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RESEARCH ARTICLE

EFFECT OF ALOIN (ALOEVERA EXTRACT) ON THE LEVELS OF PORPHYROMONAS GINGIVALIS AND AGGREGATIBACTER ACTINOMYCETEMCOMITANS IN CHRONIC GENERALIZED PERIODONTITIS: A CLINICAL & MICROBIOLOGICAL STUDY.

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Key words:-
Aloin gel(Aloevera extract), Scaling and root planing, Local drug delivery, Chronic Generalized Periodontitis.

Abstract

Background: Scaling and root planing is the basic treatment modality for periodontal disease. However, mechanical treatment is limited by physical impediments and biochemical considerations. Herbal agents may be used as an adjunct to overcome limitations of mechanical therapy. Thus the aim of this research was to evaluate the effect of Aloin, (aloevera extract) as a local drug delivery (LDD). LDD was used as an adjunct to scaling and root planing (SRP). Study was aimed to compare the clinical and microbiological effect of Aloin on the levels of Porphyromonas gingivalis (P.gingivalis) and Aggregatibacter actinomycetemcomitans (A.A comitans) in the treatment of chronic generalized periodontitis (CGP).

Methods: Thirty chronic generalized periodontitis patients with 90 sites were selected including both males and females in the age group of 25–55 years. Three groups were made, 30 sites in each group were assigned. Group A was treated with aloin alone as LDD. Group B was treated with SRP and Aloin, whereas Group C was treated with SRP alone. Plaque sample was collected on baseline, 15^{th}, 30^{th} day for quantitative and qualitative analysis of P.gingivalis and A.A comitans.

Results: There was a statistically significant improvement in all clinical and microbiological variables, including plaque and gingival index. However greatest improvement was evident in Group B in terms of reduction in colony forming units(CFU) of P.gingivalis and A.A comitans.

Conclusion: Aloin as LDD is a valuable adjunct to SRP in the treatment of chronic generalized periodontitis.

Introduction:-

Periodontitis is an inflammatory disease of the supporting tissues of the teeth caused by specific micro-organisms, resulting in progressive destruction of the attachment apparatus.[1] Interaction of these etiological agents with host defence is an important determinant of the onset and progression of the disease.[1] Predominance of anaerobic bacteria as perio-dontopathic agents have led to new treatment strategies aiming primarily at their suppression or elimination of periodontal diseases.[2]

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Mechanical debridement though established as the gold standard in the treatment of periodontal diseases may not eliminate the anaerobic infections at the base of pocket.[2] Additionally, recolonization of bacteria occurs from the residual reservoirs causing relapse of inflammatory status.[2] Local drug delivery acts as an adjunct in the treatment of periodontal diseases have resulted in a number of site-specific, controlled-release methods that maintains effective intra-pocket levels of antibacterial agents for extended periods of time.[3] LDD alters sub-gingival flora and influences the healing of an attachment apparatus as it offers the advantage of high concentrations delivered at the target sites, reduced systemic doses, fewer applications, and high patient acceptability[4]. Thus LDD therapy is established as simple, rapid and non-invasive treatment modality[4]. Tetracyclines hydrochloride, doxycycline, minocycline, metronidazole and chlorhexidine are established chemically based local drug delivery agents.[4]

Herbal drugs act as a renewable, non toxic and economic alternative to synthetic agents. Recent researches focused on herbs in dentistry establish that Aloevera, Turmeric gel, Centella asiatica, Azadirachta indica (neem), Triphala are affective against anaerobic and facultative organisms.[4]

Aloevera barbadensis, a popular houseplant has a long history as a multipurpose folk remedy.[5] Aloin, which is an Aloe vera extract is a source of 19 out of 20 essential amino acids which help in smooth functioning of our complex enzyme systems. Additionally its a rich source of source of vitamins A, B, C, E, and folic acid. Aloevera plant grows in mineral rich soil, and thus is an excellent source of calcium, sodium, potassium, magnesium, iron, copper and zinc.[5]

Richard L Wynn in 2005,reported anti-inflammatory effect of aloevera extract (LDD) on periodontal tissues.[6] Since SRP alone cannot predictably eliminate these tissue invasive micro organisms, this clinical study compared the clinical and microbiological effect of Aloin gel, aloevera extract (LDD) as an adjunct to SRP in the treatment of chronic periodontitis.

