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RESEARCH ARTICLE

ANTIBACTERIAL ACTIVITY OF POLYCARBOXYLATE CEMENT REINFORCED BY DIFFERENT AMOUNT OF FLUOROAPATITE AND CALCIUM FLUORIDE (IN VITRO STUDY).

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Abstract

Background: The streptococcus mutans and lactobacilli have been considered to be the most common causative bacteria for dental decay.
Antibacterial action of polycarboxylate cement reinforced by different amount of fluoroapatite and calcium fluoride is important property for the filling restoration to prevent secondary caries and to evaluate these materials to be used as base filling in which the antibacterial activity is important property for successful filling and cementing material for crowns and bridges ,thus the aim of the study was the analysis the antimicrobial activity of polycarboxylate cement reinforced by different amount of fluoroapatite and calcium fluoride.
Materials& methods: fluoroapatite materials and calcium fluoride were added to polycarboxylate cement at different ratios; 0% (Control), 5% ,10%,15%, 20%, 25% (by weight).
Agar diffusion method will used to evaluate the antibacterial effect polycarboxylate cement and polycarboxylate cement reinforced by different amounts of fluoroapatite and calicium fluoirde (5% ,10%,15%, 20% and 25%) in this study two bacterial strains are used ; streptococcus mutans and lactobacilli which are cultured. A six holes (5mm depth and 4mm diameter) in each Petri dish(20 petri dish for each of two micro organism of this study) ; 10 petri dish for polycarboxylate cement and polycarboxylate cement reinforced by different amounts of fluoroapatite (5% ,10%,15%, 20% and 25%) and 10 petri dish for polycarboxylate cement and polycarboxylate cement reinforced by different amounts of calicium fluoirde (5% ,10%,15%, 20% and 25%) . in this study the material were added in these Wells (one Well for each ratio was used) and after incubation of these Petri dishes , the inhibition zones of each microorganism of this study were measured in millimeter around each of these Wells.
Results: the conclusion n this study showed that the polycarboxylate cement and polycarboxylate cement reinforced by different amounts of fluoroapatite and calicium fluoride have strong antibacterial activity against streptococus mutans and lactobacilli Bacteria. Also the results showed that the increase the ratio of fluoroapatite will statistically increase their antibacterial activity aganist streptococcus mutans and lactobacilli as far as 20%.

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Introduction:-

factor causes both primary and secondary caries seems to be the same ,but the rapid deterioration of caries may occur due to the bad connection between tooth surface and restoration. Marginal defects of different kinds are common for most dental filling materials (Derand et al, 1984).Secondary caries formation represents the primary reason for replacement of amalgam and composite resin restorations **(Mjor 1985)**. A clinical study found that secondary caries was responsible for 72% of the total number of replacements necessary for amalgam and for 43% of composite restorations . So that, the restorative material resistance secondary caries attack and micro leakage at it's margin will determine whether the restoration will succeed or fail **(John 1986).**

Dental caries is a biofilm-related oral disease usually associated with high sugar diet intake. Where the acidogenic and aciduric bacteria in this biofilm will metabolize the sugar to organic acids and lead to low pH environment in the bio film matrix can cause dissolution of the tooth surfaces and initiate dental caries. Although additional acidogenic and aciduric bacteria can be involved, Streptococcus *mutans* is considered one of the most important microorganisms in the etiology of caries and has been used in many caries studies **(Beighton 2005; Lobo et al., 2005; Gama-Teixeira et al., 2007; Thomaz et al., 2008)**. One of the main causes of tooth decay is Streptococcus mutans which are play a major role in the initiation as well as the progression of caries lesions, Lactobacilli and other microorganisms have been involved in the development of the disease. **(Samaranayake *et al.*, 2002;Song et al., 2006). Fejerskov et al., 2015** reported that caries is not the result of fluoride deficiency but this ion is the only therapeutic agent known to effectively control caries progression, and fluoride-releasing materials maybe considered a way or vehicle of fluoride delivery. **Hamilton et al.in1996** published that, the fluoride is well documented as an anticariogenic agent and there are a variety of mechanisms involved in the anticariogenic effects of fluoride, including the reduction of demineralization, the enhancement of remineralization, the interference of pellicle and plaque formation and the inhibition of microbial growth and metabolism. Several studies showed that the fluoride released from dental materials inhibits the acid produced by bacteria by inhibition glycotic enzyme enolas, proton-translocating ATPase and intracellular acidification.

