Vol 11, Special Issue 1, 2019

INTERNATIONAL JOURNAL OF APPLIED PHARMACEUTICS

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

ISSN - 0975-7058

Research Article

EFFECT OF HONEY PROPOLIS HARD CANDY ON LACTOPEROXIDASE ACTIVITY IN UNSTIMULATED SALIVA

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Received 8 October 2018, Revised and Accepted 11 January 2018

ABSTRACT

Objective: We determined the effectiveness of honey propolis hard candy on salivary lactoperoxidase activity in unstimulated saliva.

Methods: In this pretest-posttest laboratory experimental design study, saliva collected from 120 subjects was reacted with potassium iodide, phosphate buffer, and hydrogen peroxide. Salivary lactoperoxidase activity was calculated by absorbance value using a microplate reader at wavelength of 340 nm.

Results: Honey propolis hard candy increased salivary lactoperoxidase activity, although the increase was not statistically significant ($p>0.05$).

Conclusions: Honey propolis hard candy tends to increase salivary lactoperoxidase activity.

Keywords: Propolis, Lactoperoxidase, Saliva.

 DOI: http://dx.doi.org/10.22159/ijap.2019.v11s1.159

INTRODUCTION

According to basic of health research Republic of Indonesia 2013, the prevalence of dental caries in Indonesia has increased significantly to 43.4% in 2007 and 53.2% in 2013 [1]. Dental caries is the most common disease in the oral cavity, disrupting the quality of life and health of an individual. Some treatments have been performed to prevent dental caries, but this is rare in the Indonesian people. Hence, dental caries preventions are required that are more easily available to Indonesian people. A natural ingredient that may help prevent dental caries is propolis.

Propolis is a natural resin substance that is collected by bees from plant trunk and tree bark exudates and used to close the pathway to their nests [2]. Herbal ingredients have been used as alternative medicines for many years. Propolis contains naphthoquinone epoxide, prenylated acid, medicarpin, Vitamin A, Vitamin E, Vitamin B complex, flavonoid, polyphenol, phenolic aldehyde, quinine, coumarin, amino acid, steroid, and some other inorganic contents, depending on the location and time of harvest, and plant sources [3,4].

Propolis has known antibacterial, antioxidant, antiulcer, antitumor, antifungal, and anti-inflammatory functions [2,4]. Its role as an antibacterial and antioxidant is due to the flavonoid content [5]. Some products containing propolis include toothpaste, gum, soap, cream, and lotions [6]. At present, propolis is being formulated into hard candies, one of which is honey propolis hard candy made by the University of Indonesia.

Honey propolis hard candy has been proven to decrease the prevalence of *Streptococcus mutans* [7]. To understand more about the effectiveness of honey propolis hard candy as an antibacterial, we performed a lactoperoxidase activity test in saliva.

Lactoperoxidase is an enzyme produced from the peroxidase system (a non-specific immunity system) in saliva. The main function of peroxidase is oxidation catalyzation by thiocyanate of hydrogen peroxide (H_2O_2) in saliva, so it has potential as an antibacterial. Human saliva contains two peroxidases (lactoperoxidase and myeloperoxidase) [8]. Lactoperoxidase is mostly found in human saliva [9].

Lactoperoxidase activity is affected by changes in the oral cavity, such as H_2O_2 concentration, thiocyanate ion (SCN^-) concentration, pH, and temperature [10], and also by the number of bacteria in the oral cavity. This is because pathogenic bacteria produce H_2O_2. Activity increases or decreases if the number of pathogenic microorganisms producing H_2O_2 increases or decreases [11]. Lactoperoxidase activity will increase when infection occurs. A study demonstrated higher lactoperoxidase activity in patients with than in those without dental caries [11]. Another study reported higher lactoperoxidase activity in patients with than in those without periodontitis [11]. Xylitol consumption, as an antibacterial, decreases lactoperoxidase activity [12]. Decreased lactoperoxidase activity after exposure to an antibacterial can affect oral health poorly due to the resultant decrease in the number of bacteria, which, in turn, can give other microorganisms, such as *Candida albicans*, a chance to develop. Therefore, a significant decrease in lactoperoxidase activity can disrupt the normal flora equilibrium in the oral cavity [13]. Lactoperoxidase activity can be used as an indicator for the effectiveness of propolis as an antibacterial, thus demonstrating whether an antibacterial can disrupt oral equilibrium.

We studied three types of candy: Honey propolis candy (containing propolis and honey), honey candy (containing honey only), and X propolis candy (containing propolis only but does not interfere with lactoperoxidase activity).

To the best of our knowledge, no study has been performed on the effect of propolis in a hard candy form on saliva lactoperoxidase activity. Therefore, this study was done to understand the effectiveness of propolis hard candy as an antibacterial on lactoperoxidase activity in saliva.

