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Original Article

# Evaluation of Antibacterial Effect of the Orthodontic Composite Containing Propolis Nanoparticles in Rat as an Animal Model

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## Abstract

**Aim:** The present study aimed to assess the antimicrobial effects of orthodontic primer containing nano-propolis against the cariogenic bacteria in a rat model.

**Materials and methods:** Transbond XT orthodontic primer containing 0%, 1%, 5%, and 10% nano-propolis was experimentally prepared in-house. The Wistar rats we used in the study were randomly divided into four groups and their oral cavities were colonized with *Streptococcus mutans*, *Streptococcus sanguinis*, and *Lactobacillus acidophilus*. After anesthetizing the rats, one drop (10 µL) of primer containing different concentrations of nano-propolis was applied to the labial surface of the maxillary incisor and light-cured. The orthodontic composite was applied on the primer and light-cured. One drop (10 µL) of primer containing the same concentrations of nano-propolis was again applied on the surface of composite and light-cured. The number of *S. mutans*, *S. sanguinis*, and *L. acidophilus* colonies in the saliva of rats was quantified at 24 h, at days 4 and 7 using plate counting.

**Results:** Primer containing 1%, 5%, and 10% of nano-propolis significantly reduced the *S. mutans* colony count at 24 h compared with the control group ($p<0.05$). At day 4, the mean *S. mutans* colony counts in the 5% and 10% nano-propolis groups were significantly lower than that in the control group ($p<0.05$). Primer containing 1%, 5%, and 10% (all ($p<0.05$) of nano-propolis significantly reduced the *L. acidophilus* at 24 hours. Also, at day 4 the mean *L. acidophilus* colony counts in the 5% and 10% nano-propolis groups were significantly lower than that in the control group ($p<0.05$). At 24 h and 4 days, the mean *S. sanguinis* colony count in the 1%, 5%, and 10% nano-propolis group was significantly lower than that in the control group ($p<0.05$). No significant difference was observed in the presence of all concentrations of nano-propolis at day 7 ($p>0.05$).

**Conclusions:** Orthodontic primer containing nano-propolis significantly reduced the colony count of cariogenic bacteria in a rat model.

## Keywords

cariogenic bacteria, nano-propolis, composite, orthodontic adhesive, primer, rat

## INTRODUCTION

White spot lesions (WSLs) around the brackets are a sequela of fixed orthodontic treatment, which is aggravated by poor oral hygiene.[1-3] Based on a recent meta-analysis, the frequency of new WSLs and carious lesions has been reported 68% in patients undergoing fixed orthodontic treatment which is quite alarming and necessitating the attention of both patients and orthodontists to effective caries prevention programs and techniques.[4] There is a significant change in the microbiome of the dental plaque after the insertion of orthodontic fixed appliances with higher concentrations of acidogenic bacteria including *S. mutans* and *L. acidophilus* as well as dental plaque inhabitant bacteria such as *S. sanguinis* where it modifies the environment to make it less hospitable for the cariogenic bacteria, such as *S. mutans*. *S. sanguinis* is a pioneering colonizer and a key player in dental biofilm development as well as serves as a tether for the interaction of a variety of another oral microbiome, which colonizes the tooth surface, form dental plaque, and recognized as the etiology of both dental caries and periodontal disease. It also contributes to extra-oral diseases including infective endocarditis.[5,6] A predominance of *S. sanguinis* is associated with healthy plaque biofilm without carious lesions, while *S. mutans* and *L. acidophilus* are associated with tooth decay. The relative balance between *S. sanguinis* and *S. mutans* may be an indicator of a patient's oral health and risk for dental caries.[7] Consequently, scientists and orthodontists have been especially devoted to emerging and new nanoparticle-based materials with anti-caries activities to minimize the occurrence of WSLs.[8]

