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Longevity of bonded composite restorations after dentin biomodification with neolignans obtained from *Nectandra leucantha*

Longevidade de restaurações de resina composta após biomodificação de dentina com neolignanas obtidas de *Nectandra leucantha*

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ABSTRACT

Objective: This *in vitro* study evaluated the effect of neolignan-containing solutions on dentin biomodification previously applied to the bonding procedure in adhesive restorations. **Material and Methods:** Neolignans, dehydrodieugenol B–CP1 and dehydrodieugenol B methyl ether–CP2, were isolated from *Nectandra leucantha* and two aqueous solutions containing 0.13% neolignans, 0.2% propylene glycol and 3.0% ethanol were prepared. Bovine teeth were ground flat to obtain 2-mm thick specimens which received resin composite restorations (N=10). The neolignan solutions were applied before the bonding procedure (60 s). Experimental groups were: control, untreated group, 0.12% chlorhexidine gel, 0.13% CP1 solution, and 0.13% CP2 solution. A push-out bond strength test was conducted (0.5 mm/min). Bovine tooth sections (0.5×1.7×7.0 mm) were also obtained to assess the modulus of elasticity and mass change after treatment (N=15). A three-point bending test evaluated the elastic modulus of fully demineralized dentine beams after immersion in the solutions. The data were statistically analyzed (= 0.05). **Results:** The bond strength of the restorations to dentin was significantly improved by the treatment with neolignan-containing solutions, irrespective of the evaluation time ($p<0.05$). After 6 months, a significant reduction in the bond strength was observed in the groups treated with the solutions ($p>0.05$), but the means were significantly higher than the control groups ($p<0.05$). The elastic modulus of demineralized dentin was significantly improved after the treatment with the solutions ($p<0.05$). All groups lost mass weight. **Conclusion:** The solutions improved the *in vitro* longevity of bonded restorations, possibly due to the dentin biomodification effect of the neolignans.

KEYWORDS

Dental restorations; Permanent; Dentin; Collagen type I; Plants medicinal; Lignans.

RESUMO

Objetivo: Este estudo *in vitro* avaliou o efeito de soluções contendo neolignanas na biomodificação da dentina aplicadas previamente à restaurações adesivas. **Material e Métodos:** Neolignanas, desidrodieugenol B–CP1 e éter metílico de desidrodieugenol B-CP2, foram isolados da espécie *Nectandra leucantha* e duas soluções aquosas contendo 0,13% de neolignanos, 0,2% de propilenoglicol e 3,0% de etanol foram preparadas. Dentes bovinos foram lixados para obter espécimes de 2 mm de espessura e preparos cavitários restaurados com resina composta (N=10). As soluções foram aplicadas em dentina antes do procedimento adesivo (60 s). Os grupos experimentais foram: controle, grupo não tratado, gel de clorexidina 0,12%, solução de CP1 a 0,13% e solução de CP2 a 0,13%. Foi realizado o teste de resistência de união push-out (0,5 mm/min). O módulo de elasticidade e a alteração de

massa após tratamento da dentina (0,5×1,7×7,0 mm) foram também avaliados em teste de flexão de três pontos (N=15). Os dados foram analisados estatisticamente (α=0,05). **Resultados**: A resistência de união das restaurações à dentina melhorou significativamente com o tratamento com as soluções, independentemente do tempo de avaliação (p<0,05). Após 6 meses, foi observada redução significativa da resistência de união nos grupos tratados com as soluções (p>0,05), com médias significativamente maiores do que nos grupos controle (p<0,05). O módulo de elasticidade da dentina desmineralizada aumentou significativamente após tratamento com as soluções (p<0,05). Todos os grupos perderam massa, independentemente do tratamento. **Conclusão**: As soluções melhoraram *in vitro* a longevidade das restaurações adesivas, possivelmente devido ao efeito biomodificador da dentina das neolignanas.

PALAVRAS-CHAVE

Restaurações dentárias permanentes; Dentina; Colágeno tipo I; Plantas medicinais; Lignanas.

