Published online in http://iijam.co.in
ISSN: 0976-5921
International Journal of Ayurvedic Medicine, 2014, 5(1), 91-101

Effect of Turmeric incorporation on fluoride release, Antibacterial activity and Physical properties of glass ionomer cement An in-vitro comparative study

Research article

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Abstract

Aim and Objective: To assess the effects of turmeric incorporation on the fluoride release, antibacterial efficacy and physical properties of Glass Ionomer Cement.
Methods: Turmeric was added to GIC at concentration ratios of 0.5% w/w (group II) and 1% w/w (group III) to obtain the test groups. Conventional GIC served as control (group I). The antibacterial activity of the cement specimens were evaluated against Streptococcus mutans using the agar well inhibition test. The fluoride release was evaluated using fluoride ion selective electrode. The physical parameters evaluated were net setting time, shear bond strength and microleakage.
Results: Group II and III showed significantly greater inhibition zones against S.mutans while the control group (Group I) showed only mild inhibition. Similarly group II and group III showed highly significant fluoride release compared to the control group. There was no statistically significant difference in the physical properties viz shear bond strength, microleakage and setting time of the cement between the groups.
Conclusion: Incorporation of turmeric at a concentration of 1% has the potential to enhance the antibacterial activity of GIC as well as fluoride release without compromising on its physical abilities viz shear bond strength, micro leakage and setting time.

Keywords: Turmeric incorporation, GIC, Antibacterial property, Fluoride release, Shear bond strength, microleakage.

Introduction:

Acidogenic bacteria play the main role in the development of dental caries(1,2,3) with Streptococcus mutans being the most frequently implicated organism(4,5). Microorganisms present beneath a restoration because of microleakage or incomplete caries removal during tooth preparation(4,6) contribute to secondary caries which in turn influences the longevity of dental restorations(4,7,8,9,10).

Though Glass ionomer cements (GICs) have been in use for more than 20 years(4), the search is still on for the 'ideal' bioactive material which could provide a therapeutic edge to restorative dentistry more so in children(11). Various

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commercially available antibiotics such as metronidazole, ciprofloxacin, cefaclor, chlorehexidine etc have been incorporated into GIC either individually or in varying combinations(12,13,14) with an intention of reaping some therapeutic benefit. But not only they were found to be expensive, a few of them did affect the physical properties of the cement as well(14,15).

Turmeric (*Curcuma longa L)*, a traditional Indian household herb commonly known as "HALDI" is a perennial plant with orange oblong tubers 2-3 inches in length and 1 inch in diameter, pointed or tapering at one end. When dried, it can be made into powder with a bitter, slightly acrid, yet sweet taste. It is a member of ginger family, Zingiberaceae, the Latin name *Curcuma longa L* being derived from the Persian word "*kirkum,"* which means "saffron". The active constituent of turmeric is known as "curcumin"(16). It has a long history of medicinal use especially in Ayurveda, Siddha and Unani systems(17) for its anti-inflammatory, anti-oxidant, anti-microbial and anti allergic properties along with other beneficial therapeutic effects(16,18).

Hence, this study aimed to assess if turmeric incorporation could influence the antibacterial activity and fluoride release of GIC, thus making it a potential therapeutic restorative option.

Materials and methods:

Preparation of Study Material:

A conventional restorative GIC (Ketac molar, 3M ESPE) was used as the control (Group I). In experimental groups (Group II and III), a herbal medicine turmeric (*Curcuma longa*) was added to the powder at 0.5 and 1% w/w respectively (Table 1).

Table 1 : Preparation of Cement samples

Groups	Composition of the control and experimental GIC (total weighing 10g)	Additives (w/w %)
Group I (Control group)	10 g of GIC	--------
Group II	0.05 g of turmeric and 9.95 g of GIC	0.5%
Group III	0.1 g of turmeric and 9.9 g of GIC	1%

1) Assessment of antibacterial activity:

The antibacterial property of turmeric incorporated GIC (Ten specimens from each group) was evaluated against Streptococcus mutans (MTCC 497, Institute of Microbial Technology, Chandigarh) using the agar well diffusion in a laminar airflow unit under aseptic conditions and it was expressed in terms of zones of inhibition (millimetres).