Nanotechnology and nanoscience, the use of matter with dimensions on the atomic, molecular, and supramolecular scale, has become increasingly utilized for medical and clinical applications and has recently attracted much interest as an approach to killing or reducing the virulence of numerous microorganisms.[9] While some natural antimicrobial agents, such as propolis, possess greater antimicrobial activities as particle size is decreased into the nanometer scale due to the increased surface to volume ratio, the shape and structure of a nanoparticle itself and the way in which it attaches with and penetrates into microbial cells appears to also be responsible for unique microbiocidal mechanisms.[10]

Propolis is a mixture of buds, exudates, and other parts of plants as well as beeswax substances, and bee salivary enzymes used by bees to protect the hive from cavities and intruders. It has various activities such as antibacterial, antiviral, antifungal, antiparasitic, antioxidant, anti-inflammatory, and antiproliferative effects. In terms of antibacterial effect, the content including phenolic and flavonoids compounds is important.[11]

No research has been conducted on combining the orthodontic composite with nano-propolis to obtain an antimicrobial effect in an animal model and improved fixed orthodontic treatment outcomes.

## AIM

The purpose of the current study was to explore a combinational orthodontic composite with nano-propolis to reduce cariogenic *S. mutans*, *S. sanguinis,* and *L. acidophilus* in oral cavity of rat as an animal model. It was hypothesized that there was a significant difference between the antimicrobial property of the orthodontic composite containing nano-propolis and the original orthodontic composite against *S. mutans*, *S. sanguinis,* and *L. acidophilus* in a rat model.

## MATERIALS AND METHODS

### Preparation of nano-propolis

Nano-propolis was prepared as described previously.[10] Briefly, the crude propolis was collected from honey bees from Golpayegan, Iran. Fine powder was prepared from propolis using an electric mill. An extract of propolis (10% w/v) was prepared after adding ethanol (85%) in a shaking water bath (at 37±1°C and 150 rpm) for 48 hours. Then the liquid portion was filtered through a filter paper (Whatman No. 1) and maintained at 4°C sonicated for 20 min and then evaporated in a water bath at 50°C to concentrate for the preparation of nano-propolis. The obtained nano-propolis was dried using a freeze-drying machine (Lyotrap/Plus, UK) for further usage. The morphological analysis of nano-propolis was determined using scanning electron microscopy (SEM).

### Preparation of modified orthodontic composite

Transbond XT orthodontic primer (3M Unitek, Monrovia, CA, USA) was used for the preparation of the modified orthodontic composite (MOA) containing 0%, 1%, 5%, and 10% nano-propolis. Twenty drops (50 µl ≈ 0.05 g per drop) of Transbond XT orthodontic primer were mixed with 0.00, 0.01, 0.05, and 0.1 g of nano-propolis for the preparation of the control (no nano-propolis), 1%, 5%, and 10% nano-propolis groups, respectively, using an ultrasonic bath for 30 minutes. The prepared experimental primers were transferred into microtubes, which were covered with aluminum wraps to prevent exposure to the light.

### Animal study design

The animal experiments were done in accordance with the Animal Ethics Committee of Tehran University of Medical Sciences guideline (IR.TUMS.DENTISTRY.REC.1396.2773) Male Wistar rats (200–250 g; Pasteur Institute, Tehran, Iran) were housed one rat per cage, at 22–25°C and at 12 h light/dark cycles, under sanitary conditions with free access to water and sanitized pellet food. Rats were allowed to adapt to the animal room conditions for 1 week to

the test day. All methods in the current study were carried out in accordance with relevant guidelines and regulations. To increase the accuracy of microbiological assessments, the bedding materials were autoclaved and replaced every day as well as the cages were disinfected with 10% povidone iodine solution. Based on the previous studies, the effect size (No. of rat in experimental groups) was estimated as nine rats per each nano-propolis concentrations, using power analysis with power arbitrarily set at 90%.[12,13] Initially, rats were infected with the test bacteria in this study.