that the hydrogen fluoride(un ionized fluoride) which formed in acidic PH enter the cell wall of bacteria and enhance fluoride inhibition ,thus the acid production by streptococcus mutans was inhibited due to decreasing in PH **(Curran et al., 1994; Iwami et al., 1995; Barboza-Silva et al., 2005; Nakajo et al., 2009). Forsset al., 1995)** was revealed that local fluoridation of area near restoration by fluoride-releasing materials play important role in prevention and inhibition of caries than increasing the saliva fluoride.and even low fluoride as reported by (**Pandit et al**. **in 2011**) can reduce the bacterial growth, plaque and acid production**(in vitro).** in addition to that, the plaque near or on the fluoride releasing restoration with fluoride releasing found to have high concentration of fluoride that inhibit the streptococci mutans in several study . **Silverstone 1990,Ten cate and Arends 1998**, results that the presence of fluoride ion enhances greatly the remineralization achieved and reduces the time period for this mechanism**(Silverstone, 1984)**. This was also shown by **Koulourides 1982**, who demonstrate that the addition of fluoride to a reminerlazing solution increase the rate of mineral deposition**(Koulourides, 1982). Silverstone 1984**, concluded that the exposure of small lesions to synthetic calcifying fluid in vitro results in a significant increase in remineralization and the degree of remineralization achieved depends upon the presence of fluoride ions in the calcifying fluid **(Silverston, 1984)**. Fluoride ions have great affinity for demineralized regions where free calcium and phosphate ions are available in abundance, frequently the fluoride ion binds both calcium and phosphate ions forming fluoroapatite or binds calcium ion only resulting in calcium fluoride. Although both bindings are very important for remineralization and will be accelerated in the outer most region of the lesion drawing away many of free mineral ions (calcium and phosphate) from inner part of the lesions (subsurface lesion or lesion body) and effectively slowing down diffusion toward interior of the lesion body **(Axelsson, 1999).** The aim of this study was to evaluate the possibility of using polycarboxylate cement reinforced by fluoroapatite cement and calcium fluoride as base and cementing material by evaluating their antibacterial activity against streptococus mutans and lacto basili the most cariogenic bacteria.

Materials and methods: Preparation of fluoroapatite; (Calcium nitrate 4-hydrate ($Ca(NO_3)_2 \cdot 4H_2O$, AnalarR grade, BDH Limited Company, Inc.), (diammonia hydrogen phosphate ($(NH_4)_2HPO_4$, Merck KgaA Company, Inc.) and (ammonia fluoride ,NH_4F, FlukaChemie Company, Inc.) These materials were used at the beginning of the research. 0.5 M Calcium nitrate 4-hydrate is dissolved in distilled water in order to achieve the ratio of Ca/P equal to 1/67, isolate the pure substance with the addition of diammonia hydrogen phosphate and then add the ammonia fluoride to the calcium solution with continuous stirring and to add quantities of ammonia fluoride solution to control the

value x in genral formula of (Ca10(PO4)6(OH)2-2xF2x)When the x was 0 and 1.0, in order to get sediment. We add the solution of ammonia to the mixed solution that PH equal to 9-10, continuous strring for 2hours then kept both solution and precipitates for 24hours before the precipitates was filtered by filter paper and washed with distilled water to get red the residual ions, dried by air and crushed by the mortar and pestle, followed by calcination at 600 °C again in muffle furnace under air atmosphere for 1 hour period. The calcined powder was then smashed to fine powders using a dry(ball mill). The prepared fluoroapatite was added to the powder of polycarboxylate cement at different weight percentage (5%, 10%, 15%,20% and 25%) and the mixed powders were shacked for half to one hour to get homogenous powders.[7]Also in this study the calcium fluoride(Honeywell Riedel-de Haën, Germany) was added to the powder of polycarboxylate cement at different weight percentage (5%, 10%, 15% ,20% and 25%) and the mixed powders were shacked for half to one hour to get homogenous powders.[7]

Sample grouping :-Six groups were used in this study (according to the ratio of added fluoroapatite to the polycarboxylate cement) as shown in table 1.
Also Six groups were used in this study (according to the ratio of added calcium fluoride to the polycarboxylate cement) as shown in table 2.

Antibacterial activity test:

Results were documented directly in the antimicrobial laboratory at national center of hematology, almustansiriya University. Both material(FA&CAF) were evaluated and tested against streptococcus mutans and lactobacilli .The bacteria streptococcus mutans provided from faculty of dentistry , Baghdad university ,while lactobacilli provided from teaching laboratory centers in medical city hospital –baghdad medical college . This bacteria reactivated in nutrient broth Infusion culture medium diluted to match a 0.5 McFarland turbidity standard, that is roughly equivalent to 150 million cells per mL. The medium incubated for 24h period in bacteriological incubator at 37°C, ,then in aseptic technique, place a sterile swab into the broth culture of a specific organism and then gently pressing the swab against the inside of the tube to remove the excess liquid , streak the Mueller-Hinton agar plate by the swab to form a bacterial lawn.

