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Effect Of Using Povidone Iodine and Herbal Propolis Antibacterial Mouth on Blood Pressure

Rini Sitanaya[1*], Hans Lesmana[1], Surya Irayani[1], Ellis Mirawati Hamid[1], Harun Achmad[2], Fatma Afrianty Gobel[3], Evy Adawiyah[4]

[1]Dental Nurse Dept. Ministry of Health Polytechnic, Makassar, Indonesia

[2]Pedodontic Dept., Hasanuddin University, Indonesia

[3]Public Health Dept., Universitas Muslim Indonesia

[4]Student of master dental health Polytechnic of Health Ministry Semarang, Indonesia

*__Corresponding author:__ Rini Sitanaya , Dental Nurse Dept. Ministry of Health Polytechnic, Makassar, Indonesia, Email:rinisitanayadrg96@gmail.com

ABSTRACT

Oral bacteria have a key role in inorganic nitrate bioactivation as they catalyze the conversion of salivary nitrate to the more reactive nitrite anion. Recent studies have shown that blood pressure increases with prolonged use of antiseptic mouthwashes, and demonstrated that nitrates derived from endogenous NO-synthase are recycled to nitrites and NO, sufficient to modulate cardiovascular function.

Reduction of salivary nitrate by oral bacteria may contribute to preventing oral disease, as well as increasing systemic nitric oxide levels which may improve conditions such as hypertension and diabetes.

Nitric Oxide is a substance that has the task of relaxing blood vessels, while bacteria are tasked with the Nitrite Pathway process, so the elimination of these bacteria due to the use of mouthwash can affect blood pressure.

This study used 12 adult samples (n = 6 Povidone Iodine and n = 6 Herbal Propolis) with age criteria of 17-30 years, healthy body and no history of hypertension. effects on blood pressure from the use of Povidone Iodine and Herbal Propolis antibacterial mouthwash. The highest systolic increase occurred on day 3, with a Standard Deviation of PI = 5.05964 & HP = 3.76386. The sample experienced an increase in blood pressure of approximately <5 mmHg. These results indicate that Povidone Iodine and Herbal Propolis mouthwash have an effect on reducing the microbiome of oral bacteria, in this case nitrate reducing bacteria so that it can affect blood pressure.

Keywords: *Nitrite Pathway, Nitric Oxide, nitrate reducing bacteria, blood pressure*

INTRODUCTION

In the oral cavity there are various kinds of microorganisms, both pathogenic and non-pathogenic (normal flora). Normal flora can be found on the skin and mucous membranes (mucosa) of healthy and diseased individuals.

In certain parts, the development of normal flora is affected by temperature, nutrition, humidity, and the presence of inhibitory substances. In certain parts of the body, normal flora has an important action for immunity because it produces

a substance that can inhibit the growth of bad microorganisms or bacteria that are harmful to the human body.

For the prevention of dental and oral diseases, plaque control is considered the most effective. Using mouthwash every day is considered as one of the efforts to eradicate plaque mechanically. Because gargling with mouthwash can reach the surface of the teeth that cannot be reached by a toothbrush. Suppress the growth of plaque bacteria have the potential to support the successful treatment in children because it can increase the body's immune system thus it can accelerate the healing of damaged or wounded tissue, such as gum hemorrhage, postoperative wounds, or healing process.(Achmad, 2019) Mouthwash is widely used by the public, and is believed to help reduce plaque and prevent gingivitis and other dental diseases caused by bacteria in the oral cavity. Most prescription and over-the-counter mouthwashes, including fluoride mouthwashes, which are useful for reducing caries, contain antiseptic or antibacterial ingredients that can inhibit plaque. Povidone iodine is an iodovor with polyvinyl pyrrolidone. Iodine has a broad spectrum of anti-bacterial, fungal, protozoa and viruses. This mouthwash is quite effective in reducing plaque, gingivitis and is also used for routine oral hygiene procedures.

