ORIGINAL ARTICLE

Effect of *Lactobacillus casei* Shirota strain probiotic beverage on the release of chromium ions in stainless steel brackets

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ABSTRACT

Introduction: Stainless steel brackets are often used in orthodontic treatment. Chromium is one of the stainless steel bracket elements that increases corrosion resistance. Chromium ions' release can cause allergic reactions. Acidic pH due to food and drink consumption can cause corrosion and the release of ions. One of the most widely consumed acidic pH drinks is the *Lactobacillus casei* Shirota strain probiotic because it provides health benefits. The purpose of this study was to analyze the effect of the *Lactobacillus casei* Shirota strain probiotic beverage on the release of chromium ions in stainless steel brackets. **Methods:** This study was laboratory experimental research with a posttest only control group design. The number of samples was 10 for two groups. The bracket was immersed in artificial saliva as the control group and in the probiotic drink *Lactobacillus casei* Shirota strain as the treatment group. After 18 hours and 15 minutes of immersion, in an incubator at 37°C, the immersion solutions were tested using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) to determine the release of chromium ions. The results of the study were analyzed using an independent t-test. **Results:** The average chromium ion release in the control group was 7.63 ppm, while in the treatment group it was 7.87 ppm. The results of the independent t-test showed a significant difference (p<0.05). **Conclusion:** The probiotic beverage *Lactobacillus* casei Shirota strain increased the release of chromium ions in stainless steel brackets. It is greater than that immersed in artificial saliva. The value of chromium ion release obtained was still below the average daily intake of chromium.

KEYWORDS
Lactobacillus casei, Shirota strain, probiotic, chromium ions release, brackets

INTRODUCTION

Orthodontic treatment is a treatment in dentistry that aims to correct malocclusion, re-establish periodontal health, improve physical function, and correct aesthetic components.[1,2] Orthodontic treatment is classified into two categories: removable orthodontics and fixed orthodontics.[3] Fixed orthodontic treatment is widely used by the general public today because the quality of the results obtained from fixed orthodontic is superior to that obtained from removable orthodontics.[4] Fixed orthodontic treatment requires relatively long-term treatment, ranging from 14 to 33 months.[5] One of the main components used in fixed orthodontic treatment is the bracket.[6] The bracket serves to transmit the force from wires or other power modules needed by the teeth. Brackets used in orthodontic treatment must have good biocompatibility, corrosion resistance, strength, and hardness.[7]

The materials used for orthodontic brackets include stainless steel, polymeric (polyurethane and polycarbonate), ceramic, titanium, or combinations thereof.[7] Stainless steel is widely used because it is strong, highly resistant to corrosion, and relatively economical compared to others.[8] The composition of the stainless steel bracket consists of iron (Fe), chromium (Cr), and Nickel (Ni).[9] Chromium can increase corrosion resistance through a self-healing passive surface layer. Chromium reacts with oxygen to form chromium oxide on the surface of the bracket.[7,10]

Corrosion is a chemical reaction caused by the interaction between metal and the environment. The corrosion that occurs in stainless steel is characterized by the release of metal ions.[11,12] Metal ions released due to the corrosion process can cause effects such as carcinogenicity, allergy, mutagenicity and cytotoxicity. One of the allergic reactions can be caused by chromium ions.[13,14] An acidic environment is one of the causes of corrosion, which can cause ion release. This is influenced by various factors, including the consumption of beverages that have an acidic pH.[7,15] One of the most popular beverages with an acidic pH is a probiotic beverage.[16]

The term probiotic comes from Latin, meaning "for life". Probiotics are nonpathogenic, beneficial, live bacteria and yeast. The most commonly used probiotics are Lactobacillus, *Bifidobacterium*, and *Saccharomyces boulardii*.[16] Probiotic beverage *Lactobacillus casei* Shirota strain is one of the probiotic beverages that is widely consumed by the public because it provides benefits for digestive health.[17]

