*Section A-Research paper*

# TO EVALUATE THE EFFICACY OF FIVE MOUTHWASHES IN REDUCING VIRAL LOAD/BACTERIAL COLONIES IN ORAL CAVITY

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**Abstract**

Human viral infection and transmission can occur through multiple paths, such as fecal–oral, exchange of saliva or by aerosols generated by sneezing or coughing Influenza and the common cold viruses(Rhinovirus) are among the most frequent types of human viral infections. This study is conducted to study efficacy of five mouthwashes in reducing viral load/bacterial colonies in oral cavity The Aim of this study is to evaluate the efficacy of 5 different mouthwashes for reducing viral load and bacterial colonies in oral cavity The study was initiated with 75 patients who were divided into five groups randomly and every group was assigned 15 patients and one mouthwash and 4 samples were taken for every patient . First two samples on first visit and 3rd , 4th sample on second visit. Patients were asked to collect 1st saliva sample using Eppendrof tubes then patient rinsed their mouth using the assigned mouthwash and second sample was collected after 2 hours of rinsing with mouthwash. Sample was stored at -80 degree. Interleukin 6 levels were tested using Elisa kit for Rhinovirus and colony forming unit on blood agar medium was used for streptococcus.Hydrogen peroxide followed by chlorhexidine and triclosan showed significant reduction in bacterial colonies with highest mean difference followed by hydrogen peroxide. Hydrogen peroxide showed maximum reduction in viral load followed by chlorhexidine hence proving their efficient

**Keywords**: Chlorhexidine , Triclosan, Mouthwashes, viral load, Bacterial colonies.

## Introduction

The human mouth is lined with a stratified squamous mucous membrane which consists of a superficial epithelial layer and a deeper., connective tissue layer. Although the oral mucous membrane has this basic structure in all parts of the mouth, it is modified in certain regions, according to function. The oral mucosa is interrupted by, teeth if they are present and is closely related to the tooth surface by means of the epithelial attachment. In addition, the mucosal surface is pierced not only by the ducts of the parotid, submandibular and sublingual glands, but also by the numerous small ducts of the accessory salivary glands scattered throughout the oral mucous membrane.[1]

A thin film of saliva therefore bathes the surface of the mucosa during waking hours and contained in the salivary layer are polymorphonuclear leukocytes, epithelial squames and the commensal oral microflora. The general environment of the outer layer of the oral mucosa could, therefore, be described as possessing a somewhat rough surface, interrupted by teeth and the orifices of ducts, coated with microorganisms and moistened with saliva.[2]
The main ecological components of the mouth are therefore the oral and dental tissues, saliva, and the oral microbial flora[3]. Usually the complex interactions of these components result in a state which is recognised as normal and healthy, but when the interactions become deranged a state may result which is regarded as abnormal and recognised as disease. [4]

Human viral infection and transmission can occur through multiple paths, such as fecal–oral, exchange of saliva or by aerosols generated by sneezing or coughing Influenza and the common cold viruses(Rhinovirus) are among the most frequent types of human viral infections [5]. Mouthwashes can be used for various conditions, depending on oral cavity[5]. So, the oral health practitioners should be aware of various etiologic factors and predisposing conditions affecting a particular oral lesion.[6]This study is conducted to study efficacy of five mouthwashes in reducing viral load/bacterial colonies in oral cavity [6]

**Material and method**

**Source of data:**

A sample size of 75 patients coming to the Department Of Orthodontics &; Dentofacial Orthopaedics, Inderprastha Dental College & Hospital, Sahibabad,Ghaziabad, India Undergoing orthodontic treatment were included in the study.

**Inclusion criteria:-**

1.Age 15-50 years
2.Clinical signs have been present for less than 8 days – Virological confirmation
3. Understanding and acceptance of the trial
4.Undergoing orthodontic treatment

**Exclusion criteria:**

1. Patient on medications i.e Antiviral, Antifungal, Antibiotics
2. Pregnancy
3. Breastfeeding
4. Patients with comorbidities like diabetes
5. Immuno-compromised patients
6. Inability to comply with protocol,
7. Patients using mouthwash on a regular basis (more than once week)
8. Patient at risk of infectious endocarditis
9. Uncooperative patient

**METHODOLOGY**

Mouthwashes concentration used in the study
1.chlorhexidine brand name chlorhex-0.2%

