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Evaluation of Antimicrobial efficacy of Hydrogen peroxide, Iodine and Combination of Hydrogen peroxide and Iodine – An *In Vitro* Microbiological Study

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Abstract

Topical antimicrobials are widely employed for medical therapies as well as infection and microbial control in dental care. Combining antimicrobials can increase their effectiveness through synergistic or additive effects, which can assist in overcoming bacteria acquired resistance to individual drugs. Iodine and hydrogen peroxide are oxidising substances that have been used as antimicrobials for a very long time. To determine the antimicrobial efficacy of iodine, hydrogen peroxide and hydrogen peroxide and iodine combination (Perimed) against oral pathogens. The samples were divided into 8 groups, Group A1 and A2 containing 500µl of saline, Group B1 and B2 containing 500µl 2% iodine diluted with equal parts of saline, Group C1 and C2 containing 500µl of hydrogen peroxide (H_2O_2), Group D1 and D2 containing 500µl of Perimed. 50µl of S.mutans and Lactobacillus suspension was added to respective groups and thoroughly mixed. After 1 minute 50µl of suspension from each test group was transferred to nutrient media and lawn culture streak was made. The plates were incubated overnight and the number of colony forming units was counted as per established protocols. Data collected was statistically analyzed and the significance of the study was evaluated. It was observed that Iodine and hydrogen peroxide had a strong antimicrobial activity against *Streptococcus mutans* and *Lactobacillus*. Iodine, hydrogen peroxide and Iodine and hydrogen peroxide combination may be effectively used as an antibacterial mouthwash within the parameters of the present study.

Keywords: Antimicrobial activity, Hydrogen peroxide, Iodine, *Streptococcus mutans, Lactobacillus*

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1. Introduction

Bacterial plaque on teeth is generally considered to be the main etiological agent in gingivitis and periodontal disease. Gingivitis develops within 2-3 weeks if plaque is allowed to accumulate at the gingival margin [1], When the plaque is removed and oral hygiene is reinstated, healthy gingival conditions will be obtained within 5-7 days. Bacterial plaque at the dento-gingival junction is responsible for the initiation of chronic gingivitis and probably the progression to chronic periodontitis [2]. It is also known that the removal of plaque-retentive factors like calculus, rough surfaces and defective restorations and regular removal of plaque by mechanical means will greatly reduce inflammation and progression of periodontal disease [3, 4]. An additional strategy for attaining good plaque reduction is chemical control. Miller discussed about the idea of employing antiseptics as early as 1890.[5] Since the 1950s and the beginning of the 1960s, antibiotics have been used, although they are now recognized as a subpar alternative for the treatment of plaque accumulation. In health care, industry, and the environment, antimicrobials are widely used for infection and microbial control [6]. They are also employed in medical procedures.[7] Combining antimicrobials may increase their effectiveness through synergistic or additive effects, and it may also aid in overcoming acquired microbial resistance to certain drugs [6].

Iodine (I_2) and hydrogen peroxide (H_2O_2) are oxidising substances that have been used as antimicrobials for a very long time.[6],[7],[8],[9],[10],[11] Iodine is a halogen-releasing chemical that quickly kills bacteria, fungi, viruses, and spores by blocking DNA synthesis and destroying amino acids, nucleotides, and fatty acids.It is frequently employed in iodophores (complexes) with solubilizing agents.[6],[7],[8],[9],[10],[11],[12] As H2O2 is effective against bacteria, yeasts, fungi, viruses, and spores, it has been demonstrated to have a broad spectrum of antibacterial activity.[13] H2O2 is also an oxidant that has been used to control plaque. With no negative effects on the tissues, oxygenating drugs can be used to treat acute ulcerative gingivitis and control supragingival plaque [14]. Iodine and hydrogen peroxide are both commonly used antiseptics and disinfectants for topical skin therapy[9],[15], wound healing[7],[12],[16],[17],[18], preoperative site

preparation[19], gingival plaque control[20], treating biofilm[21] and Fournier's gangrene[22], disinfecting catheters[23] and other surfaces[24], industrial treatments of fish eggs[25],[26], lowering bacterial pathogens on fruits[27], purification of water systems[28], and many other processes.

