ORIGINAL RESEARCH

Comparative Evaluation of Setting Time and Antibacterial Efficacy of Cinnamic Acid-incorporated Glass Ionomer Cement: An *In Vitro* Study

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Abstract

Aim and background: Glass ionomer cement (GIC) possess properties to fulfill the prerequisites of a restorative material for atraumatic restorative treatment (ART). Enhancing the antibacterial property of GIC remains the pressing priority to reduce the occurrence of secondary caries. Thus, this study aimed to evaluate and compare the setting time and antibacterial efficacy of cinnamic acid (CA)-incorporated GIC against *Streptococcus mutans* and *Lactobacillus acidophilus*.

Materials and methods: CA powder was incorporated into the GIC powder in varying concentrations. For testing the setting time, the total samples of 15 disks were divided into five groups, namely—I: control—unmodified GIC, II: 2.5% CA + GIC, III: 5% CA + GIC, IV: 7.5% CA + GIC, and V: 10% CA + GIC. Setting time was evaluated after 30 seconds of mixing the powder and liquid by lowering a flat-end needle indentor. Similarly, for testing the antibacterial efficacy against *S. mutans* and *L. acidophilus*, the samples were divided, and 5 disks were allocated to each subgroup. The disks were placed in agar diffusion plates, which were inoculated with cultured test species separately. After 24 hours of incubation, the zone of inhibition (ZOI) was measured.

Results: CA-modified GIC exhibited an increase in setting time and ZOI with the increase in concentration of CA, and this difference was found to be statistically significant between the groups.

Conclusion: CA-incorporated GIC has greater antibacterial efficacy against the caries-causing bacteria with an acceptable change in setting time.

Clinical significance: GIC with higher antibacterial potential can markedly decrease the occurrence of secondary caries and failure of restorations.

Keywords: Cinnamic acid, Glass ionomer cement, *Lactobacillus acidophilus*, Setting time, *Streptococcus mutans*.

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Introduction

Dental caries is a multifactorial dynamic process that occurs due to the inconsistency between demineralization and remineralization in the oral cavity.[1] *Streptococcus mutans* is a known causative bacterium for the initiation of dental caries as it metabolizes carbohydrates into organic acids, leading to a drop in pH and resultant demineralization. Furthermore, *Lactobacillus acidophilus* has been identified in deeper carious lesions and found to mediate the progression of dental caries.[2]

Due to the high cost of treatment and reduced accessibility to people in remote areas, the prevalence of untreated dental caries in permanent teeth has increased to 2 billion people, and in primary teeth to 514 million children worldwide.[3]

In the motto of improving feasibility and accessibility, atraumatic restorative treatment (ART) was introduced by Frencken in 2009 in Tanzania. Being a minimally invasive procedure, it utilizes hand instruments to separate only the softer demineralized carious tissue and an adhesive restorative material, high-viscosity glass ionomer cement (GIC), to seal the cavity prepared. It is widely used in developing countries to bring dental services to the most deprived and underserved populations, but it has also been used for very young uncooperative children, people with special health care needs, and for those with a phobia toward mechanically driven dental instruments.[4,5]

The high viscosity GIC used for the ART technique has exhibited promising results with high wear resistance, chemically induced setting reaction and adhesion to tooth structure, ease of manipulation, fluoride-releasing capacity (anticariogenic property), and aids in remineralization of affected dentin.[5–7]

However, due to the incomplete removal of carious tissue and minimal antibacterial properties of GIC, ART faces a major disadvantage of residual bacteria being shut off inside the cavity, which leads to the progression of secondary caries and failure of the treatment. In an objective to eliminate this disadvantage, many researchers are focused on enhancing the antimicrobial properties of GIC by the addition of synthetic and natural bioactive components.[5]

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Owing to their biocompatibility, reduced cytotoxicity, cost-effectiveness, ease of availability, and safety, natural plant components outweigh the synthetic materials, which may adversely affect the mechanical and chemical properties of GIC. Some of the plant products tested in the literature are extracts of clove, thyme, tea tree, and cinnamon against cariogenic bacteria such as *S. mutans* and *L. acidophilus*.[1,5,8]