Materials and Methods:-

This clinical study was conducted in the Department of Periodontics and Implantology. 30 patients including both, males and females in the age group of 25-55 years, with chronic periodontitis, with probing depth of >5 mm and radiographic evidence of bone loss were included in the study. However, patients with poor oral hygiene, smokers, and systemically compromised, pregnant and lactating mothers, history of periodontal therapy in past 6 months were excluded from the study

After selection of the subjects, written informed consent, explaining the nature of the study design and procedure for local drug delivery were obtained from the patient. Three groups were made, 10 patients with 3 sites in each patient i.e a total of 30 sites were assigned for each group. Group A was treated with aloin gel alone as local drug delivery agent, Group B was treated with SRP and aloin gel and Group C with only SRP. Plaque samples were collected at 0 (baseline), 7^{th} and 30^{th} day for quantitative and qualitative analysis of P.gingivalis and A.A comitans. Re-application of aloin gel was done on 7^{th} and 15^{th} day.

After the placement of aloin gel, the treated sites were given periodontal pack to isolate the area and to restrict the effect of aloin gel to particular sites for at least a week. The patient was then instructed not to use any other anti plaque agents other than normal brushing and rinsing. Patients were instructed not to floss and probe the area with tongue, finger or toothpick; report immediately, if the periodontal pack is dislodged before the scheduled recall visit or if any pain, swelling or irritation occurs.(Fig 1)
Aloin gel was stored in a dry place with a shelf life of 3 years, manufactured by Neelkant pharmaceutics LTD, Udaipur Rajasthan (India).

Periodontal status assessment

Clinical parameters, including Plaque (PI) (Silness. P and Loe. H), Gingival index (GI) (Loe. H and Silness. P), Periodontal Pocket depth (PPD) and Relative attachment level (RAL) were recorded at (baseline) 0^{th}, 7^{th} and 30^{th} day. A custom made acrylic stent and UNC-15 periodontal probe was used to standardize the measurement of PPD and RAL.

Collection of samples
Samples were collected aseptically. Sites were isolated with cotton rolls, carefully cleaned with sterile cotton pellets, and air-dried before two sterile paper points were inserted to the bottom of the pocket for a 20-seconds. Then they were transferred to thioglycollate medium.

Microbiological examination
The samples were processed within 24 hours for isolation of strict anaerobes, and were placed on non-selective blood agar plates (5%) supplemented with hemin and menadione. Kanamycin-Vancomycin blood agar plates were used for selective growth of obligate anaerobic gram-negative rods. The plates were incubated in vacuum desiccators at 37ºC under anaerobic conditions for 7 days. Then colonies with differing characteristics were subjected to various tests. Identification was based on cell morphology, gram stain reaction, biochemical and enzymatic tests including Indole test, Methyl red test, Voges- proskeur test, Citrate test, Carbohydrate fermentation test, Gas production test (H_2s), Catalase test, and Protease activity.(Fig 2)

Statistical analysis:
The recorded data was compiled and entered in a spread sheet computer program (Microsoft Excel 2007) and then exported to data editor page of SPSS version 20.0 (SPSS Inc., Chicago, Illinois, USA). Intergroup comparison was done using One way ANOVA test for quantitative data (mean values) followed by Post hoc test (Tukey's test) for intra-group comparison. The level of significance was set at 0.05.

Results:-

Plaque & Gingival Index (PI & GI)
PI and GI scores of Groups A, B & C at baseline revealed no significant difference among the three groups, however at 15^{th} and 30^{th}day both the scores revealed highly significant difference between group B and other two groups. Additionally, there was no significance difference observed in Group A and C, respectively. (Graph1)

Periodontal Pocket Depth & Relative attachment level
Comparative analysis of periodontal pocket depth and relative attachment level in Groups A, B & C at baseline revealed no significant difference among the three groups. At 30^{th} day P value of 0.001 and P value of 0.004 was reported for PPD and RAL respectively, indicating highly significant difference between group B and other two groups, whereas no significance difference was observed in Group A and C respectively.(Table 1) (Graph 2)

