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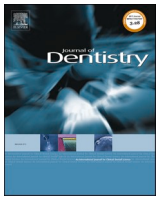

Effect of antioxidants on adhesive bond strength to bleached enamel

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ABSTRACT

Objective: To evaluate the influence of antioxidants (ATX) resveratrol, winter's bark, green tea and yerba mate on the bond strength between bleached enamel and the nanohybrid composite resin.
Methodology: Bovine incisor crowns ($n = 132$) were randomly divided into 22 groups ($n = 6$) according to the application times (5, 10, 15, 30, and 60 min) of each antioxidant. Teeth restored without previous bleaching or ATX constituted the non-bleached control group (NB Ctrl) ($n = 6$), and teeth restored after bleaching and without ATX represented the bleached control group (B Ctrl) ($n = 6$). The 35 % hydrogen peroxide was applied for 45 min (3 application of 15 min) to the buccal enamel surface. ATX was used after bleaching for the specified time of each group and removed with air-water spray. The enamel was etched with 37 % phosphoric acid (30 s) and rinsed with air-water spray. The adhesive resin was applied to the enamel dry surface. Teeth were restored using 1 mm composite resin increments (10 × 10 × 3 mm) and sectioned in test specimens of 6 mm in length and 1 mm^2 in cross-sectional area submitted to microtensile bond strength test (0.5 mm/min). The load (N) at failure was recorded, and the bond strength (σt) was calculated (MPa). The fracture area was analyzed under optical microscopy, and failures were classified as cohesive, mixed, or adhesive. Data was evaluated by Kruskal-Wallis and Dunn tests ($p \leq 0.05$).
Results: B Ctrl group presented lower σt than NB Ctrl ($p < 0.001$). Applying resveratrol for 5 or 10 min, winter's bark for 10 or 15 min, green tea for less than 15 min, and yerba mate for 15 min provided similar σt between bleached enamel and nanohybrid composite to the control group.
Conclusion: Restorative procedures performed immediately after tooth bleaching compromises adhesion. Experimental antioxidants applied to bleached enamel can increase the immediate bond strength of restorations performed directly after bleaching, with similar values to those observed in unbleached enamel.
Clinical Significance: This study presents promising results to support the use of antioxidants on the recently bleached enamel to allow adhesive tooth restorations. The immediate bonding obtained using antioxidants was similar to the one achieved in non-bleached enamel in brief application times. Green tea extract and resveratrol were able to restore the bond strength to bleached enamel in a short application time of 5 min. The reduction in the required application time holds the potential to decrease the overall duration of the clinical section, offering clinical advantages and improving the feasibility of using antioxidants on the bleached enamel prior to adhesive procedures.

1. Introduction

The active substances of bleaching agents are carbamide peroxide and hydrogen peroxide, used at different concentrations at home or in the office supervised by a dentist [1,2]. In-office bleaching is indicated for rapid results or when patients resist using the trays for at-home applications [1,2]. Hydrogen peroxide (HP) is commonly used to in-office bleaching. In contact with the dental surface, HP breaks into oxygen ions of low molecular weight and high reactivity, which penetrate the interprismatic spaces of the enamel and break the chains of high molecular weight pigments, resulting in tooth whitening [3].

The oxygen remains in the enamel structure and is completely inactivated after three weeks after bleaching [4]. This residual oxygen may interfere with the complete polymerization of adhesive systems

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applied in restorative procedures performed immediately after bleaching, compromising bonding [4,5]. Therefore, waiting at least 14 days after finishing tooth bleaching to perform restorative procedures is recommended [5]. However, this waiting time may cause discomfort to patients needing esthetic and functional teeth rehabilitation, such as restoration replacements, reshaping, and color corrections.

Despite the confirmation that tooth bleaching damages the bonding to the enamel, there is no consensus regarding the ideal interval between bleaching end and adhesive restorative procedures. Studies suggest that adhesive restorative procedures should be performed after 7 to 14 days [6] or three weeks [7] after bleaching.