METHODS

This laboratory experimental pretest-posttest study was done *in vivo* on 120 students of the Faculty of Dentistry, Universitas Indonesia, from the 1st to 6th year classes of 2013/2014. Inclusion criteria were good general health, good oral health, age 17–23 years, and provision of a signed informed consent to participate in the study until finish. Exclusion criteria were wearing orthodontic appliances, periodontal

disease, smoking, wearing dentures, other systemic diseases, taking antibiotics, drinking alcohol, and allergy to propolis. The samples included unstimulated saliva before and after treatment. The study was done at the Oral Biology Laboratory, Faculty of Dentistry, Universitas Indonesia, from July to November 2014.

The subjects were divided into three groups. Groups A (40 subjects), B (40 subjects), and C (40 subjects) consumed honey propolis, honey, and X propolis hard candies, respectively, 2 times a day for 7 consecutive days. The candies were consumed for the full number of days.

Before sample taking, patients were instructed to brush their teeth, without using mouthwash and without eating or drinking (unless mineral water) anything for a minimum of 1.5 h before treatment. Unstimulated saliva was collected in 20 mL microcentrifuge tube.

Then, 1.5 mL sample of this saliva was centrifuged at 15.000 g at 4°C for 20 min until the supernatant was obtained [14]. 250 µL supernatant was placed in a 96-well plate, and 500 µL buffer solution containing potassium phosphate (KH_2PO_4) pH 6.0, 250 µL potassium iodide (KI), and 100 µL H_2O_2 40 mM were added [15]. All samples were made in triplicate. A microplate reader was used with maximum capacity of 250 µL in each microwell in the 96-well plate. Therefore, we used 50 µL supernatant with 100 µL KH_2PO4 buffer solution pH 6.0, 50 µL KI 50 µmol, and 100 µL H_2O_2 40 mM added.

The microplate reader was calibrated and connected to the computer, so the results could be seen on the monitor. The 96-well plate was inserted in the microplate reader to measure the activity. The Microplate Manager program with the New Endpoint Protocol was used. Enzyme activity was read at 340 nm wavelength, and the results were displayed on the monitor as the absorbancy score for each microwell. This absorbancy score was used as a base to obtain data for further analysis.

Collected data were analyzed using SPSS 17 (SPSS, Inc., Chicago, IL, USA). Before processing, a Shapiro–Wilk normality test was done to determine the statistical values, due to data <50. A dependent t-test and the Wilcoxon test were used for normally and abnormally distributed data, respectively. To examine changes between before and after treatment among the three groups, one-way analysis of variance and Kruskal–Wallis tests were used for normally and abnormally distributed data, respectively.

RESULTS

The results of the Shapiro–Wilk normality test on lactoperoxidase activity data indicated that the data did not have a normal distribution. Therefore, a non-parametric statistical test was used. The Wilcoxon test was used to compare the average before-after treatment data in each group and the Kruskal–Wallis test was test used to compare the average before-after data from the three treatment groups.

Lactoperoxidase activity increased by approximately 0.00575 (nmol/L) in Group A (average before vs. after treatment, 0.01528 vs. 0.02103; Fig. 1, Table 1). The Wilcoxon test showed a significance of 0.706 ($p>0.05$). Therefore, there was no significant difference in lactoperoxidase activity between before and after treatment.

Lactoperoxidase activity increased by 0.00240 (nmol/L) in Group B (average before vs. after treatment, 0.01190 vs. 0.01430; Fig. 1). The Wilcoxon test showed a value of 0.700 ($p>0.05$), which also was not significant (Table 1).

Lactoperoxidase activity decreased by 0.00783 (nmol/L) in Group C (average before vs. after treatment, 0.02083 vs. 0.01300; Fig. 1). The Wilcoxon test showed a value of 012 ($p<0.05$), so this difference was significant. Then, the Kruskal–Wallis test revealed a value of 0.0219 ($p>0.05$), which demonstrated no significant difference in lactoperoxidase activity among the three groups.

DISCUSSION

We evaluated the effect of the consumption of candy containing propolis on lactoperoxidase activity. There was no significant difference in lactoperoxidase activity in Groups A (honey propolis candy, 0.00575 increase) and B (honey only candy, 0.0024 increase). Meanwhile, a significant difference was noted in Group C (X propolis candy, 0.00783 decrease).

Lactoperoxidase activity can be affected by SCN^-, H_2O_2, pH, and temperature [10], as well as by excessive carbohydrate intake because carbohydrate intake, especially sucrose, can block the accumulation of SCN^- [16]. In addition, the presence of glucose in saliva stimulates more H_2O_2 formation because glucose causes bacteria to produce more H^+ from fermentation [9].

Honey propolis and honey hard candy did not significantly affect lactoperoxidase activity, so both hard candies were good for the oral cavity because they did not disrupt the normal flora equilibrium. However, these candies had a tendency to increase lactoperoxidase activity, which can be due to the honey and glucose contents. Honey can increase lactoperoxidase activity by raising H_2O_2 in saliva. Honey contains 1 mm/L H_2O_2. Glucose in honey also can affect glycolysis by bacteria, so more H^+ is produced and more H_2O_2 is formed. Glucose also can affect the glucose oxidation process, which also produces H_2O_2.

