Section A-Research paper

A microbiological study to evaluate the effect of different concentrations of coenzyme q10 in inhibiting key pathogens of periodontitis

First Author: Dr. Anukrati Katariya
Post graduate Student
Department of Periodontology
Teerthanker Mahaveer Dental College and Research Centre.
Phone no: 7798689689
Email Id: anukratikatariya@gmail.com
Second Author: Dr. Bhavna Jha Kukreja
Preventive Dental Sciences Department,
College of Dentistry,
Gulf Medical College University,
Ajman, UAE.
Phone no: +971583092899
Email Id: dr.bhavna@gmu.ac.ae
Third Author: Prof. S.C. Dinda
Professor
College of Pharmacy
Teerthanker Mahaveer University
Phone no: 8249036854
Email Id: subasdinda@gmail.com
Fourth Author: Dr. Shruti Singh
Post graduate Student
Department of Periodontology
Teerthanker Mahaveer Dental College and Research Centre.
Phone no: 9838007447
Email Id: singhshruti928@gmail.com
Fifth Author: Dr. Kishore G. Bhat
Director,
Maratha Mandal's Central Research Laboratory,
Maratha Mandal's NHG Institute of Dental Sciences & Research Centre
Phone no: 08312477682
Email Id: drkgbhat@yahoo.com
Corresponding author: Dr. Bhavna Jha Kukreja
Preventive Dental Sciences Department,
College of Dentistry,
Gulf Medical College University,
Ajman, UAE.
Phone no: +971583092899
Email Id: dr.bhavna@gmu.ac.ae

ABSTRACT:

Purpose: The aim of this study is to test the efficacy of three different concentrations of cones of coenzyme Q10 on the activity of key periodontal pathogens.

Material and methods: CoQ10 cones of three concentrations i.e. 1%, 2% and 3% were formulated using combination of different polymers and their microbiological activity were tested on four periodontal pathogens including P. gingivalis, A. actinomycetemcomitans, P. intermedia, and T. forsythia. Zone of inhibition and MIC were performed. Statistical analysis was done for intergroup comparison.

Results: All three concentrations showed effectiveness against the periodontal pathogens with maximum zone of inhibition shown by 2% cone. There was statistically significant difference observed with 2% cone when compared to other groups.

Conclusion: The study concluded that all three concentrations coenzyme Q10 are effective in the non surgical treatment of chronic periodontitis.

KEYWORDS: Chronic Periodontitis, Coenzyme Q10, Minimum Inhibitory Concentration, Periodontal Pathogens, Zone of Inhibition.

1. INTRODUCTION: Periodontal disease is defined as a destructive process where specific microorganisms cause inflammation in the supporting tissues around the teeth like gingiva, bone and pdl leading to the advanced destruction of these tissues along with formation of gingival recession and pocket formation or both. [1] Superoxide pathogenic microorganisms produce ROS that are capable of disrupting cell membranes and related biomolecules, causing collagen and periodontal cell breakdown. [2]

In the diseases affecting periodontium, the choice of antimicrobial agents should depend on the bacterial etiology causing the disease. Quite a few antibiotics agents have indeed been studied in periodontal disease for both clinical as well as microbiological effectiveness. It has been found that so far only a few numbers of antimicrobial agents have been used in local delivery formulations. [3] One of such agent is Coenzyme Q10.

A person's diet/ food impacts his/her body and its tissues. A naturally found quinine - Coenzyme Q10 (CoQ10 or ubiquinone) acts as a lipid antioxidant, preventing the formation of ROS. Free radicals can be neutralized by antioxidants like CoQ10, which can help mitigate or even eliminate the caused damage. CoQ10 strengthens the immune system by boosting vitality and protects the body from free radicals. [4]

There have been several clinical trials including the oral delivery of CoQ10 to periodontal disease patients. According to the findings, oral administration of CoQ10 results in high concentrations of CoQ10 in disease ridden gingiva and efficiently suppresses progressed periodontal inflammation. [5] Hence, present microbiological study was done to compare and assess the impact of different concentrations of Coenzyme Q10 in the cure of chronic periodontitis.