INTRODUCTION

It has been extensively reported that the collagen fibrils within the interfacial area of bonded restorations can be exposed after the bonding procedures because of the difference between the depth of acid demineralization and the ability of resin monomers to infiltrate the demineralized dentin [1]. These exposed, unprotected collagen fibrils are susceptible to proteolytic degradation by endogenous matrix-bound metalloproteinases (MMPs) or cysteine cathepsins, which are intensively activated in acidic environments [2]. These enzymes are responsible for the time-dependent degradation of the collagen of the interfacial area, which usually starts at the bottom of the hybrid layer [3]. Exposure of unprotected collagen fibrils after the bonding procedure is regarded as the main problem that impacts the restoration longevity due to the deterioration that occurs at the hybrid layer [4]. As a consequence, there is a decrease in the bond strength of resin-based materials to dentin, a decrease in the sealing of the restoration margins, and ultimately restoration loss [5].

Recently, a modification of the tooth substrate was proposed to increase the resistance to general proteolysis by improving the mechanical properties of the triple helical collagen component within the hybrid layer [6-8]. It has been pointed out that collagen is strengthened in biological tissues when native cross-links are formed, which seems to increase the fibrillar resistance to enzymatic degradation [9] and improve the tensile properties [10]. In this manner, synthetic and natural compounds have been proposed to induce collagen crosslinking, enhancing the dentin matrix's biological stability, the tensile bond strength, and viscoelasticity of the collagen fibrils [11]. Several plant-derived polyphenols, such as tannins, are thought to inhibit proteases and have affinity for dentin collagen, inducing crosslinking and thus preventing dentin matrix loss [12,13].

Recently, other bioactive compounds from medicinal plants have been used in order to obtain a more durable and effective crosslinking effect [6]. Further plant extracts from Lauraceae, one of the major groups of flowering plants, such as *Nectandra leucantha* [14,15], have potential for application as dentin biomodifying agents. Extracts from leaves and branches of this species collected in the Atlantic Forest area of Brazil contain miscellaneous natural product classes, such as alkaloids, phenylpropanoids, lignans, neolignans, nitro derivatives, benzyl esters, pyrones, and flavonoids [16]. Among these compounds, dehydrodieugenol type neolignans have been isolated and chemically characterized [14-17]. Neolignans, especially dehydrodieugenol derivatives – dehydrodieugenol B and dehydrodieugenol B methyl ether (Figure 1),

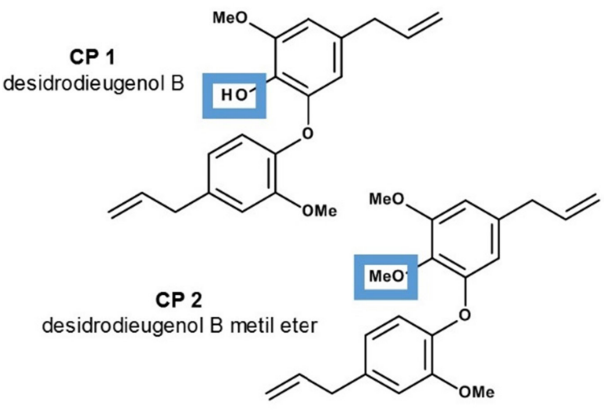

Figure 1 - Chemical structures of dehydrodieugenol B (R = OH), designated as CP1, with a high structural similarity to dehydrodieugenol (R = MeO) – CP2, neolignans isolated from Brazilian plant *Nectandra leucantha*.

express significant *in vitro* activities [16], such as antiparasitic (against Leishmaniasis and Chagas Disease), and antitumor [14,15,17].