Cinnamon leaf and bark contain many bioactive components, mainly cinnamaldehyde and cinnamic acid (CA). CA belongs to the group of organic phenolic acids and exhibits numerous pharmacological properties, such as antibacterial, antioxidant, antiviral, and antifungal. Owing to the high antimicrobial activity attributed to the composition of small lipophilic molecules, they are known to be bactericidal against cariogenic bacteria. By increasing the hydrophobicity of the cell surface, CA hinders *S. mutans* aggregation and its mechanism of acid production from fermentable carbohydrates.[5,9]

The present study was done to evaluate and compare the antibacterial efficacy of CA-incorporated GIC against the main causative microorganisms of dental caries, *S. mutans* and *L. acidophilus*. Also, the basic physical property, setting time of the modified GIC, was assessed.

Materials and Methods

For the present *in vitro* study, ethical clearance was acquired from the Institutional Ethics Committee (VDCW/IEC/355/2023). CA (Nice Chemicals (P) Ltd, Kerala, India) was procured in powder form and mixed with GIC powder (GC Gold Label Type IX, GC International, Tokyo, Japan) at varying concentrations of 2.5, 5, 7.5, and 10%. The samples were divided accordingly for evaluation of setting time and antibacterial efficacy.

For Evaluation of Setting Time

The samples were divided into five groups with three disks in each group—I: control (unmodified GIC); II: 2.5% CA + GIC; III: 5% CA + GIC; IV: 7.5% CA + GIC; V: 10% CA + GIC.

The procedure for setting time evaluation was done according to the International Organization for Standardization (ISO) guidelines 9917-1: 2003 using the Gilmore needle apparatus (Fig. 1A).[10]

The powder (control and modified GIC) was mixed with the liquid using an agate spatula in a nonpermeable mixing pad. The mixed cement was filled into a mold of diameter 10 mm and height 3 mm. About 90 seconds after the end of mixing, the indentor [mass (400 ± 5) gm, with a needle having a flat end of diameter (1.0 ± 0.1) mm] was carefully lowered vertically onto the surface of the cement and allowed to remain there for 5 seconds. This was repeated at 30 seconds intervals until the needle failed to make a complete circular indentation in the cement (Fig. 1B).[10]

The net setting time was recorded as the time elapsed between the end of mixing and the time when the needle failed to make a complete circular indentation in the cement.[10]

For Evaluation of Antibacterial Efficacy

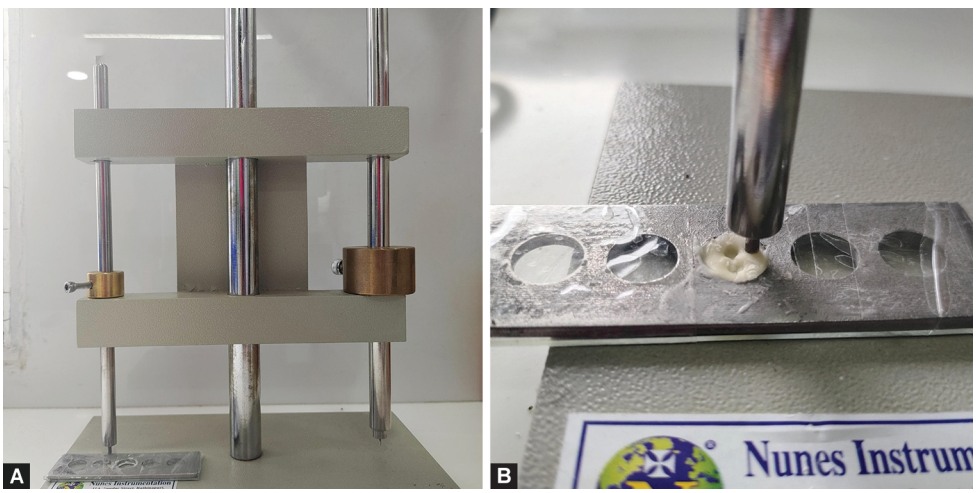
The samples were divided into two groups, which were further divided into subgroups based on the microorganisms, group I—*S. mutans*: S1—control–unmodified GIC, S2—2.5% CA + GIC, S3—5% CA + GIC, S4—7.5% CA + GIC, S5—10% CA + GIC; group II: L1—control–unmodified GIC, L2—2.5% CA + GIC, L3—5% CA + GIC, L4—7.5% CA + GIC, L5—10% CA + GIC.

