The Effectiveness of Flouride on Zam Zam Water on Inhibition of Bacterial Growth Causes of Dental Plaques

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ABSTRACT

One of the good waters on this earth is Zam Zam water. Springs that are in the area of the Grand Mosque are not only clean but also have considerable benefits for health. The uniqueness of this spring also never runs out or is dry. Although every year millions of pilgrims take water from the Zam Zam well, the water continues to flow profusely. Zam Zam water is sterile because it has a high content of calcium, magnesium and fluoride minerals which are capable of functioning as an antimicrobial in a proportional amount and do not have the effect of poisoning the human body. The content of fluoride compounds in Zam Zam water has an antibiotic role. The purpose of this study was to determine the effectiveness of Zam Zam water fluoride in reducing the growth of *Streptococcus sp* and *lactobacillus sp bacteria* as a determinant of the causes of dental plaque. The method in this study was an *experiment* with design *post-test with control group design*. The results showed that Zamzam water fluoride was able to inhibit the growth of *Streptococcus sp* and *Lactobacillus bacteria* with water fluoride concentrations of Zam Zam 20% (0.12 ppm), 40% (0.22ppm), 60% (0.35 ppm), 80% (0.52ppm) and 100 % (0.68ppm). The decrease in the number of germ numbers occurred 100% in both Streptococcus sp and *Lactobacillus sp* at a concentration of 100% fluoride (0.68ppm). It is recommended as a basis for further research to compile and develop a model of Zam Zam water use as a mouthwash ingredient.

Keywords: Zam Zam water, Fluoride concentration, Streptococcus sp, and Lactobacillus.

Introduction

One of the good waters on this earth is Zam Zam water. Based on the results of the European laboratory tests on Zam Zam water samples showed that Zam Zam water has special physical properties that make it has many benefits and advantages. The main difference is Zam Zam, and other water is sterile Zam Zam water has a high content of calcium, magnesium and fluoride minerals so that it can function as an antimicrobial in a proportional amount and does not have poisoning effects on the human body^{1,2,3}.

Corresponding Author: Ahmad Muhlisin Medical Laboratory Technology Poltekkes, Kemenkes Banjarmasin Mistar Cokrokusumo Street, 4a Banjarbaru, Indonesia Email: muhlisin01121968@gmail.com The content of the fluoride compound in Zam Zam water is one of the important ions which have the role of antibiotics. Therefore most toothpaste products prioritize the presence of fluoride content in their packaging which is used to fend off the accumulation of bacteria causing dental plaque. Flouride is a chemical compound that naturally exists in Zam Zam water at various concentrations. Proportional concentration is very beneficial for health, especially dental health because it can prevent tooth decay². Fluoride compounds serve to strengthen enamel by making it resistant to acids and inhibiting bacteria from producing acid. Fluorine is antibacterial, but its weakness can make gray stein in teeth^{4,5}.

Dental plaque is one of the problems in dental and oral health, which is a soft deposit that is firmly attached to the teeth, consisting of microorganisms that multiply if someone neglects the cleanliness of their teeth and mouth. In plaque there are various kinds of bacteria and their metabolism, for example, the results of carbohydrate metabolism by acidogenic bacteria will result in the formation and accumulation of acid which results in decalcification and destruction of tooth surfaces resulting in carries^{6,7}.

The classic research of Keyes (1960) shows that plaque which is dominated by the bacteria *Streptococcus mutan* and *Lactobacillus* causes caries to form. *Streptococcus mutan* and *Lactobacillus* are cariogenic germs because they can form acid from carbohydrates that can be distributed immediately. These germs can thrive in an acidic atmosphere and can stick to the surface of the teeth because of their ability to make extra cell polysaccharides. These extra cell polysaccharides consist mainly of glucose polymers which cause the plaque matrix to have a consistency like gelatin. As a result, the helpful bacteria attach to the teeth and stick together. Plaque is getting thicker, so it will inhibit the function of saliva from carrying out its antibacterial activity⁸.

Zam Zam water composition which contains high fluoride is thought to be able to suppress the growth of bacteria causing plaque. On herbal toothpaste containing fluoride can stimulate early icariin remineralization and reduce the ability of bacteria to produce acid and fluoridecontaining toothpaste to show antibacterial activity^{2,9}.

