ORIGINAL ARTICLE

Bali Medical Journal (*Bali MedJ*) 2022, Volume 11, Number 3: 2052-2056
P-ISSN.2089-1180, E-ISSN: 2302-2914

Brazilian propolis wax as an anti-type-IV-hypersensitivity agent in metal bracket orthodontic treatment

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Received: 2022-07-24
Accepted: 2022-09-27
Published: 2022-12-30

ABSTRACT

Background: Nickel (Ni^{2+}) allergy on the mucosa and gingiva is frequently found in patients with fixed metal orthodontic treatment as the manifestation of type-IV hypersensitivity reactions.
Method: This study aims to observe the effect of Brazilian propolis wax on salivary Ni^{2+} ion levels and blood serum IFN-γ and IL-10 levels during fixed metal bracket orthodontic treatment. A total of 34 New Zealand rabbits were divided into three groups. Before the treatment, Ni^{2+}sensitization was done using a 125 µL $NiCl_2$ injection. Metal brackets were placed on the four anterior teeth for 28 days. Saliva was carried out for Ni^{2+} ion level analysis by inductively coupled plasma-optical emission spectrometry (ICP-OES), and ELISA measured peripheral blood serum for IFN-γ and IL-10 level analysis.
Result: Ni^{2+} ion release in the control group showed a higher expression than in the wax and propolis-wax groups. IFN-γ expression level is reduced in the wax and propolis-wax group than in the control group. Moreover, a significantly different IL-10 expression was also observed in the wax and propolis-wax groups.
Conclusion: The combination of propolis into wax orthodontic inhibits Ni^{2+} ion release by promoting anti-inflammatory IL-10 expression.

Keywords: wax, propolis, Ni^{2+}, IFN-γ, IL-10.
Cite This Article: Aditya, G., Santoso, O., Suromo, L.B. 2022. Brazilian propolis wax as an anti-type-IV-hypersensitivity agent in metal bracket orthodontic treatment. *Bali Medical Journal* 11(3): 2052-2056. DOI: 10.15562/bmj.v11i3.3583

INTRODUCTION

Type IV hypersensitivity reactions caused by Ni^{2+} ions can occur in patients with fixed metal orthodontic treatment. About 8% of Ni^{2+} and other materials based on Ni^{2+}-titanium (Ti) alloys are contained in metal orthodontic brackets. Metal allergic reaction triggers a harmful systemic reaction through immunologically or non-immunologically mediated that manifests on the face and the neck, oral mucosa, and gingiva.[1–3] Oral mucosa and gingiva are the immunocompetent sites that display many immune cells, such as antigen-presenting cells (APC) and lymphocytes.[4] The continuous metal contact on this site may impact the increased level of toll-like receptor (TLR)-4 and intracellular adhesion molecule (ICAM)-1.[4,5]

Ni^{2+} ions are released into the oral cavity due to the corrosion process caused by the oxygen concentration difference.[6] The releases of metal ions contact surrounding epithelial cells and tissues that enter the body through the small intestine. A low concentration of Ni^{2+} ions that penetrate hard and soft tissues can induce damage to the mucosal cells as oral mucosal lesions, allergies, metallic sensation on the taste buds, and burning mouth sensation.[7–9] Therefore, severe complications caused by metal bracket allergy will interfere with and prolong the orthodontic treatment.[10]

Type IV hypersensitivity reactions due to Ni^{2+} ions are known to be mediated by haptens binding to larger proteins to become antigens. Hapten binds to peptide-major histocompatibility complex (MHC) molecules that migrate to lymph nodes, introducing hapten-specific naive-T-cells differentiate into CD4+ and CD8+ cells.[11] IL-10 expression was detected in the subjects with a negative patch test when stimulated with metal ions. The release of IFN-γ after metal ion stimulation increases its level along with IL-10 responses. IFN-γ produced by CD4+ and CD8+ T-cells act as the main effector on sensitization and is directly responsible for clinical manifestations. However, IL-10 is predicted to suppress inflammatory responses. The detrimental effects of CD8+ T-cells may be reduced in this regulation. Therefore, IL-10 and IFN-γ expression has been identified as significant inflammatory cytokines in hypersensitivity reactions.[3,12,13]