Rats were randomly assigned to either test (modified orthodontic composite containing 1, 5, and 10% of nano-propolis) or control groups (same as test without nano-propolis). Cariogenic bacteria-infected rat receiving original Transbond XT orthodontic primer instead of modified orthodontic composite containing nano-propolis served as controls (group A). The control group was set up with no nano-propolis (0%) applied. Test groups (B-D) were exposed to different concentrations of nano-propolis (1, 5, and 10%, respectively), while a control group (A) was not exposed **(Fig. 1)**.

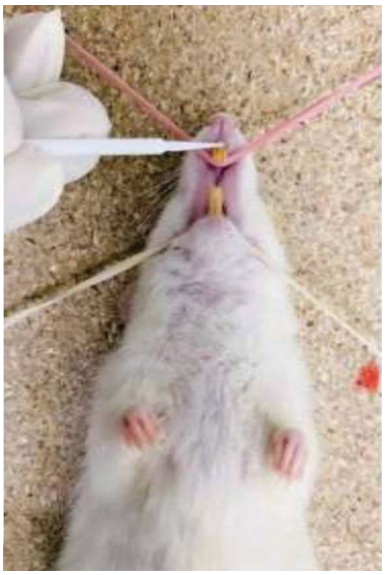

**Figure 1.** An animal model for assessment of the antimicrobial activity of orthodontic primer containing nano-propolis.

Since the oral microbiome of the rats is different from that of human, the microbiome of the rats was removed based on the previous study.[14] After that, a suspension of three test bacteria was prepared containing *S. sanguinis* (ATCC 10556), *S. mutans* (ATCC 25175), and *L. acidophilus* (ATCC 4356) in an amount of $3\times10^8$, $3\times10^8$, and $3\times10^9$ colony forming units per milliliter (CFUs/mL), respectively. The oral cavity of the rats was infected with the bacterial suspension using sterile swabs for three consecutive days as described previously.[15] To confirm the colonization of test bacteria in oral cavity of rats, after 24 h, saliva swab samples were collected and cultured on modified medium 10-sucrose agar, Man Rogosa and Sharpe-clindamycin ciprofloxacin, and agar Mitis Salivarius-Mutans valinomycin agar, and to determine the presence of *S. sanguinis*, *L. acidophilus*, and *S. mutans*, respectively as described previously.[16,17] The rats harboring all of three test bacteria in their oral cavity (n=36) were entered to the next phase of the study.

## Application of orthodontic primer and adhesive

Rats were anesthetized using intraperitoneal injection of a ketamine-xylazine cocktail. The rats were then fixed on an operating table in the supine position, their maxillary central incisor was etched with phosphoric acid (37%) for 20 s after which the central incisor was washed gently, and then dried with a cotton pellet. Next, 10 µL of primer containing nano-propolis was applied on the labial and proximal surface of central incisor and cured for 20 s using LED irradiation. A thin layer (2×2 mm) of adhesive (Transbond XT; 3M Unitek, Monrovia, CA, USA) was then applied to the area of the tooth that was primed and cured for 20 s using LED irradiation. Next, 10 µL of primer containing nano-propolis was again applied over the layer of adhesive and cured for 20 s using LED irradiation.[14]

To prevent separation of the adhesive from the surface of central incisors in occlusion, the central incisors of the mandible were shortened by 2 mm. The presence of adhesive on the surface of the teeth was checked after 24 h, 4 days, and 7 days, and saliva samples were collected from all rats at the designated time points (24 h, 4 days, and 7 days). To count the test bacteria (CFU/mL), plate counting method using brain heart infusion (BHI) agar (Merck, Germany) was done as described previously.[12]

## Statistical analysis

One-way ANOVA was run to compare the CFU/mL of test bacteria at each time point. Tukey's post hoc test was applied to compare each two means on each dependent variable for pairwise comparisons. Data were analyzed using SPSS version 23.0 (SPSS Inc., IL, USA) and a $p$-value of 0.05 was considered statistically significant.

