy (SEM) analysis were used. Each step of the experiment was performed by the same observer as shown in Figure 1.

## TOOTH COLLECTION AND STORAGE

The extracted teeth were kept in 0.1% thymol solution for no longer than 1 month and divided into three groups as follows: Negative control group (Group 1): Water n = 22, Sensodyne Fresh Mint<sup>®</sup> (Group 2) n = 24, and Sensitive Herbal Toothpaste (Group 3) n = 20. The experiment was accomplished at the Faculty of Dentistry's Laboratory, Mahidol University, Bangkok, Thailand, for 6 months.

#### TOOTH EXTRACTION AND DISC PREPARATION

The extracted noncarious and nonoccluded human mandibular third molars from 18- to 22-year-old patients were gathered using a protocol approved by the Ethics Committee of Rangsit University, Pathumthani, Thailand (COA No. RSUERB 2020-018).

A sixty-six  $1000 \pm 100 \ \mu m$  thick dentine discs were sectioned at the deep coronal dentine perpendicular to the long axis of the teeth above the pulp cavity using a water-cooled diamond saw (Accutom 50, Struers, Copenhagen, Denmark).

## MEASUREMENT OF DENTINE PERMEABILITY

A standard smear layer was performed by polishing the occlusal dentine disc with 600-grit SiC paper for 30 s. The 37% phosphoric acid was put on the dentine disc for smear layer removal.

Each disc was connected to a fluid filtration system as mentioned by Sauro *et al.*<sup>[7]</sup> under the hydrostatic water pressure used in a previous study.<sup>[8]</sup> The linear displacement of adjusted 3 mm in length of an air bubble



**Figure 1:** Schematic representation of methodology. A-E: Dentine disc preparation, F-G: SEM evaluation before and after  $H_3PO_4$  application, H: Three experimental groups, I: Dentine permeability measurement, J: SEM evaluation. AS = artificial saliva

in the capillary tube after 3 min indicated the hydraulic conductance of a specimen. The measurement of each specimen was repeated three times and set a value of 100% permeability.

## **GROUPING AND ALLOCATION: DENTINE BRUSHING**

The randomization controlled trials method with no bias and dropout rates of 20% was used for dividing the grouping of dentine discs for the experiment. Two experimental groups were brushed with nonherbal and herbal toothpastes, whereas the deionized water as the control group was replaced with the toothpaste during brushing. Sensodyne Fresh Mint<sup>®</sup> (nonherbal toothpaste, GlaxoSmith Kline) contained potassium nitrate and sodium fluoride as the active ingredients.

Another toothpaste, Sensitive Herbal Toothpaste (Twin Lotus Co. Ltd.) contains Little Ironweed, Java Tea, Toothbrush Tree, Orange Jessamine, *Clinacanthus nutans*, Cuttlefish bone, Mangosteen, and *Hydrocotyle*.

Each experimental group was brushed with the slurry of individual toothpaste (0.1 g of toothpaste mixed with 100  $\mu$ L of deionized water). Each specimen was brushed with an automated toothbrush (Braun Oral-B Vitality<sup>TM</sup> Precision Clean D12.013, Braun GmbH Co, Kronberg, Germany), 7600 rpm for 2 min. Then, the permeability of each dentine disc was determined as mentioned above.

#### **A**RTIFICIAL SALIVA AND ACID IMMERSION

Both experimental and control groups were brushed with Sensodyne Fresh Mint<sup>®</sup>, Sensitive Herbal Toothpaste, and deionized water, respectively. The specimens of each group were divided into two equal subgroups for immersion in artificial saliva (AS) and 6% citric acid respectively.