Propolis extracts have been demonstrated to have anti-allergic effects against allergic rhinitis, inflammation, atopic dermatitis, and asthma.[14,15] Propolis is also effective in reducing oxidative stress and acting as an immunomodulator. One of the types of propolis that is well-researched is Brazilian propolis.[16] Brazilian propolis also has an anti-corrosion effect on mild steel caused by artepillin-C or 3,5-diprenyl-4-hydroxycinnamic cid (DHCA) components.[17] The corrosion inhibitors from Brazilian propolis are expected to provide beneficial and non-toxic effects compared to synthetic compounds.[18,19] The immersion of mild steel coated with Brazilian propolis shows a significant difference in the corrosion quantity.[20,21] Moreover, Brazilian propolis is known to increase IL-10 expression in

the lower respiratory system and reduce IL-5 in asthmatic mice.[22,23] Based on the descriptions, the author intends to observe the effectiveness of Brazilian propolis wax as a type IV anti-hypersensitivity agent in animal models with fixed metal orthodontic appliances by the observation of the level of IL-10 and IFN-γ expression.

MATERIALS AND METHODS

Animal model

Thirty-six experimental New Zealand rabbit strains (*Oryctolagus cuniculus*) were divided into the control group, wax group, and propolis-wax group. Before the treatment, sensitization was carried out by 125 µL $NiCl_2$ intraperitoneal injection. The treatment was observed for 28 days. The food, temperature, and body weight are controlled and monitored every two days to ensure no significant injuries. The ethical clearance number 18/EC/H/FK-UNDIP/III/2020 from Diponegoro University was approved to conduct this experiment.

Wax propolis production

The Brazilian propolis *Baccharis dracunculifolia* wax 11% was designed and produced in the Pharmacy laboratory of Universitas Islam Sultan Agung, Indonesia. The wax group without any propolis fillers was formulated with components the same as the propolis-wax group without any element of propolis.

Fixed orthodontic bracket installation and measurement of surface roughness

The rabbits were anesthetized intraperitoneally using ketamine 50 mg/kg body weight and xylazine 5 mg/kg body weight. The four front teeth of the experimental animals were etched and placed on stainless steel brackets (American Orthodontics, Washington, US) with light cure bonding. The wax without filler and propolis wax was manually attached and covered on the entire bracket in all treatment groups (Figure 1a). The wax attachment was observed every day. After 28 days, the rabbits are stunned with chloroform. The saliva were collected by filter paper strips. Blood serum samples were taken 2 cc from the femoral vein and collected in the capillary tube. Mice were then euthanized using sodium phenobarbital 150 mg/kg body weight intravenously. The bracket surface was observed by scanning electron microscope-energy dispersive X-ray (SEM-EDX) (Phenom Pro X, Netherlands) to measure the surface roughness.

Measurement of salivary Ni^{2+} ion and blood serum level of IL-10 and IFN-γ

Saliva was collected by pilocarpine injection to stimulate and collected into the saliva tube. Blood samples were collected from the salivary. The inductively coupled plasma-optical emission spectrometry (ICP-OES) (Lampun Optima 8300® Germany) measured Ni^{2+} ion level. IL-10 and IFN-γ level were measured using the ELISA method (Elabscience kit®, Texas).

Statistical Analysis

The analysis of the results was carried out using SPSS (version 19) software. The