2. MATERIAL AND METHODS:

The study was approved by local ethical committee of Teerthanker Mahaveer Dental College (ref no. TMDCRC/IEC/19-20/PERIO2)

2.1. Cone Formulation:

Cones were formulated using combination of polymers such as Ethyl cellulose (60%), carbopol (20%), carboxymethyl cellulose (10%), guar gum (5%), sodium alginate (5%), coenzyme Q10 (1%, 2%, 3%), and water. Drugs and polymers were mixed together to form a dough and cones were made using the mixture. (Figure 1)

2.2. MIC Test [6] (Figure 2)

1. For MIC, each medication were diluted nine times in Thio-glycollate broth.

2. First tube: 20 microliters of medication mixed with 380 microliters Thioglycollate broth.

3. For diluted sample, 200 microliters Thioglycollate broth were added individually to the following 9 tubes.

4. Then, 200 microliters were moved to second tube, which contained 200 microliters of Thioglycollate broth. This was regarded as a 10-1 dilution.

5. To create a 10-2 dilution, 200 microliters of 10-1 diluted tube were transferred to a second tube.

6. For every drug, the dilution was performed up for 10-9 times.

7. 5 microliter collected from the maintained stock cultures of the needed organisms and added to 2ml Thioglycollate broth.

8. Every serially diluted tubes received 200 microliters of the aforesaid culture solution.

9. The tubes were cultured in an anaerobic jar at 37°C for 48-72 hours and their turbidity was measured.

2.3. Zone of Inhibition Test Procedure: [6] (Figure 3)

01) Media Used: - Blood agar

02) Temperature: - Before using agar plates, bring them to room temp.

03) Inoculum preparation: - a. Move the colonies to the plates with a loop or brush. b. Adjust the turbidity with broth to match that of a vortexed 0.5 McFarland turbidity standard. Alternatively, use a photometric instrument to standardize the suspension.

04) a. in 15 min of incorporating the inoculum to McFarland 0.5 turbidity standard, put a sterile cotton swab in inoculum & swirl it alongside the tube wall over the liquid removing excess inoculum. b. To achieve uniform dispersion, swab the whole surface of the agar plate 3 times, turning plates about 60º b/n streaks. Avoid making aerosols by striking the sides of the petriplate. c. Allow at least 3 minutes, but no more than 15 minutes, for the infested plate to set before creating wells.

05) Stock solution preparation: - Create the stock solution by dissolving 10mg of chemical in 1ml of DMSO.

06) a. Heat the 5mm hollow tube. Press it on top of the infected Agar plate and quickly remove it by forming a well-like space in plate. Similarly, form five spaces on every plate. b. Using micropipette, add 75µl, 50µl, 25µl, 10µl and 5µl in each well.

07) Incubation: - a. Plates should be incubated within 15 minutes following chemical administration. b. Plates should be inverted and stacked and over 5 high. c. Incubate for 18-24 hours at 37 degrees Celsius in an incubator.

08) Reading plates: - a. Only read plates if the field of growth is concurrent or almost concurrent. b. Holding the measuring equipment, measure the diameter of the inhibitory zone to the closest full millimeter.

2.4. Statistical analysis:

The current study's data was imported into Excel Spreadsheets 2007 and analyzed with the SPSS statistical programme 19.0 Version. Mean and standard deviation were among the descriptive statistics. The threshold of significance for the current study was set at 5%. The Kruskal-Wallis test was used to do an intergroup comparison for the difference in mean scores among independent groups.

3. RESULTS:

Two microbiological tests i.e. minimum inhibitory concentration test (Figure 1) and zone of inhibition test (Figure 2) were done to check the efficacy of cones on Prevotella Intermedia, Porphyromonas Gingivalis, Aggregatibacter Actinomycetemcomitans, and Tannerella forsythia.

These groups of microorganisms are high risk periodontal pathogens that are prevalent in periodontitis.

3.1. MIC: The results have shown that all three concentrations of cones i.e. 1%, 2% and 3% are sensitive to periodontal pathogens at different dilutions. Sensitive (S) means that the pathogen is found to inhibit by the drug's serum concentration attained with the usual dosage; and resistant means that the pathogens are resistant (R) to the drug's serum concentration attained with the usual dosage (Table 1).