In the present study, the influence of bioactive agents isolated from *Nectandra leucantha* in the dentin biomodification was evaluated. Neolignans were extracted from *N. leucantha*, purified, and then applied as dentin biomodification agents before the bonding procedures in direct bonded restorations. The results were compared to those of controls, untreated group (negative control), and a group treated with 0.12% chlorhexidine gel. In addition, the mechanical properties and resistance against degradation of the demineralized dentin specimens after biomodification with these solutions were also evaluated. The research hypotheses tested were: (i) the bond strength of restoratives will be negatively affected by the dentin biomodification with neolignan solutions, irrespective of the evaluation time; (ii) the solutions containing neolignans would negatively influence the elastic modulus of demineralized dentin, with no protection against degradation.

MATERIAL AND METHODS

Experimental design

In this *in vitro* study, the bond strength of restorations to dentin was evaluated according to the factors: (1) neolignan solutions at two levels: dehydrodieugenol B – CP1 and dehydrodieugenol B methyl ether – CP2; (2) evaluation time, at two levels: control (24 h after restorative procedure) and hydrolytic degradation (6 months of storage in water at 37°C). The elastic modulus of demineralized dentin was evaluated according to the factors: a) immersion in distilled water (control, untreated dentin); b) immersion in a supersaturated calcium phosphate solution; c) dentin biomodification with neolignan solutions, followed by an immersion in a supersaturated solution. Experimental groups were obtained among factors under study (Figure 2).

Purification process of the neolignans

The purification process for these target metabolites was previously described [16]. Briefly, 16 g of the acetonitrile phase of *n*-hexane extract from leaves of *Nectandra leucantha* (Lauraceae), the neolignan enriched fraction (NEF), was separated by means of a high speed centrifugal countercurrent chromatography (HSCCC), using a n-hexane-ethyl acetate-methanol-water (HEMWat) (7:3:7:3) solvent system at a reverse phase mode. Along with biseugenol (dehydrodieugenol), the target neolignan derivatives (dehydrodieugenol B – CP1; 3.5 g and dehydrodieugenol B methyl ether – CP2; 1.7 g) were purified from NEF, whose structures were confirmed through nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS) [16]. Figure 1 displays the molecular structure of the target neolignans CP1 and CP2.

Preparation of neolignan solutions

To assess the influence of the neolignans CP1 and CP2 on the longevity of the adhesive restorations, these compounds were solubilized in ethanol and propylene glycol, and diluted with water to obtain aqueous solutions containing the respective neolignan compound (0.13%, propylene glycol - 0.2% and ethanol - 3.0%).

Compressive bond strength test

Eighty bovine incisor teeth were selected, cleaned, and stored in a 0.5% Chloramine T solution at 4° C. The teeth were ground flat to obtain 2-mm thick specimens. Conical preparations were made in the specimens with margins in dentin with the following dimensions: top diameter 4.0 mm, bottom diameter 3.0 mm, thickness 2.0 mm [18]. The preparations were acid etched for 15 s with 35% phosphoric acid (Scotchbond Etchant, 3M ESPE, St. Paul, MN, USA) and rinsed for 20 s. Then, the neolignan solutions and controls were applied with a microbrush for 60 s. The excess solution was removed using absorbent paper. Then, an adhesive system (Adper Single Bond 2, 3M ESPE, St. Paul, MN, USA) was applied and photoactivated for 10 s, following the manufacturer's instructions. The specimens were then positioned over a polyester strip. Preparations were filled with a resin composite (Filtek Z350XT, 3M ESPE, St. Paul, MN, USA), covered with a Mylar strip, and compressed with a glass slide. The composite was cured for 20 s (Bluephase, Ivoclar Vivadent, Schaan, Liechtenstein, 1200 mW/cm^2). The specimens were then stored in physiological saline solution at 37° C for 24 h. The excess of the restorations was removed and then finished and polished using abrasive discs (Sof-Lex, 3M ESPE, St. Paul, MN, USA).