About 25 mL of molten Mueller Hinton agar was poured into sterile Petri plates and allowed to solidify. After which, the 18-hour-grown 100 µL of *S. mutans* and *L. acidophilus* bacteria were transferred onto separate plates, and a culture lawn was made using a sterile cotton swab spreader. After 5 minutes of setting, the test samples were placed on the agar surface.

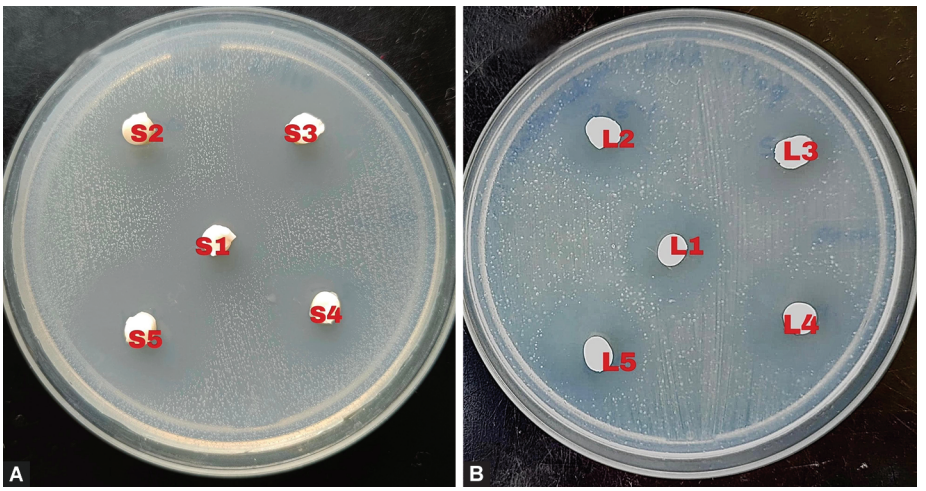
The conventional GIC and CA-modified GIC powder were mixed for 30 seconds and placed into cylindrical molds of 6 mm diameter and 3 mm height. The cement was allowed to set for 30 minutes after being covered with a glass slide at room temperature (37°C).[11]

The test samples were placed at a distance of 24 mm between each other on the Petri plates. The plates inoculated with *S. mutans* were incubated at 37°C in a bacteriological incubator for 24 hours. The plates with *L. acidophilus* were incubated under anaerobic conditions. The activity was determined by measuring the diameter of the zone of inhibition (ZOI) around the disk using an antibiotic zone scale (Figs 2A and B).[12]

The data obtained was entered into a Microsoft Excel spreadsheet and subjected to statistical analysis. All analyses were carried out using the Statistical Package for Social Sciences (version 24, IBM, Chicago, USA). Continuous variables were expressed as mean ± standard deviation (SD). The data was subjected to a normality test using the Shapiro–Wilk test.



Figs 1A and B: Gillmore needle apparatus and setting time evaluation



Figs 2A and B: CA + GIC—ZOI formed after 24 hours incubation against *S. mutans* and *L. acidophilus*

Table 1: Comparisons of setting time of control group and various concentrations of CA + GIC groups

		Setting time		
		Median	*IQR*	*p-value*
Unmodified GIC	Control (I)	4 minutes 30 seconds	10 seconds	<0.001*
CA + GIC	2.5% CA + GIC (II)	5 minutes	10 seconds	
	5% CA + GIC (III)	5 minutes 30 seconds	0 seconds	
	7.5% CA + GIC (IV)	6 minutes	0 seconds	
	10% CA + GIC (V)	6 minutes 30 seconds	30 seconds	

CA, cinnamic acid; GIC, glass ionomer cement; IQR, interquartile range; Kruskal–Wallis test; *p-value < 0.001, statistically significant

Nonparametric tests of significance were used for comparison. The level of significance was set at ≤0.05.

Kruskal–Wallis test was used to compare the setting time of CA-modified GIC with various concentrations. As significance was obtained, multiple pairwise comparisons were made between all concentrations to find the exact statistical significance. *Post hoc* Bonferroni test was used for this purpose.

Results

The obtained data were statistically analyzed, and the results were tabulated.