In Aa 70 percent were sensitive in 1% group, 90 percent in the 2% and 90% in the 3%. Discrepancy b/n groups was statistically non-significant at p less than 0.05 is significant. In Pg 40 percent were sensitive in 1% group, 50 percent in the 2% and 60% in the 3% group. Discrepancy b/n groups was statistically non-significant at p less than 0.05 is significant. In Pi 90 percent were sensitive in 1% group, 1000 percent in the 2% and 3% . Discrepancy b/n groups was statistically non-significant at p less than 0.05 is significant. In Tf, 50 percent were sensitive in 1% group, 60 percent in the 2% and 80 percent in 3% group . The difference between the groups was statistically non-significant at p less than 0.05 is significant (Table 2).

3.2. Zone of Inhibition: The results have shown that the cones have shown inhibition zone against all periodontal pathogens except for one. Resistance towards Prevotella Intermedia was shown by all three concentrations of cones (Table 3).

In the Aa group the mean zone of inhibition was highest in 2 percent group followed by 3 percent and 1 percent groups The difference between the groups was statistically significant. In the Pg group the mean zone of inhibition was highest in 2 percent group followed by 3 percent

and 1 percent groups. The difference between the groups was statistically significant. In the Tf group the mean zone of inhibition was highest in 2 percent group followed by 3 percent and 1 percent groups (Table 4). The difference between the groups was statistically significant.

4. DISCUSSION:

In the current study, the efficacy of cones containing different concentrations of coenzyme Q1O on periodontal pathogens was evaluated. The impact of 1%, 2% and 3% cones on four periodontal pathogens, via. Prevotella Intermedia, P. Gingivalis, Aa, and T. forsythia, were evaluated and compared using microbiological analysis using zone of inhibition test and MIC.

The major part of periodontal degenerative changes is induced by an unsuitable host immune reaction to both periodontopathogens as well as their products, which includes excessive production of ROS and free radicals, as well as MMPs, which roots to breakdown of periodontal cell and collagen during the inflammatory process. [7]

Several studies have attempted to link microorganisms to disease as well as health. The particular investigations have produced sufficient data to associate a select group of bacteria to periodontal disease. These said groups of bacteria are designated as important periopathogens which includes Aa, T. forsythia and P. gingivalis. [8]

CoQ10 is a potent lipid antioxidant that inhibits forming free radicals & the oxidation of protein. The total amount of CoQ10 in the body is only approximately 500-1500 mg, and it diminishes with age. Capsules, oral spray, and tablets are just a few of the CoQ10 supplement options. CoQ10 is often taken in split dosages of 30 to 90 mg per day, although the suggested quantity might be as much as 200 mg daily. [9]

The main purpose of present research was to evaluate the efficacy of coQ10 cones of different concentrations on four periodontal pathogens.

The present research demonstrated significant results with all three concentrations of coenzyme Q10 cones and most significant results were achieved with 2% cone as it has shown greater area of zone of inhibition when compared with 1% and 3% cones.

In a clinical trial in patients having severe chronic periodontitis conducted by Raut & Sethi, they discovered a substantial decrease in gingival bleeding in locations administered with coenzyme Q10. Later, the same authors found a reduction in gingival bleeding index from baseline to three months in smokers with chronic periodontitis, indicating that co-enzyme Q10 had an anti-inflammatory and immunomodulatory impact. [10]

Sale et al. (2014) observed comparable results in their investigation, finding that locally administered coQ10, either directly in the sulcus or topically, had shown considerable improvement in both cases. [11]

Manthena and associates, on the other hand, found no major difference in reducing probing depth as well as enhanced clinical attachment levels between the coenzyme Q10 and scaling and root planning groups. Sharma et al. reported no variation in plaque scores in areas receiving coQ10 in a clinical investigation. This shows that coenzyme Q10 may not provide any further protection against plaque formation. [12]

When compared to participants treated with SRP alone, Saini 2018 found that subjects treated with SRP combined with adding supplement of CoQ10 in the diet, demonstrated a highly

considerable reduction in all periodontal clinical indicators. He came to the conclusion that "long-term regular consumption of nutritious dietary supplement of CoQ10 is more favourable in nonsurgical periodontal disease treatment outcomes". [13]

Jin et al. (2014) found that systemic supplementation of CoQ10 inhibits the expression of tumour necrotic factor (TNF-) and promotes the development of interlukin-10 (IL-10) gingival tissues in periodontitis rats in an experimental investigation. Furthermore, a human investigation found a substantial difference in GCF-(TNF-) levels in individuals treated with CoQ10 gel/SRP versus SRP alone. [14]

Current microbiological research was centred on assessing and comparing efficacy of different concentrations of coenzyme Q10 cones on periodontal pathogens. No research, to our knowledge, has been undertaken evaluating the same. As a result, the current study's findings cannot be compared directly to the prior studies. However, the current study's good findings show that using co-enzyme Q10 as an adjuvant to SRP in individuals with chronic periodontitis may give further therapeutic advantages.