Colony forming units of P. gingivalis
Comparative analysis of Groups A, B & C at baseline revealed mean scores of 223.16, 174.5 and 176.2 respectively with p value of 0.075 indicating no significant difference among the groups. At 7^{th} day mean values were 83.7, 48.16 and 81.8 with p value of 0.008 indicating significant difference between group B and other two groups, whereas there was no significance difference observed in Group A and C respectively. At 30^{th} day the values showed a mean of 11.0, 0.73 and 14.7 with p value of 0.001 indicating no significant difference among the groups. (Table 2)(Graph 3)(Fig 3)

Colony forming units of A.A comitans
Comparative analysis of Groups A, B & C at baseline revealed mean scores of 213.8, 164.33 and 193.43 respectively with p value of 0.216 indicating no significant difference among the groups. At 7^{th} day mean values were 87.56, 40.3 and 82.76 with p value of 0.005 indicating significant difference between group B and other two groups, whereas there was no significance difference observed in Group A and C respectively. At 30^{th} day the values showed a mean of 16.36, 1.43 and 22.3 with p value of 0.001 indicating no significant difference among the groups respectively. (Table 2) (Graph 3)(Fig 4)

Thus management of periodontal disease could be done most effectively by using Aloin gel (Group B) as an adjunct and scaling and root planing.

Discussion:-

The prevalence and severity of periodontal disease can be reduced by eliminating pathogenic microflora using either systemic or locally applied anti-bacterial agents as an adjunct to SRP.[1]

Herbosomes are recently introduced herbal formulations that have better bioavailability, low toxicity and better theurapetic actions than the conventional botanical extracts.[7] Aloin, (10-glucopyranosyl-1,8-dihydroxy-3-hydroxymethyl-9(10H)-anthracenone), an active compound obtained from *Aloevera*, which is known as miracle herb. Aloin when undergoes semi-synthetic modifications shows better antimicrobial activity than the unmodified one.[8]

The inner gel of aloevera leaf from which aloin is obtained is surrounded by polysaccharides, which are able to defend our body from various infections.[5] The extracts of gel have an inhibitory action on the arachidonic acid pathway via cyclo-oxygenase (COX) inhibiting inflammation.[5] Aloin is proved to be beneficial in periodontal treatments, by increasing the activity of fibroblasts, additionally it improves healing by increasing the blood circulation, and oxygenation. Aloe vera gel has an active growth substance, as mannose-6-phosphate.[5]

Thus, this clinical study was aimed to determine the clinical and the microbiological efficacy of Aloin gel in the treatment of patients with periodontitis. The clinical parameters were recorded at the baseline (0 day), 15th and 30th day; and microbiological parameters at 0, 7th and 30th. This follow-up period also permits for both epithelial and connective tissue healing and to assess the rebound phenomenon after various treatments advocated in this study.

PI and GI scores in all the three groups showed a statistically significant improvement over a duration of 30 days. While comparing from baseline to 30th day; PI in Group A improved by 76.69%, in Group B by 86.2% and 75.74% in Group C; While comparing GI from baseline to 30th day a improvement in Group A was 59.28% in Group B was 76.66% and in Group C was 60% indicating a more significant reduction in Group B when compare to other two groups. However, it was observed that no statistically significant difference existed among the groups for both the scores.

Probing pocket depth (PPD) in all the three groups showed a statistically significant improvement over a duration of 30 days. The mean pocket depth reduction on comparing from baseline to 30th day in Group A;(32.45%) Group B;(49.73%) Group C;(31.63%) indicating a statistically highly significant reduction in Group B when compare to other two groups.

Relative attachment level (RAL) in all the three groups showed a statistically significant improvement over a duration of 30 days. The mean Relative attachment level on comparing from baseline to 30th day a in Group A;(1.80%) Group B;(16.48%) Group C; (0.48%) indicating a statistically significant increment in Group B when compare to other two groups respectively.

Similar findings were observed in the studies conducted by Bhatt G, Kudva in (2011)[9] and Virdi HK **(**2012).[10]

Microbial parameters were assessed by collection of plaque sample on 0 (baseline), 7th and 30th day for quantitative and qualitative analysis of P.gingivalis and A.Acomitans**.** On comparison of P.Gingivalis from baseline to 7th day a mean change in all the groups reveal Group A;(62.49%) Group B; (72.40%) Group C;(53.57%) indicated a statistically significant reduction in Group B when compared with the other two groups. On comparing from baseline to 30th day a mean change in Group A; (95%) Group B;(99.58%) Group C; (91.6%) indicated, no significant reduction among the groups.