# RESULTS

As shown in **Fig. 2**, the uniform shapes of nano-propolis are nano-sized particles, approximately 80-90 nm in diameter, which confirms the successful synthesis of nano-propolis. One-way ANOVA revealed a significant difference in *S. sanguinis* CFU/mL in the presence of different concentrations of nano-propolis at 24 h ($p<0.001$) and day 4 ($p<0.001$). According to the data in **Fig. 3**, 13% ($p=0.002$), 54% ($p<0.001$), and 63% ($p<0.001$) reduction was shown in *S. sanguinis* CFU/mL following exposure to 1%, 5%, and 10% concentrations of nano-propolis, respectively, in comparison with the control group at 24 h. The *S. sanguinis* CFU/mL in the presence of 1% nano-propolis was significantly higher than that in the presence of 5% ($p<0.001$) and



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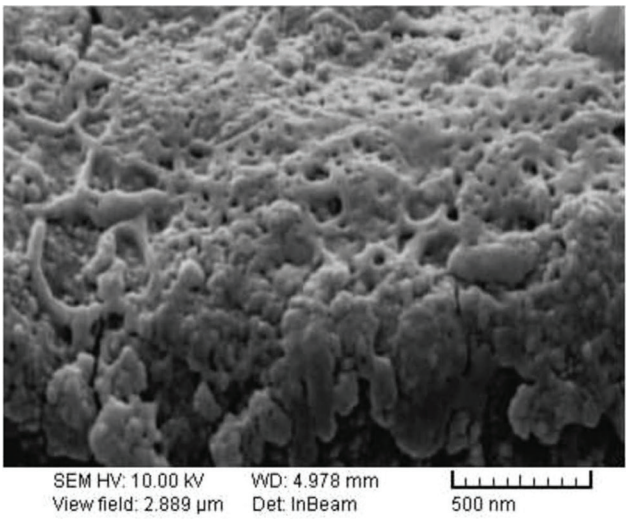


**Figure 2.** SEM image of synthesized nano-propolis.

10% ($p$<0.001) nano-propolis. At day 4, 17% ($p$=0.40), 41% ($p$=0.003), and 54% ($p$<0.001) reduction in *S. sanguinis* CFU/mL was observed in the presence of 1%, 5%, and 10% nano-propolis, respectively, in comparison with the control group **(Fig. 3)**. Also, 2% ($p$=0.998), 29% ($p$=0.206), and 23% ($p$=0.412) reduction in *S. sanguinis* CFU/mL was displayed in the presence of 5% and 10% nano-propolis, respectively, in comparison with the control group at day 7. Over time, the CFU concentrations of *S. sanguinis* showed a tendency of nonsignificant decrease in all control groups at days 1 ($9.10\times10^5$), 4 ($8.30\times10^5$), and 7 ($7.20\times10^5$) ($p$>0.05).

According to the data in **Fig. 4**, 14% ($p$=0.022), 53% ($p$<0.001) and 62% ($p$<0.001) reduction was shown in *S. mutans* CFU/mL following exposure to 1%, 5% and 10% concentrations of nano-propolis, respectively, in comparison with the control group at 24 h. The *S. mutans* CFU/mL in presence of 1% nano-propolis was significantly higher than that in the presence of 5% ($p$<0.001) and 10% ($p$<0.001) nano-propolis. At day 4, 17% ($p$=0.409), 29% ($p$=0.003), and 54% ($p$<0.001) reduction in *S. mutans* CFU/mL was observed in the presence of 1%, 5%, and 10% nano-propolis, respectively, in comparison with the control group **(Fig. 4)**. Also, 2% ($p$=0.998), 29% ($p$=0.206), and 23% ($p$=0.412) reduction in *S. mutans* CFU/mL was displayed in the presence of 5% and 10% nano-propolis, respectively, in comparison with the control group at day 7. Throughout the study period, *S. mutans* CFU/mL revealed a tendency of nonsignificant decrease in all control groups at day 1 ($9.20\times10^5$), day 4 ($8.50\times10^5$), and day 7 ($7.40\times10^5$) ($p$> 0.05).