The main limitation of the present study is lack of clinical application of coenzyme Q10 cones as a localized intrapocket medicament in patients with chronic periodontitis.

5. CONCLUSION

In the current study, the microbiological effect on periodontal pathogens of three different concentrations of coenzyme Q10 cones were evaluated and compared. It may be inferred, within the scope of the investigation, that coenzyme Q10 can be used in the treatment of periodontal pockets. Although, all three concentrations have shown positive results in inhibiting the bacterial

growth, it was discovered that statistically there was a significant difference on comparing results of 1%, 2% and 3% cones showing the better efficacy of 2% concentration than others.

All three concentrations of coenzyme Q10 cones used in the study are effective against key periodontal pathogens i.e. Prevotella Intermedia, Porphyromonas Gingivalis, Aggregatibacter Actinomycetemcomitans, and Tannerella forsythia. Therefore, further clinical research to check the effect of cones on various periodontal tissues as a local drug delivery is suggested.

REFERENCES:

1. Saini R, Saini S, Saini SR. Periodontitis: A risk for delivery of premature labor and low birth weight infants. J Nat Sci Biol Med 2011;2:50-2.
2. Battino M, Bullon P, Wilson M, Newman H. Oxidative injury and inflammatory periodontal diseases: the challenge of anti-oxidants to free radicals and reactive oxygen species. Crit Rev Oral Biol Med 1999;10:458–76.
3. Rajpoot AS, Parihar AS, Thakur S, Choudhary K, Rajput P, Chaudhary A. Local Drug Delivery in Periodontics. Int J Res Health Allied Sci 2017;3:63-67.
4. Saini R. Coenzyme Q10: The essential nutrient. J Pharm Bioallied Sci 2011;3:466-7.
5. Hanioka, M. Tanaka, M. Ojima, S. Shizukuishi and K. Folkers. Effect of Topical Application of Coenzyme Q10 on Adult Periodontitis. Mol Aspects Med 1994;15:241-248.
6. Henry D Isenberg. Clinical microbiology procedures handbook. American society for microbiology. Washington, D.C Volume 1. 1992.

7. Silva N, Abusleme L, Bravo D, Dutzan N, Garcia-Sesnich J, Vernal R et al. Host response mechanisms in periodontal diseases. J Applied Oral Science 2015;23:329-55.
8. Phaniendra A, Jestadi D and Periyasamy L. Free radicals: properties, sources, targets, and their implication in various diseases. Indian J Clin Biochem 2015;30:11-26.
9. Jaykaran DS, Yadav P, Kantharia ND. Nonsignificant P values cannot prove null hypothesis: Absence of evidence is not evidence of absence. J Pharm Bioallied Sci 2011;3:465.
10. Raut CP, Sethi KS. Comparative evaluation of co-enzyme Q10 and Melaleuca alternifolia as antioxidant gels in treatment of chronic periodontitis: a clinical study. Contemp Clin Dent 2016;7:377–381.
11. Sale ST, Parvez H, Yeltiwar R, Vivekanandan G, Pundir AJ, Jain P. A comparative evaluation of topical and intrasulcular application of coenzyme Q10 (Perio QTM) gel in chronic Periodontitis patients: a clinical study. J Indian Soc Periodontol 2014;18:461–465.
12. Manthena S, Rao MV, Penubolu LP, Putcha M, Harsha AV. Effectiveness of CoQ10 Oral supplements as an adjunct to scaling and root planing in improving periodontal health. J Clin Diagn Res 2015;9:26–28.
13. Saini R. A clinical and microbiological study to evaluate the effect of dietary supplement of coenzyme Q10 in nonsurgical treatment outcome of chronic periodontitis patients after phase 1 periodontal therapy. Eur J Dent 2014;3:194-8.
14. Jin HJ, Xue Y, Chen G. Effect of coenzyme Q10 on the expression of tumor necrosis factor-α and interleukin-10 in gingival tissue of experimental periodontitis in rats. Chin J Dent Res 2013; 48:660-663.