2. hydrogen peroxide brand name hydroxyl-3%
3.cetylpyridinium chloride brand name cpc-0.07%
4. Povidine-iodine brand name betadine-7%
5.triclosanThe study was initiated with 75 patients who were divided into five groups randomly and every group was assigned 15 patients and one mouthwash and 4 samples were taken for every patient . The saliva samples collected in the before the rinse for each group
1 st group containing 15 subjects were given chlorhex containing chlorhexidine 0.2%
2 nd group containing 15 subjects were given Purexa mouthwash hydrogen peroxide 3%
3 rd group containg 15 subjects were given cetylpyridinium chloride brand name cpc-0.07%
4 th group containing 15 subjects were given Povidine-iodine brand name betadine
5th group containing 15 subjects were given colgateplax containing triclosan


**Fig1:Mouthwashes**

**Methodology**

The study was initiated with 75 patients who were divided into five with 15 patients in every group. These group were named as group 1,group 2, group 3 , group 4 and group 5

Group 1- 15 subjects were given Chlorhex containing Chlorhexidine 0.2%.

Group 2- 15 subjects were given Purexa mouthwash Hydrogen Peroxide 1.5%.

Group 3- 15 subjects were given Cetylpyridinium chloride brand name cpc-0.07%.

Group 4- 15 subjects were given Povidine-iodine brand name Betadine.

Group 5- 15 subjects were given Colgate plax containing Triclosan.

Patients were informed abount the procedure and consent form was signed .Four samples were collected from each patients

Sample1-first sample on first day of visit

Sample2-second sample after rinsing with mouthwash

Sample3-third sample on second visit before rinse one month post bonding

Sample 4-fourth sample on second visit after rinse one month post bonding

Patients were asked to collect saliva sample-1 using Ependroff tubes then patient were asked to rinse their mouth using the mouthwash and second sample- 2 was collected after 2 hours of rinsing with mouthwash.

Then Patient were recalled one month post bonding , saliva sample-3 was collected before rinsing and then patient were asked to rinse their mouth with same mouthwash used for collecting sample-2 and then sample-4 was collected .

These Sample were then stored at -80 degree .samples were then transported to lab where Interleukin 6 levels were tested using Elisa kit for Rhinovirus and colony forming unit were observed on blood agar medium for streptococcus.

**Fig 2 Dry Ice and Eppendrof tube**

**Fig 3 Sample collection**

## Result and observation

Table 1 Shows the Mean difference in IL-6 levels between sample 1 and 2 (before bonding). When sample was subjected to t test, Chlorhexidine and Betadineshowed non significant difference in Interleukin levels pre and post rinsing with the mouthwash. Significant difference in the levels of IL-6 were observed with Betadine ,Hydrogen peroxide and Triclosan. Highest mean difference , of 0.14 units was seen with Hydrogen peroxide with p-value 0.0001.when this data was subjected to ANOVA analysis ,It says it was seen that there was a significant difference among all the groups.

Table 2 Shows the Mean difference in IL-6 levels between sample 3 and 4 (After bonding). When sample was subjected to t test, CPC andTriclosan showed non significant difference in Interleukin levels pre and post rinsing with the mouthwash. Significant difference in the levels of IL-6 were observed with Betadine ,Hydrogen peroxide and Chlorhexidine. Highest mean difference , of 0.20 units was seen with Hydrogen peroxide with p-value 0.0001.when this data was subjected to ANOVA analysis ,it was seen that there was a significant difference among all the groups.

Table 3 Shows the Mean difference in Bacterial load between sample 1 and 2 (Before bonding). When sample was subjected to t test, CPC andTriclosan showed non significant difference in Colony forming unit in pre and post rinsing with the mouthwash. Significant difference in the levels of Colony forming unit were observed with Betadine ,Hydrogen peroxide and Chlorhexidine. Highest mean difference , of 250 units was seen with Hydrogen peroxide with p-value 0.0001.when this data was subjected to ANOVA analysis.

Table 4 Shows the Mean difference in Bacterial load between sample 3 and 4 (After bonding). When sample was subjected to t test, CPC, andTriclosan showed non significant difference in Colony forming unit in pre and post rinsing with the mouthwash. Significant difference in the levels of Colony forming unit were observed with Betadine ,Hydrogen peroxide and Chlorhexidine. Highest mean difference , of 160 units was seen with Hydrogen peroxide with p-value 0.0001.when this data was subjected to ANOVA analysis.
Mean change in IL-6 levels in all mouthwashes using post –hoc analysis (table 6), it was observed that significant difference was seen between group 2(hydrogen peroxide) and group 3 ( Cetylpyridinium) and Group 2(Hydrogen peroxide) and group 4( Betadine).