In order to obtain uniform growth, streak the plate with the swab in one direction, rotate the plate 90° and streak the plate again in that direction. Repeat it 3 times then allow the plate to dry for approximately 5 minutes. (The media used in Kirby-Bauer testing must be Mueller-Hinton agar at only 4 mm deep, poured into either 100m or 150mm Petri dishes). The pH level of the agar must be between 7.2 and 7.4.five plates for each microorganism are prepared.

Antibacterial Activity Assessment:-

Antibacterial activity of both material were determined through disk diffusion method (Kirby- Bauer testing) on solid medium. The product was inserted into wells made-up in Petri plates containing Müeller Hinton agar, six wells (5mm depth and 4mm in diameter) were made-up in each plate by using sterile disposable tips. The polycarboxylate cement reinforced by Fluoroapatite and Calcium fluoride were manipulated on a sterile glass board in accordance with manufacturers' guidelines[8] and then inserted into each well until its complete filling, The plates were incubated at 37°C in bacteriological incubator for 24 hours and the reading of results was done afterwards. The antibacterial activity was evaluated by using a caliper to measure the diameter of halos of growth inhibition of the strains assayed. The zone around polycarboxylate cement reinforced by Fluoroapatite and Calcium fluoride disk that has no growth is referred to as the zone of inhibition. the diameter of this zone is then measured in mm and compared with each other groups of this study by statistical analysis (Descriptive statistic; tables and figures) and infernicial statistic (one way ANOVA test and LSD test).

Results:-

1-Antibacterial activity of polycarboxylate cement and polycarboxylate cement reinforced by different amounts of fluoroapatite

A:-against streptococcus mutans

The results showed (figure 1 and table 3) that the group VA and group VIA has the highest diameter of inhibition zones of culture media of Streptococcus mutans . while the group IA has the lowest diameter of inhibition zones of culture media of Streptococcus mutans.

One-way ANOVA test (Table 4) showed that there was statistically significant difference among all the groups at the P value less than 0.01

LSD test (table 5) showed that there was highly statistical significant differences between Group IA as compared with Group IIA, Group IIIA, Group IVA, Group VA and Group VIA , while there was no statistical significant differences between Group IIA as compared with Group IIIA also there was highly statistical significant differences between Group IIA as compared Group IVA, Group VA and Group VIA, While there was no statistical significant differences between Group IVA as compared with Group VA and Group VIA while there was no statistical significant differences between Group VA as compared with Group VIA.

B- against lactobacilli bacteria:
The results showed (figure 2 and table 6) that the group VA has the highest diameter of inhibition zones of culture media of lactobacilli bacteria . while the group IA has the lowest diameter of inhibition zones of culture media of against lactobacilli bacteria:

One-way ANOVA test (Table 7) showed that there was statistically significant difference among all the groups at the P value less than 0.01

LSD test (table 8) showed that there was highly statistical significant differences between Group IA as compared with Group IIA, Group IIIA, Group IVA, Group VA and Group VIA , while there was no statistical significant differences between Group IIA as compared with Group IIIA, Group IVA and Group VIA also there was highly statistical significant differences between Group IIA as compared Group VA.While there was no statistical significant differences between Group IVA as compared with Group VA and Group VIA while there was no statistical significant differences between Group VA as compared with Group VIA.

A-against streptococcus mutans:-
The results showed (figure 3 and table 9) that the group VB and group VIB has the highest diameter of inhibition zones of culture media of Streptococcus mutans . while the group IB has the lowest diameter of inhibition zones of culture media of Streptococcus mutans .

One-way ANOVA test (Table 10) showed that there was statistically significant difference among all the groups at the P value less than 0.01

LSD test (table 11) showed that there was highly statistical significant differences between Group IB as compared with Group IIB, Group IIIB, Group IVB, Group VB and Group VIB , while there was no statistical significant differences between Group IIB as compared with Group IIIB also there was highly statistical significant differences between Group IIIB as compared Group IVB, Group VB and Group VIB, While there was no statistical significant differences between Group IVB as compared with Group VB and Group VIB .Also there was no statistical significant differences between Group VB as compared with Group VIB.