The AS was composed of methyl-*p*-hydroxybenzoate, sodium carboxymethyl cellulose, KCl, MgCl<sub>2</sub>· $6H_2O$ , CaCl<sub>2</sub>· $2H_2O$ , K<sub>2</sub>HPO<sub>4</sub>, KH<sub>2</sub>PO<sub>4</sub>, and KOH, pH = 6.25. Each subgroup was individually immersed in AS 1 h,

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Table 1: Hydraulic conductance (LP) after tooth brushing with the different treatments			
Treatment	Water ( <i>n</i> = 22)	Sensodyne Fresh Mint® ( <i>n</i> = 24)	Sensitive Herbal Toothpaste ( $n = 20$ )
37% phosphoric acid	100 <sup>Ab</sup>	100 <sup>Ab</sup>	100 <sup>Ab</sup>
Tooth brushing	$114.40 \pm 40.57^{Aa}$	61.65±38.95 <sup>Bb</sup>	$59.65 \pm 36.97^{\text{Bb}}$
Immersion in AS	$71.57 \pm 36.48^{Ca}$	48.04 ± 36.26 <sup>cb</sup>	50.17±45.01 <sup>Bb</sup>
Tooth brushing	$127.72 \pm 40.72$ <sup>Ba</sup>	$60.29 \pm 27.42^{\text{Bb}}$	58.85±21.91 <sup>Bb</sup>
Acid challenge	105.44±37.62 <sup>ва</sup>	64.74±25.94 <sup>Bb</sup>	75.29±38.55 <sup>Bb</sup>

The same lowercase letters and uppercase letters indicate P > 0.05 within the rows and the columns, respectively

 $37^{\circ}$ C at 100 rpm vibration. Whereas the other subgroups of dentine discs were also individually immersed in 1 mL of 6% citric acid, pH 1.5 for 1 min at room temperature. Then, the dentine permeability of each specimen was determined again.

#### SCANNING ELECTRON MICROSCOPY ANALYSIS

The dentine discs in each group after the procedure of brushing, AS, and acid immersion were prepared for SEM observation (JSM 6610 LV, JEOL, Akishimashi, Japan) with an accelerating voltage of 20 kV and 12 mm of working distance at  $2000 \times$ .

## STATISTICAL ANALYSIS

The linear displacement of the air bubble in each specimen was converted into volume flow ( $\mu$ L/min). The fluid flow across each dentine disc was transformed into hydraulic conductance (Lp,  $\mu$ /min/cm<sup>2</sup> using the formula LP = Q/ At (where Q is the fluid flow in  $\mu L$ , A is the area of the dentine in  $cm^2$ , and t is the time in minutes.<sup>[7]</sup> The data are presented as the means and standard deviation values of the %LP. Repeated measures analysis of variance (ANOVA) was used to compare the mean permeability at three separate time points (prebrushing, after brushing, and postimmersion in AS/acid). Tukey's post hoc test was used to determine significant differences between the means when the ANOVA test result was significant. Statistical analysis was performed using the Statistical Package for the Social Sciences 26.0 for Windows (SPSS Inc., Chicago, IL, USA) at  $P \le 0.05$ .

## RESULTS

## **DENTINE PERMEABILITY MEASUREMENTS**

The results of dentine permeability are indicated as percentages of the maximum permeability, (100%) after 37%  $H_3PO_4$  etching. The hydraulic conductance after brushing, AS, or citric acid immersion is shown in Table 1.

## **A**FTER BRUSHING

The hydraulic conductance of Sensodyne Fresh Mint<sup>®</sup> and Sensitive Herbal Toothpaste groups significantly reduced (P = 0.000) after brushing the etched dentine discs. In the control group, the hydraulic conductance was not significantly different from that measured before brushing (P = 0.177) in the AS immersion

group but was significantly different in the citric acid immersion group (P = 0.045).

#### **ARTIFICIAL SALIVA IMMERSION**

The brushed dentine discs in the Sensodyne Fresh Mint<sup>®</sup> or Sensitive Herbal Toothpaste groups demonstrated decreased dentine permeability, however, the difference between the groups was not significant (P = 0.247). The toothpaste groups demonstrated a significantly lower dentine permeability compared with the control group (P = 0.000).