Based on the results of Household Health Survey (SKRT) in 2011 the Ministry of Health of Indonesia shows dental and oral diseases including caries and periodontal disease is a problem often complained by the community with a high percentage of incidents as much as 60% that because of plaque. (Achmad, 2018)

Propolis is a resinous substance that bees collect from the buds of leaves and tree bark. Propolis has antibiotic activity to protect against viruses, bacteria and other organisms. Compounds that have antimicrobial and anti-plaque action are Flavonoids. Two mechanisms of antiplaque content in propolis, namely antimicrobial performance against cariogenic bacteria and propolis inhibition of glucosyltransferase enzymes and glucosyltransferase enzymes and can convert salivary sucrose into extracellular polysaccharides (PSE) through the glycosylation

process. This extracellular polysaccharide will form a matrix in the plaque where other bacteria can attach (Prity, 2014)

In 2017, 203 million (62%) Americans used mouthwash, and 17 million used it twice daily. Nearly two-thirds of a representative sample in the US used mouthwash to treat dental disease or dental problems in the past seven days, and 36% used it daily (Joshipura et al, 2019).HoweverJoshipura et al (2019)also reported that the use of mouthwashes has an adverse effect on the oral microflora, as it drastically inhibits the beneficial nitrate-reducing activity, leading to a sharp reduction in salivary nitrites, even after nitrate loading.

Oral bacteria have a key role in inorganic nitrate bioactivation as they catalyze the conversion of salivary nitrate to the more reactive nitrite anion. Recent studies have shown that blood pressure increases with the use of antiseptic mouthwashes, indicating that nitrates derived from endogenous NO-synthase are recycled to nitrites and NO, which are sufficient to modulate cardiovascular function. (Sundqvist, 2016)

The use of mouthwash not only kills pathogenic bacteria in the oral cavity but also eradicates the normal flora in the mouth. So this can disrupt the balance of the microbiome in the mouth. One of the bacteria that is affected by the use of this mouthwash is a bacteria that plays a role in converting nitrates into nitrites and then into Nitric Oxide (NO) which plays a role in relaxing blood vessels.

MATERIALS AND METHODS

The type of research used in this research is qualitative research, with the type of qualitative experimental research using a cross-sectional study, as well as using a Pre-Post Experimental Design (Nondesigns). Samples were taken using the quota sampling method from this study consisting of 12 adults, aged 17-30 years. In good health and have no history of hypertension (high blood pressure). The sample used in this study were adults with age criteria of 17-30 years and no history of high blood pressure (hypertension). Samples were asked to rinse their mouth using a mouthwash containing Povidone Iodine and

Herbal Propolis twice a day routinely as much as 10 ml. The research sample filled out informed consent. The researcher checked the blood pressure results on day 3. Then recorded the results of the data obtained. Furthermore, the researchers will come back on days 7, 14, and 21 to find out the progress of the blood pressure results of the samples. then it will produce Post-Test data by analyzing any changes that occur on the day of the pressure check. Analysis of the differences obtained from the results of the Pre-Test and Post-Test with the Two Way Anova Test.

RESEARCH RESULT

This study used a sample of 12 adults who were divided into 2 groups, namely group A and B where the number of each group consisted of 6 people. Group A is a group that uses Povidone Iodine as a mouthwash and group B is a group that uses Herbal Propolis as a mouthwash. By gargling 2 times a day routinely as much as 10 ml.

TABLE 1: Results of blood pressure measurements before and after using mouthwash containing Povidone Iodine (PI)

Povidone Iodine (A)	Before	After			
		Day-3	Day-7	Day-14	Day-21
PI1(P)	99/80	112/80	114/80	116/80	120/80
PI2 (P	99/80	100/80	106/80	116/80	118/80
PI3(P)	100/80	102/80	106/80	109/80	117/80
PI4(P)	103/80	106/80	106/80	114/80	115/80
PI5(L)	96/80	98/80	100/80	105/80	106/80
PI6(P)	99/80	106/80	109/80	109/80	114/80

TABLE 2: Results of blood pressure measurements before and after using Herbal Propolis (HP) mouthwash.

