COMPARATIVE EVALUATION OF ANTI-MICROBIAL EFFICACY OF A NOVEL HERBAL GEL CONTAINING ORANGE PEEL EXTRACT AND GINGER EXTRACT WITH CHLORHEXIDINE GEL ON PERI-IMPLANTITIS PATHOGENS – AN INVITRO STUDY.

Dr. Mitali Pandit[1], Dr. Anandkumar G Patil[2] , Dr. Dipa Patil[3], Dr. Swapnil Shankargouda[4]

[1]Post Graduate,

Department of Prosthodontics and Crown and Bridge,

KAHER'S KLE V.K. Institute of Dental Sciences, Belagavi-590010. Karnataka, India

[2]Professor and Head of the Department,

Department of Prosthodontics and Crown and Bridge,

KAHER'S KLE V.K. Institute of Dental Sciences, Belagavi-590010. Karnataka, India

[3]Assistant professor

Jawaharlal Nehru Medical College

Department of microbiology, belagavi-590010. Karnataka, India

[4]Reader,

Department of Prosthodontics and Crown and Bridge,

KAHER'S KLE V.K. Institute of Dental Sciences, Belagavi-590010. Karnataka, India

ABSTRACT

OBJECTIVE: The aim of this present study is to evaluate the efficacy of novel herbal gel containing aqueous orange peel extract and alcoholic ginger extract when compared with chlorhexidine gel against periimplantitis pathogens. **METHODS:** A total of 68 discs of commercially available pure Titanium grade 5 fabricated of diameter 10 mm and a width of 2 mm. The discs were subdivided into groups as control(chlorhexidine) and experimental (herbal gel). The extracts used was alcoholic ginger extract and aqueous orange peel extract. The prepared extract was subjected to antibacterial assay (MIC and MBC) using serum

dilution and disk diffusion method to check the zone of inhibition. Once the MIC and MBC values were achieved the formulation of the herbal gel was carried out. The gel formulation was carbopol based Once the herbal gel was formulated, the Titanium discs were subjected to antibacterial testing in vitro using disk diffusion method. **RESULT:** The collected data was subjected to statistical analysis using Kolmogorov Smirnov test and parametric tests. Herbal gel showed comparable results with chlorhexidine gel on day 4. **CONCLUSION**: This present study showed that herbal gel showed antibacterial activity against early peri implant pathogens in Titanium implant material. On the other hand when compared to the Chlorhexidine (CHX) gel control group, the experimental group(herbal gel) showed comparable zone of inhibition on P.intermedia and *P.gingivalis*. **KEYWORDS**: Chlorhexidine gel, ginger,orange peel, Porphyromonas gingivalis, Prevotella intermedia , peri-implantitis.

INTRODUCTION

Dental implants can be defined as any artificial, biocompatible material which is partially or completely inserted or grafted into the body for therapeutic, diagnostic, prosthetic, or experimental purposes. It has become most popular and common treatment plan for replacing missing tooth or teeth in completely or partially edentulous patients with high success rate (95-98%) and patient acceptability.[1]

They have a higher success rate than traditional methods, there are still a number of things that might cause an implant to fail.[1]

An inflammation and destruction of soft and hard tissues around dental implants is referred to as mucositis and peri-implantitis. This condition is analogous to gingivitis and periodontitis, which affect the periodontium of natural teeth.

Peri-implantitis is a disease that affects the hard and soft tissues that surround implants. It is a degenerative condition that cannot be reversed, and its symptoms include bone resorption, impaired osseointegration, increased pocket development, and purulence.[2]

According to Paulo et al ,there are 5 main Gram negative anerobes that are seen in peri implantitis which are *Porphyromonas gingivalis* ,*Treponema denticola* and *Tannerella forsythia* which belongs to red complex and *Prevotella intermedia* ,*Camylobacter rectus* which belongs to orange complex .Gram-positive aerobic bacteria such as *Staphylococcus aureus* is also a part of the early colonizing bacterias.[3-4]

Porphyromonas gingivalis is one of main periodontal pathogen which has severe virulence factors and causes periodontal destruction . Another most commonly seen organism which is present in peri implantitis is Prevotella intermedia which affects host immune response and periodontal destruction .[5]

Same treatment protocols that are utilised in the management of gingivitis and periodontitis have been utilised in the management of peri-implant mucositis and peri-implantitis respectively. Both non-surgical and surgical treatments are available for the management of peri-implantitis. Non surgical treatment includes scaling and root planning , air abrasive system, ultrasonic devices and lasers, topical application of therapeutic gels, irrigation solutions have also been suggested as a way to improve the results of nonsurgical debridement and/or rinses, as well as in a variety of formulated combinations. Whereas surgical treatment includes flap surgery, apically repositioning flaps, regenerative techniques etc. [6]

In the management of peri-implantitis, chlorhexidine gluconate, also known as CHX, is frequently prescribed as a non-surgical antimicrobial agent. Chlorhexidine has side effects such as discoloration of teeth, tongue and restorative materials, dysgeusia, desquamative

gingivitis, burning of mucous membrane and sometimes allergic reactions.[6] Unlike the commonly available CHX gel, which may cause unpleasant side effects and is harmful, herbal compounds are safe to use and do not cause any adverse reactions. Ginger extract and orange peel extract have shown antibacterial properties against Porhyromonas gingivalis and Prevotella intermedia.[7]