| Groups | Before | | After | | Mean difference | p-value |
|---|---|---|---|---|---|---|
| | Mean sample 1 | S.D. | Mean sample 2 | S.D. | | |
| Chlorhexidine | 0.85 | 0.1 | 0.78 | 0.01 | 0.07 | 0.006 |
| CPC | 0..78 | 0.2 | 0.70 | 0.04 | 0.08 | 0.009 |
| Betadine | 0.67 | 0.1 | 0.60 | 0.02 | 0.07 | 0.001 |
| Hydrogen peroxide | 0.84 | 0.1 | 0.68 | 0.12 | 0.14 | 0.0001 |
| Triclosan | 0.87 | 0.13 | 0.81 | 0.18 | 0.06 | 0.0008 |

**Table 1 Mean difference in IL6 levels between sample 1 and 2 (before banding)**

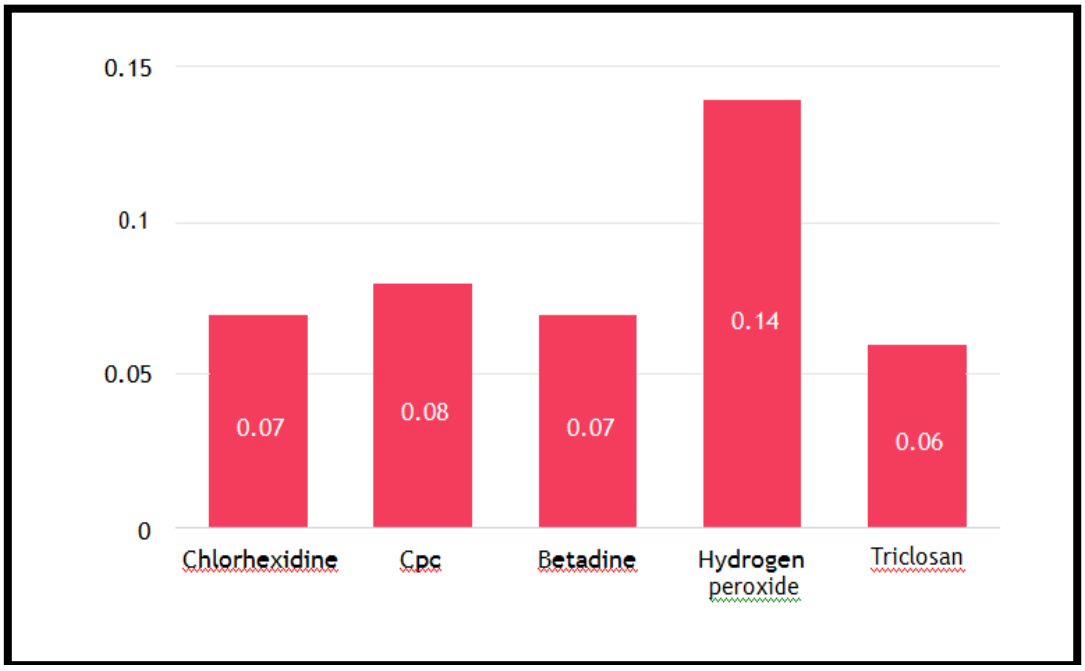


**Fig 4 Graph showing Mean difference between sample 1 and sample 2 showing (Before bonding)**

| Groups | Before | | After | | Mean difference | p-value |
|---|---|---|---|---|---|---|
| | Mean sample 3 | S.D. | Mean sample 4 | S.D. | | |
| Chlorhexidine | 0.99 | 0.1 | 0. | 0.13 | 0.12 | 0.003 |
| CPC | 0.93 | 0.2 | 0.92 | 0.13 | 0.15 | 0.006 |
| Betadine | 0.75 | 0.12 | 0.73 | 0.12 | 0.08 | 0.0004 |
| Hydrogen peroxide | 0.94 | 0.1 | 0.72 | 0.01 | 0.20 | 0.0001 |
| Triclosan | 0.92 | 0.1 | 0.90 | 0.12 | 0.02 | 0.03 |