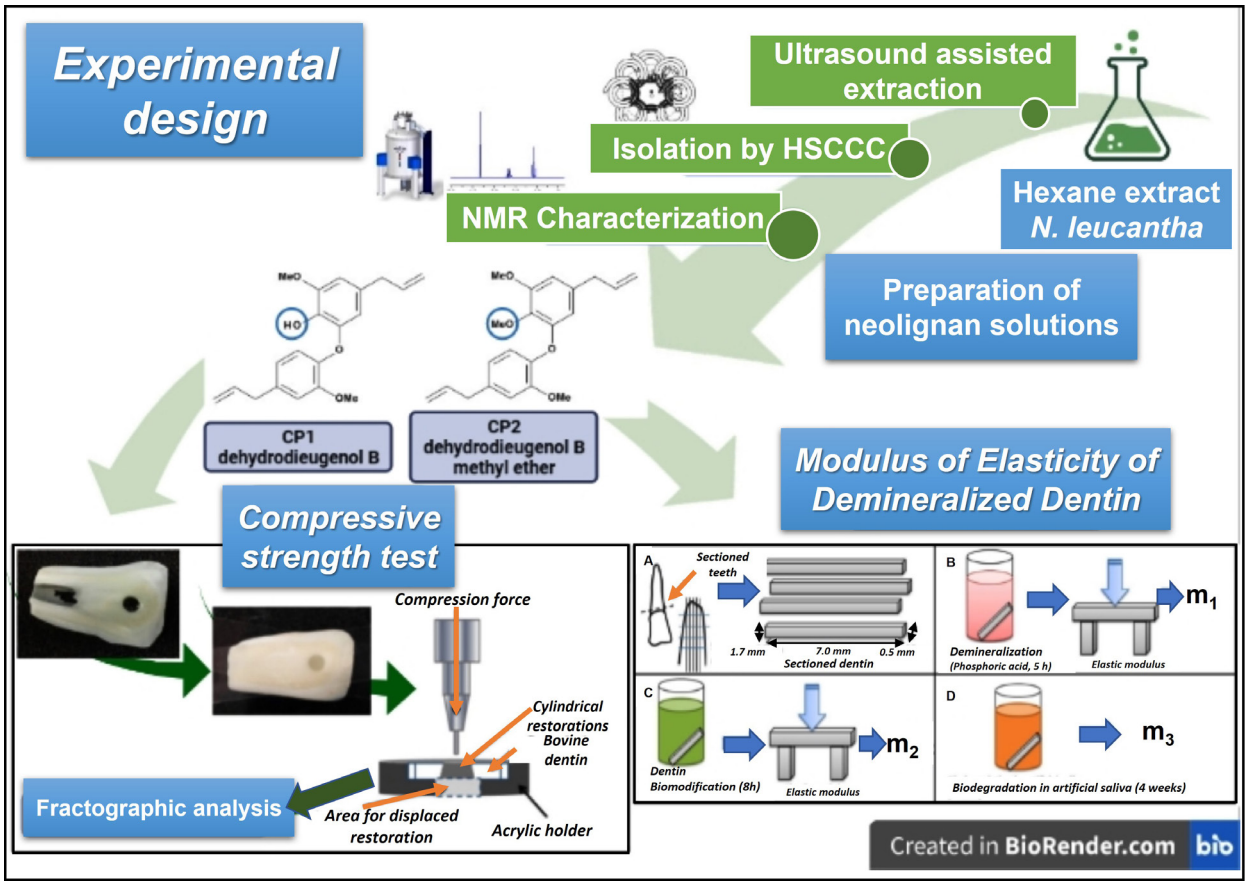

Figure 2 - Summarized experimental design of the present study. Abbreviations: HSCCC - High speed centrifugal countercurrent chromatography; NMR - nuclear magnetic resonance spectroscopy; m – mass

The compressive push-out bond strength of bonded restorations was evaluated according to the factors: (1) dentin pretreatment at four levels: negative control - untreated dentin, positive control - 0.12% chlorhexidine gel, and dentin biomodification with two neolignan solutions; and (2) storage time at two levels: immediate evaluation (24 h) and evaluation after 6 months. Eight experimental groups were categorized (n=10). The push-out bond strength test was conducted at a crosshead speed of 0.5 mm/min, as previously described [18]. The compressive force of the probe was applied at the bottom surface of the restoration. The means were expressed in MPa [18]. After the test, a fractographic analysis was also performed at 5x magnification using a dissecting microscope (Stereozoom, Bausch & Lomb, Rochester, NY, USA) as previously described [18].