Zingiber officinale, more commonly known as ginger, is a member of the Zingiberaceae family. The pungent chemicals found in ginger, namely gingerols, in its Rhizomes offer properties that are advantageous to one's health, including those that are antioxidant, anti-inflammatory, antibacterial, and antifungal, anti-emetic.[7]

Orange peel (Citrus Reticulata), contains substances likes tannins, saponins, flavonoids, terpenoids, cardiac glycosides ,alkaloids and phenols they exhibit antimicrobial and antioxidant properties. [8]

So, this study was conducted to compare the synergistic effect of aqueous orange peel extract and alcoholic extract of ginger with chlorhexidine against Prophyromonas gingivalis and Prevotella intermedia.

MATERIALS ANS METHODS

1. MATERIALS:

- Grade V Titanium discs (10mm x 2mm).-Type V (Ti-6Al-4V) -Special metals, Mumbai
- 1% Chlorhexidine gel- Hexi gel 1%- ICPA health products
- Orange peel extract -Herbo Nutra, Delhi
- Ginger extract- KLE Ayurvedic college belgaum
- Carbopol gel base- Carbopol 940- OEM manufacturers
- Brain Heart Infusion Agar- Hi media
- Porphyromonas gingivalis

- Prevotella intermedia
- Mice Fibroblasts – NCCS Pune.
- Ethanol- Changshu Hongsheng Fine Chemicals Co.Ltd
- DMEM Media- Hi media.
- MTT reagent- Hi media, Mumbai
- Tryphan blue- Hi media, Mumbai

2. ARMAMENTARIUM USED IN STUDY:

- Weighing machine
- Beakers
- Petri dish- Hi-Media, Mumbai
- Water bath
- Water evaporator
- Incubator - Eppendoff
- Anaerobic jar- Hi-media ,Mumbai
- 96 Well Plates
- Falcon Tubes.
- Micro pipettes- Riveria Glass Pvt, Ltd, Mumbai

METHODOLOGY:

METHOD OF EXTRACTION:

A) Preparation of aqueous orange peel extract.

Powder form of peel of citrus reticulata(Herbo Nutra) was used as a source of the extract.As a solvent, distilled water that was kept at room temperature was used.The combination of peel powder and solvent was placed in a water bath and heated to an extraction temperature of 40 degrees Celsius for a period of four hours.The liquid extract was sterilised in an autoclave after being filtered using Whatman No.1 filter paper to remove any solid particles that could have been present. The aqueous extract was collected in a vail and stored in a refrigerator .

Figure 2: Aqueous orange peel extract.

B) Preparation of alcoholic ginger extract.

The alcoholic ginger extract is prepared by mixing 40mg of ginger powder to 500ml 99.9 ethanol alcohol was added and mixed well. The container was then sealed airtight with cotton and foil to prevent any alcohol from escaping, and it was allowed to sit out at room temperature for twenty-four hours. The contents will be filtered after being concentrated, and this will be accomplished by evaporating the solvent (alcohol) in a hot air furnace at a temperature of 40 degrees Celsius for 24 hours (Nweze and Okafor ,2010).After this procedure , alcoholic ginger extract was collected and was stored in a refrigerator.

Figure 2: Alcoholic ginger extract.

To check the antibacterial sensitivity test for aqueous orange peel and alcoholic ginger extract ,MIC(minimum inhibitory concentration) and MBC (Minimum bactericidal concentration) was done.

A standard procedure for testing the MIC was followed. Micro broth dilution method was used to determine the MIC. MBC test was done from the MIC dilutions tubes, first 3 or 5 tubes were plated (which was sensitive in MIC) and then incubated for 24 hrs then next day the colony count was taken.

MIC of aqueous orange peel extract on P.gingivalis was 25µg/ml and of P.intermedia was 50µg/ml whereas, MIC of alcoholic ginger extract on P.gingivalis was 0.2µg/ml and of P.intermedia was 0.4µg/ml. Similarly, MBC of aqueous orange peel extract on P.gingivalis was 25µg/ml and of P.intermedia was 50µg/ml and MBC of alcoholic ginger extract on P.gingivalis was 0.2µg/ml and of P.intermedia was 0.8µg/ml.

Combination MIC, MBC obtained was 0.4 % .Herbal gel was formulated using this concentration.

TO CHECK THE ZONE OF INHIBITION OF HERBAL GEL AND CHLORHEXIDINE GEL.

Disc diffusion test was carried out to check the zone of inhibition . Brain heart infusion agar(BHI) was used. Inoculum preparation was done using a loop or swab, transfer the colonies to the plates. Then it was adjusted visually to see the turbidity with broth to equal that of a 0.5 McFarland turbidity standard that was been vortexed. Alternatively, standardize the suspension with a photometric device.

TO CHECK CYTOTOXICITY OF THE GEL.

MTT solution (stock solution) of 5 mg in 1 ml of PBS was prepared.

Cell culture:

The cell line used for the study was L929, Mouse Fibroblasts (procured from NCCS, Pune). The cell line was maintained in 96 wells micro titer plate containing DMEM media supplemented with 10% heat inactivated fetal calf serum (FCS), containing 5% of mixture of Gentamicin (10ug), Penicillin (100 Units/ ml) and Streptomycin (100µg/ml) in presence of 5% CO_2 at 37ºC for 48-72 hours.