On comparison of A. A comitans from baseline to 7th day a mean change in all the groups reveal Group A; (59.04%) Group B;(75.47%) Group C;(57.21%) indicated a statistically significant reduction in Group B when compared with the other two groups. On comparing from baseline to 30th day a mean change in Group A;(92.34%) Group B;(99.12%) Group C;(88.47%) indicated, no significant reduction among the groups respectively.

There was statistically significant reduction found in the colonies of P.Gingivalis and A.A.comitans in Group B when compared to the other groups, on comparing from baseline to 7th day and on comparing from 7th to 30th day, there was no significant reduction found among the groups respectively.
At the end of 30th day the recolonization of micro-organism was observed ,this is due to the lack of bioavailability of the drug because the efficacy of aloin gel in different concentrations were analysed on pure culture of P.gingivalis and A.Acomitans ATCC 33277 using blood agar medium under strict anaerobic conditions. The zone of inhibition was observed for 10 days and were maintained upto 7 days. After that, effectiveness of aloin gel reduced

significantly. It is advisable that during treatment, the gel should be administered in seven days interval for the inhibition of P.gingivalis and A.Acomitans

Thus as compared with SRP alone, there were greater benefits in terms of reductions in PPD, gains in RAL, and reductions in levels of P.gingivalis, and A.Acomitans when aloin was used as an adjunct to SRP.

The possible limitations of this study were small sample size and 'carry-cross' effects, which precludes use of this design for equivalence trials because of potential contamination across the randomized segments.

Conflicts of interest
No conflict of interest.

Table 1:-Comparison between the outcomes of different treatment regimes on the basis of Pocket depth and Clinical attachment level.

	Pocket Depth				**Clinical attachment level**			
Interval	Group A (Mean±SD)	Group B (Mean±SD)	Group C (Mean±SD)	P value	Group A (Mean±SD)	Group B (Mean±SD)	Group C (Mean±SD)	P value
0 day	6.07±1.2	5.63±0.99	6.10±1.21	0.216	14.4±2.1	13.53±1.25	14.4±2.23	0.136
30th day	4.1±1.15[a]	2.83±1.36[b]	4.17±1.14[a]	0.001**	14.66±2.22[a]	15.76±1.16[b]	14.33±1.88[a]	0.004*
Significance 0 day v/s 30th day	t=19.372; p≤0.001**	t=34.106; p≤0.001**	t=18.154; p≤0.001**	-	t=10.269 p≤0.001**	t=10.269; p≤0.001**	t=11.564; p≤0.001**	-

*p≤0.05 (Significance); **p value≤0.001 (Highly significant)

Table 2:-Comparison between the outcomes of different treatment regimes on the basis of Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans counts.

Interval	**Porphyromonas gingivalis**				**Aggregatibacter actinomycetemcomitans**			
	Group A	Group B	Group C	P value	Group A	Group B	Group C	P value
0 day	223.16±86.32	174.5±86.92	176.2±103.21	0.075	213.8±113.5	164.33±93.43	193.43±116.97	0.216
7th day	83.7±48.43[a]	48.16±52.46[b]	81.8±44.08[a]	0.008*	87.56±60.66[a]	40.3±47.43[b]	82.76±70.52[a]	0.005*
30th day	11.0±9.24[a]	0.73±1.48[b]	14.7±11.38[a]	0.001**	16.36±17.3[a]	1.43±2.32[b]	22.3±25.1[a]	0.001**
Significance 0 day v/s 7th day	t= 9.208; p≤0.001**	t=9.587; p≤0.001**	t=7.930; p≤0.001**	-	t= 8.490; p≤0.001**	t=7.685; p≤0.001**	t=8.195; p≤0.001**	-
Significance 0 day v/s 30th day	t=13.694; p≤0.001**	t=8.621; p≤0.001**	t=8.949; p≤0.001**	-	t=9.879; p≤0.001**	t=7.557; p≤0.001**	t=8.648; p≤0.001**	-

*p≤0.05 (Significance); **p value≤0.001 (Highly significant)

Figure 1 a:-Collection of subgingival plaque sample using sterile paper points.
Figure 1 b:-Placement of aloevera gel in between 36 & 37
Figure 1 c:-Periodontal dressing placed