Significant reductions were seen in *L. acidophilus* CFU/mL in the presence of 1%, 5%, and 10% nano-propolis at 24 h ($p$<0.05) in comparison with the control group. Exposure to 5% and 10% nano-propolis at day 4 had no significant reduction in *L. acidophilus* CFU/mL when compared with the control group. No significant reduction was observed in *L. acidophilus* CFU/mL in the presence of 1%, nano-propolis at day 4 ($p$>0.05), and in the presence of 1%, 5%, and 10% nano-propolis at day 7 ($p$>0.05) in comparison with the control group. As shown in **Fig. 5**, there was 56% ($p$<0.012), 43% ($p$<0.001), and 30% ($p$=0.12) reduction in *L. acidophilus* count (CFU/mL) in the presence of 10% nano-propolis at 24 h, days 4 and 7, respectively, in comparison with the control group. The difference between 1% nano-propolis and 10% nano-propolis groups was also significant at all examined times ($p$<0.05) except day 7 ($p$<0.554). During the study time, the *L. acidophilus* CFU/mL showed a tendency of nonsignificant decrease in all control groups at day 1 ($9.50\times10^5$), day 4 ($9.00\times10^5$), and day 7 ($8.30\times10^5\pm6.0$; $p$>0.05).

## DISCUSSION

There are some concerns about the incidence of white spots as well as dental caries lesions during fixed orthodontic treatment.[18,19] Fixed orthodontic appliances are always in direct contact with the enamel surface of tooth. Depending on the treatment plan, the duration of treatment and patient oral hygiene, the accumulation of microbial biofilm and the load of acidogenic bacteria including *Streptococcus* species and *L. acidophilus* increases in orthodontic patients. These bacteria reduce the pH of the biofilm structure in orthodontic patients. At the same time, topical application of sodium fluoride mouthwashes as well as enhancing oral hygiene behaviors have not had sufficient effects in preventing white spot lesions and dental caries.[20] In this regard, due to the possible antibacterial properties of propolis nanoparticles in preventing the occurrence of caries lesions, the present study was conducted to determine the antibacterial effects of propolis nanoparticles in composites used in orthodontics in a rat model.

The main constituents of propolis associated with antimicrobial effects include flavonoids and cinnamic acids.[21] The other compounds of propolis such as aldehyde, aliphatic acid ester, carboxylic acids, cinnamic acid and its esters, ketone, terpene, alcohol, ether, hydrocarbon and phenolic, each of which exhibits antibacterial properties.[22] In addition, the synergies between these compounds, along with the unique effects of the components themselves, are effective in counteracting the antibacterial effects of propolis. In addition, it has been shown that each of the compounds of propolis alone is effective against microorganisms and that propolis has more effects against pathogenic microorganisms than each of its components.[22-24]

In the present study, three bacteria *S. mutans*, *S. sanguinus*, and *L. acidophilus* were used to evaluate the effects of different concentrations of nano-propolis used in orthodontic composites. *S. mutans* is usually involved in the onset of dental caries, and *L. acidophilus* is rarely seen in the early stages of caries. *S. sanguinus* is also associated with plaque biofilm and is one of the bacteria that is colonized in the oral cavity and helps to bind other microorganisms and plays a key role in the development of oral biofilm.[25,26] This bacterium is associated with non-cariogenic plaques and competes with *S. mutans* to colonize the enamel surface.[27,28]