B- against lactobacilli bacteria:

The results showed (figure 4 and table 12) that the group VIB has the highest diameter of inhibition zones of culture media of lactobacilli bacteria . while the group IB has the lowest diameter of inhibition zones of culture media of against lactobacilli bacteria:

LSD test (table 14) showed that there was highly statistical significant differences between Group IB as compared with Group IIB, Group IIIB, Group IVB, Group VB and Group VIB , Also there was statistical significant differences between Group IIB as compared with Group IIIB. While there was no statistical significant difference between Group IIB as compared with Group IVB, group VB and group VIB also the results showed that there was highly statistical significant differences between Group IIIB as compared Group VB and group VIB .While there was no statistical significant differences between Group IVB as compared with Group VB and Group VIB while there was statistical significant differences between Group VB as compared with Group VIB.

Discussion:-
Enamel, dentin and plaque are known as primary sites of fluoride retention in the month. Additionally, oral soft tissues may act as fluoride retention sites after application of fluoride components. As fluoride directly contacts oral tissues and is retained in the oral cavity for a long period of time, it should be characterized by a low cytotoxicity

and high pharmacological safety in the applied closes in order to provide maximum benefit without side effects **(Dogan et al., 2002)**.The secondary caries formation has been shown to be the major reason for failure and replacement of restorations. The fluoride has been incorporated in dental restorative materials, because it can exhibit an anticariogenic activity by increasing dentin and enamel resistance to acids present in the oral cavity, protecting the dental structure against caries attack.**(Itota et al.,2002)**. Fluoride is usually classified as a trace element and belongs to the halogen group (fluoride, chlorine, iodine and bromine) and it exists only in combination with other elements as a fluoride compound. This trace element fluoride is present in the body almost entirely in bone and teeth **(Ekstrand et al., 1988)**. The fluoride's best-known effect is to serve as an aid for both the mineralization of developing tooth enamel prior to tooth eruption and for remineralization of surface enamel. The combination of these fluoride effects greatly reduces occurrence of dental caries **(Inoue et al., 2005)**.

Secondary caries usually caused by lactic acid formation produced by bacteria which lead to low PH . commomly by streptococcus mutans and lactobacilli .material with high fluoride releases in acid condition of benefit in inhibition caries around restorations .but high scale study need to estimate if the glass filler with polycaroxlyase cement give an increase fluoride release if exposed to acid media. But the type of glass filler in different products may affect the releasing of fluoride and its antibacterial activity **(Toshiyuki et al 2005) .**

The A number of approaches have been used to evaluate the antimicrobial
effectiveness in the laboratory. These include; incubation of broth cultures of the selected microorganisms with the agent under the test, growth of the selected microorganisms as lawns on agar surfaces using the disc diffusion method, and the artificial infection of extracted teeth with the selected organisms **(Ayhan et al 1999).**

The following results was gain from this in vitro study:
1-Antibacterial activity of polycarboxylate cement and polycarboxylate cement reinforced by different amounts of fluoroapatite against streptococcus mutans and lactobacilli Bacteria.
The result of this study showed that the Polycarboxylate cement and polycarboxylate reinforced by different ratios of fluoroapatite have strong antibacterial activity against streptococus mutans and lactobasili bacteria ,thus the fluoroapatite will increase antibacterial activity, also the results showed that the increase the ratio of fluoroapatite will statistically effect on their antibacterial activity.

2-Antibacterial activity of polycarboxylate cement and polycarboxylate cement reinforced by different amounts of Calcium fluoride against streptococcus mutans and lactobacilli Bacteria.
The result of this study showed that the Polycarboxylate and polycarboxylate reinforced by different ratios of fluoroapatite have strong antibacterial activity against streptococcus mutans and lactobacilli Bacteria but the addition of the 20%, and 25% will not increase antibacterial activity as compared with lower ratio This results can be explained by the antimicrobial activity of Polycarboxylates and Polycarboxylate reinforced by different amounts of fluoroapatite is assumed to rely on fluoride ion release, the quantities of fluoride released may be sufficient to reach a concentration that effectively kills microorganisms (Yoshimine *et al*.2003). Toshiyuki et al in 2005) showed that in the early 10 weeks, the fluoride containing cement give a greater fluoride release because these materials undergo dissolution of the inorganic fluorides.