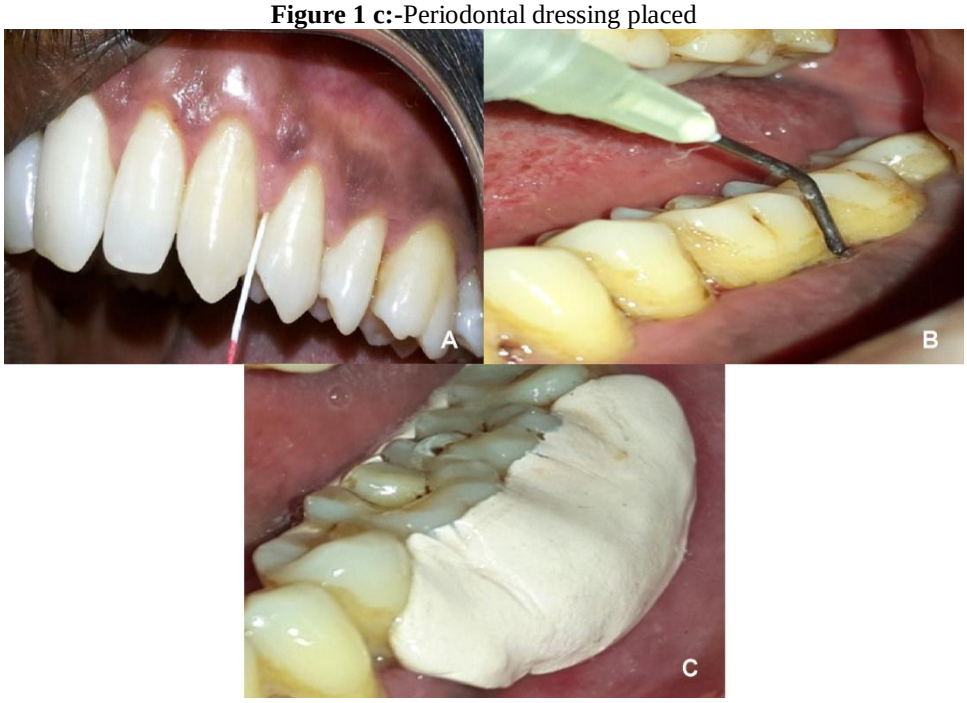


Figure 2 a:-Incubator
Figure 2 b:-Vacuum desiccator
Figure 2 c:-Thioglycollate media
Figure 2 d:–Petridish

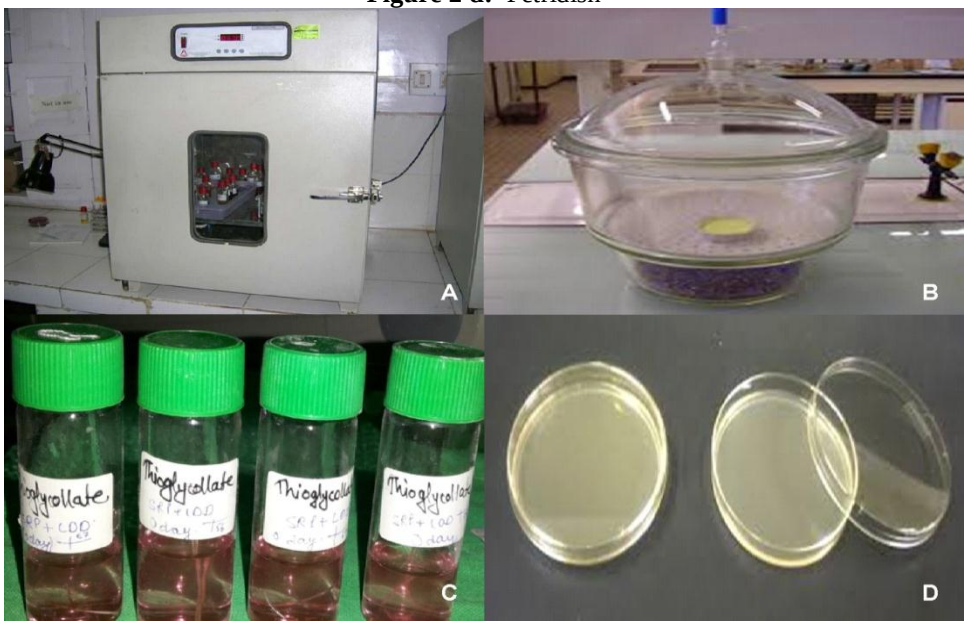


Figure 3:-Biochemical test for confirmation of P.Gingivalis,Microbial culture at baseline,Microbial culture at 7th day and Microbial culture at 30th day