Antioxidants may neutralize the reactive oxygen and reduce the waiting time for restorative procedures [8–12]. Several antioxidant agents are available at different concentrations and potential protocols are being studied to allow immediate restorations of bleach teeth. The literature confirms that winter's bark, resveratrol, green tea, and yerba mate extracts have antioxidant potential that could overcome this clinical problem [13–17]. However, the substances concentration and application protocols that would result in bonding improvement to bleach enamel need to be clarified.

Therefore, this study evaluated the influence of resveratrol, winter's bark, green tea, and yerba mate antioxidants at 10 % on the bond strength to the enamel bleached with 35 % hydrogen peroxide. The hypothesis was that antioxidants applied immediately after tooth bleaching increase adhesive bonding to the bleached enamel.

2. Methodology

The present research evaluated in vitro two antioxidants (ATX) previously used in Dentistry (resveratrol and green tea) [14,15,18–20] and two experimental substances (winter's bark and yerba mate) [13,21,22]. The antioxidant potentials of the four substances were measured by the calibration curves obtained using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method [23]. Radical samples were reacted in an ethanol solution with a stable DPPH. The reaction was performed by adding 60 μL of the sample to 2.960 mL of 0.5 mM DPPH solution dissolved in methanol. Methanol (3 mL) was used as a blank. The negative control was 60 lL of water added to 2.960 mL of 0.5 mM DPPH solution dissolved in methanol. DPPH reduces when reacts with an antioxidant, changing the solution color from deep violet to light yellow. After 60 min of the reaction, the absorbance [Abs] was read at 517 nm using a UV–VIS spectrophotometer and converted to a percentage of antioxidant activity (AA), using Eq. (1). [Mensor LL et al. 27]

$$AA\% = 100\left[\left(Abs_{sample} - Abs_{white}\right) / Abs_{control} \times 100\right] \quad (1)$$

The experiment was performed in triplicate for each antioxidant and a mean value was obtained. The absorbance analysis revealed the antioxidant potential for each ATX (from the highest to the lowest): winter's bark (0.235; 0.219; 0.365), resveratrol (0.265; 0.313; 0.371), green tea (0.614; 0.775; 0.740) and yerba mate (0.632; 0.671; 0.644).

Sound bovine incisors ($n = 132$) with preserved enamel surfaces were collected from a licensed distributor. The sample size was calculated using the G-Power program for ANOVA, with the parameters of effect size f, power (1- B err prob), the number of groups, the noncentrality parameter, critical F, numerator df, denominator df, total sample size resulting in a minimum of 6 specimens per group.

The bovine teeth were manually scraped with McCall periodontal scalers to remove organic residues. After cleaning, the teeth crowns were separated from the roots at the amelocemental junction using a double-sided diamond disc (KG Sorensen, 010 × 22 mm) under water cooling. The buccal enamel of the crowns were abraded with #400 #600, #800 and #1000-grit silicon carbide sandpaper in a polishing machine (Abramin, Struers, Copenhagen, Denmark) to obtain a flat and standardized enamel surface of approximately 1 cm^2. The teeth were cleaned in sonic bath for five minutes under distilled water at 24 °C, stored in 5 °C distilled water for a week before the experiments.

Table 1 describes the antioxidant substances and dental materials used in the present study. The samples were randomly divided into five experimental groups ($n = 6$) for different application times (5, 10, 15, 30, and 60 min) of each ATX, and two control groups of bleached teeth (B Ctrl) and unbleached teeth (NB Ctrl) without ATX (Fig. 1).

The bleaching agent 35 % hydrogen peroxide (Whiteness HP®, FGM, Joinville, SC, Brazil) was manipulated according to the manufacturer's instructions (3 parts of bleaching for 1 part of thickener). The bleaching gel was applied to the enamel surface using a disposable syringe (approximately 0.5 mL of bleaching gel per tooth) and spread on the flat area using a microbrush. The bleaching agent was applied once every 15 min, and reapplied to 2 times (3 applications of 15 min, totaling 45 min). After each application, the enamel was rinsed with air-water spray and air-dried.

Winter's bark, green tea, yerba mate, and resveratrol extracts were prepared in a concentration of 10 % (NatuPharma, Passo Fundo, RS, Brazil) and stored at 5 °C for 1 week before the experiments. Immediately after bleaching, the substances were applied to the enamel surface using a cotton pad according to the time specified for each experimental. The substance was reapplied every minute due to solution evaporation. After final application time, the surface was rinsed with air-water spray and air-dried.