A loopful of bacterial inoculum from the lyophilized culture was transferred to 10ml of Brain Heart Infusion (BHI) broth and was incubated at 37^0C for 24 hours. After a 24 hour incubation period, a loopful of S.mutans were spread onto a BHI agar plate and left for 30 minutes.

Six millimetre diameter wells were cut from the agar by using sterile glass made pipettes attached to a vacuum pump. The cement pastes were prepared by mixing powder and liquid from each group (P/L ratio: 3/1) and wells were filled with cement pastes(14).

The plates were then incubated at 37± 0.5ºC and the diameters of zones of inhibition produced around the specimens were measured at three different points with a digital caliper after 48 hours and seven days on the same set of specimens. The sizes of inhibition zones were calculated by subtracting the diameter of

the specimen (6mm) from the average of the three measurements of the halo(13).

2) Assessment of Fluoride release:

Ten specimens were prepared from each group, using cylindrical brass mould (10mm×2mm), which were kept in a 100% humid environment for one hour. After removing the specimens from the moulds, they were immersed in individual plastic vials containing five ml double deionised water at 37^0C for seven days. The double deionised water was changed daily. Fluoride ions released was measured after 24hours and on seventh day using Ion sensitive electrode (membrane electrode) method (19).

3) Assessment of Physical properties :

Setting time:

The test was carried out at 37^0C, using a Vickers needle. Five specimens per group were prepared in a brass mould of standard dimension (10mm×4mm). The brass mould was positioned on an aluminum foil and filled to its brim with the mixed cement (P/L ratio: 3/1). The upper surface was made flat by pressing with a glass slide. The assembly comprising of mold, foil and cement was then placed into the Vicat cabinet. The indenter was carefully lowered vertically onto the surface of the cement every 15sec. The net setting time was recorded as the time elapsed between the end of mixing and the time when needle failed to make complete circular indentation on the surface of the cement(20).

Shear Bond Strength:

Fifteen non carious extracted human premolar teeth were selected. Teeth were sectioned in mesio-distal direction using a diamond disc. The 30 specimens obtained out of 15 teeth were randomly assigned to the control and experimental groups. Each specimen was embedded in acrylic resin and a flat surface of dentin was obtained using a polishing lathe. The cement for each group was manipulated according to manufacturer's instructions and was placed on smoothened dentinal surface by using Teflon mould of standard dimension (3mm×4mm). The samples were stored in deionized water for 24 hours and were subjected to shear bond strength testing using the Universal Testing Machine(21).

Microleakage:

Thirty non carious extracted human premolar teeth (ten teeth per group) stored in a solution of 1% sodium hypochlorite were used. After surface debridement with a hand instrument and cleaning with a rubber cup and pumice slurry, standardized buccal surface class V cavities, approximately 4mm wide×2mm high×1.5mm deep(22) were prepared with a no. 329 carbide bur in a high-speed hand piece. A template was used to obtain a uniform kidney-shaped outline. Subsequently the teeth were randomly assigned to control and experimental groups.

After restoring with their designated material(23) (depending on their group) all the teeth were thermocycled. The cycles were set at 200 cycles, between 5 and 55°C ± 2°C, with a dwell time of 60 seconds at each temperature. The specimens were then sealed with a coating of nail polish, barring a 1 mm margin around the restoration and were immersed in 2% methylene blue dye for 24 hours. Subsequently they were washed under running water, dried and mounted on a stone cast with an orientation such that the occlusal surface was visible outside the plaster. The teeth in the mounted cast were sectioned longitudinally in a buccolingual direction through the center of the restoration with a slow speed diamond disk(24). The cut surfaces were then examined under stereomicroscope and scored based on following criteria(25)-

0=No marginal leakage
1=Dye penetration up to one-half of the cavity depth
2=Dye penetration greater than one-half of the cavity depth
3=Dye penetration extending to the axial wall of the cavity

Statistical analysis

One way ANOVA was used for multiple group comparison. For marginal leakage Kruskal Wallis was used. POST HOC Tukey's test was used for group wise comparison if significant differences were found between groups.

Results:

Antimicrobial activity:

All the groups exhibited zones of inhibition against S.mutans after 48 hours. But group II and group III showed highly significant (<0.001) zones of inhibition than the control group (Group I). However there was no statistically significant difference between groups II and III. (p=0.28) (Fig 1, 2 & 3, Graph 1, Table 2). When the inhibition zones were measured again after 7 days, there was no observable difference with the readings obtained at 48 hrs.