Cytotoxicity assay was carried out

RESULTS

Comparison between two gels (Herbal gel and Chlorhexidine) against two microorganism (P. gingivalis and P.intermedia) using Titanium implant material was assessed using statistical tests which determined the mean zone formation for the Control group and Experimental group was found to be statistically significant ($p = <0.05$).

Statistical Analysis was done by using Kolmogorov Smirnov tests and Parametric tests

Independent t test was done to compare two independent groups in order to determine whether they are statistically significant.

When herbal gel was compared with chlorhexidine gel on day 4, chlorhexidine gel showed more zone of inhibition with the mean of 27.35±0.70 when compared with herbal gel that is 25.59 ±1.54

On day 7, chlorhexidine gel showed 27.24±0.66 and herbal gel showed 24.24±0.66, similarly on day 10, chlorhexidine gel showed increased zone of inhibition than herbal gel that is 25.65±0.70 and 20.41±0.94 respectively.

When intergroup comparsion was done of herbal gel (day 4^{th} to day 7^{th}) was done , mean was 1.35±1.54 when compared with chlorhexidine gel which was 0.12±0.93.

From day 7^{th} to day 10^{th} mean of zone of inhibition of herbal gel was 3.82±1.13 and in chlorhexidine gel was 1.59±0.87.Similarly from day 4^{th} to day 10^{th}, mean of zone of inhibition in herbal gel increases when compared with chlorhexidine gel that is 5.18±1.91 and 1.71±1.05 respectively .This increase in mean difference values was due to the result of decreased antimicrobial efficiency .

Microbial efficiency decreased from day 4 to day 10 in herbal gel. But in chlorhexidine group from 4^{th} to 10^{th} day the mean difference is significantly less and there is no much difference from day 4 to day 10. Thus there is a significant difference in between herbal gel group and chlorhexidine group. (Table 1)

Dependent test was used to compare the sample means from two related groups.It helps to determine the % of change from one group to other.

As the zone of inhibition decreased at different time intervals,the % of change in herbal gel was more when compared with chlorhexidine gel that is 20.23% and 6.24% respectively.(Table 2)

Comparison of herbal gel and chlorhexidine gel on Prevotella intermedia , was done on the bases of different time zones that was on 4^{th} 7^{th} and 10^{th} day. It showed that the mean gradually decreased from 15.53±1.62 to 11.71±0.85 that is from 4^{th} day to 10^{th} day respectively, when compared to chlorhexidine gel that is 21.76±1.25 to 21.35±1.22 from 4^{th} day to 10^{th} day respectively.

On intergroup comparison from day 4 to day 7 , mean of zone of inhibition in herbal gel was 0.76±0.83 and in chlorhexidine gel was 0.53 ±1.42. Similarly, when compared from day 7 to day 10 mean of zone of inhibition increased in herbal gel than chlorhexidine gel group that is 3.06±1.48 and -0.12 ±1.50 respectively. Whereas from day 4 to day 10 , mean of zone of inhibition showed significant increase in the mean values on comparsion with chlorhexidine gel that is 3.82±1.94 and 0.14±1.58 respectively. This increase in the mean difference was due to reduced antimicrobial efficiency as the day increases.

It indicates that microbial efficiency reduced from day 4 to day 10. In chlorhexidine group from day 4 to day 10 mean difference is significantly less.(Table 3)

Dependent test was used to compare the sample means from two related groups .It helps to determine the % of change from one group to other.

As the zone of inhibition decreased at different time intervals , the % of change in herbal gel was more when compared with chlorhexidine gel that is 24.62% and 1.89% respectively from 4^{th} to 10^{th} day. (Table 4)

Cytotoxicity of the gel:

After checking the antimicrobial property of the herbal gel, cytotoxicity of the herbal gel was done. Cytotoxicity of the gel was checked on L929 mice fibroblasts. It showed that there was total 37% cell proliferation and 63% cell death.

DISCUSSION

People with completely or partially edentulous jaws are the most common candidates for oral implant treatment, and numerous reports have been published demonstrating successful long-term outcomes with a variety of oral implant systems.[1]

In spite of their enormous success, the number of complications and rates of failure have been steadily increasing. According to **Salah et al**, keys reasons for early and late implant failure in majority of the cases are lack of primary stability surgical stress and infection. Variations in the bone's marginal level following early remodelling, together with bleeding on peri-implant probing (BOP), are considered as diagnostic indicators for this condition. Even though bacteria are the primary cause of peri-implantitis, the likelihood of acquiring the condition may be increased by a number of other variables. The identification of these determinants, regardless of whether they are innate or can be changed, is essential not only for the prevention but also the treatment of the disease. Regionally specific risk factors such as insufficient management of plaque, mucositis, malposition of the implant, and

inadequately constructed prosthesis or presence because of the extra cement ,certain hereditary variables, cardiovascular and autoimmune illnesses, high-dose blood pressure medication, and hormone replacement therapy may enhance the susceptibility to peri-implantitis.