Fluoride has various antimicrobial effects; it may exert an effect on bacterial adhesion which is essential if a bacterial population is to colonize the tooth**(Rolla, 1977**). A major effect for fluoride on oral bacteria is in modification of metabolism. Fluoride will reduce the ability of plaque bacteria to produce acid and reduce carbohydrate metabolism. Fluorides can inhibit the process of glycolysis by interfering with the enzyme enolase. Also, the first intermediate of the glycolytic pathway can rapidly be inhibited by fluoride which affects the glucose transport system**(Geddes et al., 1993**). Another antimetabolic effect of fluoride is its reducing effect on intra and extra cellular polysaccharides**(Hamilton et al., 1988).**

The use of fluoride containing products has been the major factor for reduction of caries prevalence. there is overwhelming evidence that the primary caries preventive mechanisms of action of fluoride are post eruptive topical effects for both children and adults **(Featherstone et al., 1999)**. **Formon et al. 2000**, suggested that the predominant beneficial effect of fluoride occur locally at the tooth surface and that systemic effect of fluoride are of less importance as fluoride incorporated during tooth development is insufficient to play a significant role in caries protection.

Freedman et al., 2003 concluded that, an initial fluoride "burst" effect is desirable, as it will reduce the viability of bacteria that may have been left in the inner carious dentin and induce enamel/dentin remineralization.

The a pronounced cytotoxic effect in the early phase of the setting reaction of these materials and the strong inflammatory reactions and high toxicity caused by Polycarboxylate is due to the high initial acidity ,the addition of Fluoroapatite and calcium fluoride to Polycarboxylate cement may reduce its cylotoxcity of any living cells and may reduced the inflammatory response of body tissues because these additives is basic compound reduce the initial acidic PH during setting reaction of polycarboxylate cement.

Table 1:- The control and The experimental groupA of this study.

Groups	Ratio of fluoroapatite added to polycarboxylate cement
Group IA (control)	0%
Group IIA	5%
Group IIIA	10%
Group IVA	15%
Group VA	20%
Group VIA	25%

Table 2:- The control and The experimental groupB of this study.

Groups	Ratio of calcium fluoride added to polycarboxylate cement
Group IB (control)	0%
Group IIB	5%
Group IIIB	10%
Group IVB	15%
Group VB	20%
Group VIB	25%

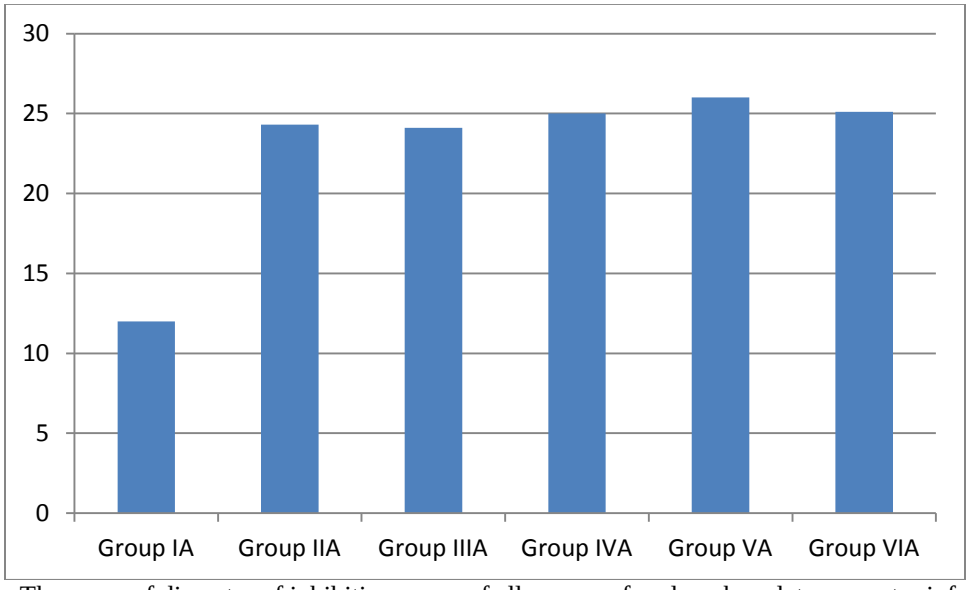

Figure 1:- The mean of diameter of inhibition zones of all groups of polycarboxylate cement reinforced with Fluoroapatite of this study of culture media in the streptococcus mutans

Table 3:- Mean and standard deviation (mm) of diameter of inhibition zones of all groups of polycarboxylate cement reinforced with Fluoroapatite of this study of culture media in the streptococcus mutans .

