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Research Article

EFFECT OF PROPOLIS HONEY CANDY CONSUMPTION ON THE ACTIVITY OF LACTOPEROXIDASE IN STIMULATED SALIVA

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ABSTRACT

Objective: This study aimed to analyze the effect of propolis honey candy consumption on lactoperoxidase (LPO) activity in stimulated saliva.

Methods: Stimulated saliva samples were collected from subjects before and after propolis honey candy consumption twice a day for 7 days, and the LPO activity was measured by optical density using a microplate reader.

Results: The LPO activity before and after propolis honey candy consumption was found to be 0.010 and 0.013, respectively.

Conclusions: A statistically significant increase in the LPO activity after propolis honey candy consumption was observed (Wilcoxon test; $p < 0.05$).

Keywords: Propolis, Lactoperoxidase, Saliva, Flavonoids.

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INTRODUCTION

Propolis (beeswax) is a sticky, dark brown, mixed resin material that is collected by honey bees from various plants. Propolis is not only used to create beehives, but also has many benefits for human health [1,2]. In the past, propolis was used to make medicine in Egypt and as a chemical in the mummification process for corpses [2]. Propolis is proven to have various biological activities such as antibacterial, antifungal, antiviral, anti-inflammatory, antitumor, and antioxidant activities [3,4].

Propolis consists of 50% resin, 30% wax, 10% essential and aromatic oils, 5% pollen, and 5% other substances [4]. Flavonoids have antibacterial activity, which inhibits bacterial growth through destruction of cell walls, microsomes, and lysosome permeability, as a result of the interaction between flavonoids and the host DNA. This leads to decreased production of hydrogen peroxide (H_2O_2) by bacteria. This decreasing amount of H_2O_2 can inhibit enzyme activities such as increasing lactoperoxidase (LPO) in saliva [5].

Saliva consists of 99.5% water and 0.5% inorganic and organic materials, one of which is protein (non-immunoglobulin and immunoglobulin) [6]. One of the non-immunoglobulin proteins is peroxidase, which forms the peroxidase system [7]. There are two types of peroxidase in saliva, LPO and myeloperoxidase that exhibit bacteriostatic effects [8]. Both of the enzymes use H_2O_2 as the substrate, whereas they use different cosubstrate ions [8].

LPO is an important oxidative enzyme in saliva because saliva itself does not have antimicrobial activity. However, a combination of LPO, thiocyanate ion (SCN^-, cosubstrate from saliva), and H_2O_2 (bacterial product) can produce a hypothiocyanate ion ($OSCN^-$), which acts as an antibacterial agent [7]. The mechanism of LPO is based on the amount of H_2O_2. If this amount is decreased, then SCN^- cannot be oxidized. This leads to an ineffective antibacterial system for LPO. Antibacterial application, such as that exhibited by propolis, can inhibit H_2O_2 production as well as inhibit LPO activity [9].

Many studies have demonstrated the application of propolis in dentistry. However, to the best of our knowledge, there is no research about propolis candy and LPO activity in stimulated saliva. Therefore, this research was conducted to determine the effect of propolis candy on LPO activity in stimulated saliva and its effectiveness in decreasing LPO activity compared with honey candy and X candy.

METHODS

This research was a clinical experiment in the laboratory at Faculty of Dentistry, Universitas Indonesia, from August to November 2014. A total of 120 subjects participated. The inclusion criteria were as follows: 19–23 years old, good medical condition, good oral condition, and willingness to participate in this research and provide informed consent. The exclusion criteria included fixed orthodontic appliances, periodontal disease, smoking, dentures, systemic disease that manifested in oral condition, bad oral hygiene, allergic to propolis, alcohol consumption, and medications such as antibiotics.

Subjects were screened to examine oral health. The stimulated saliva was obtained in the morning before consuming candy and 1 week later after routinely consuming candy twice a day for 7 days. Before collecting sample, subjects were instructed not to eat and drink (except mineral water) for a minimum of 1.5 h after brushing teeth, then subjects chewed paraffin chewing gum, and saliva was collected in a 50 ml tube with a funnel for 10 min. Each tube was coded and put into a cooler to be processed at the laboratory.