**Table 2 Mean difference In IL6 levels between 3 and 4 sample( after bonding)**

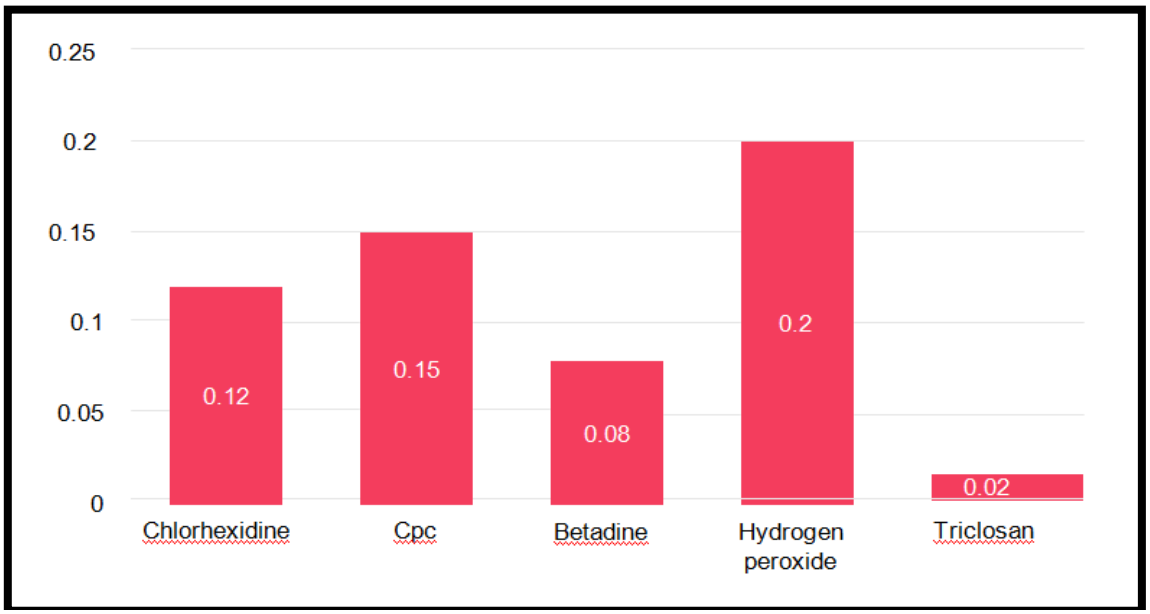


**Fig 5 Mean IL-6 levels between sample 3 and sample 4 among the five mouthwash**

| Groups | Before | | After | | Mean difference | p-value |
|---|---|---|---|---|---|---|
| | Mean sample 3 | S.D. | Mean sample 4 | S.D. | | |
| Chlorhexidine | 0.99 | 0.1 | 0. | 0.13 | 0.12 | 0.003 |
| CPC | 0.93 | 0.2 | 0.92 | 0.13 | 0.15 | 0.006 |
| Betadine | 0.75 | 0.12 | 0.73 | 0.12 | 0.08 | 0.0004 |
| Hydrogen peroxide | 0.94 | 0.1 | 0.72 | 0.01 | 0.20 | 0.0001 |
| Triclosan | 0.92 | 0.1 | 0.90 | 0.12 | 0.02 | 0.03 |