Method and Materials

Type of research is experimental with a *pretest* and *posttest design with control group design*, which measures the effect of Zam Zam water on the inhibitory forces of *Streptococcus sp* and *Lactobacillus sp* in vitro. The research sample was Zam Zam water made with a concentration variation of 20%, 40%, 60%, 80%, 100%. Preparation of bacterial stock; cultures of *Streptococcus sp* and *Lactobacillus sp were* scratched on blood agar media, incubated for 18-24 hours at 37 ° C, and stored in the refrigerator. Preparation of bacterial suspension; inoculation of 1 colony of *Streptococcus sp* and *Lactobacillus sp* bacteria from bacterial stock and dipped

in 10 ml 0.9% sterile NaCl solution, equated with turbidity with standard *Mc. Farlan* 0.5 (estimated cell number 108), carried out repeatedly until the turbidity is the same.

The Minimum Inhibition Concentration (MIC) measurement was determined qualitatively based on whether or not each sample in the test tube was cloudy compared to the control. If clear, bacterial growth is inhibited and if cloudy, bacterial growth is not inhibited. Determination of Minimum Bactericidal Concentration (MBC) carried out the distribution of TSB suspension as a result of the MIC test on Nutrient Agar media, then incubated at 37 ° C for 24 hours. It is viewed whether or not bacterial growth (qualitative) and the number of colonies (quantitative) were calculated. The lowest concentration/level that is not overgrown with bacteria is MBC¹⁰.

Results and Discussion

Table 1: Results of Examination Fluoride

No.	Packaging	Fluoride level (ppm)
1.	100 ml	0.38
2.	100 ml	0 (Undetectable)
3.	100 ml	0.12
4.	100 ml	0.35
5.	100 ml	0.37
6.	5000 ml	0.69
7.	5000 ml	0.67

Table 2: Levels of fluoride Zam Zam

No.	Concentration	Ν	Concentration fluoride (ppm)
1.	20%	7	0.12
2.	40%	7	0.22
3.	60%	7	0.35
4.	80%	7	0.52
5.	100%	7	0.68
Number		35	-

No	Media Code	Ν	Average ∑ Germs <i>Streptococcus sp</i>	Media Code	Ν	Averagev ∑ Germs <i>Lactobacillus sp</i>
1.	Z1.1	7	1031	Z2.1	7	2053
2.	Z1.2	7	1028	Z2.2	7	2054
3.	Z1.3	7	1031	Z2.3	7	2050
4.	Z1.4	7	1032	Z2.4	7	2039
5.	Z1.5	7	1033	Z2.5	7	2053
Total/Average		35	1031		35	2050

Table 3: Growth of bacteria Streptococcus sp and Lactobacillus sp on Media Control

No.	Fluoride Concentration (%)	N	Average Germ Number of Streptococcus sp	N	Average Number of Germs <i>Lactobacillus sp</i>
1.	20 (0.12 ppm)	7	570	7	1440
2.	40 (0.22 ppm)	7	294	7	756
3.	60 (0.35 ppm)	7	193	7	292
4.	80 (0.52 ppm)	7	92	7	119
5.	100 (0.68 ppm)	7	0	7	0
Total/Average		35	230	35	521

Table 4: Number of Figures for Streptococcus sp and Lactobacillus sp on Zam Zam Water

Table 5: Fluoride Concentration as Minimum Inhibitory Power of Streptococcus sp and Lactobacillus sp

No.	Fluoride Concentration (%)	Inhibiting Power Against Streptococcus sp.	Inhibitory Power Against Lactobacillus sp.	Control (bacteria)
1	20 (0.12 ppm)	Turbid	Turbid	Turbine
2.	40 (0.22 ppm)	Clear	Crystal Clear	Turbid
3.	60 (0.35 ppm)	Clear	Crystal	Cloudy
4.	80 0.52 ppm)	Clear	Crystal	Cloudy
5.	100 (0.68 ppm)	Clear	Crystal	Cloudy

Table 6: The ability of Zam Zam Water Flouride in Reducing Growth of Streptococcus sp and Lactobacillus sp

No.	Zam Zam Water Flouride	The decrease in Number of Germ Number			
	Concentration (%)	Streptococcus sp. (%)	Lactobacillus sp (%)		
1.	20 (0.12 ppm)	30	45		
2.	40 (0.22 ppm)	63	71		
3.	60 (0.35 ppm)	86	81		
4.	80 (0.52 ppm)	94	91		
5.	100 (0.68 ppm)	100	100		



Figure 1: Increased Germicidal Power Based on Fluoride Concentration

Zamzam used in this study is 5000 Zam of Zam Zam pack water with 0.68 ppm fluoride content. In a previous study conducted by Muhlisin found a fluoride content of 0.7 ppm^{1,3}.