There is a wide variety of microorganisms that might cause problems for the periodontium that surrounds an implant. Gram negative organisms such as Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola and Prevotella intermedia and fusobacterium nucleatum are seen in peri implantitis cases as per the research study don by gloria et al. Porphyromonas gingivalis is one of the primary periodontal pathogens, and it is characterised by high levels of virulence and the ability to destroy periodontal tissue. Another organism that is present in peri implantitis that is observed rather often is Prevotella intermedia, which has an effect on the immunological response of the host and may cause periodontal damage.[3-4]

Chlorhexidine gluconate (CHX) is commonly prescribed as non-surgical antimicrobial agent in management of peri-implantitis.CHX works against Gram-positive and Gram-negative anaerobic and aerobic bacteria, fungi, yeasts as well as several viruses. It is prescribed as a rinsing solution (0.2%) or in gel topical form (1%).[9]

Neem, tulsi, aloe vera, ginger, turmeric, chamomile, tea tree, eucalyptus, orange peel extract showed antibacterial activity against various organisms such as Porphyromonas gingivalis, Provotella intermedia, Staphylococcus aureus etc.[8]

Ginger contains gingerols, in its rhizomes and has beneficial health activities including antioxidant, anti-inflammatory, anti-microbial and antifungal properties.[14] Similarly Orange peel (citrus reticulata) contains high levels of phenolic compounds including several flavonoids.

So, in the present study,a herbal gel was formulated using aqueous orange peel extract and alcoholic ginger extract at 0.4% concentration, to check the anti-microbial property against P.gingivalis and P intermedia . It was found that there was significant increase in mean difference values of zone of inhibition from day 4 to day 10 in herbal gel against Porphyromonas gingivalis as compared to chlorhexidine gel which was 5.18±1.91 and 1.71±1.05 respectively. But on day 4, the mean of zone of inhibition of herbal was comparable with chlorhexidine group that is 25.59 ±1.54 and 27.35±0.70 respectively. This indicates that zone of inhibition decreased with increased time. Similarly on Prevotella intermedia, day 4 to day 10, mean of zone of inhibition showed significant increase in the mean values on comparsion with chlorhexidine gel that is 3.82±1.94 and 0.14±1.58 respectively. This increase in the mean difference was due to reduced antimicrobial efficiency as the day increases.

In this present study, chlorhexidine was used as positive control and showed better anti-microbial activity against P.gingivalis and P.intermedia in terms of MIC and a greater zone of inhibition, establishing itself as gold standard .

A study done by **A. NALBANTSOY et al** to check antimicrobial and cytotoxicity of ginger.The ethanol extract on the L929 cells showed a cytotoxic impact on both human cervical cancer (HeLa) cell-lines and mouse fibroblast (L929) cell-lines. The amount of cytotoxicity is proportional to the amount of ethanol extract present.[11]

KEERTHANA T et al evaluated the cytotoxicity of citrus sinensis against fibroblasts. The peel extract from Citrus sinensis showed much reduced cytotoxic activity.[10]

When cytotoxicity of herbal gel was checked on L929 mice fibroblasts, cell viability was found to be less. This may be due to the higher concentration which was used to make the gel or due to the interaction of the components present in the extracts which were used. The

present study only evaluated the cytotoxicity of the acquired concentration, so cytotoxic effect can be reduced by further alterations of the concentrations.

Although chlorhexidine remains to be the gold standard , the formulated herbal gel showed comparable antimicrobial activity against peri implantitis pathogens and it can be used as an alternative for maintenance of early peri-implantitis.

CONCLUSION

This study concluded the anti-bacterial property of herbal gel inhibited the complex strain P.ginigvalis and Prevotella Intermedia which lead. to early peri-implantitis when compared with commercially available topical CHX gel. The formulated herbal gel reported toxicity on the acquired concentration so, further research can be done by reducing the concentrations . This opens the door to further investigations. Even more widely, as a standard clinical treatment modality.

ACKNOWLEDGEMENT

I would like to acknowledge Dr S. B. Javali for the statistical analysis and also Department of microbiology, JNMC ,Belgaum.