#### ACID IMMERSION

The brushed dentine in the two toothpaste groups resulted in increased dentine permeability, however, no significant difference was observed from that after brushing (P = 0.105 and 0.824, respectively). The dentine permeability also decreased in the control group but remained significantly higher than that of the toothpaste groups (P = 0.0000).

## **SEM** EVALUATION

## Smear layer creation and smear layer removal

A thick uniform smear layer covered the entire surface with most dentinal tubule openings obliterated with smear plugs. After applying 37% phosphoric acid solution on the dentine surface, the smear layer on the surface and smear plugs dissolved. The dentinal tubule orifices were open and widened [Figure 2].

## Brushing with each toothpaste or water

Brushing with water generated a smooth surface with open dentinal tubules as shown in Figure 3A. In contrast, in the Sensodyne Fresh Mint® group images, individual or agglomerated fine granular crystal-like structures were observed. After brushing with Sensitive Herbal Toothpaste, fine granular round structures were present that were slightly smaller than those on the surface brushed with Sensodyne Fresh Mint® is shown in Figures 3B and C, respectively.

## Immersion in artificial saliva

A clean surface with longitudinal scratches and slightly distorted and open dentinal tubules were seen in the water group [Figure 3D]. The Sensodyne Fresh Mint<sup>®</sup> group exhibited fine granular-like deposits in approximately



Figure 2: (A) Dentine disk after smear layer creation. (B) After 37% phosphoric acid treatment. 2000×



Figure 3: (A–C) Scanning electron microscopy of the dentine surfaces after brushing. (D–F) After immersion in artificial saliva. (G–I) After acid challenge.  $2000\times$ 

half of the dentinal tubules [Figure 3E] with few granular particles present on the surface. In contrast, the surface brushed with Sensitive herbal toothpaste images demonstrated fine round crystal-like structures on the dentin surface and in the tubules with some open tubules present [Figure 3F].

## Immersion in citric acid

A smooth surface without any debris remnants or open dentinal tubules was observed on the dentine surfaces in the water group [Figure 3G]. Similar findings were seen in the toothpaste groups, the granular precipitate was removed from most dentinal tubule orifices with increased tubular opening diameters, indicating more extensive demineralization in these groups [Figure 3H and I].

## DISCUSSION

The present study demonstrated that the hydraulic conductance was reduced by  $\sim 40\%$  after brushing with

nonherbal and herbal toothpastes. However, in the control group, the hydraulic conductance increased by 14%–27%. These results agreed with those of previous studies indicating that brushing with toothpaste reduces dentine permeability better compared with brushing with deionized water.<sup>[8,9]</sup> The dentine surface after brushing with water in this study was smooth with open dentinal tubules; however, the other two groups demonstrated small granular crystal-like and round particles on the dentine surfaces and dentinal tubules. Our observations were similar to those of a previous study<sup>[10]</sup> where the SEM images of the dentine surfaces brushed with herbal or nonherbal toothpastes revealed fine granular crystal-like structures on the dentine surface and closed dentinal tubules. Moreover, the dentine permeability was reduced by 40% after brushing with these toothpastes.

The active component of Sensodyne Rapid Relief® is strontium acetate. An in vitro study showed that after brushing the dentine disc with this desensitizing toothpaste, strontium salts formed a thin occluding layer on the surface of the dentine that did not extend into the dentinal tubules.<sup>[11]</sup> In the present study, the active ingredients in Sensodyne Fresh Mint® are potassium nitrate and sodium fluoride, sodium fluoride promoted greater dentinal tubule occlusion than stannous fluoride, however, both increased the roughness of the eroded dentine<sup>[12]</sup> Markowitz and Kim<sup>[13]</sup> reported that potassium nitrate reduces dentinal sensory nerve activity due to the depolarizing action of the K<sup>+</sup> ions. Moreover, potassium nitrate is more effective in occluding dentinal tubules when applied twice daily in a toothpaste compared with a mouthwash.<sup>[14]</sup> A clinical study in China demonstrated that potassium nitrate dentifrice is an effective and quick-acting agent for daily home treatment of dentine hypersensitivity.<sup>[15]</sup>