Kruskal–Wallis comparison of median values of setting time of control GIC (group I) and various concentrations of CA-incorporated GIC (groups II, III, IV, V) was found to be statistically significant (*p*-value = <0.001) (Table 1).

The pairwise intergroup comparison using the Mann–Whitney test was done between the control and study groups. This revealed a statistically significant difference (*p*-value = <0.05). The pairwise comparison between the study groups (II, III, IV, V) using the *post hoc* Bonferroni test also yielded a statistically significant difference (Table 2).

Kruskal–Wallis test comparison of the ZOI values of the control GIC and the test groups with different CA concentrations against *S. mutans* and *L. acidophilus* revealed a statistically significant difference (*p*-value = <0.001) (Table 3).

Mann–Whitney and *post hoc* Bonferroni pairwise comparison tests were performed and found that the control and the test groups had statistically significant differences (*p*-value = <0.05) (Table 4).

The multiple pairwise comparisons between control and each test group for the ZOI values against *S. mutans* were found to be statistically significant (*p*-value = <0.001) when 2.5% CA + GIC was compared with 7.5% CA + GIC and 10% CA + GIC. Comparison of 7.5% CA + GIC with 5% CA + GIC and 10% CA + GIC was also found to be statistically significant (*p*-value = <0.001). The comparison of control GIC with 2.5% CA + GIC, 5% CA + GIC, 7.5% CA + GIC, and 10% CA + GIC was found to be statistically significant (*p*-value = <0.001). The multiple pairwise comparison between control and each test group for the ZOI values against *L. acidophilus* was found to be statistically significant (*p*-value = <0.001) (Table 2).

Table 2: Multiple pair wise comparisons of setting time between the groups

	CA + GIC	
Comparison	*Median difference*	*p-value*
I vs II	–30 seconds[a]	0.006*
I vs III	–1 minute[a]	0.001*
I vs IV	–1 minute 30 seconds[a]	<0.001*
I vs V	–2 minutes[a]	<0.001*
II vs III	–30 seconds[b]	0.43
II vs IV	–1 minute[b]	<0.001*
II vs V	–1minute 30 seconds[b]	0.01*
III vs IV	–30 seconds[b]	0.06
III vs V	–1 minute[b]	<0.001*
IV vs V	–30 seconds[b]	0.03*

CA, cinnamic acid; GIC, glass ionomer cement; a–comparison using Mann–Whitney test; b–comparison using *post hoc* Bonferroni test; *p-value < 0.05, statistically significant

Table 3: Comparison of ZOI of control group and various concentrations of CA + GIC groups against *S. mutans* and *L. acidophilus*

		ZOI		
		Median	*IQR*	*p-value*
S. mutans	Control (S1)	23	1	<0.001*
	2.5% CA + GIC (S2)	24	1	
	5% CA + GIC (S3)	26	1	
	7.5% CA + GIC (S4)	27	1.5	
	10% CA + GIC (S5)	29	3.5	
L. acidophilus	Control (L1)	13	1	<0.001*
	2.5% CA + GIC (L2)	14	2.0	
	5% CA + GIC (L3)	16	2.5	
	7.5% CA + GIC (L4)	16	3.0	
	10% CA + GIC (L5)	18	3.0	

CA, cinnamic acid; GIC, glass ionomer cement; IQR, interquartile range; Kruskal–Wallis test; *p-value < 0.001, statistically significant

Table 4: Multiple pairwise comparison of ZOI between control GIC and various concentrations of CA + GIC against *S. mutans* and *L. acidophilus*

	S. mutans		*L. acidophilus*	
Comparison	*Median difference*	*p-value*	*Median difference*	*p-value*
Control vs 2.5% CA + GIC	−1[a]	<0.001*	−1[a]	0.02*
Control vs 5% CA + GIC	−3[a]	<0.001*	−3[a]	<0.001*
Control vs 7.5% CA + GIC	−4[a]	<0.001*	−3[a]	<0.001*
Control vs 10% CA + GIC	−6[a]	<0.001*	−5[a]	<0.001*
2.5% CA + GIC vs 5% CA + GIC	−2[b]	0.07	−2[b]	<0.001*
2.5% CA + GIC vs 7.5% CA + GIC	−3[b]	<0.001*	−2[b]	<0.001*
2.5% CA + GIC vs 10% CA + GIC	−5[b]	<0.001*	−4[b]	<0.001*
5% CA + GIC vs 7.5% CA + GIC	−1[b]	<0.001*	0[b]	<0.001*
5% CA + GIC vs 10% CA + GIC	−3[b]	0.07	−2[b]	<0.001*
7.5% CA + GIC vs 10% CA + GIC	−2[b]	<0.001*	−2[b]	<0.001*