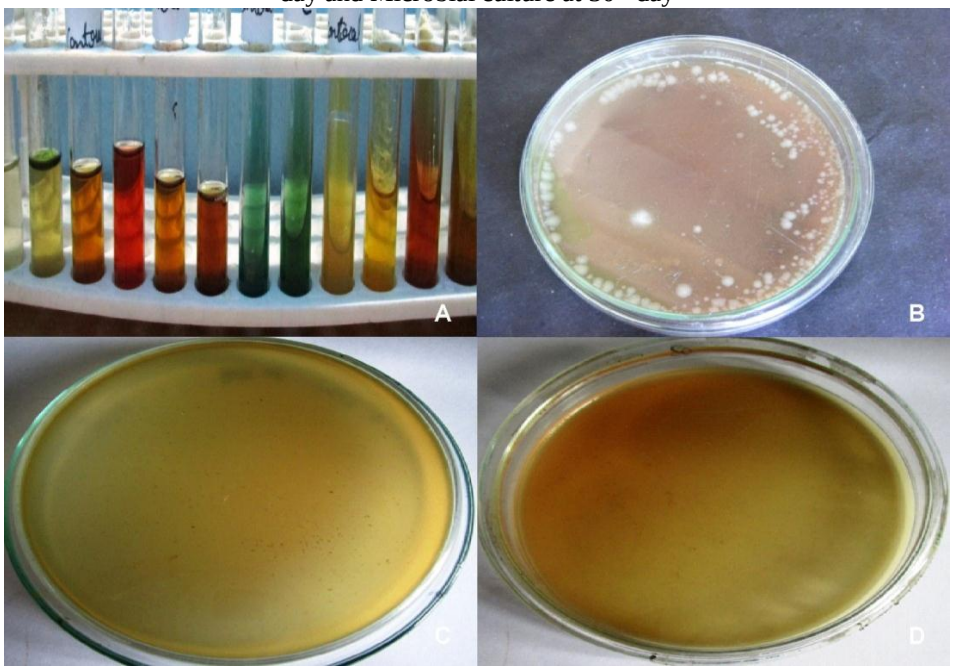
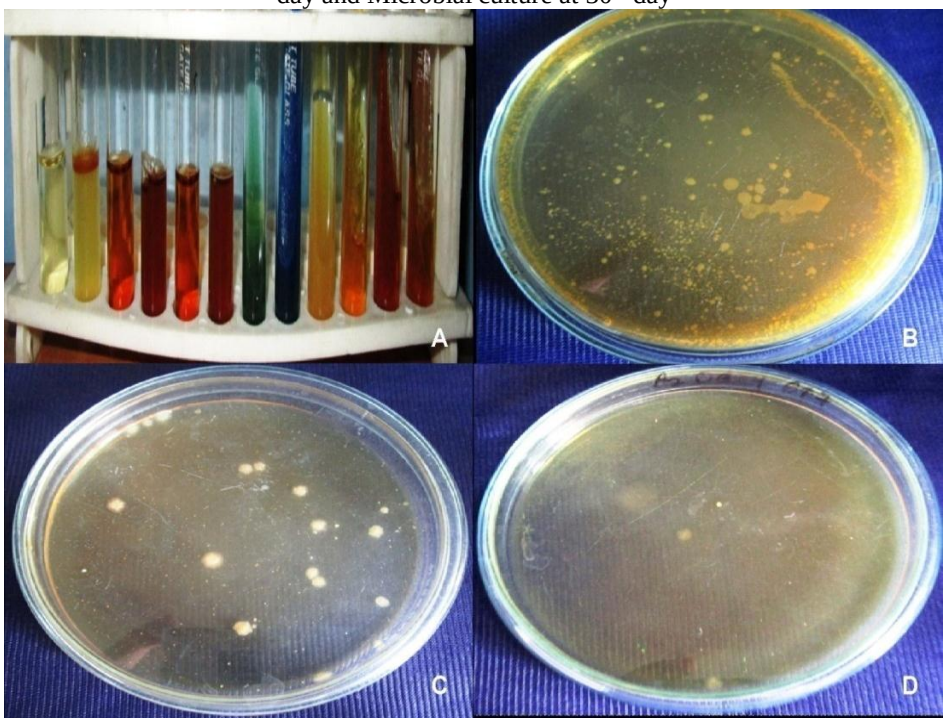
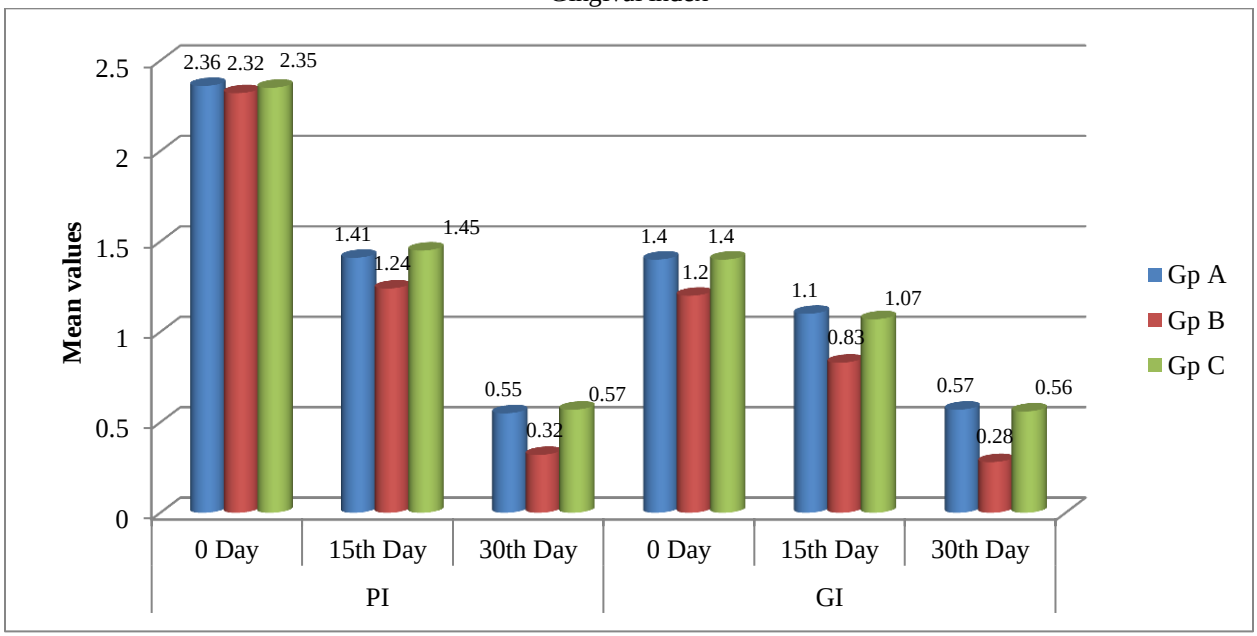