Groups	**Mean**	**±Sd**
Group IA	12	2.58
Group IIA	16.4	2.01

Group IIIA	15.8	1.62
Group IVA	21.1	2.96
Group VA	22.4	2.17
Group VIA	22.1	2.28

Table 4:- ANOVA test for of diameter of inhibition zones (mm) of all groups of polycarboxylate cement reinforced with Fluoroapatite of this study of culture media in the streptococcus mutans

	Sum of square	D F	Mean square	F	P(value)
Between groups	886.4	5	177.28	33.217	0.000
Within groups	288.2	54	5.337		
Total	1174.6	59			

D.F=degree of freedom P-value=probability

Table 5:- LSD test to compare diameter (mm) of inhibition zones of all groups of polycarboxylate cement reinforced with Fluoroapatite of this study of culture media in the streptococcus mutans.

Comparison (I)Group X (J)Group	Mean differences(I-J)	Significance
Group IA X Group IIA	-4.4	0.000*
Group IA X Group IIIA	-3.8	0.001*
Group IA X Group IVA	-9.1	0.000*
Group IA X Group VA	-10.4	0.000*
Group IA X Group VIA	-10.1	0.000*
Group IIA X Group IIIA	0.6	0.564
Group IIA X Group IVA	-4.7	0.000*
Group IIA X Group VA	-6.0	0.000*
Group IIA X Group VIA	-5.7	0.000*
Group IIIA X Group IVA	-5.3	0.000*
Group IIIA X Group VA	-6.6	0.000*
Group IIIA X Group VIA	-6.3	0.000*
Group IVA X Group VA	-1.3	0.214
Group IVA X Group VIA	-1.0	0.337
Group VA X Group VIA	0.3	0.773

* significant at (P<0.05)

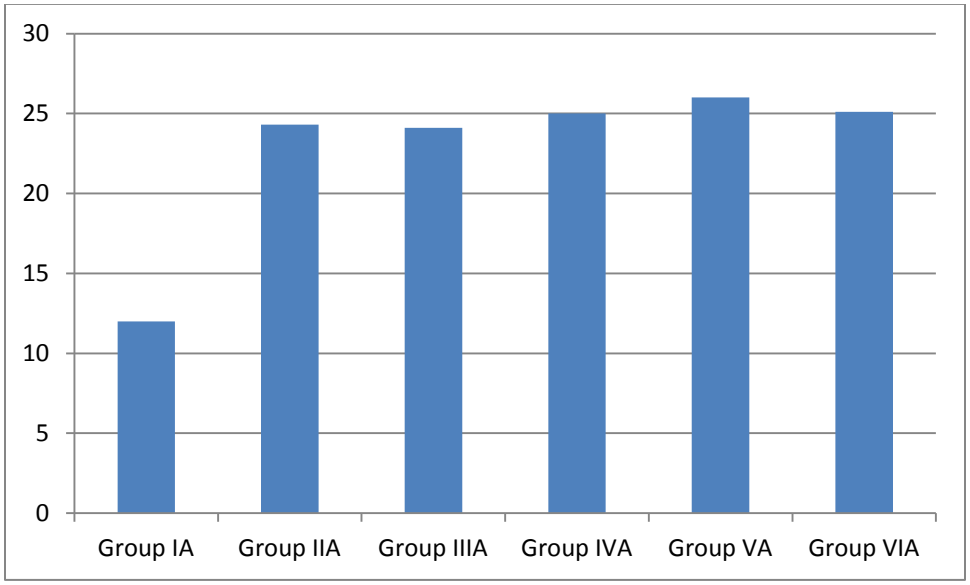

Figure 2:- The mean of diameter of inhibition zones of all groups of polycarboxylate cement reinforced with Fluoroapatite of this study of culture media in the lactobacilli bacteria.

Table 6:- Mean and standard deviation (mm) of diameter of inhibition zones of all groups of polycarboxylate cement reinforced with Fluoroapatite of this study of culture media in lactobacilli bacteria.

Groups	**Mean**	**±Sd**
Group IA	12	2.58
Group IIA	24.3	1.77
Group IIIA	24.1	1.79
Group IVA	25	0.82
Group VA	26	1.15
Group VIA	25.1	

Table 7:- ANOVA test for of diameter of inhibition zones (mm) of all groups of polycarboxylate cement reinforced with Fluoroapatite of this study of culture media in the lacto bacilli bacteria.

	Sum of square	DF	Mean square	F	P(value)
Between groups	1409.35	5	281.87	108.799	0.000
Within groups	139.9	54	2.591		
Total	1549.25	59			

DF.=degree of freedom P-value=probability

Table 8:- LSD test to compare diameter (mm) of inhibition zones of all groups of polycarboxylate cement reinforced with Fluoroapatite of this study of culture media in the lactobaCilli bacteria.