CA, cinnamic acid; GIC, glass ionomer cement; a–comparison using Mann–Whitney test; b–comparison using *post hoc* Bonferroni test; *p-value < 0.05, statistically significant

Discussion

Dental caries, even though being a worldwide public health problem, can always be treated if intercepted at the early stage of progression. ART is a unique technique, which serves to intervene in this process by removal of carious tissue and sealing the cavity with high-viscosity GIC. But the residual bacteria left behind after excavation and microleakage of oral contents through the interface persist as a significant cause of secondary caries and treatment failure. Thus, it is paramount to eliminate the residual bacteria by restoring the cavity with GIC that has better antibacterial efficacy than the conventional GIC.[5,6,8,13]

Conventional GIC depends on the fluoride ion release for imparting anticariogenic property to inhibit the scarce amount of bacteria left beneath. The addition of natural antibacterial agents to GIC is gaining popularity due to their biocompatibility and minimal cytotoxicity. The overall success of the antibacterial GIC relies on the improved antibacterial efficacy against cariogenic bacteria without compromising its basic physical characteristics.[13,14]

There are only minimal studies in the literature that have evaluated the antibacterial efficacy of GIC modified by the addition of plant-derived bioactive components. This study was done to assess the antibacterial property and setting time of CA-modified GIC.[13,14]

Type IX GIC (GC Gold Label Type IX) was used in this study as it is a high-viscosity GIC commonly used for ART. It is known to have smaller glass particles with a high powder-to-liquid ratio. Thus, it confers the advantages of higher strength, wear resistance, and flexural strength. CA was used due to its known antibacterial properties against cariogenic bacteria.[15] Also, there are no studies in the literature that have evaluated the physical and antimicrobial properties of GIC incorporated with CA. Thus, in this study, GIC powder was modified by the incorporation of CA at varying concentrations of 2.5, 5, 7.5, and 10%.

In the present study, it was found that the setting time of modified GIC prolonged with an increase in the concentration of CA when compared to the control GIC. This may be due to the fact that CA is a weak monocarboxylic acid with a higher pKa (dissociation constant = 4.37) value when compared to the acids in GIC liquid. The higher the pKa, the lesser the solubility and reactivity of the acid. This slower rate of leaching of glass particles may have retarded the setting reaction and prolonged the setting time of the modified GIC.[2,16–18]

Though there is a statistically significant increase in the setting time of CA-modified GIC, the obtained results fall under the acceptable limit of net setting time of 90–480 seconds according to the ISO 9917-1:2007 specifications. This may be considered favorable to the dentist in terms of easy handling and manipulation of the GIC in a clinical scenario.[13]

Prentice et al. assessed the effectiveness of oxalic acid on the initial setting time, working time, 24-hour hardness, and

compressive strength of conventional GIC. He modified the liquid content of GIC by adding 0.5, 1, 2, 3, 5, and 7% of oxalic acid. It was found that there was a decrease in both initial setting time and working time when >2% oxalic acid was added.[16] These opposing results in setting time may be due to the fact that oxalic acid is a relatively stronger dicarboxylic acid when compared to CA, which might have lowered the pH and enhanced the leaching of ions from the glass surface, leading to an accelerated setting reaction.