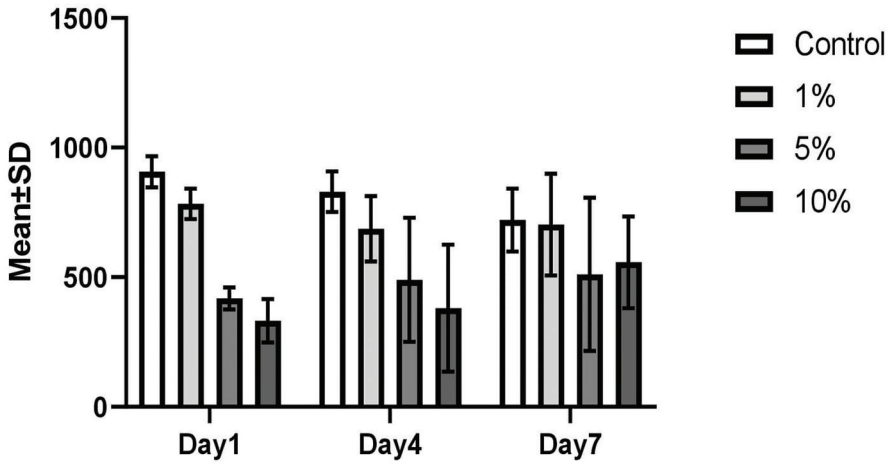


**Figure 3.** Mean and standard deviation of the number of *S. sanguinus* CFU/mL in terms of nanopropolis concentration and evaluation time.

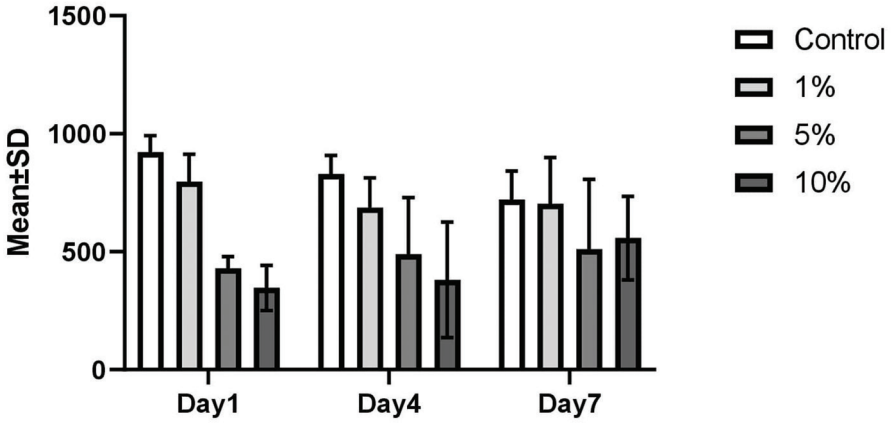


**Figure 4.** Mean and standard deviation of the number of *S. mutans* CFU/mL in terms of nanopropolis concentration and evaluation time.

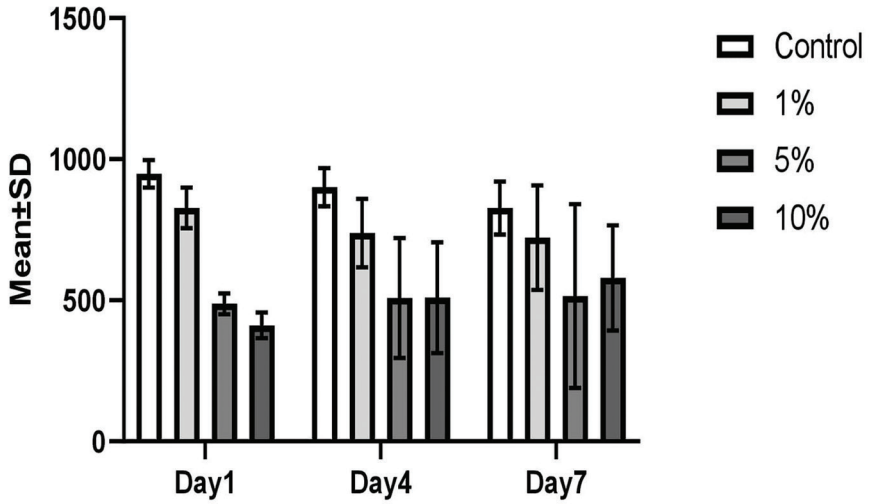


**Figure 5.** Mean and standard deviation of the number of *L. acidophilus* CFU/mL in terms of nanopropolis concentration and evaluation time.