Probiotic beverages will pass through the oral cavity to the stomach and attach to the intestinal mucosa, preventing the attachment of pathogenic bacteria to the epithelium. Some bacteria, especially *Lactobacillus* and *Bifidobacterium*, produce lactic acid, acetic acid, and propionic pathogens.[16] The pH of the probiotic Lactobacillus casei Shirota strain ranged from 4.8 to 5.14.[18] The acidic pH of beveragescan affect the oral cavity environment.[15]
In the research conducted by Kuhta *et al*[19], orthodontic appliances were immersed in artificial saliva with a pH of 6.75 and 3.5. The release of chromium ions was greater in pH 3.5 than 6.75. These results prove that a low pH will cause a greater release of chromium ions. Another study conducted by Sumule *et al*[20], showed that stainless steel brackets immersed in carbonated drinks with an acidic pH released more chromium ions than the control group. As far as we know, there are no other researchers who conduct research on the effect of the probiotic beverage Lactobacillus casei Shirota strain on the release of chromium ions on stainless steel brackets. The purpose of this study was to analyze the effect of the *Lactobacillus casei* Shirota strain probiotic beverage on the release of chromium ions in stainless steel brackets

METHODS

This research was a laboratory experimental study with a posttest only control group design. The research was carried out from May 2022 until completion at the Microbiology Laboratory, Faculty of Medicine and Environmental Engineering Water Laboratory, Andalas University. The number of research samples was 10 with 2 groups, namely artificial saliva as the control group and probiotic beverage *Lactobacillus casei* Shirota strain as the treatment group.

The research procedure began with the preparation of stainless steel bracket samples (mini roth stainless steel 0,018 from Protect, China) using 1 set of brackets containing 20 brackets consisting of 10 maxillary brackets and 10 mandibular brackets. The soaking solution used for each sample was 50 ml of *Lactobacillus casei* Shirota strain and artificial saliva. The artificial saliva based on the Afnor method contains Na_2HPO_4, KSCN, NaCl, KH_2PO_4, KCl, $NaHCO_3$, and HCl.
Samples from each group were put into the immersion solution. Each bottle contained one set of brackets and was then incubated at 37˚C. Samples were immersed for 18 hours and 15 minutes. This duration was calculated from the consumption of Yakult®, the probiotic beverage containing *Lactobacillus casei* Shirota strain, once a day. the beverage exposed in the oral cavity for about 1 minute, and the average use of brackets for 3 years. After 18 hours and 15 minutes, all bracket samples were removed from the immersion solution and tested for nickel ion release using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). Data from the instrument was collected and statistical analysis was performed. Parametric statistical analysis is an Independent t-test that is used to analyze the mean comparison of two independent groups.

RESULTS

The results of measuring the release of stainless steel bracket chromium ions in the probiotic beverage *Lactobacillus casei* Shirota strain and artificial saliva after 18 hours and 15 minutes of immersion showed that there were differences in the release of chromium ions in the two groups. The average release of chromium ions was greater in the group of the probiotic *Lactobacillus casei* Shirota strain than in the artificial saliva group. The average value of ion release for each group can be seen in Table 1.

Table 1. The mean release of stainless steel bracket chromium ions after immersed in artificial saliva and *Lactobacillus casei* shirota strain probiotic beverage

Group	n	Mean ± SD (ppm)	Minimum (ppm)	Maximum (ppm)
Control-artificial saliva	5	7.63 ± 0.04	7.57	7.70
Treatment-*Lactobacillus casei shirota strain* probiotic beverage	5	7.87 ± 0.12	7.73	8.05

The mean of chromium ion release in the control group after immersion in artificial saliva was 7.63 ppm. The average release of stainless steel bracket chromium ions in the treatment group after being immersed in the *Lactobacillus casei* Shirota strain probiotic beverage was 7.87 ppm.

Table 2. Independent t-test results of stainless steel bracket chromium ion release after immersion in artificial saliva and *Lactobacillus casei* Shirota strain probiotic beverage

Group	n	Mean ± SD (ppm)	*p*
Control-artificial saliva	5	7.63 ± 0.04	0.004
Treatment-*Lactobacillus casei* shirota strain probiotic beverage	5	7.87 ± 0.12	

The results of the normality test by Shapiro-Wilk with a significance value of $p > 0.05$ in both groups showed that the data were normally distributed. The results of the Independent t-test in Table 2 showed a value of 0.004 ($p<0.05$), which indicated that there was a significant difference in the

value of chromium ion release between the two groups, namely the artificial saliva group and the *Lactobacillus casei* Shirota strain probiotic beverage group.