CORRESPONDING AUTHOR : DR ANANDKUMAR G PATIL

Professor and Head of the Department,

Department of Prosthodontics and Crown and Bridge,

KAHER'S KLE V.K. Institute of Dental Sciences, Belagavi-590010.
Karnataka, India

REFERENCES

1. Misch CE. Prosthetic options in implant dentistry. Contemporary Implant Dentistry. 2007; Nov 26:92.
2. Shankar, T & Gowd, M & Rajeev, Ranjan & Jalaluddin, Mohammad & Kummar, Dhiirrendra & Kore, Shobha. (2016). Peri-implantitis: A Concern for Implant Failure. International Journal of Oral Care and Research. 1. 10.5005/jp-journals-10051-0045.
3. Pérez- Chaparro PJ, Duarte PM, Shibli JA, Montenegro S, Lacerda Heluy S, Figueiredo LC, Faveri M, Feres M. The current weight of evidence of the microbiologic profile associated with peri- implantitis: a systematic review. Journal of periodontology. 2016 Nov;87(11):1295-304..
4. Lafaurie GI, Sabogal MA, Castillo DM, et al. Microbiome and Microbial Biofilm Profiles of Peri-Implantitis: A Systematic Review. *J Periodontol*. 2017;88(10):1066-1089.
5. Mombelli A. Microbiology and antimicrobial therapy of peri- implantitis. Periodontology. 2002 Jan;28;177-89
6. Figuero E, Graziani F, Sanz I, Herrera D, Sanz M. Management of peri- implant mucositis and peri- implantitis. Periodontology. 2014 Oct;66;255-73
7. Lourenço R, Samuel J, Samuel LJ. The Invaluable Role of Phytotherapeutic Agents in the Adjunctive Treatment of Periodontal Disease.
8. Mankar, Chetana Dilip et al. Evaluation of antimicrobial activity of orange peel extract against oral biofilm forming organisms: an in vitro microbial study and scanning electron microscopic assessment. International journal of basic & clinical pharmacology, [S.l.], 2017 ;5:1917-1923.
9. Fiorillo L. Chlorhexidine gel use in the oral district: A systematic review. Gels. 2019 Jun 11;5(2):31.

10. Karaboz I. Antimicrobial and cytotoxic activities of Zingiber officinalis extracts. Fabad J. Pharm. Sci. 2010;33:76-85.

11. Albantsoy a, tamiş da, akgün ih, yalçin tö, gürhan id, Karaboz I. Antimicrobial and cytotoxic activities of Zingiber officinalis extracts. Fabad Journal of Pharmaceutical Sciences. 2008;33(2):77-86.

FIGURE LEGENDS :

Figure 1: Aqueous orange peel extract.

Figure 2: Alcoholic ginger extract.

Figure 3: (A) Zone of inhibition on P.gingivalis and chlorhexidine on day 4.

Figure 3: (B) Zone of inhibition on P.gingivalis and chlorhexidine on day 7.

Figure 3: (C) Zone of inhibition on P.gingivalis and chlorhexidine on day 10.

Figure 4: (A) Zone of inhibition on P.intermedia and chlorhexidine on day 4.

Figure 4: (B) Zone of inhibition on P.intermedia and chlorhexidine on day 7.

Figure 4: (C) Zone of inhibition on P.intermedia and chlorhexidine on day 10.

-

Figure 3: (A) Zone of inhibition on P.gingivalis and chlorhexidine on day 4.