Comparison (I)Group X (J)Group	Mean differences(I-J)	Significance
Group IA X Group IIA	-12.3	0.000*
Group IA X Group IIIA	-12.1	0.001*
Group IA X Group IVA	-13	0.000*
Group IA X Group VA	-14	0.000*
Group IA X Group VIA	-13	0.000*
Group IIA X Group IIIA	0.2	0.782
Group IIA X Group IVA	-0.7	0.335
Group IIA X Group VA	-1.7	0.022*
Group IIA X Group VIA	-0.8	0.271
Group IIIA X Group IVA	-0.9	0.217
Group IIIA X Group VA	-1.9	0.011*
Group IIIA X Group VIA	-1	0.170
Group IVA X Group VA	-1	0.170
Group IVA X Group VIA	-0.1	0.890
Group VA X Group VIA	0.9	0.217

* significant at (P<0.05)

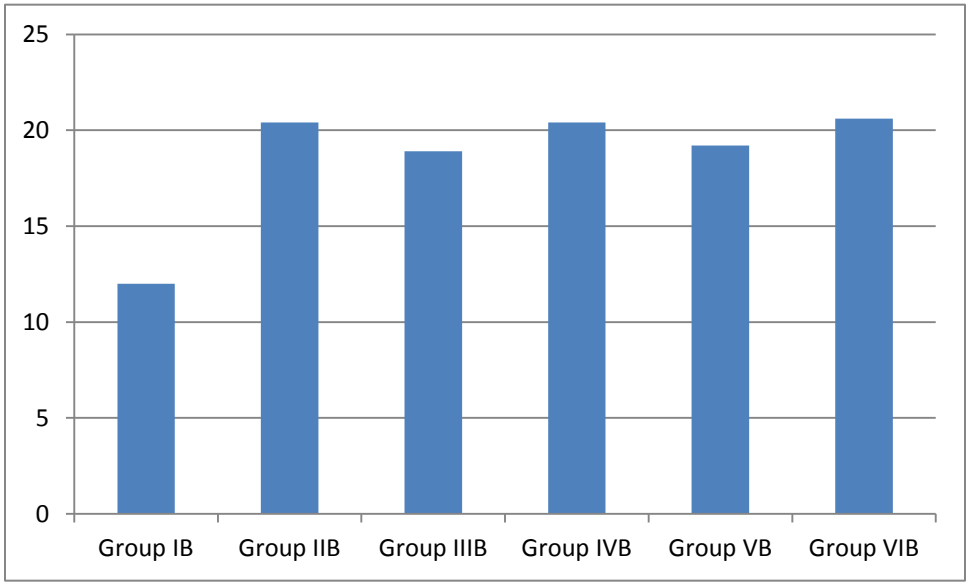

Figure 3:- The mean of diameter of inhibition zones of all groups of polycarboxylate cement reinforced with Calcium fluoride of this study of culture media in the streptococcus mutans

Table 9: Mean and standard deviation (mm) of diameter of inhibition zones of all groups of polycarboxylate cement reinforced with Calcium fluoride of this study of culture media in the streptococcus mutans.

Groups	**Mean**	**±Sd**
Group IB	12	2.58
Group IIB	16.4	2.01
Group IIIB	15.8	1.62
Group IVB	20.3	2.54
Group VB	20,7	1.32
Group VIB	20.5	0.69

Table 10:-ANOVA test for of diameter of inhibition zones (mm) of all groups of polycarboxylate cement reinforced with Calcium fluoride of this study of culture media in the streptococcus mutans

	Sum of square	DF	Mean square	F	P(value)
Between groups	607.573	5	121.515	33.338	0.000
Within groups	196.827	54	3.645		
Total	804.4	59			

DF=degree of freedom P-value=probability

Table 11:-LSD test to compare diameter (mm) of inhibition zones of all groups of polycarboxylate cement reinforced with Calcium fluoride of this study of culture media in the streptococcus mutans..