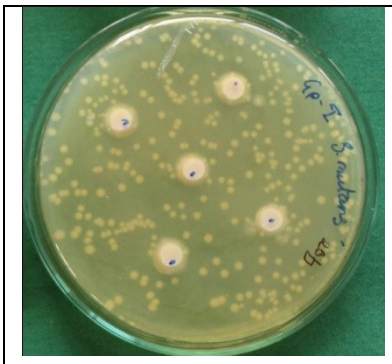

Fig 1

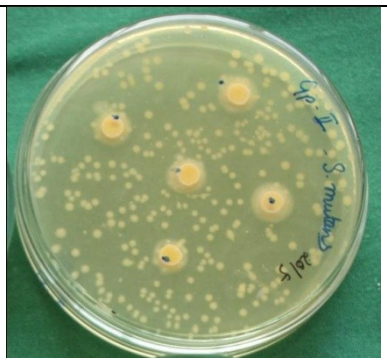

Fig 2

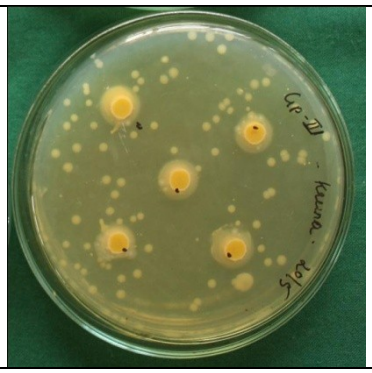

Fig 3

Graph 1

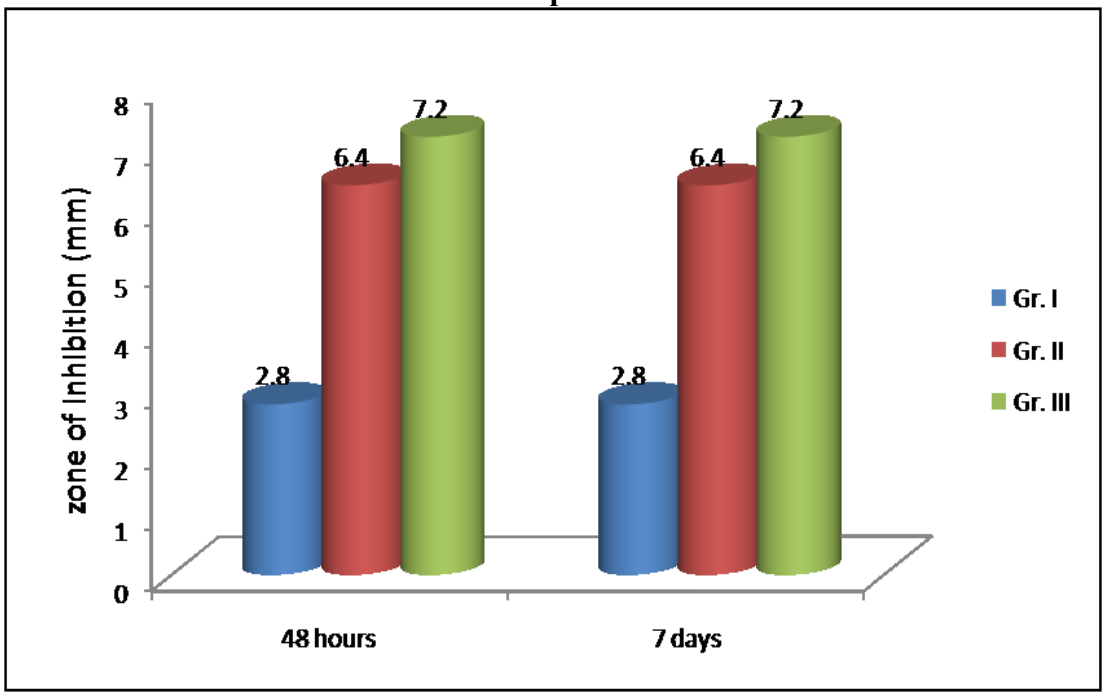

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Table 2 : Antibacterial property- Mean and levels of significance(p value)			
Groups		**48 hours**	**7 days**
I		2.8±0.9	2.8±0.9
II		6.4±1.6	6.4±1.6
III		7.2±0.8	7.2±0.8
ANOVA		F=41.66, p<0.001	F=41.66, p<0.001
Difference between the groups (p values)	I-II	<0.001	<0.001
	II-III	<0.001	<0.001
	II-III	0.28	0.28