Saliva was transferred from the 50 ml tube to a 1.5 ml microtube. The saliva was then subjected to centrifugation at 15.000×g for 20 min at 4°C. The supernatant from natant (pellet) was transferred into another 1.5 ml microtube. The microplate was prepared, 100 µl phosphate buffer+50 µl KI+50 µl stimulated saliva supernatant+2 µl H_2O_2 were added into well plate (by triplo), and 100 µl aquades mixed with same reagent were added by triplo into the well plate as a blank (control). Then, the plate was incubated for 30 min in dark. The microplate reader was observed at 340 nm wavelength, the mixture was shaken for 10 s, and well mapping was set. The optical density (OD) of LPO in saliva was measured with the following formula: Saliva absorbance – blank absorbance=OD.

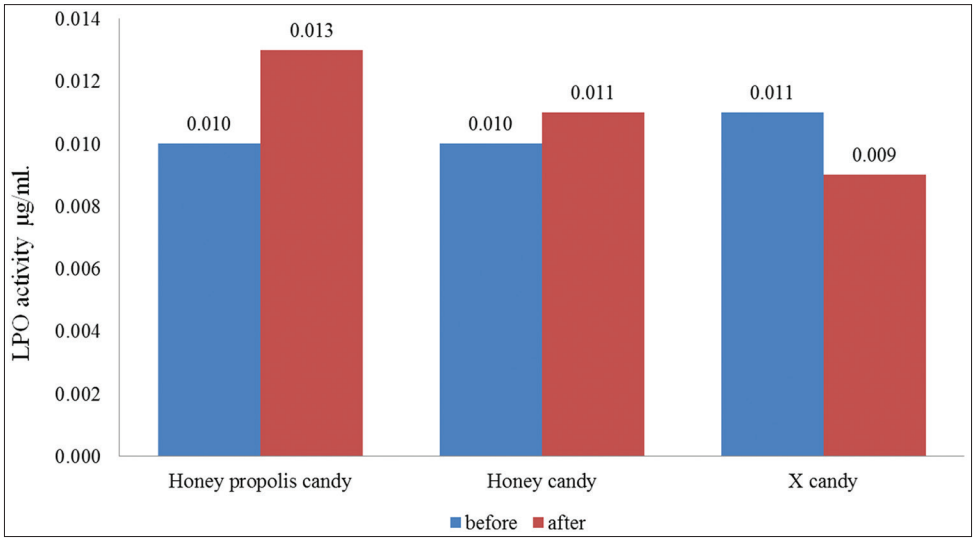

Fig. 1: Comparison of lactoperoxidase activity before and after consuming honey propolis candy, honey candy, and X candy

Table 1: LPO activity in stimulated saliva before and after treatment

Group N=40	Average of before	Average of after	Wilcoxon (Sig.)
Group A Mean±SD	0.00973±0.003714	0.01340±0.007334	0.001*
Group B Mean±SD	0.00975±0.003747	0.01133±0.004233	0.107
Group C Mean±SD	0.01138±0.006274	0.00865±0.005390	0.043*

*p<0.05, LPO: Lactoperoxidase

Table 2: LPO activity before and after treatment

Group n=40	Average change of LPO activity before and after treatment
Group A Mean±SD	0.00373±0.007285
Group B Mean±SD	0.00160±0.005486
Group C Mean±SD	0.00273±0.008262
Kruskal–Wallis Sig.	0.001*

*p<0.05, LPO: Lactoperoxidase

Statistical analysis

Data were analyzed with SPSS 17. Data normality test was conducted using Shapiro–Wilk test. If data had normal distribution, parametric analysis was used. Statistical tests included paired *t*-test, one-way analysis of variance, and *post hoc* tests. If data were not normally distributed, then non-parametric analysis was used, such as Wilcoxon, Kruskal–Wallis, and Mann–Whitney tests.