The results of the present study showed that the use of composites with concentrations of 1%, 5%, and 10% of nano-propolis had specific antibacterial effects against S. *mutans, S. sanguinis,* and *L. acidophilus* at each time of day 1, day 4, and day 7. Also, the antibacterial effects of composites with concentrations of 5% and 10% nano-propolis were stronger than those of composites with concentrations of 1% nano-propolis, but the effects of composites with concentrations of 5% and 10% nano-propolis were estimated to be equal to each other, which indicates that the effects are dose dependent. These results are consistent with the results reported by Akhavan et al.[29], which show the effects of nano-propolis on the antimicrobial properties of Transbond XT composite containing 1%, 2%, 5%, and 10% nano-propolis were investigated against S. *mutans, S. sanguinis*, and *L. acidophilus*. According to the results of their study, the lowest CFU/mL of *S. mutans* and *S. sanguinis* was observed at 15 days, which decreased significantly at 2%, 5%, and 10% concentrations of nano-propolis and the CFU/mL of *L. acidophilus* colonies at all concentrations (except 1%) was significantly reduced at day 30.[29]

Over time, the CFU concentrations of test bacteria showed a tendency of nonsignificant decrease in all control groups at days 1, 4, and 7. It is possible that the exposure to environmental microbial strains and reversion of the natural oral microbiome in rats during the study period interfere with the prior implanted *S. mutans*. On the other hand, we cannot exclude the possibility that the change in rat saliva composition throughout the study period in the presence of orthodontic composite was responsible for the changes in CFU concentrations of test bacteria.[30] Besides aiding in the mechanical clearance of the oral microbiome, it has been shown that certain components of saliva may specifically influence the attachment and accumulation of different oral bacteria including *S. mutans* on oral surfaces, these include glycoproteins which adsorb to the tooth surface leading to the formation of the "acquired pellicle" and continually bathe the oral surfaces.[31,32] This suggests that a tendency of nonsignificant decrease of CFU concentrations of test bacteria in all control groups during the study period may be associated with changes in the quantity and quality of the rat saliva in presence of orthodontic composite. Moreover, the role of environmental- and host-specific factors that dictate implanted S. mutans populations in oral rat, remains to be investigated. Most research on the antibacterial effects of propolis on products such as propolis mouthwash and toothpaste has been done, and according to researchers, except in one case and differently[29], no study has been done on the effects of nanopropolis used in different concentrations on composites. Orthodontics has not been performed on cariogenic microorganisms.

Most research on the antibacterial effects of propolis has resulted in products such as mouthwash and toothpaste, and to our knowledge, this is the first report that attempts to show the antimicrobial effect of an orthodontic composites containing different concentrations of nano-propolis against cariogenic microorganisms.

Vanni et al.[33] reported that mouthwash containing propolis did not have a significant effect on reducing the number of bacterial colonies in multi-bacterial biofilms, which is not consistent with the present study. The reason for this difference can be related to the type of material used to induce antibacterial effects, which in the current study was nano-propolis in the composition with orthodontic adhesive and in the research of Vanni et al., it was a mouthwash and toothpaste products containing propolis in non-nano form.

In another study, Netto et al.[34] showed that propolis mouthwash in comparison to chlorhexidine mouthwash has clear and superior effects in suppressing active carious lesions. Despite the differences in the protocols used in the two studies, the results of the present study are in line with the results of the study of Netto et al., in which the addition of 2% non-alcoholic propolis enhanced the antimicrobial activity of the mouthwash against *S. mutans*.

