Efficacy of Mouthwash Containing Curcuma xanthorrhiza on Lactoperoxidase System Activity and Halitosis Risk

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Objective: The use of mouthwash may increase the activity of lactoperoxidase system in saliva and may reduce the risk of halitosis. The research was conducted to determine the effect of mouthwash containing Curcuma xanthorrhiza on lactoperoxidase system activity in relation to halitosis risk.

Methods: The research design was a before after clinical trial, the 5 ml saliva samples were taken from 10 subjects age 20-23 years old at 22.00 WIB at night and at 04.00 WIB in the morning. Lactoperoxidase enzyme activity was measured using the method of Chance and Maehly while the concentration of thiocyanate ion was measured using Thomas and Aune method.

Results: The mean±SD of the baseline lactoperoxidase activity absorbance in the evening 0.2463±0.1260 and then decreased in the morning to 0.1994±0.0593 and the mean±SD baseline thiocyanate ion from 1.9328±0.1872 in the night decreased in the morning to 1.819±0.5106. After intervention with mouthwash containing Curcuma xanthorrhiza, the increasing of average value of baseline Lactoperoxidase activity absorbances was indicated from 0.2121±0.1079 in the evening becomes 0.2402±0.0919 in the morning, while the average of thiocyanate ion from 1.0531±0.1088 in the evening becomes 1.0903±0.2858 in the morning. There were no significant differences between the activity of lactoperoxidase enzyme and thiocyanate ion concentrations in both treatments (p > 0.05).

Conclusion: The use of mouthwash containing Curcuma xanthorrhiza has the potential to raise the lactoperoxidase system activity.

Keywords: saliva, lactoperoxidase, thiocyanate, halitosis, mouthwash

Introduction

Oral malodor or halitosis become one of the reason why the patient visits the dentist, in addition to dental caries and periodontal (1). Halitosis is not a disease but rather a symptom of underlying oral, systemic, or physiological conditions, but it can increase someone’s confidence and can affect interpersonal social communication (1,2). The true prevalence of halitosis is unknown, however the available evidence suggests that halitosis is common and can affect people of all ages (1). In the oral cavity, there are various inter-related components to maintain oral health, such as teeth, gingiva, saliva, tongue, and mucosa. Saliva is one contributing factor toward halitosis. Natural protection by saliva obtained from its function as a lubricant, flushing or rinsing, antimicrobial, and buffering (3,4). In saliva there are various kinds of enzymes such as liozyme, lactoperoxidase, amylase, lipase, etc. Enzymes is also an antibacterial, one of the body’s defense system against microorganism (3). This journal will discuss about lactoperoxidase enzyme. Lactoperoxidase enzyme could be founded naturally in cow’s milk, or in tear, gastric fluid, and saliva. There is a sys-
tem in lactoperoxidase enzyme called lactoperoxidase system (LPO), it is an oxidation reaction between thiocyanate ion (SCN) and hydrogen peroxide (H₂O₂) from bacteria, with lactoperoxidase enzyme as the catalyst, resulting hipothiocyanate ion (OSCN⁻) that has bacteriostatic effect. This bacteriostatic effect can help improve oral health and reduce the bacteria, including bacteria that cause halitosis (3).

As a preventive for halitosis, people usually use mouthwash after brushing teeth. In this experiment, a herbal ingredient called Curcuma xanthorrhiza is the main ingredient in the mouthwash. Some of the advantages of herbal medicine is: cheaper; easy to get, especially Indonesia; and no side effects. Curcuma xanthorrhiza known as an antibacterial, antifungal, and anti-inflammation agent. Its has no toxic effect even if used for a long term (5,6).

Jae Kwan H proves that Curcuma xanthorrhiza can reduce halitosis (7). It can fight against Streptococcus and Porphyromonas gingivalis. Xanthorrhizol stimulate denaturation on bacteria’s cell and it makes proteins come out from bacteria (6).

The research is focusing the effect of Curcuma xanthorrhiza mouthwash on lactoperoxidase system in human’s saliva. It is expected that the use of mouthwash containing Curcuma xanthorrhiza can be optimized and can be used by all levels of society.

Material and Methods

The research method is “before after clinical trial”. There are 10 subjects (7 woman and 3 man), age 20-23, all of them have signed the informed consent. The exclusion criteria are:

1. Have systemic disease
2. Have tooth and mouth disease
3. Smoking and drinking alcohol
4. Wearing orthodontics
5. Wearing dentures

All subjects should consume same food for dinner to avoid the bias data. After dinner, subjects must brushed their teeth with same toothpaste and didn’t allowed to eat or drink again at least for 2 hours. During the research time, subjects are not allowed to do the scaling, root planning, fasting, or consuming any medicine especially anticiotics because it has bacteriostatic effect.