Modulus of elasticity of demineralized dentin after dentin biomodification

The specimens were obtained as previously described [19]. Briefly, bovine incisor teeth were cut with a slow-speed water-cooled diamond saw to obtain sections from the middle coronal dentin with dimensions of 0.5×1.7×7.0 mm. Sixty beams were obtained (n=15). The specimens were then immersed in 10% phosphoric acid solution (LabChem, Pittsburgh, PA, USA) for 5 h, and then thoroughly rinsed with distilled water for 10 min. After the demineralization process, the specimens were immersed in distilled water for the baseline measurements of the modulus of elasticity. Then, the specimens were immersed in both neolignan solutions for 8 h. The specimens were then immersed in supersaturated solutions (2.5 mM $CaCl_2$ and 1.5 mM K_2HPO_4: Ca/P = 1.67) for 7 days. The modulus of elasticity of the specimens before and after dentin biomodification was tested in a universal testing machine (Instron 4484; Instron Inc., Canton, MA, USA) with a three-point bend fixture, using a 5 N load cell at 0.5 mm/min crosshead speed. Load–displacement curves were converted to stress–strain curves. The width and thickness of the specimens were measured and the apparent elastic modulus was calculated at 3% strain, as previously described [1,19,20]. In this manner, the modulus of elasticity of demineralized dentin was evaluated according to the factor "dentin

treatment", at four levels: specimens immersed in distilled water (control, untreated dentin); specimens immersed in a supersaturated calcium phosphate solution; dentin biomodification with two neolignan solutions followed by an immersion in a supersaturated solution. Data were expressed in MPa, and the percentage variation of the elastic modulus was calculated as the ratio of the final mean (after treatment with both solutions) to the initial means (baseline).

Mass weight change of demineralized dentin after dentin biomodification

Demineralized dentin beams were weighed before (M_1) and after (M_2) dentin biomodification with both neolignan-containing solutions with an analytical balance (0.00001 mg precision, Ohaus Analytical Plus, Florham Park, NJ, USA). The mass was assessed after degradation in artificial saliva for 4 weeks (M_3). For that, the specimens were previously dried in a vacuum desiccator containing silica gel beads for 24 h at room temperature. Mass variation (W_{mc} %) was determined as the percentage of gain or loss in mass in each specimen [19], as follows:

$$Wmc(\%) = ((M2\,x100)/M1) - 100 \quad (1)$$

where M_1 is the mass of the demineralized dentin beam before dentin biomodification and M_2 is the mass of biomodified dentin specimens. To calculate the biodegradation percentage mass variation, the formula was used as follows:

$$Wde(\%) = [(M3 - M1)\times 100]/M1) \quad (2)$$

where M_1 is the mass of the demineralized dentin beam before dentin biomodification, and M_3 is the mass of dentin specimens after 4-week artificial saliva immersion.

Statistical analysis

Means and standard deviations of the bond strength test were calculated and statistically analyzed with two-way ANOVA and Tukey's test (5%). Data of elastic modulus and mass weight variation were analyzed with ANOVA/Tukey's test (5%).

RESULTS

Table 1 describes the results of the bond strength test according to the experimental groups.

The bond strength means for the immediate evaluation ranged between 34.2 MPa (0.12% chlorhexidine gel) and 56.8 MPa (CP1 0.13%). After 6 months, the means varied depending on the treatment, from 34.8 MPa (0.12% chlorhexidine gel) to 44.8 MPa (CP1 0.13%). The bond strength means were statistically evaluated by two-way ANOVA: the factors 'dentin treatment', 'storage time', and the interaction 'dentin treatment' *vs.* 'storage time' were significant ($p < 0.05$). The bond strength means were significantly higher when the dentin was treated with both neolignan solutions, compared to the means observed for the control groups (untreated and chlorhexidine) ($p<0.05$). After 6 months, the bond strength means of the groups treated with both solutions were also significantly higher than those of observed in both control groups ($p<0.05$). When the results of both evaluation times were compared, a significant reduction in the bond strength mean was observed in both experimental groups treated with the neolignan solutions after 6 months ($p<0.05$). On the other hand, no significance was observed when the results of both evaluation times were compared to the control groups (untreated group and the group treated with 0.12% chlorhexidine gel) ($p>0.05$).