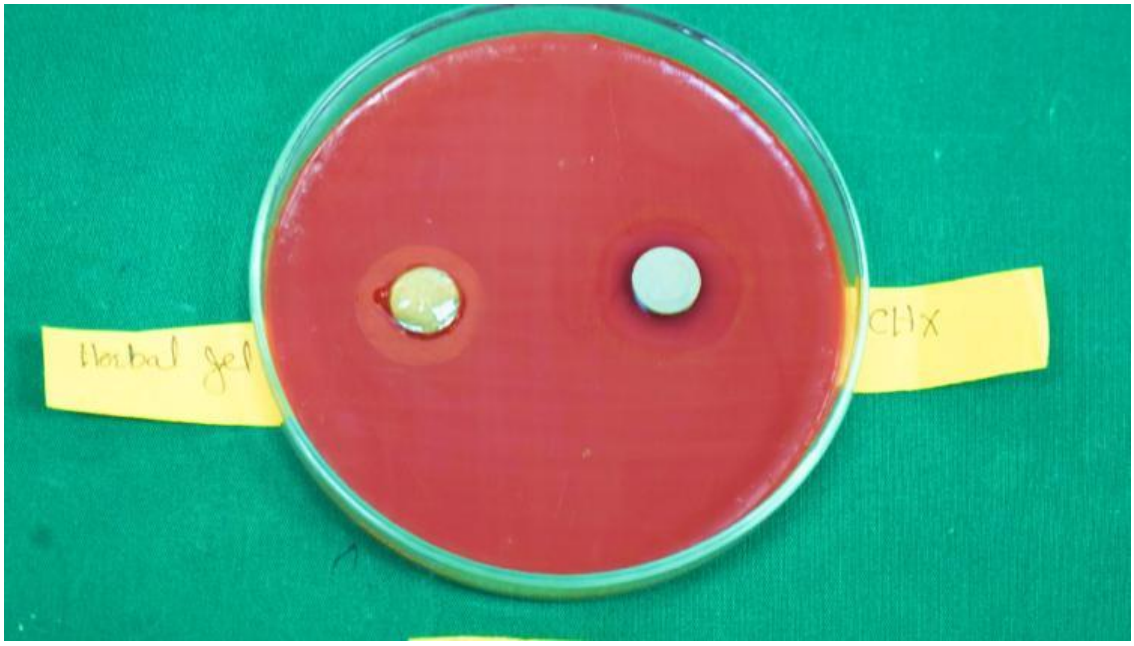

Figure 3: (B) Zone of inhibition on P.gingivalis and chlorhexidine on day 7

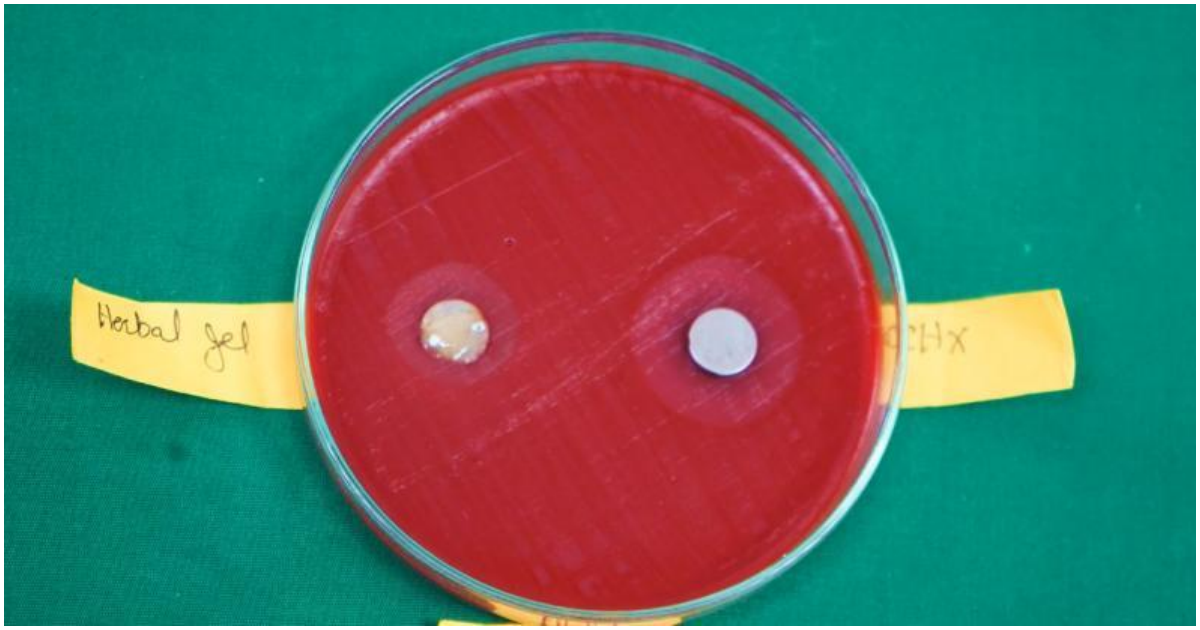

Figure 3: (C) Zone of inhibition on P.gingivalis and chlorhexidine on day 10.

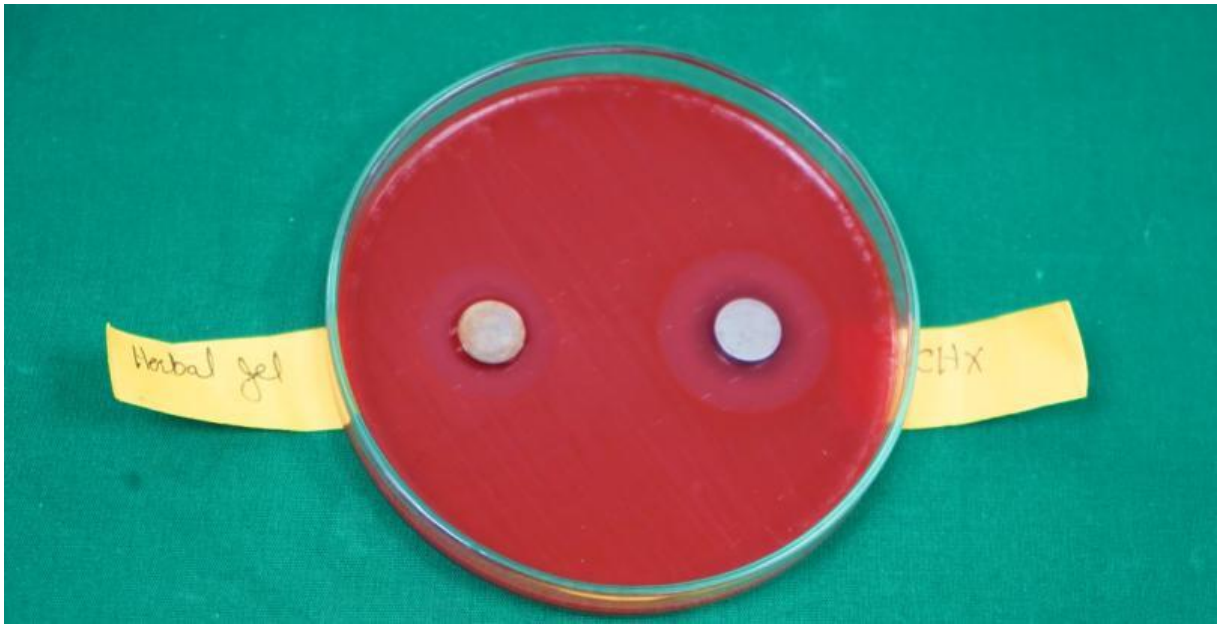

Figure 4: (A) Zone of inhibition on P.intermedia and chlorhexidine on day 4.