There is evidence to suggest that the majority of patients may not have the motivation or skills necessary to use oral hygiene tools including toothbrushes, dental floss, toothpicks, and interdental brushes. Inadequate oral hygiene is a concern for some groups of people, such as those who are temporarily impaired, non-ambulatory patients and handicapped people.[29] Therefore, a different approach to controlling plaque, such as a chemical control strategy, would be desired and pertinent. Thus, the present study was undertaken to evaluate and compare the antimicrobial effect of iodine, hydrogen peroxide and combination of iodine and hydrogen peroxide.

2. Materials and Methods

This study is conducted to analyze the antibacterial activity against the two most common facultative anaerobe associated with periodontal lesions, lactobacilli and S. mutans as a member of viridans streptococci. The method is broth dilution method. Pure culture of standard strains of *Streptococcus mutans* and *lactobacillus* were used. The organisms were grown on appropriate media S.mutans on blood agar and lactobacillus on MacConkey agar. The organisms were suspended in a sterile cuvette to a concentration matching 0.5 Mcfarland standard in a sterile cuvette containing 1ml saline. Culture Plates were examined for sterility before use by incubating at 37ºC for 48 hrs. The iodine used in the study was the commercially available 2% Povidone - Iodine mouthwash diluted with equal part of saline as instructed by the manufacturer. The hydrogen peroxide (H_2O_2) used in the study was commercially available 1% (H_2O_2) and the Perimed combination was obtained by mixing equal parts Iodine with hydrogen peroxide.

The study samples were divided into 8 groups, including both Control group (Group A1 and A2) and Test group (Group B1, B2, C1, C2, D1 and D2). Group A1 and A2 includes 2 sterile cuvettes containing 500µl of saline, Group B1 and B2 includes 3 sterile cuvettes containing 500µl 2% iodine diluted with equal parts of saline, Group C1 and C2 includes 3 sterile cuvettes containing 500µl of 1% hydrogen peroxide (H_2O_2), Group D1 and D2 includes 3 sterile cuvettes containing 500µl of Perimed. 50µl of S.mutans suspension was added to Group A1, B1, C1 and D1 and 50µl of Lactobacillus suspension was added to Group A2, B2, C2 and D2 using a micropipette and thoroughly mixed. After 1 minute of resting period 50µl of suspension from each test group was transferred to the respectively labeled Brain Heart Infusion Agar and lawn culture was made with a sterile inoculation loop. The culture plates were incubated at 37ºC for 24 hrs and the number of colony forming units was counted as per established protocols. Data collected were tabulated, and statistically analyzed and the significance of the study was evaluated.

3. Results and Discussion

The microbial analysis showed that among the streptococcus species the mean number of colony growth was more than 1 lakh colonies in control group, 180 colonies in Iodine group, 120 colonies in hydrogen peroxide group and 46.67 colonies in combination group; similarly, among the Lactobacillus species the mean number of colony growth was more than 1 lakh colonies in control group, 86.67 colonies in Iodine group, 60 colonies in hydrogen peroxide group and 160 colonies in combination group (Figure 1-3 and Table 1). On intergroup comparison among the Streptococcus species there was a slightly higher antimicrobial activity exhibited by hydrogen peroxide group compared to iodine group and furthermore antimicrobial activity was exhibited by the combination group and the difference was not statistically significant (P value > 0.05) and among the Lactobacillus species there was a slightly higher antimicrobial activity exhibited by hydrogen peroxide group compared to iodine group but a lower antimicrobial activity was exhibited by the combination group compared to iodine and hydrogen peroxide groups and the difference was not statistically significant (P value > 0.05)(Table 2 and Table 3). Mechanical plaque removal with assorted devices remains the primary and most widely accepted means of controlling plaque and preserving good oral hygiene. As an adjunct to mechanical techniques, chemical plaque control comprising of a variety of chemotherapeutic agents, have been beneficial and advisable.[14],[30] The findings of the present study show that iodine, hydrogen peroxide tested alone and in their combination had a significant antimicrobial activity against *Streptococcus mutans* and *lactobacillus* compared to control (P value < 0.05). On intergroup comparison there was slightly higher antimicrobial activity expressed by hydrogen peroxide than iodine between both streptococcus and lactobacillus species and the combination group exhibited a slightly higher antimicrobial activity than iodine and hydrogen peroxide among streptococcus species but a slightly lower antimicrobial activity than iodine and hydrogen peroxide among lactobacillus species which may be due to the decrease in the individual chemical concentration due to the dilution of the compounds in combination group. Gusberti et.al[31] in his study said that when compared to the placebo group, among the group using 1% hydrogen peroxide showed a marginal reduction in gingivitis incidence of 15% and a 28% reduction in bleeding sites, but reduction in the plaque scores was not statistically significant.