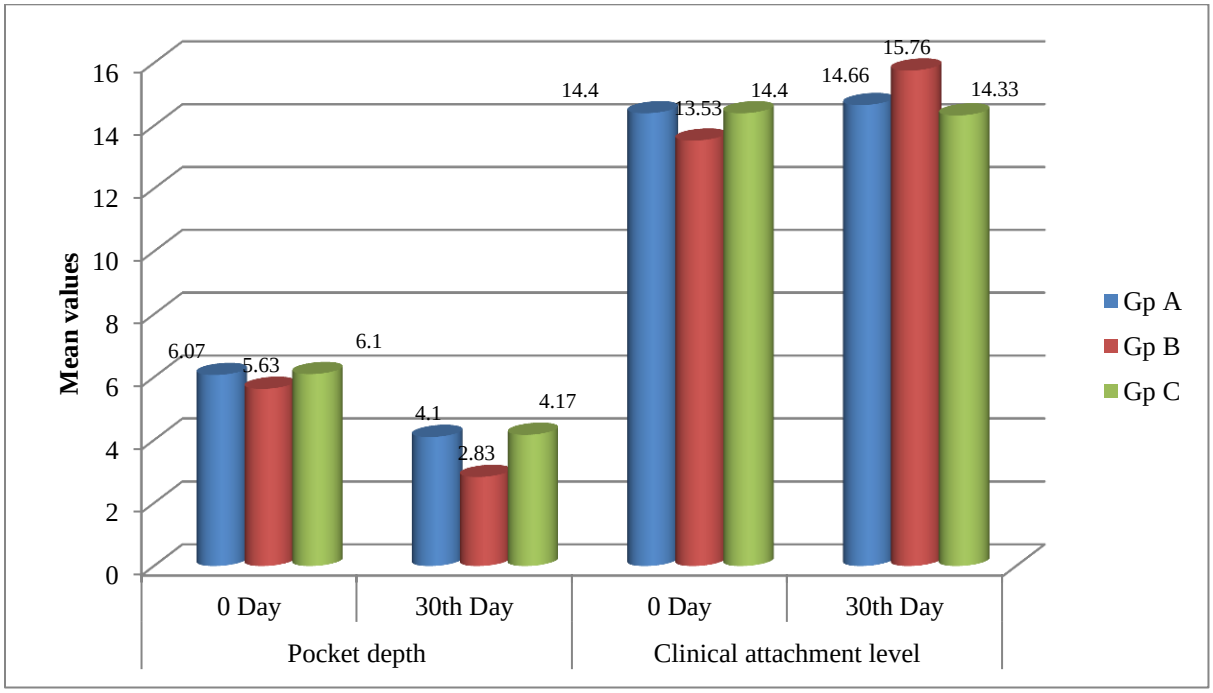
Figure 4:-Biochemical test for confirmation of A.Comitans, Microbial culture at baseline, Microbial culture at 7th day and Microbial culture at 30th day