The increased lactoperoxidase activity was greater in Group A than in Group B because the propolis content in honey propolis hard candy formed an H_2O_2 compound as an inductor of DNA destruction in bacteria. The flavonoid propolis acts as a temporary electron carrier, which is accepted from metal transition ions and is forwarded to oxygen molecules to form superoxide (O_2^-) and H_2O_2 [5]. If the H_2O_2 concentration increases, lactoperoxidase activity also increases [10]. The increased H_2O_2 can have a good impact on the oral cavity because it can cause a potential host defense by increasing lactoperoxidase capacity.

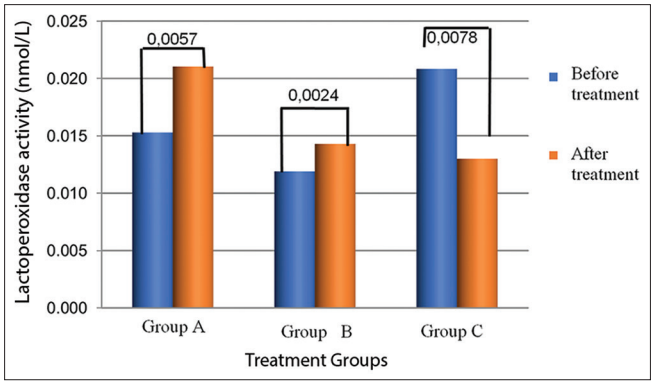

Fig. 1: Lactoperoxidase activity before and after treatment comparison in three groups

Table 1: Saliva lactoperoxidase activity score

Group	Before treatment average	After treatment average	p-value (Sig.)
Group A (n=40) Mean±SD	0.01528±0.015159	0.02103±0.042073	0.706
Group B (n=40) Mean±SD	0.01190±0.011050	0.01430±0.018509	0.700
Group C (n=40) Mean±SD	0.02083+0.028162	0.01300±0.013640	0.012

However, our results are not in agreement with those of another study reporting that propolis functions as an antibacterial. Propolis functioning as an antibacterial also may decrease H_2O_2 concentration. Decreased numbers of bacteria result in decreased toxins from the bacteria such as H_2O_2, H_2S, H^+, and others. When salivary H_2O_2 concentration decreases, lactoperoxidase activity also decreases. Another study on honey propolis hard candy reported a decreased prevalence of *S. mutans* and *C. albicans* biofilm formation. A study on the effects of xylitol and mouthwash with betel water showed a change in lactoperoxidase activity [17,18]. Xylitol and betel have the same antibacterial effect as propolis. According to this study, lactoperoxidase activity should decrease with the use of honey propolis hard candy.

Our results also showed that honey propolis hard candy exposure in Group A did not significantly affect lactoperoxidase activity (although activity tended to increase), while activity decreased significantly in Group C (X propolis hard candy). Such a decrease could also disrupt the normal flora equilibrium in the oral cavity. The different effects of both candies also can be due to the honey content in honey propolis candy. Group A (honey propolis hard candy) contained honey, whereas Group C (X propolis hard candy) did not. The flavonoid and H_2O_2 in honey act as an antibacterial and antioxidant. As mentioned above, flavonoid can bind with Fe^{2+} ion and form H_2O_2. The concentration of H_2O_2 is high in honey (1 mmol/L) [19], and honey also has high osmolarity and low acidity (pH 3.2–4.5) [20]. $OSCN^-$ accumulation is more optimal at lower pH [20].

Honey propolis hard candy contained glucose syrup and sugar, whereas X propolis hard candy contained polydextrose. Glucose normally can be fermented by bacteria in the oral cavity, so it produces acid (H^+), which can bind with O_2^- and form H_2O_2. Polydextrose is an artificial sweetener that often is used in foods and drugs. This artificial sweetener can disrupt reproduction, acid production, cell aggregation, and plaque formation of *S. mutans*, as well as its adherence to the teeth surface. Polydextrose also produces 20% lower acid than glucose, so it forms less H_2O_2 [21].

Propolis with fluoride showed good result in inhibit *Streptococcus mutans* and *Enterobacter faecalis* [22]. Soekanto *et al* showed that after mastication simulation using chewing gum of Casein Phosphopeptide-Amorphous Calcium Phosphate -Propolis will increased calcium and phosphate ion level in caries-free saliva and decreased of *S. mutans* biofilm mass [23]. With this finding, it is necessary to further study and analyze the effectiveness of propolis in different combination to fight caries.

CONCLUSIONS

Honey propolis hard candy and honey candy did not affect lactoperoxidase activity significantly, but it had a tendency to increase activity. Meanwhile, X propolis hard candy decreased lactoperoxidase activity significantly.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

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