Following ATX application, the tooth enamel was etched with 37 % phosphoric acid for 30 s and cleaned with air-water spray. The water excess was eliminated with absorbent paper discs. Next, the universal adhesive resin was applied to the etched enamel using a microbrush. The solvent was evaporated with air spray for 10 s, and the adhesive was light-cured (Radii-Cal SDI™1200 mW/cm^2) for 10 s. A second layer of adhesive was then applied as described for the first layer. The composite resin restoration was performed applying 2 mm^3 increments inside a pre-made silicone mold with internal dimensions of 10 × 10 × 3 mm of thickness. Each increment was light-cured for 20 s.

The teeth were cut (metallographic cutting machine, Biopidi™, São Carlos, SP, Brazil) in cross-sectional and longitudinal directions with a diamond disc (Extec model 12205, Extec Corp., Enfield, CT, USA) at 250

Table 1
Materials used to produce the study specimens.

Name	Manufacturer	Classification	Composition
Opallis™	FGM	Nanohybrid composite resin	Bis (GMA) and (EMA), UDMA, TEGDMA, silanized barium aluminum silicate glass and silicon dioxide nanoparticles, camphorquinone, accelerants, stabilizers, and pigments
Ambar™	FGM	Universal adhesive 5 ml	MDP, methacrylate monomers, photoinitiators, co-initiators and stabilizers
Whiteness HP™	FGM	In-office bleaching agent	35 % hydrogen peroxide, thickeners, pigments, neutralizing agents, calcium gluconate, glycol and deionized water
37 % Condac	FGM	Enamel and dentin acid conditioner	Water base, 37 % phosphoric acid
10 % winter's bark extract	Natupharma	Antioxidant agent	10 g of winter's bark + 100 mL of distilled water
10 % resveratrol extract	Natupharma	Antioxidant agent	10 g of resveratrol+ 100 mL of distilled water
10 % green tea extract	Natupharma	Antioxidant agent	10 g of green tea + 100 mL of distilled water
10 % yerba mate extract	Inovamate	Antioxidant agent	10 g of yerba mate + 100 mL of distilled water