Tables:

Table 1: Results of Minimum Inhibitory Concentrations of 1%, 2%, and 3% cones

Sl. No.	Samples AND Name of the organisms	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml	0.4 µg/ml	0.2 µg/ml
Q-10 CONES P.i‡											
1	1%	S	S	S	S	S	S	S	R	R	R
2	2%	S	S	S	S	S	S	S	S	S	R
3	3%	S	S	S	S	S	S	S	S	S	R
Q-10 CONES P.g†											
4	1%	S	S	S	S	R	R	R	R	R	R
5	2%	S	S	S	S	S	R	R	R	R	R
6	3%	S	S	S	S	S	S	R	R	R	R
Q-10 CONES A.a*											
4	1%	S	S	S	S	S	S	S	S	S	R
5	2%	S	S	S	S	S	S	S	S	S	S
6	3%	S	S	S	S	S	S	S	S	S	S
Q-10 CONES T.f§											
4	1%	S	S	S	S	S	**R**	R	R	R	R
5	2%	S	S	S	S	S	S	R	R	R	R
6	3%	S	S	S	S	S	S	S	S	R	R

* Aggregatibacter Actinomycetemcomitans

†Porphyromonas Gingivalis

‡Prevotella Intermedia

§Tannerella forsythia

Table 2: intergroup comparison of Minimum Inhibitory Concentration results in Aa* vs Pg† Vs Pi‡ Vs Tf§

TYPE	Groups	Resistant	Sensitive	Chi square value	P value
Aa*	1%	30.00%	70.00%	1.82	0.383 (Non-Significant)
	2%	10.00%	90.00%		
	3%	10.00%	90.00%		
Pg†	1%	60.00%	40.00%	0.8	0.670 (Non-Significant)
	2%	50.00%	50.00%		
	3%	40.00%	60.00%		
Pi‡	1%	10.00%	90.00%	2.01	0.366 (Non-Significant)
	2%	0.00%	100.00%		
	3%	0.00%	100.00%		
Tf§	1%	50.00%	50.00%	2.065	0.355 (Non-Significant)
	2%	40.00%	60.00%		
	3%	20.00%	80.00%		

Where, $p \leq 0.05$ is considered significant.

* Aggregatibacter Actinomycetemcomitans

†Porphyromonas Gingivalis

‡Prevotella Intermedia

§Tannerella forsythia

Table 3: Results of Zone of Inhibition of 1%, 2%, and 3% cones

Sl. No.	Samples	75µl/ml	50µl/ml	25µl/ml	10µl/ml	5µl/ml
Q-10 CONES A.a*						
1	1%	16	14	8	R	R
2	2%	17	14	10	R	R
3	3%	18	15	11	R	R
Q-10 CONES P.g†						
5	1%	11	10	9	R	R
6	2%	12	10	8	R	R
7	3%	15	11	9	R	R
Q-10 CONES P.i‡						
8	1%	R	R	R	R	R
9	2%	R	R	R	R	R
10	3%	R	R	R	R	R
Q-10 CONES T.f§						
11	1%	10	8	R	R	R
12	2%	10	9	R	R	R
13	3%	11	9	5	R	R