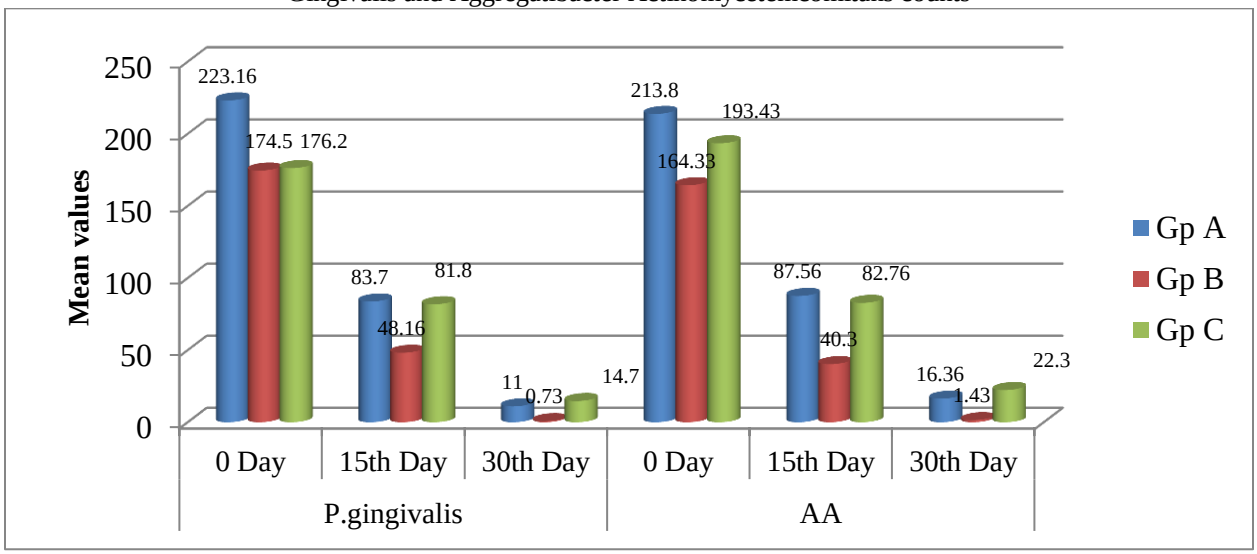
Graph 1:- Comparison between the outcomes of different treatment regimes on the basis of Plaque index & Gingival index



Graph 2:-Comparison between the outcomes of different treatment regimes on the basis of Pocket depth & Clinical attachment level



Graph 3:-Comparison between the outcomes of different treatment regimes on the basis of Porphyromonas Gingivalis and Aggregatibacter Actinomycetemcomitans counts



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