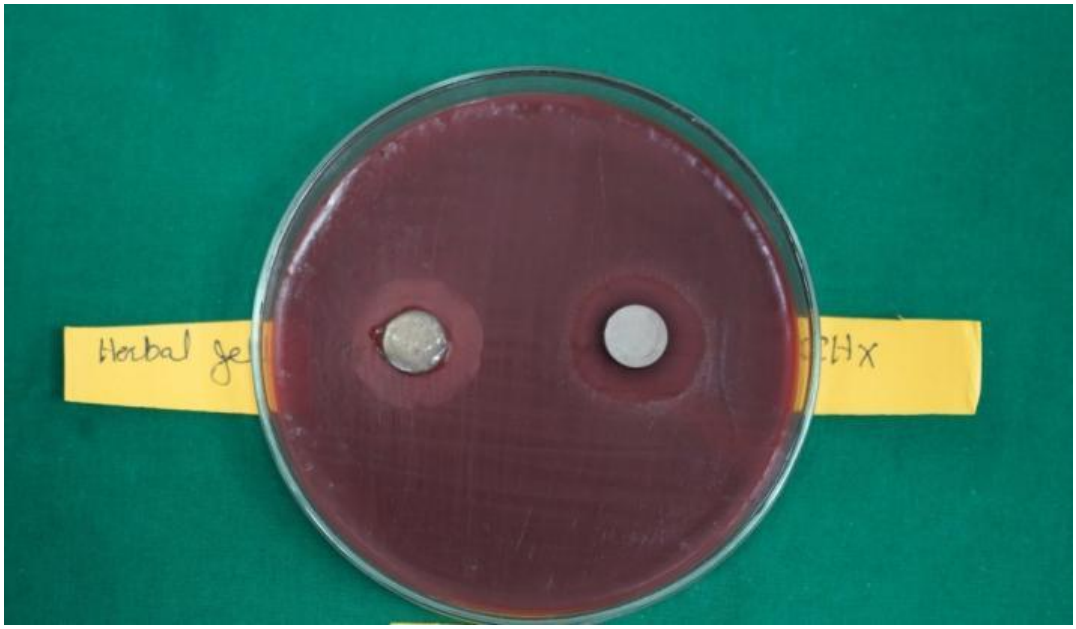

Figure 4: (B) Zone of inhibition on P.intermedia and chlorhexidine on day 7.

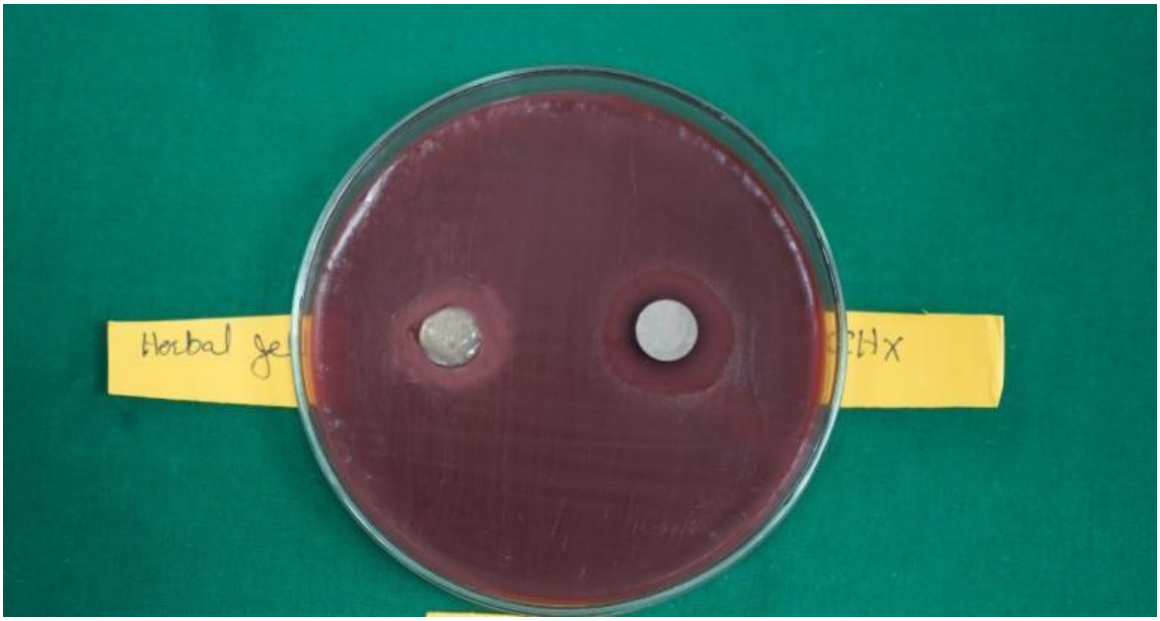

Figure 4: (C) Zone of inhibition on P.intermedia and chlorhexidine on day 10.