Figure 3 describes the fractographic analysis of the specimens after the push-out bond strength test.

In the groups evaluated after 24 h, there was a clear predominance of adhesive failure (type 1) in the control groups, whereas in the groups treated with the solutions, there was a predominance of type 2 fracture (mixed: adhesive and cohesive in composite resin). After 6 months,

Table 1 - Push out strength means as a function of the evaluation time

	Control	0.12% chlorhexidine gel	CP1 0.13%	CP2 0.13%
24 h	39.1 b,A (15.7)	34.2 b,A (11.8)	56.8 a,A (22.3)	50.5 a,A (14.1)
6 months	36.2 b,A (14.0)	34.8 b,A (18.1)	44.8 a,B (16.3)	41.7 a,B (14.6)

Means followed by different small letters in row and capital letters in column indicate significant differences ($p < 0.05$). Values within the parenthesis are standard deviations.

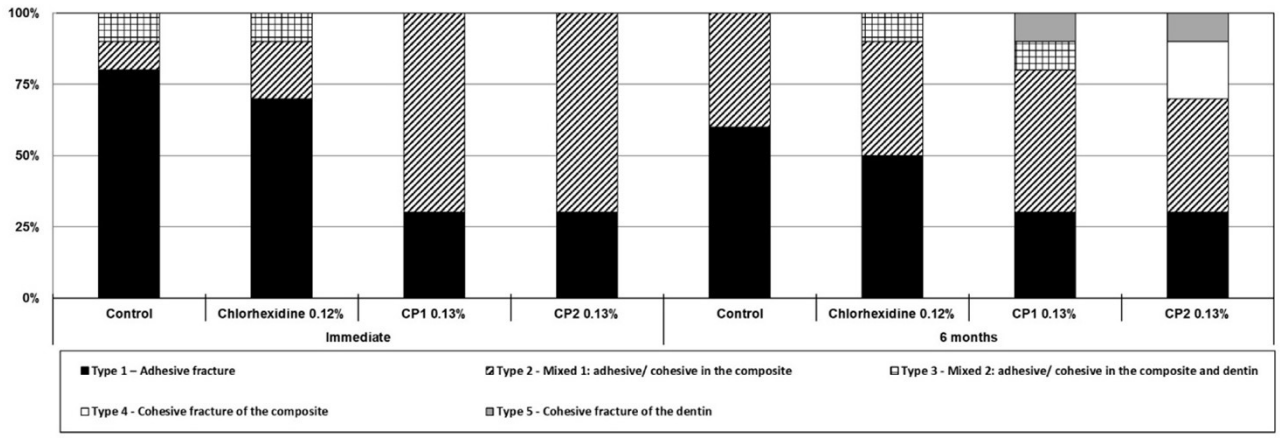

Figure 3 - Incidence of failure modes (%) according to the experimental groups.

Table 2 - Mean (SD) of elastic modulus (MPa) of demineralized dentin specimens as a function of the treatment

	Initial modulus (MPa)	Modulus after treatment (MPa)	Elastic modulus variation (%)
Control (H_2O)	1.47 (1.17) a	1.17 (0.78) b	-20.4 (0.85) B
Ca/P (1.67)	1.43 (0.93) a	1.28 (0.75) b	-10.5 (0.45) B
CP1 0.13%	1.65 (0.84) a	2.50 (0.95) a	51.5 (0.42) A
CP2 0.13%	1.68 (0.68) a	2.12 (0.67) a	26.2 (0.23) A

N=15. Ca/P: supersaturated calcium phosphate solution. CP1 and CP2: neolignan solutions. Different capital letters depict significant differences in percentage variation ($p < 0.05$), whereas different small letters indicate statistical differences in elastic modulus means ($p < 0.05$).

there was still a predominance of type 1 and type 2 fractures in the controls, but other more catastrophic types of failure were observed in the groups treated with the solutions (types 4 and 5).