Propolis herbs (B)	Before	After			
		Day-3	Day-7	Day-14	Day-21
HP1(P)	109/80	111/80	115/80	117/80	120/80
HP2(P)	90/80	107/80	108/80	112/80	119/80
HP3(P)	108/80	110/80	111/80	111/80	113/80
HP4(P)	103/80	109/80	110/80	115/80	119/80
HP5(P)	117/80	118/80	118/80	119/80	120/80
HP6(P)	110/80	110/80	113/80	113/80	114/80

The table above is the result of blood pressure measurements before using Povidone Iodine & Herbal Propolis mouthwash and after using mouthwash. Blood pressure checks were carried out periodically on days 0, 3, 4, 7 and 21.

TABLE 3: Descriptive analysis of the PI and HP samples

Descriptives					
Results	N	Average (Mean)		Standard Deviation	
		Povidone Iodine	Propolis herbs	Povidone Iodine	Propolis herbs
Day 0 (Before)	6	99.3333	106.1667	2.25093	9.10860
3rd day	6	104.0000	110.8333	5.05964	3.76386
7th day	6	106.8333	112.5000	4.57894	3.61939
14th day	6	111.5000	114.5000	4.50555	3.08221
21st day	6	115.0000	117.5000	4.89898	3.14643
Total	30	107.3333	112.3000	6.91990	6.09777

From the table above, it can be seen that the average and standard deviation for each blood pressure check, starting from day 0, namely the day before the intervention or before the sample gargling with mouthwash routinely continued on the 3rd, 7th, 14th and 21 after the intervention (giving mouthwash), it was seen that the biggest change in blood pressure occurred on day 3.

TABLE 4 : Hypothesis Testingusing the Two Way Anova test (ANOVA)

Povidone Iodine Anova					
Results	Sum of Squares	df	MeanSquare	F	Sig.
Between Groups	909,000	4	227,250	11,844	.000
Within Groups	479,667	25	19,187		
Total	1388667	29			

From the table above it can be seen the value . Because of value $Sig. = 0.000$ $Sig. = 0.000$ smaller than then it gives a conclusion to reject H0 $\alpha = 0.05$ and accept H1. Thus, it can be concluded thatthere was a change in blood pressure in the sample using Povidone Iodine mouthwash routinely 2x a day for 21 days. Where the biggest change in blood pressure occurred on day 3 after gargling with Povidone Iodine mouthwash.

TABLE 5: ANOVA Test (Two Way Anova) Herbal Propolis Mouthwash

Propolis Herbal Anova					
Results	Sum of Squares	df	MeanSquare	F	Sig.
Between Groups	430,133	4	107,533	4.148	010
Within Groups	648,167	25	25,927		
Total	1078,300	29			

From the table above it can be seen that with the value $Sig. = 0.010$ smaller than then it gives a conclusion to reject H0 $\alpha = 0.05$ and accept H1. Thus, it can be concluded thatthere was a change in blood pressure in the sample using Herbal Propolis mouthwash routinely 2x a day for 21 days. Where the biggest change in blood pressure occurred on day 3 after rinsing with Herbal Propolis mouthwash.

DISCUSSION

The antibacterial content contained in Povidone Iodine and Herbal Propolis mouthwash can kill bacteria in the oral cavity without distinguishing

between pathogenic bacteria and non-pathogenic bacteria. Recent evidence suggests that reduction of salivary nitrate by oral bacteria can contribute to preventing oral disease, as well as increasing systemic nitric oxide levels which can improve conditions such as hypertension and diabetes (Rosier, 2020). This is because Nitric Oxide bacteria in the Nitrite Pathway which are useful for relaxing blood vessels are also eliminated. When the nitrate-reducing bacteria are reduced or due to the use of anti-bacterial mouthwashes, the Nitrite Pathway process will be hampered. When blood vessels are not elastic, the heart will work harder to pump blood throughout the body, when the heart has to work harder than usual, this can cause blood pressure to rise. If this happens for a prolonged time it can cause hypertension. Lower bioavailability of nitric oxide (NO) is associated with the development of hypertension, through loss of direct vascular vasodilatory abilities and secondary effects that increase oxidative stress and inflammation, which can lead to vascular injury and the risk of developing hypertension. Treatment with mouth rinses containing chlorhexidine effectively reduced the conversion of nitrate to nitrite orally but had no effect on plasma levels of these anions or plasma cGMP. RMR and 24-hour outpatient blood pressure were not affected by the intervention. In healthy young women, Antiseptic mouthwashes were effective in interfering with oral bacterial conversion of nitrates to nitrites, but this was not associated with changes in plasma nitrites, RMR or blood pressure. (Sundqvist, 2016).