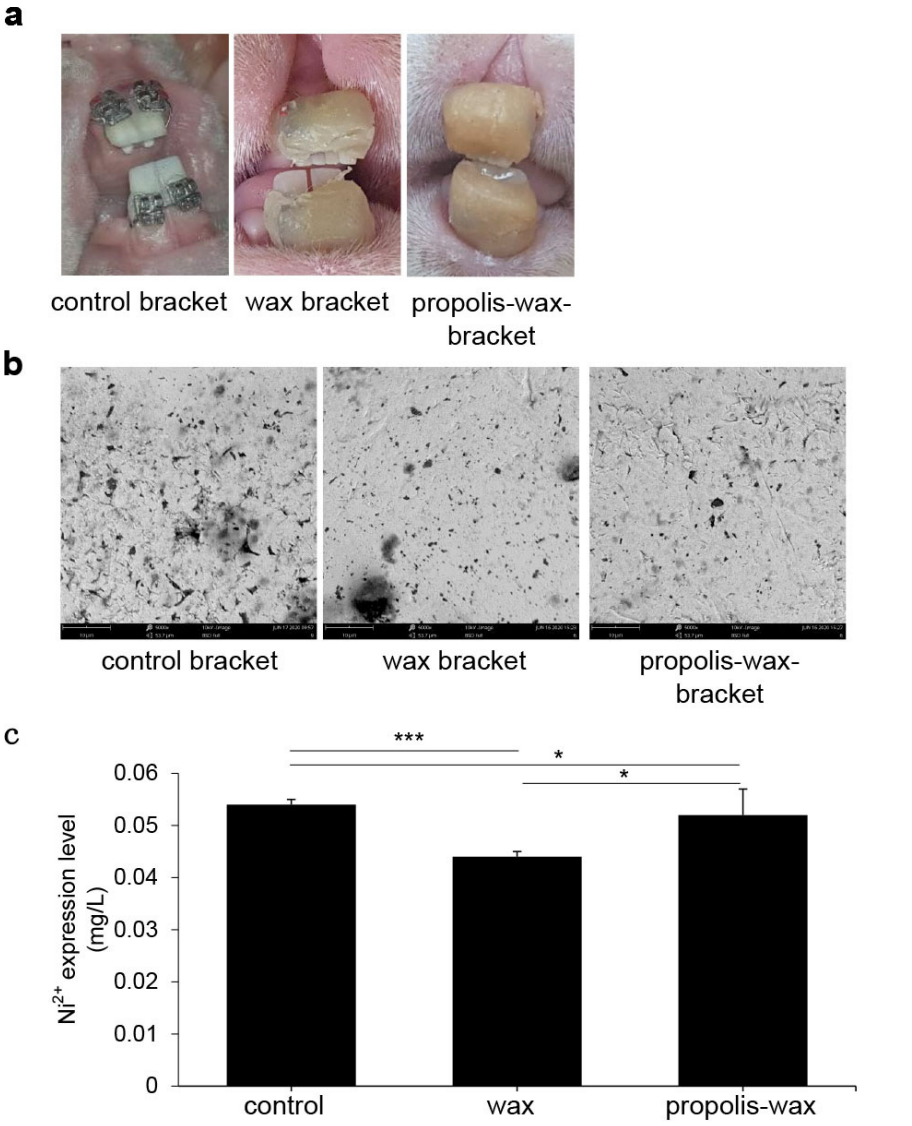

Figure 1. The metal bracket surface and measurement of salivary Ni^{2+} ion release. **(a)** 11% propolis wax produced in combination with the wax orthodontic. **(b)** SEM analysis showed the irregular surface of the metal bracket surface after 28 days of application. Due to the release of metal ions, there are more irregular dots on the bracket surface of the control group than in wax and propolis-wax groups. (n=4 each group; scale bar: 10 µm) **(c)** Salivary Ni^{2+} ion release measured by spectrometry analysis. Wax and propolis wax groups showed a decreased level of Ni^{2+} ions. (Data as mean±SD; 3-4 individual animal each group; *** =p<0.001, **p<0.01, *p<0.05).