DISCUSSION

Table 1 shows the amount of chromium ions released after being immersed in artificial saliva and the *Lactobacillus casei* Shirota strain probiotic beverage. The release of chromium ions that occured in the stainless steel bracket after immersion happened because of the components found in each group. Saliva in the oral cavity acts as an electrolyte for transferring electrons and metal ions.[21] Saliva contains 99,5% water, 0.4–0.6% organic components, and 0.2–0.9% inorganic components that trigger electrochemical reactions. The inorganic components are Na^+, Cl, HCO_3^-, and K^+ ions. The release of chromium ions occurs as a result of this reaction, an indication of the corrosion process.[22,23]

The release of chromium ions after immersion in the probiotic *Lactobacillus casei* Shirota strain occurred due to the low pH, which was 3.8. Table 1 shows that the mean of chromium ions released in *Lactobacillus casei* Shirota strain probiotic beverage is higher than artificial saliva. The content of lactic acid ($C_3H_6O_3$) produced from the fermentation of lactic acid bacteria causes a decrease in pH so that it has a higher concentration of H^+. Increased concentration of H^+ will accelerate the reduction reaction. The reduction process involves the capture of electrons by H^+ ions, which result in the production of hydrogen gas. Electrons are obtained from the release of chromium ions as an oxidation reaction. This reaction will accelerate corrosion rate and the release of chromium ions.[24] The process and corrosion rate are also affected by time. Exposure in a longer time to an environment that has a low pH will further affect the process of corrosion.[19]

The stainless steel surface is protected by a passive layer, which is formed due to the passivating effect to prevent corrosion. This layer can be damaged by several things, including brushing your teeth and acidity levels. Low pH will reduce the stability of the passive layer on the surface of the bracket. The reduced corrosion resistance as a result of the passive layer instability will induce the release of more chromium ions.[22,25]

The data of this study indicated that the release of chromium ions immersed in the probiotic *Lactobacillus casei* Shirota strain, with a pH of 3.8 was greater than that of artificial saliva with a pH of 6.8. Table 2 shows that there is a significant difference in the value of chromium ion release between the two groups. The difference in pH between the two immersion groups has led to a significant difference in the measurements of chromium ion release. These results are in line with research conducted by Kuhta et al[19], who immersed orthodontic appliances in artificial saliva with a pH of 6.75 and pH 3.5. The release value of chromium ions was greater 37-fold in pH 3.5 than 6.75. These results indicate that low pH will cause an increase in H^+ ions and induce greater release of chromium ions.

In line with a study conducted by Sumule et al[20], immersed stainless steel brackets in carbonated drinks with acidic pH showed that the release of chromium ions was greater than that in the control group. Another study by Sfondrini, et al[26] conducted immersion of stainless steel brackets at various acidities, which are pH 4.2, 6.5, and 7.6. The pH 6.5 and 7.6 represent the range of human natural salivary pH, and the pH 4.2 represent a condition that can occur when people consume acidic foods and beverages. The release value of chromium ions was greater than those achieved at pH 6.5 and 7.6.

The presence of metal ions, such as nickel and chromium in stainless steel brackets, can act as allergens. The release of chromium ions will cause potential allergies and may have adverse effects, such as headaches, diarrhea, irritation, and vomiting if in larger intakes. The average daily intake of chromium is 280 µg. Based on the the study findings, the amount of chromium ion released after immersion in artificial saliva and the probiotic beverage *Lactobacillus casei* Shirota strain is still far below the average daily intake of chromium.[14,27,28]

This research is in line with previous studies, which concluded that orthodontic appliances are potentially corrosive. Fluctuations in pH and temperature, enzyme and microbial activity, as well as various chemicals that enter the oral cavity are conductors of corrosion. When the corrosion process occurs, metal ions are released in the oral cavity. The amount of metal ion released according to most studies is below the average daily intake, but this is not a guarantee of safety. Chronically, low metal ion levels can disrupt cell metabolism and morphology, and cause inflammation. Orthodontic devices made from materials that have the highest degree of biocompatibility, such as titanium, can be an alternative.[11,12,21,29]

CONCLUSION

The probiotic beverage *Lactobacillus casei* Shirota strain increases the release of chromium ions of stainless steel brackets. It is greater than that immersed in artificial saliva. The value of chromium ion release obtained is still below the average daily intake of chromium. Fixed orthodontic users are advised not to consume probiotic drinks excessively because they allow for more ion release.

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