Similarly, Crisp and Wilson studied the setting reaction of GIC by adding 5% tartaric acid to the polyacrylic acid. It was concluded that tartaric acid accelerated the extraction of ions from the glass powder, leading to higher availability of ions to react with the polyacrylic acid, resulting in an acceleration of setting time without affecting the working time. This contrasts with the results of our study. It may be attributed to the stronger acidic nature of tartaric acid (dicarboxylic, pKa_1 = 3.02, pKa_2 = 4.54) when compared to CA.[19,20]

The antibacterial efficacy of the CA-modified GIC exhibited in this study may be explained by the logP (partition coefficient), pH value, and constituents of the CA. The values of ZOI of various concentrations of CA-incorporated GIC against *S. mutans* and *L. acidophilus* revealed greater antibacterial efficacy with an increase in concentration and were statistically significant when compared to the control group. This might be because of the lower logP value of CA (<3, logP = 2.1), which suggests the minimal lipophilicity of the acid. This leads to passive diffusion of the small lipophilic molecules through the cell membrane of the Gram-positive bacteria in undissociated form, leading to acidification of the cytoplasm and denaturation of the intracellular proteins, thereby causing eventual cell death. The increase in concentration of CA might have caused a rise in the available undissociated acid forms intracellularly, contributing to its antibacterial efficacy.[2]

Here, the CA group had a better ZOI against *S. mutans* when compared to *L. acidophilus*. This difference in inhibition between the two Gram-positive bacteria may be due to the dissimilarity in the pH tolerance of the microorganisms (*S. mutans*–4.5–5; *Lactobacillus* species–below 3). When there is an extracellular rise in acidification, the bacteria activate the acid tolerance response (ATR), which includes the efflux of protons and acidic end products of metabolism to maintain the neutral pH in the cytoplasm. When this decrease in pH progresses beyond the critical limits of optimum pH for a longer period, there is a disruption of the cell membrane, the ATR, and subsequent change in the neutral intracellular pH. Intracellular pH is known to regulate the physiological processes of the cell, including glycolysis, protein and nucleic acid synthesis, nutrient transport, and metabolism.[21–23] This might have caused the higher inhibition of *S. mutans* when compared to *L. acidophilus*.

Ribeiro et al. evaluated the antimicrobial activity of certain phytochemicals, including CA, against *S. mutans* in planktonic and biofilm states. Also, the cytotoxicity of the compounds was assessed. It was concluded that CA demonstrated significant antibacterial activity (MIC 5 mM) and lower cytotoxicity. This was substantiated with the weak acidic nature and lipophilicity of the CA.[2]

Elsharkawy et al. added gallic acid (a polyphenolic monocarboxylic acid) at varying concentrations of 125, 62.5, and 31.25 mg/mL to the GIC liquid and assessed the antibacterial effect against *S. mutans*, fluoride release, and setting time. It was concluded that gallic acid improves the antibacterial effect of GIC with an increase in concentration. Also, there was an increase in the setting time and working time within the limits of ISO 9917-1:2007 guidelines. This similarity in results when compared to our study may be due to the weak acidic nature of gallic acid, which is nearly similar to that of CA.[13]

In our study, the acquired results suggest the effective inhibition of *S. mutans* and *L. acidophilus* by the CA-incorporated GIC, with greater ZOI against *S. mutans*. As *S. mutans* initiates the dental caries process, its inhibition by CA may be considered more favorable for the disruption of the process of dental caries at an early stage, suggesting its usefulness in dentistry. Though there is an increase in setting time with the increase in concentration of CA, it was within the acceptable limits of ISO specifications.

Considering the limitations of an *in vitro* study, further studies must be done to shed light on the cytotoxicity of these phytochemicals at different concentrations, leaching of ions from the modified GIC, and the physical properties such as compressive and bond strength, fracture resistance, and fluoride release to assess the clinical applicability and usage in ART.

Conclusion

Based on the observations and results of our study, there was a statistically significant increase in the antibacterial efficacy of CA-incorporated GIC against *S. mutans* and *L. acidophilus*, with the highest ZOI noted against *S. mutans*. Also, the setting time evaluation revealed a statistically significant increase in the setting time in modified GIC when compared to the control GIC, which was within the limits of the ISO specifications. This property of relatively higher inhibition of primary cariogenic bacteria (*S. mutans*) by the CA-modified GIC can be considered beneficial from the aspect of clinical and minimally invasive restorative dentistry.

Clinical Significance

Conventional GIC has anticariogenic properties attributed to the fluoride release from the cement, which is limited. Thus, a substantial increase in its antimicrobial property against cariogenic bacteria can be considered essential for the prevention of secondary caries in ART. This, ultimately, will reflect on the success of the treatment in clinical practice.

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