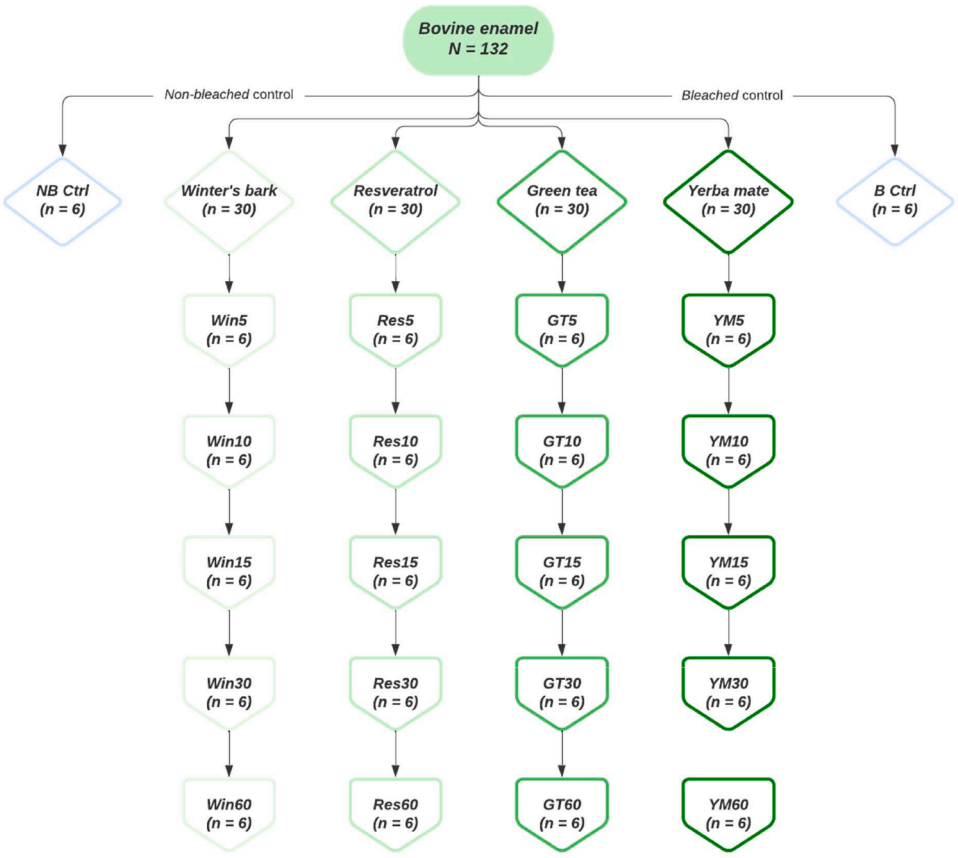

Fig. 1. Distribution of specimens in the experimental groups.

rpm under water cooling. That resulted in specimens of 1 mm^2 cross-sectional area and 6 mm in length (3 mm of enamel and 3 mm of resin). Each specimen dimensions were checked with a digital caliper (Digimess™, São Paulo, SP, Brazil). Damaged or specimens out of the established standards were discarded. The specimens were stored in distilled water at 37 °C for up to one week after the microtensile bond strength test.

The adhesive interface area (A, in mm^2) was based on the width and thickness of each specimen, obtained with a digital caliper (Digimess™, São Paulo, SP, Brazil). Each specimen was fixed to a microtensile device (Odeme Biotechnology, Joaçaba, SC, Brazil) by its ends using cyanoacrylate resin (Super Bonder™, Henkel Loctite Adesivos Ltda, Itapevi, SP, Brazil), and submitted to the microtensile test (0.5 mm/min) in a universal testing machine (EMIC™, São José dos Pinhais, PR, Brazil) until failure. The load at failure was recorded (L, in Newtons) and the bond strength (σt) was calculated (MPa) according to the equation: $\sigma t = L/A$. A σt value equal to the lowest obtained for the respective group was attributed to the pre-test failed specimens.

The fracture area of the samples was analyzed under optical microscopy (Zeiss Axiostar binocular microscope) at 200x magnification. Failures were classified as cohesive in resin or enamel, mixed (cohesive and adhesive), or adhesive (complete resin debonding from the enamel surface). Data failed the Shapiro-Wilk normality test ($p < 0.050$). Therefore, non-parametric Kruskal-Wallis and Dunn tests (α=0.05) (Sigmaplot v. 12.0 software) were used to analyze the differences on the bond strength between experimental groups to the control group (not bleached control).

3. Results

Table 2 presents bond strength results and the statistical analysis. The bleached group, restored after bleaching without antioxidant (B Ctrl), showed significantly lower bond strength than the non-bleached control group (NB Ctrl).

Table 2
Bond strength (MPa) results obtained for the experimental groups.

Group	Mean	Standard deviation	Median	Statistical grouping	25 %	75 %
NB CTRL	23.6	10.0	17.9	A	17.9	26
B CTRL	14.9	10.1	9.1	B	9.1	17.2
WIN5	15.9	9.5	9	B	9	22.3
WIN10	25.9	8.0	26.1	A	20	32.4
WIN15	28.4	12.4	27.4	A	19.5	37.2
WIN30	4.6	3.6	2.8	B	2.8	2.8
WIN60	10.5	5.9	7.2	B	7.2	10.8
RES5	21.6	9.4	21	A	13.4	28.5
RES10	27.7	12.7	28.3	A	12.7	36.4
RES15	19.1	11.5	10.4	B	10.4	25.7
RES30	8.0	5.7	5.1	B	5.1	8.9
RES60	15.6	7.7	11.3	B	11.3	17.7
GT5	27.1	14.5	22.8	A	12.8	37.4
GT10	17.8	14.2	17.3	A	5.7	37
GT15	23.7	12.2	24.3	A	14	33.7
GT30	9.3	7.1	9	B	2.2	14.5
GT60	9.1	7.7	5.3	B	5.3	7.8
YM5	20.8	9.8	15	B	15	25.1
YM10	13.5	5.5	11.1	B	11.1	11.6
YM15	19.4	7.0	16.3	A	16.3	17.7
YM30	14.3	6.3	11.4	B	11.4	11.4
YM60	13.8	5.0	13.1	B	13.1	13.1