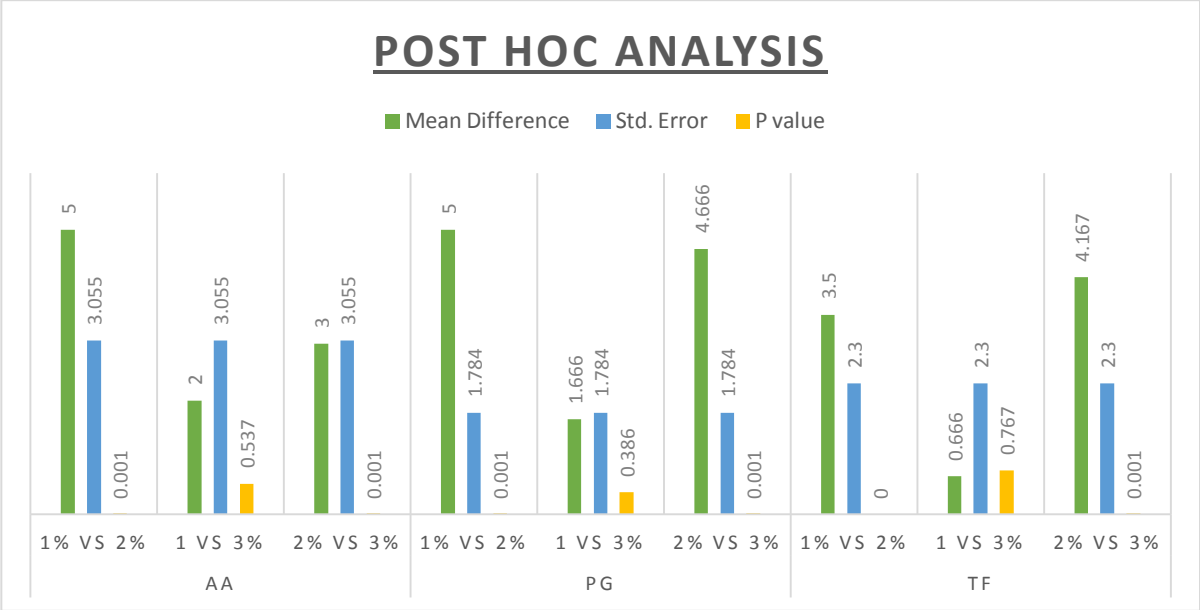
* Aggregatibacter Actinomycetemcomitans

†Porphyromonas Gingivalis

‡Prevotella Intermedia

§Tannerella forsythia

Table 4: Intergroup comparison of post hoc analysis in Aa* vs Pg† vs Tf§



Where, p≤0.05 is considered significant.

* Aggregatibacter Actinomycetemcomitans

†Porphyromonas Gingivalis

§Tannerella forsythia

FIGURES:

Figure 1: Coenzyme Q10 cone.

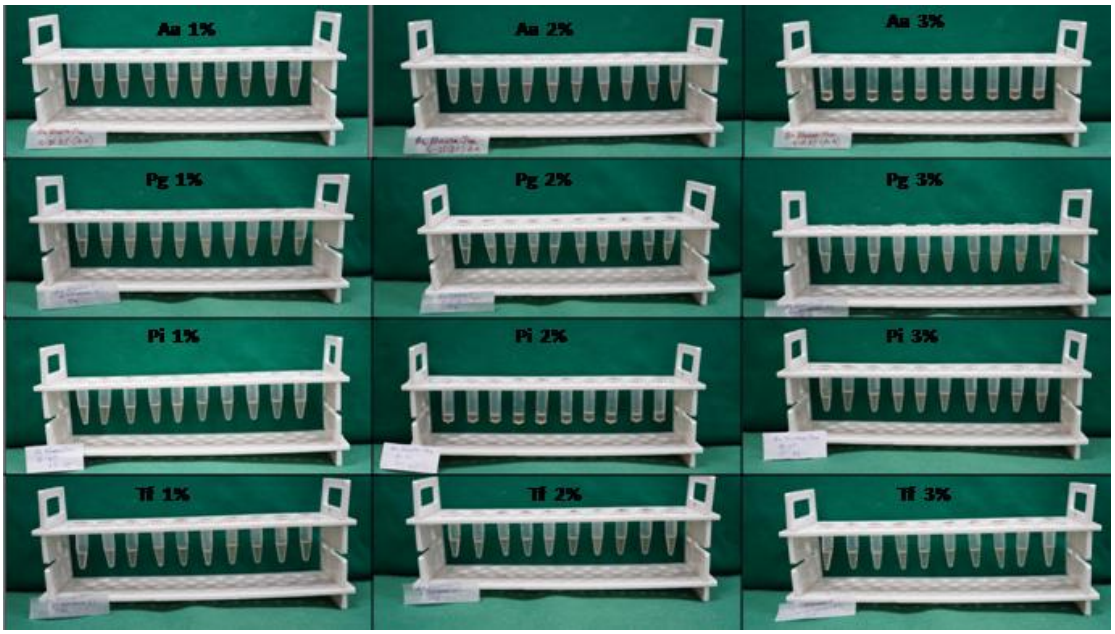


Figure 2: Minimum Inhibitory Concentration test done of 1%, 2%, and 3% cones to check its effect on four periodontal pathogens i.e. Aa[*], Pg[†], Pi[‡], and Tf[§].