In the variation of the concentration of fluoride content in Zam Zam water which was used to inhibit the growth of *Streptococcus sp* and *Lactobacillus sp*, MIC was obtained at a concentration of 40% (0.22 ppm). The decrease in the number of bacteria *Streptococcus sp* and *Lactobacillus sp* occurs at the time Fluoride concentration in Zam Zam water is increasing. This condition shows the influence of fluoride in inhibiting the growth of *Streptococcus sp* and *Lactobacillus sp*. Fluoride plays a role in inhibiting the growth of *Streptococcus sp*. High concentrations of fluoride in the oral cavity can inhibit acid production by bacteria and can reduce the number of certain bacterial species¹¹.

The Zam Zam water fluoride concentration has a significant effect on the germ numbers of both bacteria *Streptococcus sp* and *Lactobacillus sp* (P<0.05). Fluoride is one of the substances that can provide pressure on environmental factors that have an impact on the oral bacterial community, besides providing beneficial effects on caries prevention, it also affects the enamel and demineralization¹². Fluoride inhibits bacterial metabolism so that it affects the rate of catabolism of carbohydrates which can be fermented into acid. The acid pH produced from carbohydrate fermentation leads to an increase in oral microbial communities in dental plaque-causing bacteria^{11,12}.

The mechanism of action of fluoride on bacteria *Streptococcus sp* and *Lactobacillus sp* can inhibit the enzyme *enolase* which plays arole in bacterial metabolism so that it cannot form plaques¹³. *Enolase* catalyzes glycerate-2-phosphate to phosphoenolpyruvate. Phosphoenolpyruvate is further metabolized into acids such as lactic acid, formic acid, pyruvic acid which can cause demineralization of tooth enamel. Phosphoenolpyruvate is also an essential substrate for the phosphotransferase sugar transport system that is dependent on phosphoenolpyruvate and is found in many bacteria, including *Streptococcus* and *Lactobacillus*^{13,14}.

In addition to containing fluoride, Zam Zam also contains bicarbonate, which can neutralize acids produced by bacteria that cause dental plaque (*Streptococcus* and *Lactobacillus*) and with hypertonic properties can cause hypotonic components of bacterial cells to lose water. The loss of water causes bacteria to dehydrate and die. Bicarbonate compounds can damage the bacterial matrix structure and weaken the bond between bacteria and tooth surfaces².

The bacteria that can cause dental plaque, in general, are mutant Streptococcus and Lactobacillus. The two bacteria will then be able to cause caries formation. Interactions between bacterial plaque, food, and teeth can cause dental caries¹⁵. Fluoride can inhibit the activity of bacterial plaque enzymes including enolase, phosphatase, proton extruding ATPase, and pyrophosphatase; this activity will inhibit the process glycotransferase that forms extracellular polysaccharides plaque and interferes with plaque attachment^{16,17}. Therefore the results of this study which show the ability of fluoride Zam Zam-containing water to dental plaque bacteria have the potential to be used as a mouthwash to prevent the development and decrease bacteria in the mouth that cause dental plaque. Sterile Zam Zam water contains high fluoride which has proportional antimicrobial properties and does not give poison to the body.

Conclusion

Inhibitory effectiveness of the growth of *Streptococcus sp* and *Lactobacillus* begins at dilution of water fluoride Zam Zam 40% (0.22 ppm). The ability of fluoride in Zam Zam water to inhibit the growth of *Streptococcus sp* and *Lactobacillus sp* respectively 0.12 ppm (30%); (45%), 0.22 ppm (63%); (71%), 0.35 ppm (86%); (81%), 0.52 ppm (94%); (91%) and 0.68ppm (100%); (100%).

Ethical Clearance: Received from the Ethics Committee of the Banjarmasin Health Polytechnic Health Research.

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