The baseline data was taken at 10.00 p.m (after subjects brushed their teeth and wait for 2 hours). All subjects collected their saliva in saliva tube 5 ml each person, then they went to sleep. At 6.00 a.m, all subjects should collected their saliva again in saliva tube, 5 ml each person. All saliva tubes were kept in a portable freezer to keep the enzyme composition.

The lactoperoxidase enzyme activity was measured in laboratory based on Chance and Maehly method (1954):

1. The saliva tubes were sentrifugated with sentrifugator for 10 minutes, with 1,000 rpm speed and 4°C temperature to get the suspension of the saliva (Figure 1).
2. Take 125 µl supernatant and put into microplate well. Then put buffer phosphate 0,01 mM 250 µl, guaiakol 20 mM 125 µl, and H₂O₂40 mM 5 µl into the microplate well, and mixed them all with pippetting technique (Figure 2).
3. Put the microplate well into the microplate reader and wait for a few seconds to read the result, use 450 nm wave length (Figure 3).

The concentration of thiocyanate ion was measured base on Thomas and Aune method (9):

1. The saliva tubes were sentrifugated with sentrifugator for 10 minutes, with 1,000 rpm speed and 4°C temperature to get...
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**Results**

Baseline concentration of lactoperoxidase activity absorbance value at night was 0.246333±0.126053 and then decreased in the morning to 0.1994999±0.059258. After intervention with mouthwash containing *Curcuma xanthorrhiza*, the average of baseline concentration of lactoperoxidase activity absorbance value was indicated increased from 0.2121±0.107991 at night and becomes to 0.240233±0.091862 in the morning. The T-paired test for lactoperoxidase enzyme absorbance value shows that the decreasing value in baseline data and increasing value in intervention data is not significance (p > 0.05) (Figure 4).

Baseline concentration of thiocyanate ion was 1.9328±0.187181 at night and decreased in the morning to become 1.819±0.510573. After intervention with mouthwash containing *Curcuma xanthorrhiza*, the average of baseline concentration of thiocyanate ion was increased from 1.0531±0.108838 at night becomes to 1.090267±0.285835 in the morning. The Wilcoxon test for thiocyanate ion concentration absorbance value shows that the decreasing value in baseline data and increasing value in intervention data is not significance (p > 0.05) (Figure 5).

**Discussion**

During sleep, human’s cortex activity is decreasing. Saliva production rate fell to its lowest point during sleep, this is supported by research Scheneyer et al. (1956) who said the salivary flow rate is negligible during sleep. The absence of activity in the mouth during sleep make the salivary glands unstimulated (10). Therefore, the volume of saliva is reduced and resulting xerostomia, which one cause of halitosis (1). This is consistent with Miyazaki (1999) who said that bad breath occurs when there is no oral activity more than 2 hours, especially during wake up time in the morning.

Naturally, saliva contains enzymes lactoperoxidase and thiocyanate ion. So, it was concluded that if the amount of saliva is reduced, it means the concentration of lactoperoxidase activity absorbance of thiocyanate ion is also decrease. Lactoperoxidase system has antimicrobial effects, characterized by its nature is...
capable of inhibiting bacterial growth and production of acid produced by several microorganisms. If the value of absorbance decrease, the antimicrobial effect will also decrease (3,11).

On the other hand, when human is sleeping, the microorganisms continue their activity to degrade the proteins in oral cavity. Bacteria are not the part of human’s body, so their activities did not decrease even when human is sleeping, it prove through with an example: a coating on the tongue when wake up in the morning. This layer can be colored yellow or white depending on the severity and the amount of sulfur that the bacterial produced. The presence of tongue coating appears to be related to several factors of which oral hygiene is the strongest (12).

Therefore, when wake up in the morning, the state of human’s mouths contain a high acid content of the bacterial metabolism, and one of them is Porphyromonas gingivalis that cause halitosis. This is exacerbated by saliva as a flushing function is diminished by xerostomia. This is why the “morning breath” happened.

Rhizome extractions could inhibit the growth of all Gram positive bacteria. And Jae Kwan et al proves that Curcuma xanthorrhiza can reduce halitosis (7,8). The content of ingredients in mouthwash, such as lactoferrin, glucose oxidase, lysozyme, and Curcuma xanthorrhiza have certain functions that are bacteriostatic and even bactericidal. Glucose oxidase catalyzes the oxidation of glucose to gluconic acid with the production side of hydrogen peroxide. With the increasing amount of hydrogen peroxide, hipothyocianate anion production was also increase (13).

Because of the limited time, this research only tested the mouthwash once, so it cannot observe the effects of long-term use that is expected to provide a more meaningful improvement.

**Conclusion**

The use of mouthwash containing Curcuma xanthorrhiza has the potential to increase the lactoperoxidase system activity and reduce the halitosis risk.

**References**