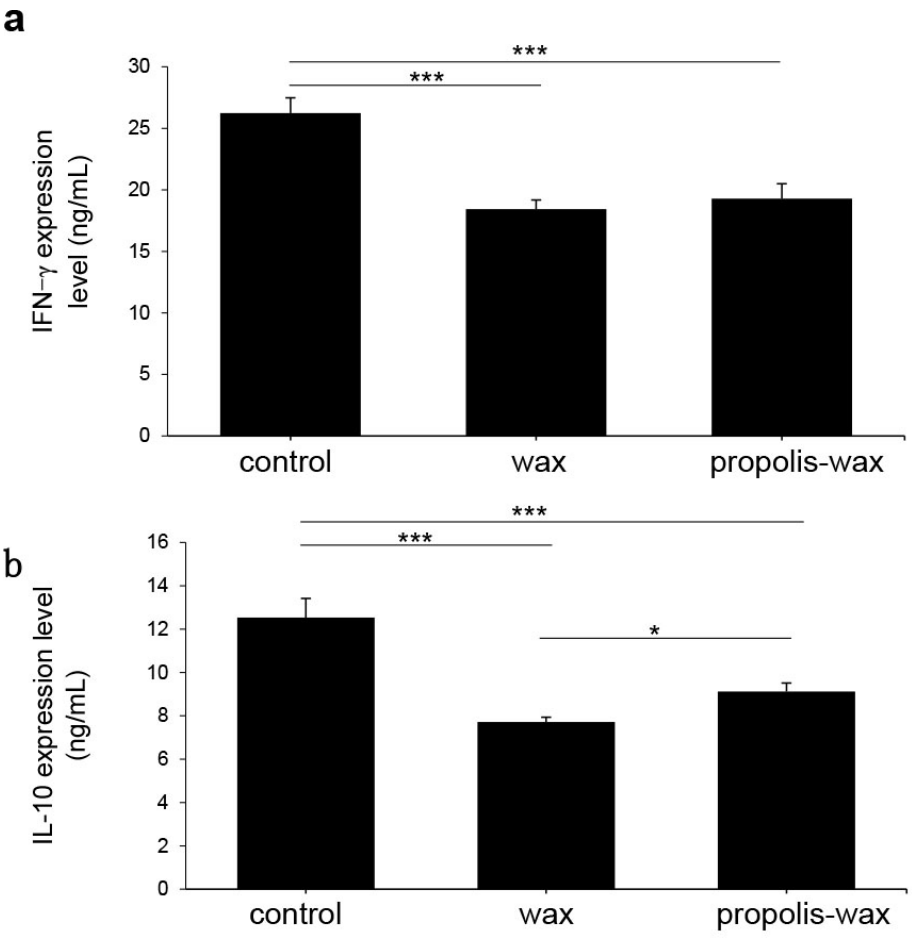

Figure 2. Characteristic of the immune regulator of Ni^{2+} sensitivity by the systemic blood serum IFN-γ and IL-10 cytokines. A decreased level of IFN-γ **(a)** and IL-10 **(b)** was observed in the wax and propolis-wax group than in the control group. The propolis-wax group increased more IL-10 expression significantly than the wax group. (Data as mean ± SD; 3-4 individual animal each group; *** =p<0.001, **p<0.01, *p<0.05).

normal and homogeneous distribution data was analyzed using ANOVA, followed by post hoc Least Square Difference (LSD) test or *Tamhane* T2 test analysis. Kruskal-Wallis and Mann-Whitney test was also used to analyze the data distribution and normality.

RESULTS

Wax propolis reduces surface roughness

SEM analysis showed the morphology of the bracket surface. Surface roughness was observed on all of the metal bracket surfaces. However, the control bracket group has a rougher surface than the propolis-wax bracket and wax bracket group (Figure 1b). This data suggested that the oral environment (saliva and oral pH) impacted the stability of the metal bracket, thus corroding the metal surface and releasing metal ions that are harmful to surround tissues.[24] While wax coating prevents the metal ions from being released into the oral cavity.

Propolis wax bracket reduced the number of Ni^{2+} ions released in saliva

Saliva is easy to collect and painless. The chemical component and pH of saliva affects the immune system in the oral cavity.[25] The Ni^{2+} ions are immediately released after bracket placement in the oral cavity and increase up to a specific period into the saliva.[26] The wax and propolis-wax group showed a decreased level of salivary Ni^{2+} ions than the control group (Figure 1c).

Propolis wax reduces type IV hypersensitivity reactions

Identifying the effect of propolis on the type IV hypersensitivity mediators using IFN-γ and IL-10 markers were measured from the peripheral blood of experimental animals. The expression of IFN-γ was reduced in both the wax and propolis-wax groups. The decreased level of IFN-γ indicates that wax and propolis wax effectively inhibit anti-inflammatory reactions in fixed metal orthodontic braces treatment. Moreover, the expression of IL-10 in the propolis wax group is increased significantly than in the wax group only. These findings indicate that propolis is more effective as an anti-inflammatory agent than wax only under hypersensitivity conditions.

DISCUSSION

Immune response in metal allergy is associated with the increased expression of T-cells induced by thymic stromal lymphopoietin (TSLP), increasing TNF-α in the oral epithelium.[27] Wax-coating metal orthodontic brackets are designed to relieve pain caused by orthodontic treatment as a barrier to prevent metal irritation and secondary infection, reduce patient discomfort, and allow tissues to heal. Kumarasinghe et al. revealed that coated metal brackets with bioactive plasma effectively inhibit the release of metal ions *in vitro* study.[24] While superamphiphobic coating in orthodontics, stainless steel effectively inhibits wear resistance and cytotoxicity.[28] However, another approach to improve the metal ions reduction mechanism and immune response in metal bracket orthodontics remain to be further analyzed. Our study provides novel evidence based on combining propolis in the wax orthodontic to treat and prevent Ni^{2+} ions allergy on the metal bracket orthodontic treatment.