The substances of higher ATX potential Winter's bark and Resveratrol provided similar bond strength to NB Ctrl when applied for 10 to 15 min (Win10; Win15) and for 5 to 10 min (Res5; Res10). Green tea (intermediate ATX potential) applied for 5 to 15 min (GT5; GT10; GT15)

restored the bond strength of the bleached enamel to values similar to the NB Ctrl. Yerba mate presented values similar to NB Ctrl only when acting at the enamel for 15 min (YM15). Extended application times (30 min and 60 min) compromise the bond strength values compared to lower times of application.

The failure analysis revealed a higher frequency of mixed failures in the experimental groups which presented higher bond strength values, and adhesive failures prevailed in the other groups, including the pre-test failed specimens.

4. Discussion

Bleaching techniques result in tooth whitening through oxidative interactions with long chain pigments within the dental structures. Reactive oxygen can remain in the enamel for an extended time period, damaging the bonding between adhesive resins to the dental surface [4–7]. The present study supported this statement, showing that a significant lower bond strength was obtained with the bleached enamel compared to the non-bleached. The literature recommends waiting from 7 to 14 days after the bleaching treatment to perform adhesive restorations [4–7].

Investigations have been conducted on substances that could neutralize the bleaching residual oxygen to reduce the waiting time between the end of bleaching procedures and adhesive bonding [8–12, 20]. Thus, using natural plant extracts has been encouraged as a potential alternative to chemical and synthetic antioxidants (ATX) [15,16].

This study selected substances with high antioxidant potential for evaluating their effectiveness in restoring the bond strength to bleached enamel. Resveratrol, winter's bark, green tea, and yerba mate extracts at 10 % were applied to enamel immediately after bleaching and effectively reversed the compromised bond strength at different application times, partially confirming the hypothesis of this study. The redox potential of the bleached surface is changed by electron donation to free radicals, thus, neutralizing the bleaching agent effect on the bond strength [15,24].

Resveratrol (RES) is a synthetic substance previously used to investigate the effects on enamel physical, mechanical, and adhesive properties after tooth bleaching [25]. Although relatively new in dental research, resveratrol has shown positive outcomes in past studies [25]. Brigantini et al. [25] demonstrated that resveratrol increased the adhesive bond strength of bleached enamel, corroborating to the bond strength results obtained by the present study, and showed no further damage to the surface roughness and microhardness.

The literature acknowledges the antioxidant potential of natural substances with antioxidant effects recognized in other fields, such as yerba mate and winter's bark [13,21,22]. Winter's bark is a frost-tolerant conifer named Drimys winteri, commonly found in temperate forests. WIN extract was obtained from the plant bark and presented the highest antioxidant potential between the studied substances. Despite having ATX potential values very close to RES, a time of application of 5 min is inadequate for this substance, which increased the bond strength values significantly when applied to the bleached enamel for 10 and 15 min. YM showed the lowest ATX potential and resulted in bond strength increase only when applied for 15 min.

Green tea (GT) derives from the Camellia sinensis plant [15-17,19, 20], affecting the inactivation of residual oxygen left by the bleaching agents. Despite presenting a lower antioxidant potential than RES and WIN, the present study confirmed the benefit of GT in restoring the adhesive bond strength to bleached enamel at the values found for non-bleached substrate. Similar results were found in recent studies [24, 26], which successfully reversed the bond strength after bleaching to baseline (unbleached) values using 10 % GT for 10 to 15 min. In the current study, the efficacy of GT and RES was observed even when applied for a brief duration of 5 min. The reduction in the required application time holds the potential to decrease the overall duration of the clinical section, offering clinical advantages and improving the feasibility of using antioxidants on the bleached enamel prior to adhesive procedures.

There is no consensus on the literature regarding the application time required to reestablish the bond strength. Our study demonstrated that maximum application times of 15 min for green tea, yerba mate, and winter's bark, and 10 min for resveratrol have a positive effect on the bond strength to bleached enamel. A number of studies states 10 min as an adequate time of application to restore bond strength [27–29], while others suggest a greater time to assure improved adhesive strength to bleached enamel [15,30,31]. The ATX substances, concentration and testing method significantly differs between studies resulting on different outcomes.