RESULTS

In this study, the effect of propolis candy on LPO activity in stimulated saliva was analyzed. Saliva was sampled before and after consuming candy. Saliva was centrifuged to remove the supernatant and then reacted with K_2HPO_4, KI, and H_2O_2 in a microwell and read with a microplate reader. LPO activity was calculated for the average triplo absorbance score of saliva minus the average of blank absorbance. The results are shown in Fig. 1. The LPO activity before and after consuming honey propolis candy increased by 0.004. It increased by 0.002 after consuming honey candy and decreased by 0.003 after consuming X candy (Fig. 1).

Data were analyzed with SPSS 17. Normality testing using Shapiro–Wilk test showed that data did not have normal distribution because it had a significant score ($p<0.05$). Therefore, non-parametric tests were used. Wilcoxon and Kruskal–Wallis tests were used to analyze differences among honey propolis candy, honey candy, and X candy.

For Wilcoxon test, there was a significant difference in LPO activity before and after consuming X candy and honey propolis candy, shown by the significance score for X candy ($p=0.043$) and the significance score for honey propolis candy ($p=0.001$). Meanwhile, LPO activity before and after consuming honey candy was not significantly different ($p=0.107$) (Table 1).

According to Kruskal–Wallis test, there was a significant difference in LPO before and after consuming candy, which was significant ($p=0.001$) (Table 2). *Post hoc* testing was used to determine which group had significant differences. *Post hoc* analysis for Kruskal–Wallis test was Mann–Whitney U-test. Mann–Whitney U-test showed differences between groups: X candy and propolis honey candy ($p=0.000$); X candy and honey candy ($p=0.022$); and propolis honey candy and honey candy ($p=0.166$). Therefore, significant differences in LPO activity were noted between the groups consuming X candy and propolis honey candy, and between the groups consuming X candy and honey candy.

DISCUSSION

This study showed that LPO activity increases after the consumption of honey propolis candy and honey candy (Fig. 1). This may be due to the honey and glucose in the candies, with glucose composition being 41%. The previous studies have showed that a honey solution activated glucose oxidase that produced H_2O_2 and increased $OSCN^-$ product, leading to increased OD scores [8,10].

LPO activity after consuming honey propolis candy showed a significantly increasing score (Table 1), which is expected because propolis contains flavonoids. This is supported by a previous study stating that flavonoids contain H_2O_2 that initiates oxidative destruction of bacterial DNA [11]. H_2O_2 increases LPO activity. LPO activity after consuming honey candy does not show a significant difference (Table 1). It is suspected that this occurs because honey candy does not have propolis, and this supposition is supported by a previous study [12].

In addition, LPO activity after consuming X candy significantly decreases ($p=0.043$) when comparing activity before and after consuming candy. This decrease in activity was observed in other studies showing that flavonoids can inhibit the growth of bacteria by destructing the permeability of cell walls, microsomes, and lysosomes of bacteria [13].

This leads to decrease in H_2O_2 as a bacterial product, indicating that SCN^- in the LPO mechanism will not be oxidized. In other words, LPO activity will be decreased [8]. A previous study has also stated that propolis inhibits H_2O_2 production by bacteria [14].

Mann–Whitney U-test showed a significant difference in the LPO activity between X candy and honey propolis candy (p=0.001) and between X candy and honey candy (p=0.022). It is thought to be due to the candy composition. Although both have propolis, honey propolis candy has a honey and glucose composition, which forms H_2O_2. In X candy, glucose is replaced by low-calorie artificial sweeteners such as polydextrose, lactitol, and acesulfame-k. These are synthetic glucose components, and thus, they will not oxidize glucose. This means that no H_2O_2 products are produced in X candy, decreasing LPO activity [10].

Recent study showed that Propolis fluoride had good result in inhibit *Streptococcus mutans* and *Enterobacter faecalis* [15]. Soekanto *et al* showed that calcium and phosphate ion level in caries-free saliva after mastication simulation using chewing gum of Casein Phosphopeptide-Amorphous Calcium Phosphate -Propolis will increased and decreased of *S.mutans* biofilm mass [16]. With this finding, it is necessary to further study and analyze the effectiveness of propolis in different combination to fight caries.

CONCLUSIONS

Based on this research, it can be concluded that honey propolis candy may increase LPO activity in stimulated saliva. It is also concluded that X candy is more effective in decreasing LPO activity in stimulated saliva than honey propolis candy and honey candy.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

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