Table 2 displays the mean dentin elastic modulus and the standard deviation. The statistical analysis demonstrated significant differences between the treated groups in comparison to the control groups ($p<0.05$). In comparison to the control groups, the modulus was significantly increased after dentin treatment with the solutions, irrespective of the content of neolignans. The treatments with both CP1 and CP2 allowed significantly higher increases of 51.5% and 26.2%, respectively. Conversely, a negative impact on the elastic modulus was observed in both control groups, with a significant difference when compared to the treated groups ($p<0.05$).

All experimental groups exhibited an equivalent mass reduction, irrespective of the treatment (Table 3). On the other hand, after biodegradation in artificial saliva, the groups treated with both neolignan solutions exhibited significantly higher dentin mass reduction compared to the control groups (Table 3).

Table 3 - Mean (SD) of mass variation (%) of dentin specimens as a function of the treatment

Mass variation (%)	After treatment	4 weeks of degradation
Control (H_2O)	-12.1 (5.5) a	-16.0 (4.8) A
Ca/P (1.67)	-10.3 (5.1) a	-10.3 (10.6) A
CP1 0.13%	-9.8 (4.0) a	-21.6 (9.4) B
CP2 0.13%	-17.5 (6.2) a	-28.9 (4.2) B

N=15. Ca/P: supersaturated calcium phosphate solution. CP1 and CP2: neolignan solutions. Different letters indicate statistically significant differences ($p < 0.05$) within each column.

DISCUSSION

The first research hypothesis that the bond strength of restoratives would be negatively affected by the dentin biomodification with neolignan solutions, irrespective of the evaluation time, was not accepted. Indeed, in both evaluation times, significant higher bond strength means were observed when the neolignan solutions were applied. Although a significant reduction was observed in the bond strength after 6 months, the means in the experimental groups treated with both solutions were significantly higher compared to those of the control groups. The fractographic analysis also confirmed the results of the push-

out compression test, in which the restorations of the groups treated with both solutions, produced higher bond strength means. It was clearly demonstrated that the fracture patterns of the experimental groups treated with both neolignans solutions were entirely different from that of the control groups, in which more catastrophic types of fractures were observed, not limited to the interface of the tooth-restoration union (type I). Moreover, the second hypothesis that the solutions containing neolignans would negatively influence the elastic modulus of demineralized dentin, with no protection against degradation, was also not accepted. In general, the modulus of elasticity of the dentin significantly increased after treatment with both neolignan solutions, even after the 4-week artificial saliva immersion. In this manner, the neolignan solutions also helped to promote protection against water degradation.

It has been previously pointed out that when the collagen fibrils are exposed to tannins it is formed a vegetable tannin-aldehyde combination tannage, which is based on multiple hydrogen bonds between vegetable tannins and collagen, and on the covalent bonds between aldehydic tanning agent and the side amino groups of collagen [21]. Moreover, the presence of highly nucleophilic sites in the tannin molecules can also act as an additional crosslinking ligand of the tanning agent, increasing the stability of collagen fibrils [21]. Reasons that explain the positive effects of the solutions as dentin biomodification agents rely on the molecular structure of the neolignans (Figure 1), which favors the formation of hydrogen bonds and interactions with the ϖ bonds in the aromatic ring and allyl side chain. In this sense, the final neolignan solutions prepared may have aided type I collagen binding in some way, presumably through hydrogen bonding and hydrophobic interactions. These interactions are claimed to inhibit the breakdown of collagenase [10], thus increasing the hydrothermal stability of collagen fibrils [21].