Fluoride release:

The mean fluoride release of the control and experimental groups after 24 hours and 7 days is shown in Graph 2. The experimental groups showed higher fluoride release when compared with the control group both at 24 hours and 7 days, the difference being statistically highly significant (p<0.001). Also statistically highly significant difference in the values of fluoride release was found between group II and group III (p<0.001), Group III showing the highest fluoride release (Table 3). However the fluoride release values at 7 days were lesser than at 24 hours for all groups.

Graph 2

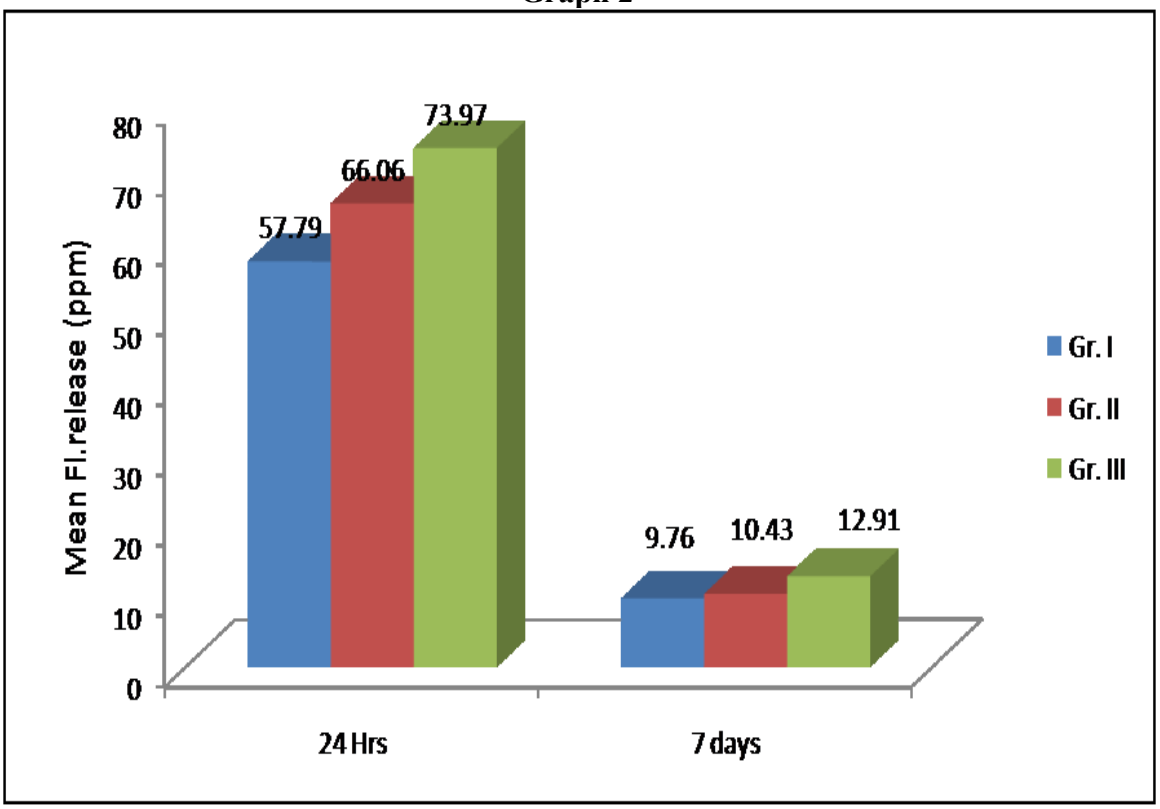
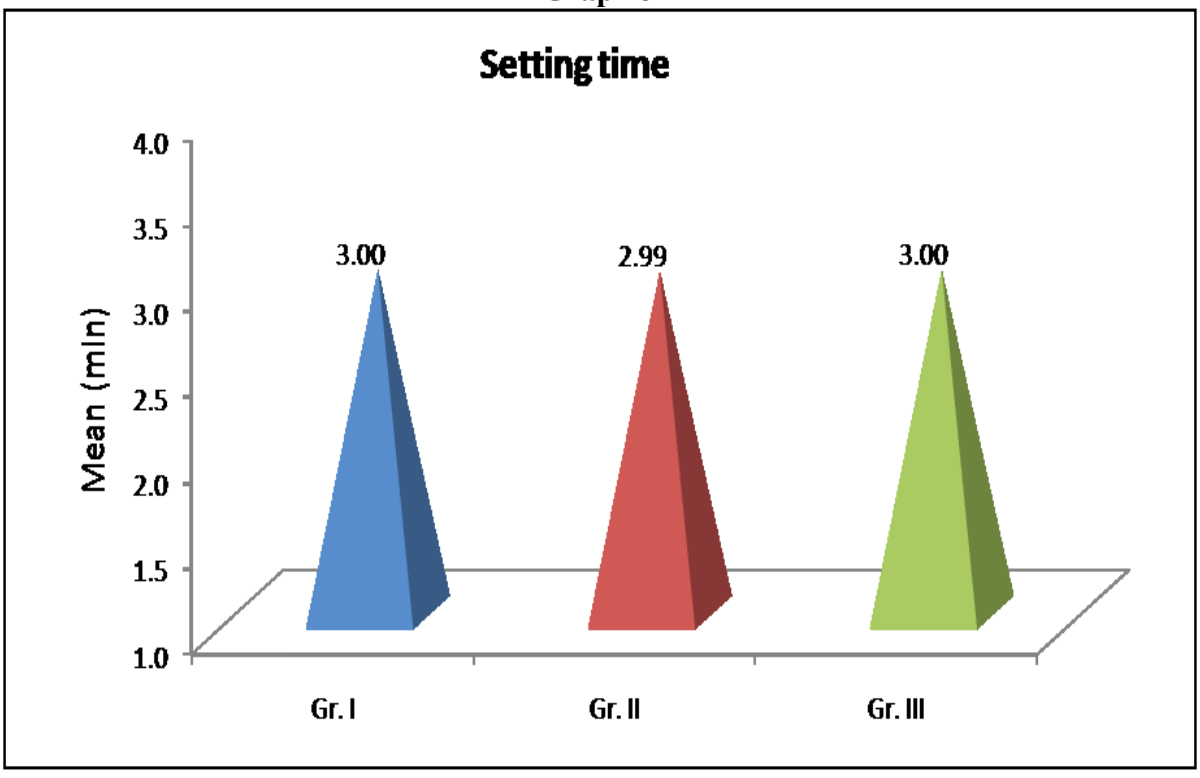