* Aggregatibacter Actinomycetemcomitans

†Porphyromonas Gingivalis

‡Prevotella Intermedia

§Tannerella forsythia

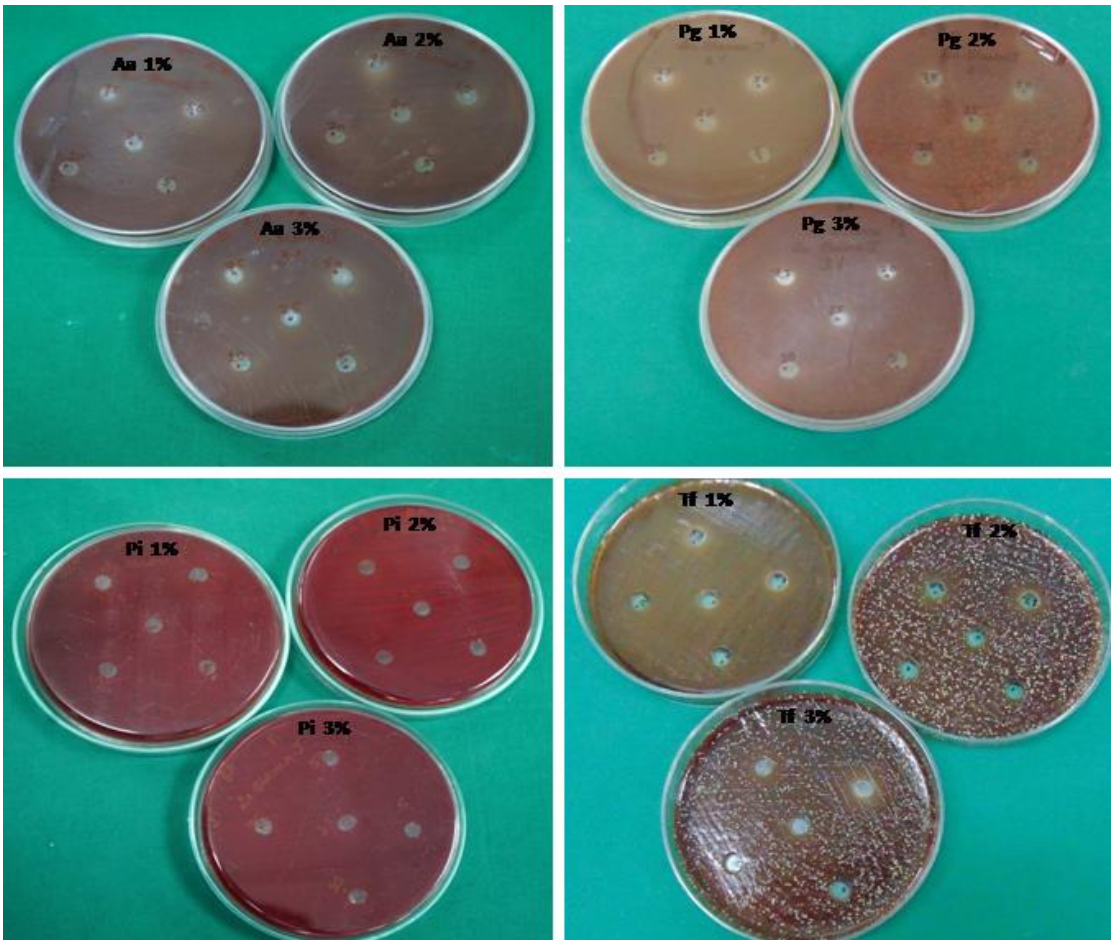


Figure 3: Zone of Inhibition test done of 1%, 2%, and 3% cones to check its effect on four periodontal pathogens i.e. Aa[*], Pg[†], Pi[‡], and Tf[§].

* Aggregatibacter Actinomycetemcomitans

†Porphyromonas Gingivalis

‡Prevotella Intermedia

§Tannerella forsythia