Previous studies proved that tannin-rich extracts act as crosslinkers, demonstrating superior results compared to chlorhexidine [22-24]. A previous study also demonstrated that tannic acid, a commercial synthetic condensed tannin, is able to reduce the enzymatic degradation to improve the mechanical properties of dentin, thus increasing the bond strength of resin-based restorations to dentin [25]. Conversely, another previous study [20] demonstrated that the use of tannin-containing extracts had a questionable effect on the longevity of bonded restorations as some of these extracts either had no effect to improve the bond strength of bonded restorations to dentin or negatively affected the apparent dentin modulus after treatment. In addition, the higher the tannin content, the more the extracts stained the dentin, which is impeditive for its clinical application. In this manner, the search for new compounds are imperative to improve the longevity of bonded restorations with no side effects [26]. In this manner, neolignan-containing solutions seem to be a promising dentin biomodification agent in a clinically relevant time.

Considering its limitations, the present study was conducted *in vitro* with solutions containing neolignans. In addition, it was not clear the mechanism by which the neolignans and other compounds present in the solutions affected the dentin collagen fibrils, such as propylene glycol and ethanol. These components can present a synergic effect in the binding mechanism of neolignans to collagen fibrils and their potential stabilization mechanism [1]. It is also important to emphasize that during the 4-week artificial saliva immersion, some of the residues entrapped in the demineralized dentin during the demineralization process (such as degraded collagen, remaining apatite crystallites inside the gap regions of the collagen fibrils, among others) can be released from the collagen mesh during this time. In this manner, this fact may interfere with the interpretation and analysis of the results. These characteristics lead the mass gains and losses to be unbalanced, with a greater tendency of mass loss, mainly due to the fact that the newly created calcium phosphate crystals during the immersion in both neolignan solutions for 8 h can be easily solubilized in an aqueous medium. In spite of these limitations, these results provided important new information regarding the application of these compounds between the steps of acid etching and adhesive bonding when applying bonded restorations. Future studies are necessary to provide more information, including the evaluation of the effect of these neolignan solutions on the nanostructure of collagen I fibrils and *in vivo* studies.

Based on the results of the analysis performed in this study, it was observed that compounds

from *Nectandra leucantha* (Lauraceae) led to an increase in the bond strength of the restorative to dentin, which was also observed in the evaluation after 6 months. Although a significant reduction in the bond strength was observed in the groups treated with the compounds after 6 months compared to the immediate results, significantly higher bond strengths were observed compared to those from the control groups. In spite of the limitations of the present study, it can be speculated that neolignans isolated from the leaves of *N. leucantha* have the potential to act as crosslinking agents for dentin collagen fibrils, which, among other factors, led to an increase immediately and after 6 months of union strength compared to the control groups. This was thought to be related to their unique molecular structure, which facilitates the establishment of various intermolecular contacts between the compounds and the collagen fibrils, which play key roles as functions of their chemical capabilities. Studies are needed to complement the results and corroborate the biomodification actions of these compounds found in *N. leucantha.*

CONCLUSION

Taken together, the data strongly suggests that the neolignan solutions obtained from the leaves of *N. leucantha* present a significant *in vitro* effect as dentin biomodification agents. The compounds positively influence the bond strength of restoratives to dentin, regardless of the evaluation time, and significantly improve the apparent modulus of elasticity of the demineralized dentin after treatment. Other *in vitro* and *in vivo* studies are necessary to support and confirm that neolignan solutions are effective dentin biomodification agents.

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Authors' Contributions

MHGG: Evaluation of modulus of elasticity and mass weight change of demineralized dentin. MHMM: Experimental analysis and collection of data in the push-out bond strength test. CFOG: Conceptualization, data analysis, statistical analysis and interpretation. AHMG: Study design; collection of data; data analysis/interpretation; Paper writing. SSG: Study design; plant extract preparation and analysis; data analysis; Paper writing. PHPD: Study design; Data analysis/interpretation; Paper writing; Corresponding author.

Conflict of Interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

Regulatory Statement

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