Table 3 : Fluoride release- Mean and Levels of significance (p value)			
Groups		**24 hours**	**7 days**
I		57.79±2.99	9.76±0.24
II		66.06±4.57	10.43±0.71
III		73.97±4.79	12.91±0.69
ANOVA		F=37.16, p<0.001	F=80.14, p<0.001
Difference between the groups (p values)	I-II	<0.001	<0.04
	I-III	<0.001	<0.001
	II-III	<0.001	<0.001

Setting time, Shear bond strength and Microleakage:

The mean setting time, shear bond strength and microleakage for the control and experimental groups are shown in Graph 3, 4 and 5. The differences observed between control and experimental groups were not statistically significant (p>0.05). Similarly there was no statistically significant difference between group II and III as well (p>0.05) (Table 4, 5).

Graph 3



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Graph 4

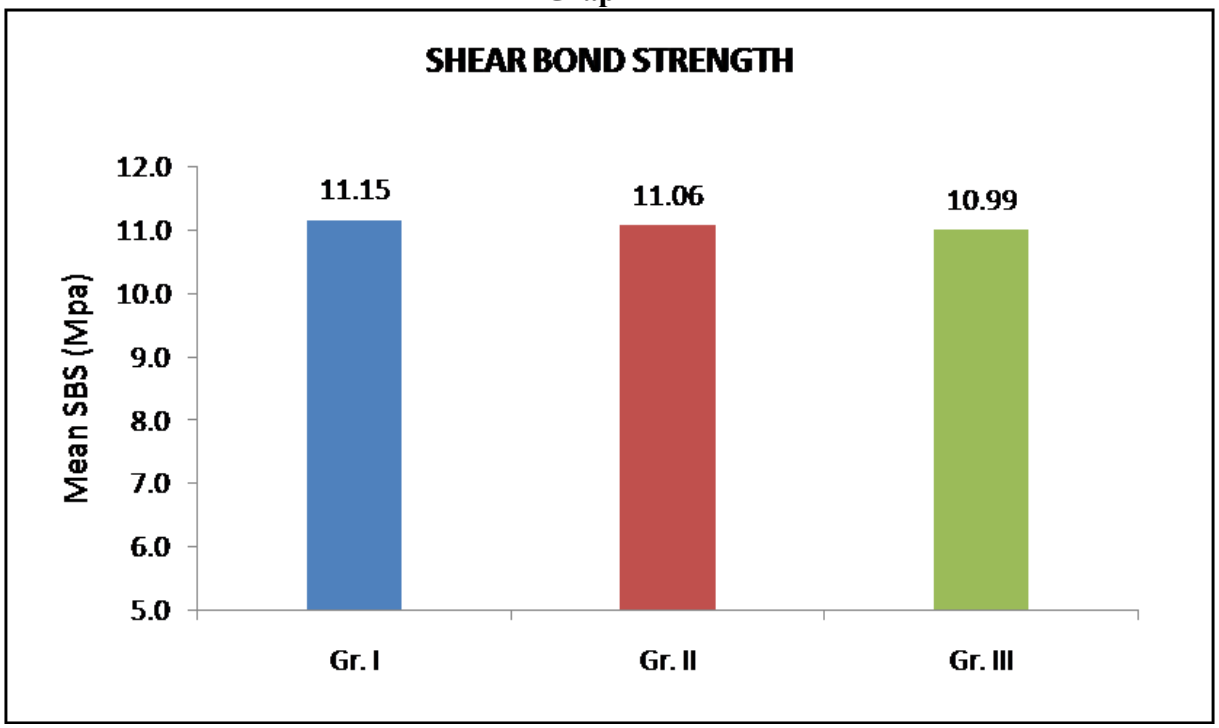

Table 4 : Mean values of Setting time and Shear bond strength		
Groups	**Setting time**	**Shear bond strength**
I	3.00±0.04	11.15±0.16
II	2.99±0.05	11.06±0.14
III	3.00±0.04	10.99±0.17
ANOVA	F=0.12, p=0.89 (Not significant)	F=1.30, p=0.31 (Not significant)