**Table 3 Mean difference In IL6 levels between 3 and 4 sample( after bonding)**

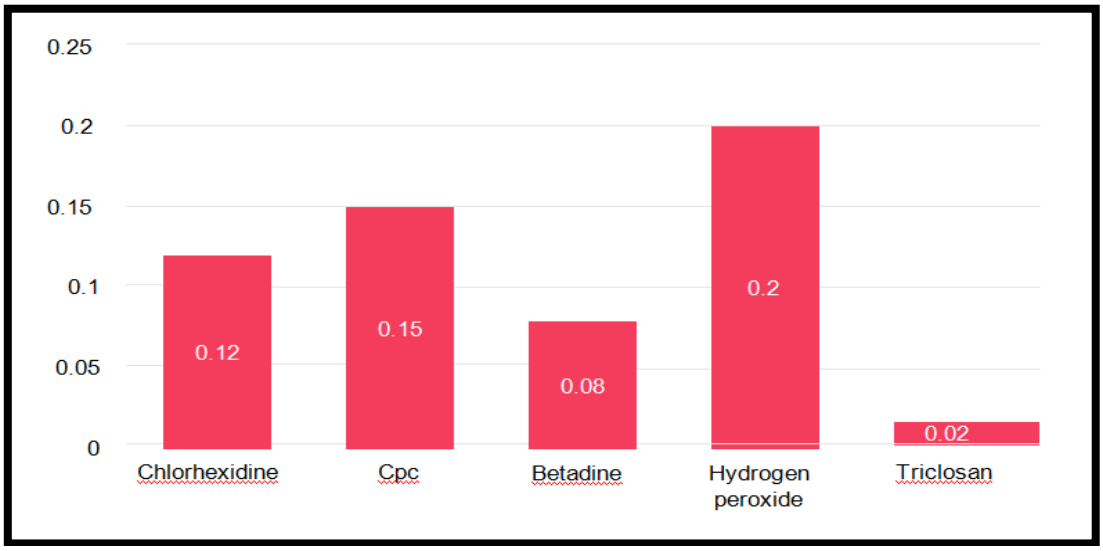


**Fig 6 Mean IL-6 levels between sample 3 and sample 4 among the five mouthwash**

| Groups | Before | | After | | Mean difference | p-value |
|---|---|---|---|---|---|---|
| | Mean sample 1 | S.D. | Mean sample 2 | S.D. | | |
| Chlorhexidine | 295 | 97.1 | 116 | 61.3 | 179 | 0.002 |
| CPC | 346 | 32.1 | 142 | 29.2 | 204 | 0.006 |
| Betadine | 408 | 032 | 176 | 23 | 232 | 0.0004 |
| Hydrogen peroxide | 412 | 25 | 162 | 60 | 268 | 0.0001 |
| Triclosan | 417 | 43 | 150 | 34 | 250 | 0.004 |

**Table 4 Mean difference in Bacterial levels between sample 1 and 2 (before banding)**

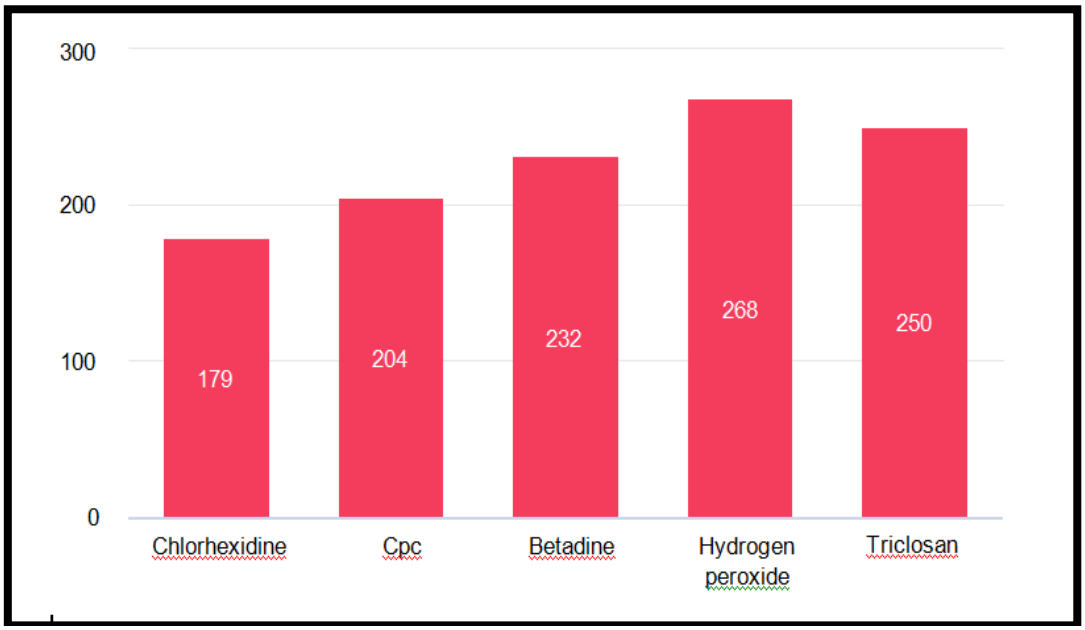


**FIG 7-comparision of mean value of reduction of bacterial load in sample 1 and 2**

| Group (I) | Group (J) | Mean difference | p-value |
|---|---|---|---|
| **Chlorhexidine** | **CPC** | 0.068 | 1.00 |
| | **Betadine** | 0.046 | 1.00 |
| | **Hydrogen peroxide** | -0.083 | 0.609 |
| | **Triclosan** | -0.024 | 1.00 |
| **CPC** | **Betadine** | -0.022 | 1.00 |
| | **Hydrogen peroxide** | -0.152 | 0.009* |
| | **Triclosan** | 0.093 | 0.383 |
| **Betadine** | **Hydrogen peroxide** | -0.13 | 0.042* |
| | **Triclosan** | -0.07 | 1.00 |
| **Hydrogen peroxide** | **Triclosan** | 0.059 | 1.00 |

**Table 5Intergroup comparison of mean change in IL-6 levels among the study group**

## Discussion

The results of the present study showed that oral aerosol/splatter from subjects who rinsed with a mouthwash containing 1.5% hydrogen peroxide, harbored a significantly lower viral and bacterial content that from their samp,le before rinse. This represents important protection for the dentist and dental hygienists, who are the main targets of the microorganisms generated during oral procedures. It is important to emphasize that the dental/surface barriers, the methods most commonly used to minimize cross-infection in the dental office, do not reduce the levels of microorganisms in the environment.