Comparison (I)Group X (J)Group	Mean differences(I-J)	Significance
Group IB X Group IIB	-4.4	0.000*
Group IB X Group IIIB	-3.8	0.000*
Group IB X Group IVB	-8.3	0.000*
Group IB X Group VB	-8.7	0.000*
Group IB X Group VIB	-8.45	0.000*
Group IIB X Group IIIB	0.6	0.485
Group IIB X Group IVB	-3.9	0.000*
Group IIB X Group VB	-4.27	0.000*
Group IIB X Group VIA	-4.05	0.000*

Group IIIB X Group IVB	-4.5	0.000*
Group IIIB X Group VB	-4.87	0.000*
Group IIIB X Group VIB	-4.65	0.000*
Group IVB X Group VB	-0.37	0.678
Group IVB X Group VIB	-0.15	0.854
Group VB X Group VIB	0.21	0.806

* significant at (P<0.05)

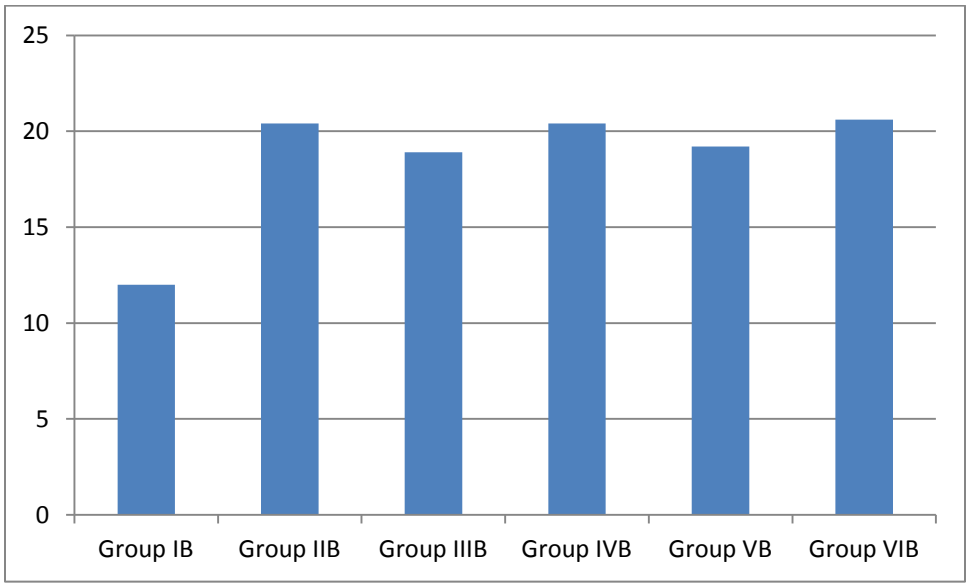

Figure 4:- The mean of diameter of inhibition zones of all groups of polycarboxylate cement reinforced with Calcium fluoride of this study of culture media in the lactobacilli bacteria.

Table 12:- Mean and standard deviation (mm) of diameter of inhibition zones of all groups of polycarboxylate cement reinforced with Calcium fluoride of this study of culture media in lactobacilli bacteria

Groups	**Mean**	**±Sd**
Group IB	12	2.58
Group IIB	20.4	0.699
Group IIIB	18.9	1.197
Group IVB	20.4	1.17
Group VB	19.2	1.2
Group VIB	20.6	0.687

One-way ANOVA test (Table 13) showed that there was statistically significant difference among all the groups at the P value less than 0.01.

Table 13:-ANOVA test for of diameter of inhibition zones (mm) of all groups of polycarboxylate cement reinforced with Calcium fluoride of this study of culture media in the lactobacilli bacteria.

	Sum of square	DF	Mean square	F	P(value)
Between groups	550.382	5	110.076	55.140	0.000
Within groups	107.801	54	1.996		
Total	658.183	59			

DF=degree of freedom P-value=probability

Table 14:- LSD test to compare diameter (mm) of inhibition zones of all groups of polycarboxylate cement reinforced with Calcium fluoride of this study of culture media in the lacto bacilli bacteria.

Comparison	Mean differences(I-J)	Significance
(I)Group X (J)Group		

Group IB X Group IIB	-8.4	0.000*
Group IB X Group IIIB	-6.9	0.000*
Group IB X Group IVB	-8.4	0.000*
Group IB X Group VB	-7.2	0.000*
Group IB X Group VIB	-8.64	0.000*
Group IIB X Group IIIB	1.5	0.021*
Group IIB X Group IVB	0.0	1
Group IIB X Group VB	1.18	0.075
Group IIB X Group VIA	-0.24	0.703
Group IIIB X Group IVB	-1.5	0.021*
Group IIIB X Group VB	-0.32	0.622
Group IIIB X Group VIB	-1.74	0.007*
Group IVB X Group VB	1.18	0.075
Group IVB X Group VIB	-0.24	0.703
Group VB X Group VIB	-1.41	0.03*

* significant at ($P<0.05$)

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