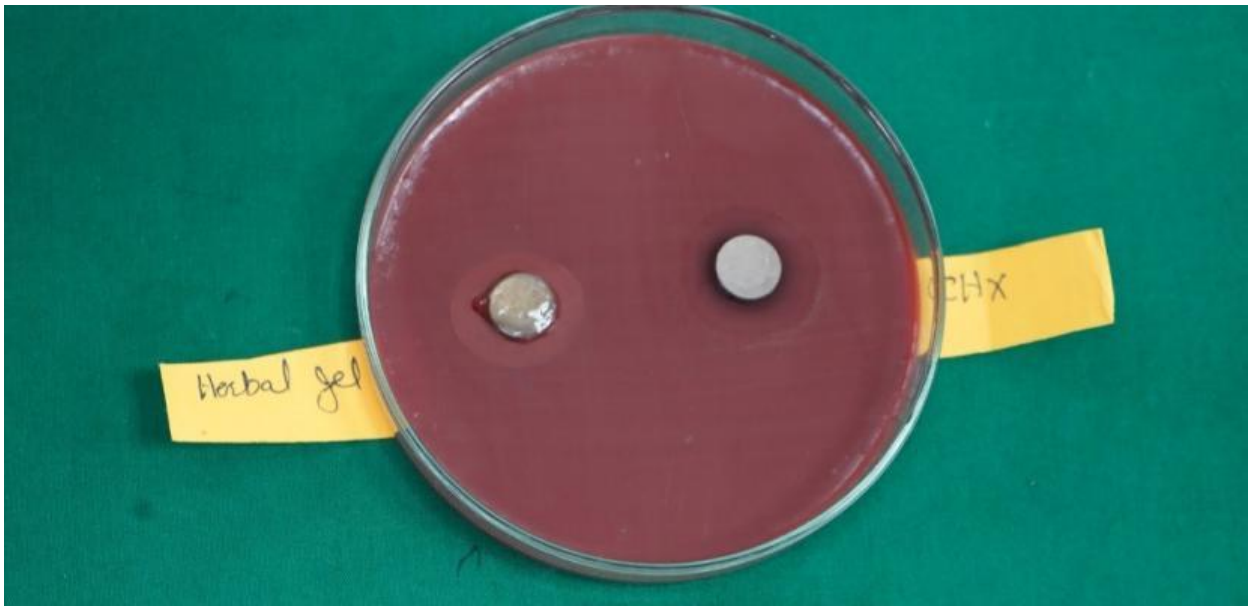

Table 1 : **Comparison of Herbal gel group and Chlorhexidine group with Zone of inhibition (mm) scores in P.Gingivalis at different treatment time points by Independent t test.**

Treatment times	Herbal gel group		Chlorhexidine group		t-value	p-value
	Mean	Std.Dev.	Mean	Std.Dev.		
Day 4	25.59	1.54	27.35	0.70	-4.2912	0.0002*
Day 7	24.24	0.66	27.24	0.66	-13.1681	0.0001*
Day 10	20.41	0.94	25.65	0.70	-18.4083	0.0001*
Day 4 to Day 7	1.35	1.54	0.12	0.93	2.8349	0.0079*
Day 7 to Day 10	3.82	1.13	1.59	0.87	6.4579	0.0001*
Day 4 to Day 10	**5.18**	1.91	**1.71**	1.05	6.5657	0.0001*

Table 2: Comparison of different treatment time points with Zone of inhibition (mm) scores in P.Gingivalis in Herbal gel group and Chlorhexidine group by Dependent t test

Groups	Changes from	Mean Diff.	SD Diff.	% of change	t-value	p-value	Effect size
Herbal gel group	Day 4 to Day 7	1.35	1.54	5.29	3.6253	0.0023*	0.8630
	Day 7 to Day 10	3.82	1.13	15.78	13.9375	0.0001*	
	Day 4 to Day 10	5.18	1.91	**20.23**	11.1648	0.0001*	
Chlorhexidine group	Day 4 to Day 7	0.12	0.93	0.43	0.5230	0.6082	0.6810
	Day 7 to Day 10	1.59	0.87	5.83	7.5247	0.0001*	
	Day 4 to Day 10	1.71	1.05	**6.24**	6.7197	0.0001*	

*$p<0.05$ indicates significant

Table 3: Comparison of Herbal gel group and Chlorhexidine group with Zone of inhibition (mm) scores P. intermedia at different treatment time points by independent t test

Treatment times	Herbal gel group		Chlorhexidine group		t-value	p-value
	Mean	Std.Dev.	Mean	Std.Dev.		
Day 4	15.53	1.62	21.76	1.25	-12.5358	0.0001*
Day 7	14.76	1.25	21.24	1.39	-14.2455	0.0001*
Day 10	11.71	0.85	21.35	1.22	-26.7366	0.0001*
Day 4 to Day 7	0.76	0.83	0.53	1.42	0.5898	0.5595
Day 7 to Day 10	3.06	1.48	-0.12	1.50	6.2302	0.0001*
Day 4 to Day 10	**3.82**	1.94	**0.41**	1.58	5.6103	0.0001*

*p<0.05 indicates significant

Table 4: Comparison of different treatment time points with Zone of inhibition (mm) scores in P. intermedia in Herbal gel group and Chlorhexidine group by dependent t test

Groups	Changes from	Mean Diff.	SD Diff.	% of change	t-value	p-value	Effect size
Herbal gel group	Day 4 to Day 7	0.76	0.83	4.92	3.7925	0.0016	0.7970
	Day 7 to Day 10	3.06	1.48	20.72	8.5343	0.0000	
	Day 4 to Day 10	**3.82**	**1.94**	**24.62**	8.1092	0.0000	
Chlorhexidine group	Day 4 to Day 7	0.53	1.42	2.43	1.5378	0.1436	0.0680
	Day 7 to Day 10	-0.12	1.50	-0.55	-0.3244	0.7498	
	Day 4 to Day 10	0.41	1.58	1.89	1.0722	0.2995	

*$p<0.05$ indicates significant