Potassium nitrate is one of the chemical constituents extracted from Little Ironweed and Java Tea.<sup>[6,16]</sup> A previous study reported only the effect of potassium nitrate on depolarizing the nerve endings.<sup>[13]</sup> An *in vitro* study using SEM micrograph analysis revealed dentinal tubules occluded by potassium nitrate.<sup>[14]</sup> Furthermore, a meta-analysis of five studies suggested that there is sufficient evidence to support the use of a potassium nitrate- and stannous fluoride-containing toothpaste for dentine hypersensitivity, but not the use of a strontium-containing desensitizing toothpaste.<sup>[17]</sup>

The mechanism of how the herbal toothpaste reduced dentine permeability was demonstrated by the %LP reduction observed in the present study. This finding indicates that the potassium nitrate in the herbal composition penetrated to occlude the dentinal

tubules. However, we observed differences between the experimental groups. Variations in the morphology of the dentine of individual dentine discs resulted in nonnormally distributed data measures of central tendency. However, increasing the sample size might result in the data, that is, normally distributed.

The %LP after brushing with Sensodyne Fresh Mint® and Sensitive Herbal Toothpaste was 13% and 7%, respectively, lower than before immersion in saliva. The reduction in %LP corresponded with the SEM images, on which increased fine granular-like and round crystal-like structures were observed on the dentine surface and in the tubules, respectively, in both groups. In contrast, the group brushed with deionized water demonstrated slightly distorted dentinal tubules. In addition, the %LP in the control group decreased from before salivary immersion but was higher compared with the toothpaste brushing groups (P < 0.05).

In the 6% citric acid immersion groups, the %LP was higher compared with before acid immersion, but not significantly different from before acid immersion in the toothpaste brushing groups (P > 0.05). The SEM images of these groups demonstrated increased dentinal tubule diameters. This study confirmed the effect of acid on dentine demineralization that was seen in previous studies.<sup>[7,10]</sup>

The guideline for the management of dentine hypersensitivity of the Indian Society of Periodontology recommends using a combination of at-home and in-office therapies starting with noninvasive treatment and progressing to more invasive interventions.<sup>[18]</sup> The first alternative utilizing a natural or synthetic desensitizing agent provides fast and long-lasting pain relief by intratubular occlusion or obliteration of neuronal action.<sup>[19-22]</sup> Increasing public concern regarding undesirable consequences from synthetic chemical agents has resulted in the exploration of various herbal agents for managing dentine hypersensitivity.<sup>[23-25]</sup> The limitation of the present study was that the experiment was performed in vitro. A clinical study to evaluate whether an herbal toothpaste containing Little Ironweed and Java Tea reduces the pain occurring from dentine hypersensitivity is needed.

# CONCLUSION

Taken together, the results demonstrated that a Thai herbal toothpaste containing Little Ironweed and Java Tea reduced dentin permeability reduction and occluded the occlusion of the dentinal tubules in all treatment conditions. The dentine permeability was similar to that of the nonherbal toothpaste containing chemical ingredients after AS and acidic environment exposure.

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## **CONFLICTS OF INTEREST**

There are no conflicts of interest.

## **AUTHOR CONTRIBUTIONS**

LV: proposal writing, experimental designing, manuscript writing, and funding application. SK: dentine permeability testing and data analysis. CH: SEM analysis. CT: dentine cutting and scanning electron microscope laboratory performing. WS: dentine brushing.

ETHICAL POLICY AND INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable.

PATIENT DECLARATION OF CONSENT

Not applicable.

## DATA AVAILABILITY STATEMENT

Not applicable.

## **R**EFERENCES

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