The antibacterial properties of hydrogen peroxide are exhibited in the elimination of gram-positive and gram-negative bacteria. When hydrogen peroxide is exposed to other compounds, it breaks down very quickly into water and oxygen. Notably, the oxygen is released in the form of a free radical and, through oxidation, destroys microorganisms, particularly those that are anaerobic.[32] More specifically, anaerobes lack the enzymes needed to detoxify products such as hydrogen peroxide. When hydrogen peroxide reacts with oxygen, a free hydroxyl radical is formed. This radical is a very potent oxidant and can attack any organic substance in the cell.[32].

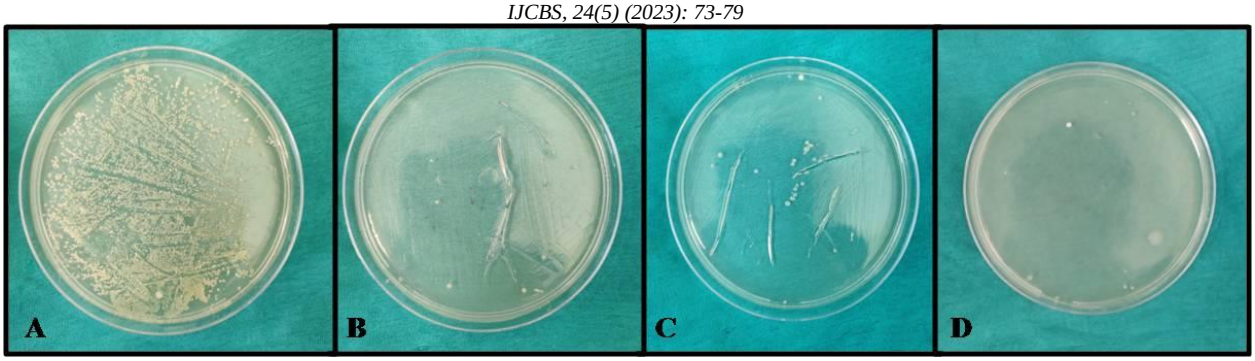

Figure 1: *Streptococcus mutans* colony growth in brain heart infusion agar; a: control group, b: h2o2 group, c: i2 group, d: combination group