Propolis generally contains calcium (Ca^{2+}), magnesium (M^{2+}), aluminum (Al^{3+}), iron (Fe^{2+}), manganese (Mn^{2+}), zinc (Zn^{2+}), and Ni^{2+} ions.[3] However, Ni^{2+} ions in propolis are very low and tend to decrease under the ethanol extract procedure.[29,30] Propolis acts as an immunomodulator by upregulating innate immunity and modulating proinflammatory responses.[31] The combination of propolis into wax

orthodontics is suggested to be an anti-corrosive and anti-inflammatory agent in metal bracket orthodontics. In this research, wax only and propolis wax reduced the surface roughness on the metal bracket orthodontic than in control (Figure 1b). In addition, wax and propolis wax act as a coating agent on the bracket to protect the surface from the oral environment. Thus, wax and propolis wax attenuate the release of metal ions from microleakage.

Analyzing the release of Ni^{2+} ions level in the saliva can detect corrosion of the metal bracket surface. Previously, the increased level of Ni^{2+} ions in the saliva was linear with the increased level of salivary pH.[26] Therefore, an increased acidity level in the saliva promotes a higher number of Ni^{2+} ions damaging the metal surface.[32] After 28 days of metal bracket installation, wax, and propolis-wax groups reduced the release of Ni^{2+} ions more than the control group. However, wax-only groups significantly decrease the salivary Ni^{2+} ions more than the propolis-wax group (Figure 1c).

Ni^{2+} ions induce allergy by activating the monocytes and suppressing the expression of intracellular adhesion molecule-1 (ICAM-1).[26] Propol, an anti-hypersensitivity agent, is known to activate anti-inflammatory macrophages, modulate lymphocytes and natural killer cells, promote antibody proliferation, and produce cytokines such as IL-10 and IFN-γ.[23] Flavonoids, prenylated phenylpropanoids, concanavalin-A, and artepillin-C as the significant components of propolis are suggested to activate peritoneal macrophages and attenuate IFN-γ expression.[33–36] Here, we found that the IFN-γ expressions decreased significantly in propolis-wax and wax-only groups (Figure 2a). Thus, coating metal bracket orthodontics effectively inhibits the inflammatory reaction caused by Ni^{2+} ions released into the oral cavity.

The effects of propolis as an anti-inflammatory agent were detected by measuring IL-10 expression. Some previous studies showed that propolis effectively inhibits the infiltration of inflammatory cells (macrophages, neutrophils, and lymphocytes), followed by the decreased concentration of TNF-α and IL-6.[37] Ni^{2+} allergy-induced IL-4 and IFN-γ to IL-10 response in human peripheral blood mononuclear cells.[38] The increased level of TGF-β and IL-10 was observed in the propolis group than in the control group of the acute pulmonary inflammation.[37] On the other hand, propolis-activated macrophage cell lines under lipopolysaccharide (LPS) and IFN-γ stimulation showed a slight decrease in IL-10 expression on the culture supernatants.[23] The induction of IL-10 in the saliva also reduces allergic symptoms.[39] After metal bracket application, the expression of IL-10 on the blood serum decreased in the wax and propolis-wax groups (Figure 2b). However, the significantly increased level of IL-10 is observed in the propolis-wax group rather than the wax group suggesting the propolis modulates anti-inflammatory effects on the Ni^{2+} induced hypersensitivity. Thus, it is expected that propolis compounds on the wax would enhance immune response, specifically for those sensitive to Ni^{2+} ions of the metal bracket orthodontic.

CONCLUSION

Applying wax on the orthodontic bracket reduces metal surface roughness in the oral cavity and effectively reduces the release of Ni^{2+} ions into the saliva. Propolis compounds on the wax orthodontic enhance immune response characterized by IFN-γ and IL-10 decrease expression on the blood level. Thus, it is suggested that propolis be adequate to combine in orthodontic wax to prevent Ni2+ sensitivity of the metal bracket orthodontic.

DISCLOSURE

The authors declare that they have no conflict of interest.

ACKNOWLEDGMENT

This work was supported by Universitas Diponegoro and Universitas Islam Sultan Agung.

AUTHORS CONTRIBUTIONS

Study design: All authors
Data acquisition: GA
Data analysis: All authors
Writing of the first draft: All Authors
Manuscript revision and approval of final manuscript: All authors

FUNDING

None.

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