Joshipura, et all in the US in 2019 in their research stated that people who use mouthwash on the market have an 85% higher risk of experiencing hypertension and conclude that long-term use of mouthwash 2 times a day or more is more at risk of developing hypertension compared to people who rarely or do not use mouthwash. However, you must pay attention to other potentials that can increase blood pressure.

The use of Povidone Iodine mouthwash is significant for reducing nitrate which affects the performance of the Veillonella dispar bacteria. According to Takahiro Mitsui, et al (2017), Nitrates from food are reduced to Nitrites and Nitric Oxides by microbial flora, and this activity is beneficial for the health of blood vessels. It has been reported that this bacterial process can be inhibited by the mouthwash Povidone Iodine & Chlorhexidine, although the effects of the other products are largely unknown. Chlorhexidine at a concentration of 0.12% or 0.2% reduced salivary nitrites drastically, whereas at a concentration of 0.0025% which was studied in Takahiro's research it was not enough to interfere with salivary nitrite production. Takahiro et al. tested the effect of several treatments on salivary nitrate/nitrite and nitrate reducing bacteria. This study involved 12 university staff and students who were asked to rinse their mouths with water (control), essential oil, 0.35% povidone-iodine, or 0.0025% chlorhexidine and then eat 100g of lettuce (110mg nitrate content). Then followed by the collection of bacteria in saliva and tongue, starting at the beginning of the study 1, 5, and 10 hours afterward. Individual treatments were separated by one week intervals. Salivary nitrate/nitrite was measured using the calorimetry method, and the species of nitrate-reducing bacteria that appeared, Veillonella dispar, was detected using the polymerase chain reaction (PCR) test. A significant increase in salivary nitrate/nitrite was observed for all treatments (all $P<0.05$). PCR test showed that water, essential oil and povidone-iodine mouthwash had little effect, whereas the Veillonella dispar DNA band was significantly inhibited after rinsing with antibacterial mouthwash. These results suggest that povidone-iodine essential oil and mouthwash have little effect on oral nitrate-reducing activity. Salivary nitrite production was not reduced by Chlorhexidine, but the weakened Veilonella dispar DNA band suggests that longer daily use may decrease this nitrate-reducing activity. These results indicate that daily use of chlorhexidine mouthwash, even at a low concentration of 0.0025%, can affect the oral microflora and inhibit its nitrate-reducing activity. These results suggest that povidone-iodine essential oil and mouthwash have little effect on oral nitrate-reducing activity. Salivary nitrite production was not reduced by Chlorhexidine, but the weakened Veilonella dispar DNA band suggests that longer daily use may decrease this nitrate-reducing activity. These results indicate that daily use of chlorhexidine mouthwash, even at a low

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Raul Bescos et al (2020), investigated the effect of 7 days of using antibacterial mouthwash on the salivary microbiome as well as several salivary and plasma biomarkers in 36 healthy people. They gargled (for 1 minute) twice daily for seven days with a placebo mouthwash and then repeated this protocol with an antibacterial mouthwash for another seven days. Saliva and blood samples were taken at the end of each treatment to analyze the accumulation and diversity of oral bacteria, as well as the concentrations of pH, lactate, glucose, nitrate and nitrite. Antibacterial mouthwash according to Raul Bescos et al, significantly increased the accumulation of Firmicutes and Proteobacteria, and reduced the content of Bacteroidetes, TM7, SR1 and Fusobacteria. This change in the state of the oral microbiome is associated with a significant decrease in salivary pH and buffering capacity, accompanied by increased salivary lactate and glucose levels. Reduced salivary flow and plasma nitrite concentration were found after 7 days of using antibacterial mouthwash, followed by a trend of increasing systolic blood pressure. Overall, this study suggests that mouth rinses containing antibacterial agents may be associated with profound changes in the salivary microbiome, leading to a more acidic environment and lower nitrite availability in healthy individuals. As such, these findings add to growing evidence that the application of antibacterial mouthwashes should be considered with more caution, and that antibacterial mouthwashes can have detrimental effects on the healthy microbiome, and risk heart health, although this still requires further investigation.