Graph 5

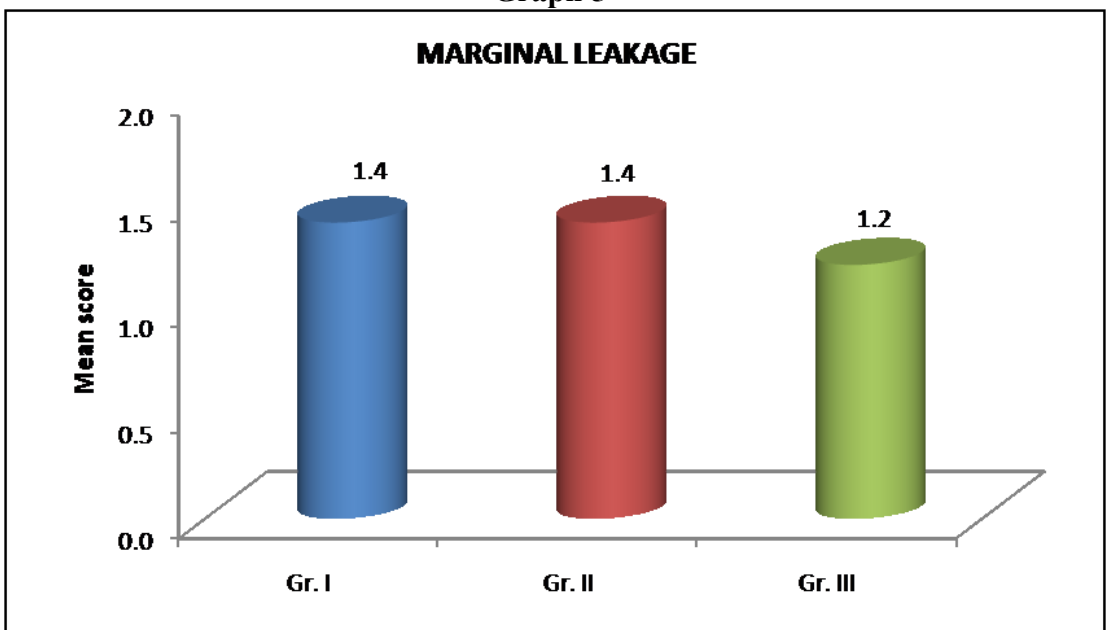

Table 5 : Microleakage

Groups	Marginal leakage scores				Mean score±SD	Median
	0	1	2	3		
I	-	8	-	2	1.4±0.9	1.0
II	-	6	4	-	1.4±0.5	1.0
III	1	4	4	-	1.2±0.8	1.0
K-W ANOVA, H=0.18, p=0.91(Not significant)						

Discussion

Clinical studies have demonstrated that one of the main factors responsible for replacement of dental restorations is the presence of secondary caries, thereby emphasizing the need for restorative materials with anticariogenic properties(26).

GICs have been in use for more than 30 years, and have been subject to numerous interventions attempting to improve the antibacterial activity. Chlorhexidine hydrochloride,[13],[27] cetyl pyridinum chloride(27,28), cetrimide(26), benzalkonium chloride(27,28), triple antibiotics(14), triclosan(29), quaternary ammonium salt (PQAS)(15) have all shown to enhance the antibacterial activity of GIC, but the ease of availability, cost, effect on its' physical properties as well potential toxic side effects of the additives cannot be ruled out. Hence the use of indigenously occurring natural herb(16) such as Turmeric which is a proven antibacterial agent(18,30,31,32) seems a plausible alternative.

Turmeric or "HALDI", a commonly used Indian traditional herb, is a treasure house of antibacterial and antioxidant molecules(16) and is extremely cost effective as well. It's spectrum of activity against caries causing bacteria(18,30,31,32) was considered before selecting it for this therapeutic intervention. Since turmeric is available in powder form, it was decided to incorporate the same into the powder of conventional GIC (Ketac molar).

The results of the current study demonstrated that turmeric was effective in inhibiting bacterial growth. After 48 hours, specimens containing turmeric exhibited statistically significant inhibitory zones against S mutans. However the size of the inhibition zones were dependent upon the quantity of the turmeric incorporated into the GIC. Also, all the turmeric containing specimens showed no change in the zones of inhibition at 7 days. These results can be explained by understanding the setting mechanism of GIC materials which show more solubility initially. This observed trend of antibacterial activity could also correspond to decrease in the availability of 'free' turmeric (below minimum concentration levels). This decrease in turmeric could in turn be a result of the loss of material by elution, or perhaps may be due to the ability of turmeric to form insoluble salts with the GIC, in accordance with the suggestion made by Ribeiro and Ericson with regards to chlorhexidine(33).

However, the important thing to be noted is that turmeric could serve as an effective therapeutic agent against S mutans in the most crucial period i.e. the 1st 48 hours of restoring the tooth.

Eventually, an effective marginal seal established by the chemical bonding of the cement to dentine, could potentially suffice to keep the tooth in question, 'caries-free'.

In the present study, it was also observed that, increasing the concentration of the turmeric had no impact on the physical properties of the cement. When compared with the control groups, the setting time, shear bond strength and micro leakage of the test groups remained unaffected ($p>0.5$).

Another important finding to come out of the present study was the highly significant increase in the release of fluoride from the turmeric groups when compared to the control group both at 24 hours and 7 days intervals. The amount of fluoride ions released had a linear increase with the increase in the concentration of turmeric in the cement. However long term studies are required to understand the mechanism involved to substantiate this claim.

Also the effects of turmeric on the color stability and compressive strength of GIC need to be investigated through long term in vivo trials before proclaiming it as a therapeutic restorative material.

Conclusion

The results of this in vitro investigation demonstrated that experimental GIC's containing turmeric are effective in inhibiting S mutans, the key bacteria causing dental caries. The addition of turmeric at a concentration ratio of 1w/w% was more effective with no adverse effects on the physical properties of GIC.

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