Rinsing provides a viable mean of protection because these barriers, such as gloves, masks and glasses may have openings, smaller pores or defects, through which bacteria can pass. Furthermore, the aerosol particles may remain in the environment for up to 4 hours after a procedure31 and normally, the clinician and patients remove the protective barriers shortly after completion of the procedure. Therefore, the risk of airway contamination by these microorganisms even after the completion of the appointment is high.1 Thus, minimizing the quantity of microorganisms in the oral cavity before the aerosol/splatter is generated is essential to reduce the risk of cross-infection in the dental environment.

In addition, rinsing also represented a major benefit for the patients. In the present study, high bacterial counts, which reached an average of ≈500 CFUs in the Water and No Rinsing groups, were observed on the blood agar plates positioned.

The surface of Rhinovirus presents a spike protein (S) involved in the receptor recognition and cell membrane fusion process. The S protein mediates cell entry when it contacts the angiotensin-converting enzyme 2 (ACE2) receptors, and oral mucosa and salivary gland epithelium present a great amount of these receptors . In a study by Huang et al. RNA molecules of Rhinovirus were consistently found in ACE2-expressing ducts of salivary glands and in epithelial cells of the oral mucosa. They also proposed that the virus replicating in infected glands and the shedding of the infected oral mucosa are the sources of Rhinovirus in saliva.

The data from this study demonstrated rapid bactericidal and virucidal activity of Five commercial mouthwash against Rhinovirus and streptococcus. The surface of Rhinovirus presents a spike protein (S) involved in the receptor recognition and cell membrane fusion process. The S protein mediates cell entry when it contacts the angiotensin-converting enzyme 2 (ACE2) receptors, and oral mucosa and salivary gland epithelium present a great amount of these receptors . In a study by Huang et al. RNA molecules of Rhinovirus were consistently found in ACE2-expressing ducts of salivary glands and in epithelial cells of the oral mucosa. They also proposed that the virus replicating in infected glands and the shedding of the infected oral mucosa are the sources of Rhinovirus in saliva.

A study showed PVP-I is composed of iodine and the water-soluble polymer polyvinylpyrrolidone. PVP-I has antimicrobial activity when it dissociates and releases iodine. Iodine penetrates the microbes, oxidizes nucleic acids, and disrupts proteins. Thus, PVP-I damages the virus via the perturbation of several metabolic pathways and

disorganization of the cell membrane (Nagatake et al. 2002)[3]. PVP-I has been demonstrated to have greater antiviral activity against both enveloped and nonenveloped viruses as compared with other antiseptic agents, such as CHX (Kawana et al. 1997). In vitro studies evaluating the 50% tissue culture infective dose (TCID50) method demonstrated that PVP-I has virucidal activity against Viruses. Gargle and mouth rinse with solutions containing PVP-I at 1% achieved a virucidal activity higher than 99.99%, which corresponds to a reduction of virus load .in my study PVP showed significant result in reducing bacterial load 2 hours after rinsing[8]

Hydrogen peroxide is a substance which is degraded into oxygen and water when in contact with catalase – an enzyme present in almost all living beings, including micro-organisms within the oral microbiota – and this oxidative process would be capable of eliminating bacteria and fungi .Peng *et al*[4]. assumed that this process of oxidation might also be effective against SARS-CoV-2 by alleging that this virus would be sensitive to oxidation [9]. The work in question has been cited frequently in the literature since its publication reater than 4 $\log_{10}$, after 30 s of contact (Anderson et al. 2020)[5] my study showed maximum reduction in viral load among five groups of mouthwashes.[10]

## Conclusion

Significant reduction in viral load was seen in all five mouthwash groups .

Hydrogen peroxide showed maximum reduction in viral load followed by chlorhexidine hence proving their efficacy.

The use of mouthwash should be limited to a smaller period of time depending on the lesion present and should always be used as an adjunct to mechanical plaque control measures (tooth brushing and flossing). Long term use of alcohol based mouthwashes should be discouraged

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