This is also supported by Bodonno et al (2014), in his journal stated in samples using antibacterial mouthwash for 7 days that antibacterial mouthwashes can reduce nitrate activity thereby affecting the increase in blood pressure. Bondonno, et al (2014), involved fifteen men and women with hypertension who were being treated with an average age of 65 years. In this study it was shown that the effect of using an antibacterial mouthwash for 3 days resulted in a significant weakening of the reduction capacity of oral nitrate compared to the control group which gargled with plain water. Compared to the control, the use of antibacterial mouthwash for 3 days resulted in a significant increase in systolic of 2.3mmHg, but did not increase diastolic pressure significantly, namely less than 0.7mmHg. The results of this study indicate that the use of antibacterial mouthwash by treated hypertensive patients has a detrimental effect on blood pressure. Hypertension is associated with reduced NO production and/or bioavailability, so interruption of the nitrate-nitrite-NO pathway in hypertensive patients treated with antibacterial mouth rinses results in a slight increase in systolic blood pressure. This adds weight to the hypothesis that oral nitrate-reducing bacteria play a physiological role in blood pressure regulation. This suggests that the use of antibacterial mouthwashes in healthy subjects can attenuate the nitrite reduction activity of oral bacteria by at least 80%. This leads to lower availability of nitrites and increases blood pressure.

Meanwhile, according to Govoni (2008), the use of antibacterial mouthwash 0.2% twice a day for 7 days reduced salivary nitrite production by up

to 90% and increased systolic and diastolic blood pressure by 2-3.5 mmHg.

Slightly different from a study conducted by Tribble, et al (2019), involving 26 volunteers, gargling for 7 days, twice a day using chlorhexidine gluconate (Peridex) with a concentration of 0.12%. There are 17 girls and 10 boys; the mean age was 31.8 years, with the youngest subject being 22 years and the oldest being 71 years. The mean resting blood pressure at baseline in this group was 113/78 mmHg, and the systolic and diastolic data points corresponded to normal distributions. Two-way ANOVA repeated measurements of systolic blood pressure readings showed that there were significant differences between time points (p = 0.017), and between individual subjects (p = 0.0001), with a significant interaction component (p = 0.005). Post-hoc analysis identified significant changes in systolic blood pressure between treatment and 3-day recovery (115 vs 111.5 mm Hg) time points, as well as 3-day recovery and 7-day recovery (111.5 vs 113.3 mm Hg). Diastolic blood pressure also differed significantly at these same points. The researchers collected samples of the participants' saliva and tongue scrapings and measured their blood pressure at four different points during the study: at baseline, then 7, 10, and 14 days later. Bryan et al reported that twice-daily use of chlorhexidine was associated with a significant increase in systolic blood pressure after 1 week of use and recovery from use resulted in an enrichment of nitrate-reducing bacteria on the tongue. and 3 days recovery and 7 days recovery (111.5 vs 113.3 mm Hg). Diastolic blood pressure also differed significantly at these same points. The researchers collected samples of the participants' saliva and tongue scrapings and measured their blood pressure at four different points during the study: at baseline, then 7, 10, and 14 days later. Bryan et al reported that twice-daily use of chlorhexidine was associated with a significant increase in systolic blood pressure after 1 week of use and recovery from use resulted in an enrichment of nitrate-reducing bacteria on the tongue. and 3 days recovery and 7 days recovery (111.5 vs 113.3 mm Hg). Diastolic blood pressure also differed significantly at these same points. The

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CONCLUSION
In this study it was proven that there were changes in blood pressure in samples using Povidone Iodine and Herbal Propolis mouthwash. So that the final conclusion is that the use of Povidone Iodine and Herbal Propolis mouthwash can increase blood pressure by < 5 mmHg.

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