One research reports a minimum application time of 60 min to neutralize the whitening effects [15]. The present investigation included application times up to 60 min based on the reports of literature and considering the mean duration of a bleaching clinical session. In this study, application times longer than 30 min compromised the adhesive bonding, differing from Ozelin et al. [15], who showed that green tea applied for 30 min reduced bond strength but increased it again with a 60-minute application. However, it is important to consider the entire duration of a clinical section, thus, 60 min could reduce the viability of ATX application after bleaching or before performing adhesive restorations. In addition, longer ATX application times may accumulate sediments from the experimental substances at the bleached surface, decreasing the adhesive bond strength [8]. Green tea, yerba mate, and winter's bark extracts contain pigments that may interfere with tooth whitening results. Further studies should investigate the influence of ATX application on the tooth whiteness and color changes.

Groups presenting higher bond strength showed a prevalence of mixed failures, suggesting improved bonding quality between the enamel and the resin adhesive. Adhesive failures were prevalent in the groups with the lowest bond strength, including the pre-test failed specimens.

In-office bleaching is safe and efficient, presenting similar clinical outcomes to the at-home method [1,2]. The present in vitro study selected a high-concentration substance used for in-office bleaching to submit the specimens to the highest content of residual oxygen on the enamel. Although different adhesive systems were not accessed in the present study, a universal adhesive (Ambar™, FGM, Joinville, SC, Brazil) was selected based on the solvent composition, ethanol. Ethanol-based solvents present in some adhesive systems may interact with residual oxygen, minimizing the harms of bleaching on the adhesive bonding strength [32–34].

Bovine teeth enamel samples were chosen because they present composition, morphology and bonding performance similar to the human enamel, with the benefit of easy obtaining and standardization [35,36,37]. Bovine teeth have been used in bond testing for a long time as an alternative to human substrate. A recent systematic review and metanalysis investigated the bovine teeth suitability as a substitute for human tooth in bond strength tests. The results have shown that bond strength values obtained using bovine teeth are comparable to the human ones, for enamel and dentin substrates, confirming bovine teeth as a viable substitute for human teeth on bond strength tests. [37] Bond strength values were accessed by the microtensile test, which demands a reduced adhesive area of the specimens, with a smaller population of defects, and homogeneously distributes loading stresses at the interface which results in a more precise evaluation [34].

The present study investigated a series of antioxidants, exploring their potential impact on adhesion to bleached enamel. In this investigation, sample attrition was not considered to perform sample size and power analysis, which would be recommended in similar future studies. However, we believe that the impact of potential differences on sample size would be negligible, since the statistical analysis performed was able to show the differences between the experimental groups.

The tested substances showed promising results, supporting the application of antioxidants on recently bleached enamel to assist

immediate adhesion to tooth restorations. Adhesive interfaces are susceptible to degradation over time by water-assisted corrosion and other aging mechanisms, influencing the clinical longevity of the dental restorations. Future studies are necessary to investigate weather reduced time of application, changes on concentration and aging (water storage, thermocycling or fatigue) could affect the initially obtained bond strength when using antioxidants.

5. Conclusion

Restorative procedures performed immediately after tooth bleaching compromises the adhesion between enamel and composite resin. The immediate bonding between recently bleached enamel and composite resin obtained using the tested antioxidants for up to 15 min was similar to the one achieved in non-bleached enamel.

CRediT authorship contribution statement

Thaís Brock: Writing – original draft, Methodology, Data curation. **Andrew Bruschi Soveral:** Writing – original draft, Methodology, Data curation. **João Renato Dieterich Junior:** Writing – original draft, Methodology, Data curation. **Ana Luiza Becker:** Writing – original draft, Data curation. **Eduardo Fávero:** Writing – original draft, Methodology, Data curation. **Aline Jaeger de Oliveira:** Methodology, Data curation. **Charise Dallazem Bertol:** Resources, Methodology, Investigation, Conceptualization. **Paula Benetti:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Formal analysis, Conceptualization. **João Paulo De Carli:** Writing – original draft, Validation, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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