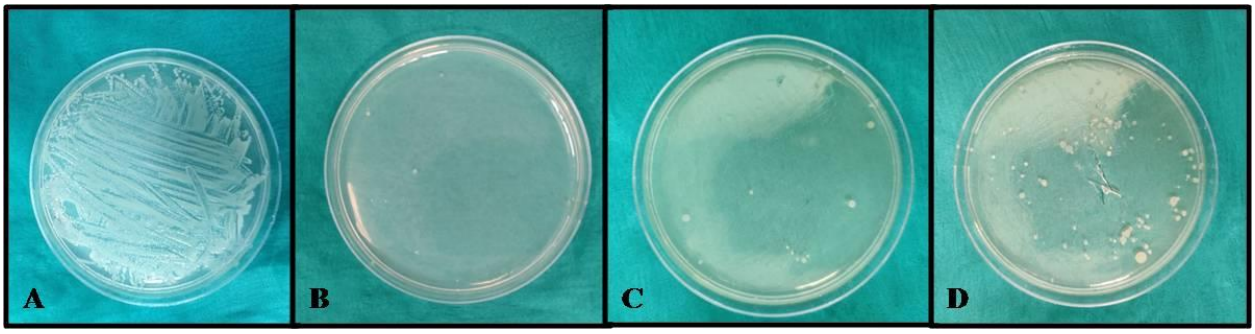

Figure 2: *Lactobacillus* colony growth in brain heart infusion agar; a: control group, b: h2o2 group, c: i2 group, d: combination group

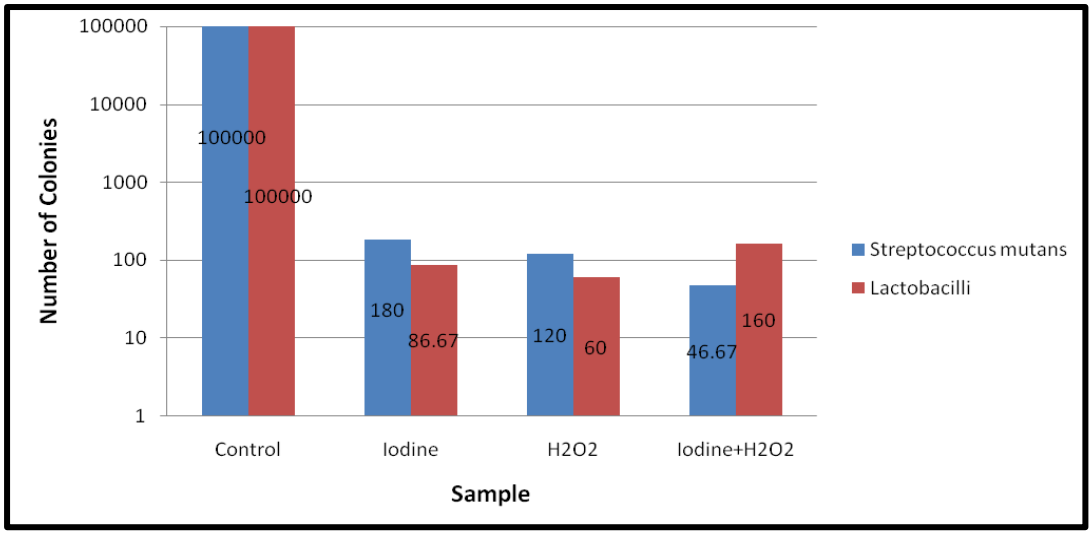

Figure 3: Mean bacterial colony count

Table 1: Mean bacterial colony count

	SAMPLE/ml	Control	Iodine	H_2O_2	Iodine+H_2O_2
Mean colonies count	*Streptococcus mutans*	100000	180	120	46.67
	Lactobacilli	100000	86.67	60	160

Table 2: Intergroup comparison of the mean bacterial colony count of *Streptococcus mutans*

Multiple Comparisons						
Dependent Variable: BACTERIAL COLONY COUNT Bonferroni						
(I) SAMPLE	(J) SAMPLE	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CONTROL	IODINE	99820.000*	23.570	.000	99738.00	99902.00
	H_2O_2	99880.000*	23.570	.000	99798.00	99962.00
	H_2O_2+IODINE	99953.333*	23.570	.000	99871.34	100035.33
IODINE	CONTROL	-99820.000*	23.570	.000	-99902.00	-99738.00
	H2O2	60.000	23.570	.206	-22.00	142.00
	H_2O_2+IODINE	133.333*	23.570	.003	51.34	215.33
H2O2	CONTROL	-99880.000*	23.570	.000	-99962.00	-99798.00
	IODINE	-60.000	23.570	.206	-142.00	22.00
	H_2O_2+IODINE	73.333	23.570	.087	-8.66	155.33
H_2O_2+IODINE	CONTROL	-99953.333*	23.570	.000	-100035.33	-99871.34
	IODINE	-133.333*	23.570	.003	-215.33	-51.34
	H_2O_2	-73.333	23.570	.087	-155.33	8.66
*. The mean difference is significant at the 0.05 level.						

Table 3: Intergroup comparison of the mean bacterial colony counts of *Lactobacilli*

Multiple Comparisons

Dependent Variable: BACTERIAL COLONY COUNT
Bonferroni

(I) SAMPLE	(J) SAMPLE	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CONTROL	IODINE	99913.333*	36.818	.000	99785.25	100041.42
	H_2O_2	99940.000*	36.818	.000	99811.92	100068.08
	H2O2+IODINE	99840.000*	36.818	.000	99711.92	99968.08
IODINE	CONTROL	-99913.333*	36.818	.000	-100041.42	-99785.25
	H_2O_2	26.667	36.818	1.000	-101.42	154.75
	H_2O_2+IODINE	-73.333	36.818	.489	-201.42	54.75
H_2O_2	CONTROL	-99940.000*	36.818	.000	-100068.08	-99811.92
	IODINE	-26.667	36.818	1.000	-154.75	101.42
	H_2O_2+IODINE	-100.000	36.818	.158	-228.08	28.08
H_2O_2+IODINE	CONTROL	-99840.000*	36.818	.000	-99968.08	-99711.92
	IODINE	73.333	36.818	.489	-54.75	201.42
	H_2O_2	100.000	36.818	.158	-28.08	228.08

*. The mean difference is significant at the 0.05 level.

According to earlier research, rinsing with a 1% hydrogen peroxide solution has very little impact on gingivitis and dental biofilm.[31],[32] Hydrogen peroxide had no positive effects on patients with mild to moderate periodontitis, according to Pihlstrom et al.[33] On the other hand, a study by Marshall et al. discovered that Actinobacillus actinomycetemcomitans, a bacteria that frequently causes periodontal disorders, was suppressed or eliminated when a 3% hydrogen peroxide solution was irrigated into periodontal pockets twice a week for six months.[32]. The findings of this study confirm earlier research by Marshall et al. that hydrogen peroxide possesses antibacterial properties.[32] The results did not support previous research that had concluded hydrogen peroxide was ineffective against oral infections.[33] On a regular basis, new oral hygiene products are released. Each one makes a variety of promises and guarantees, many of which remain untested. It is crucial to read the most recent research on oral health care products to identify those that might be safe and helpful and those that might not.

Although chlorhexidine is regarded as the greatest agent for treating gingivitis and plaque, many patients dislike its initial bitter taste, and frequent use results in staining of teeth and taste abnormalities. Therefore, it is not recommended to use chlorhexidine as a regular mouthwash. Therefore identifying an alternative chemical agent without the drawbacks of chlorhexidine is inevitable. From the present study we can infer that hydrogen peroxide, iodine and their combination prove to be very good antimicrobials against streptococcus and lactobacillus species and can be used as active agents in chemical plaque control methods. The results of present study were limited because they were studied only in vitro. Results were also limited because of the small sample size. Recommendations for future studies would be to conduct the study in vivo and to have a larger sample and longer experimental time. More studies need to be done on the effects of sodium bicarbonate and hydrogen peroxide to determine their maximum clinical significance.

4. Conclusion

2% Iodine and 1% hydrogen peroxide shows significant antimicrobial activity against *Streptococcus mutans* and *lactobacillus* species when compared with normal saline as placebo, also the combination of Iodine and hydrogen peroxide showed significant antimicrobial activity compared to normal saline and slightly higher antimicrobial activity on intergroup comparison but not statistically significant. Therefore 2% Iodine and 1% hydrogen peroxide and their combination may be effectively used as an active agent in chemical plaque control methods within the parameters of the present study.

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