In the current study, with increasing time, the antibacterial effects of composites containing different concentrations of nano-propolis have decreased. The increase in the number of cariogenic microorganisms on day 7 compared to days 1 and 4 indicates that the antimicrobial properties of nano-propolis decreased over time to day 7, due to insufficient release of propolis nanoparticles. It seems that with increasing the concentration of nano-propolis, it may continue to release and induce antimicrobial effects, although the use of high concentrations of nano-propolis can also weaken the mechanical properties and bond strength of the orthodontics composite contain nano-propolis.

According to the results of the present study, the highest levels of microbial inhibition occurred in all three bacteria, *S. mutans*, *S. sanguinus*, and *L. acidophilus*, at a concentration of 10% nano-propolis. Also, concentrations of 5% and 10% of nano-propolis were significantly different only in the first day of exposure. On other days (i.e., days 4 and 7), no significant differences were observed in *L. acidophilus* and *S. mutans CFU/mL*, in terms of bacterial inhibition. In other words, the antibacterial effects of these two concentrations were equal to each other. Since the use of various compounds such as nano-propolis can affect other physical and mechanical properties, including bond strength to the tooth, it seems that the best concentration for antibacterial effects is 5% nanoparticles.

Malhotra et al.[35] explained the antibacterial effects of mouthwashes containing synthetic propolis (made in the laboratory with a 1:5 dilution of water) against *S. mutans*, *Lactobacillus* spp and *Candida albicans*. In another report, Duailibe et al.[36] have concluded that propolis extract has antimicrobial activity against *S. mutans* and may be used as an alternative method to prevent tooth decay. The observations in the Malhotra et al.[35] and Duailibe et al.[36] studies are generally consistent with the results of the present study.

The results of this study are consistent with a recent report[14] in which the 10% chitosan nanoparticles (CNPs) caused maximum inhibition of *S. mutans* and *S. sanguinis*; 5% and 10% concentrations of CNPs had no significant dif-

ference with each other at any time point. Although the antimicrobial effects of nano-propolis with different concentrations in rat model were confirmed in the present study, it is necessary to confirm these results in a clinical trial.

## CONCLUSIONS

Our data support the finding that orthodontic composite containing 10% nano-propolis demonstrated antibacterial activity against *S. mutans*, *S. sanguinus*, and *L. acidophilus* up to day 7 in a rat model. To fully assess the viability of nano-propolis, future studies will focus on gauging the physical properties of orthodontic composite containing nano-propolis, such shear bond strength and adhesive remnant index.

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## Disclosure statement

No potential conflict of interest was reported by the authors.

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# Оценка антибактериального действия ортодонтического композита, содержащего наночастицы прополиса, на крысах в качестве животной модели

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## Резюме

**Цель:** Настоящее исследование направлено на оценку антимикробного действия ортодонтического праймера, содержащего нанопрополис, на кариесогенные бактерии в модели на крысах.

**Материалы и методы:** Ортодонтический праймер Transbond XT, содержащий 0%, 1%, 5% и 10% нанопрополиса, был экспериментально приготовлен собственными силами. Крысы линии Вистар, которых мы использовали в исследовании, были случайным образом разделены на четыре группы, и их ротовая полость была колонизирована *Streptococcus mutans, Streptococcus sanguinis* и *Lactobacillus acidophilus.* После анестезии крыс на вестибулярную поверхность верхнечелюстного резца наносили одну каплю (10 μL) праймера, содержащего различные концентрации нанопрополиса, и полимеризовали. Ортодонтический композит наносился на праймер и полимеризовался светом. Одну каплю (10 μL) праймера, содержащего те же концентрации нанопрополиса, повторно наносили на поверхность композита и светоотверждали. Количественно определяли количество колоний *S. mutans, S. sanguinis* и *L. acidophilus* в слюне крыс через 24 часа, на 4 и 7 день с помощью чашечного подсчета.

**Результаты:** Праймер, содержащий 1%, 5% и 10% нанопрополиса, значительно снижал количество колоний *S. mutans* через 24 часа по сравнению с контрольной группой ($p$<0.05). На 4-й день среднее количество колоний *S. mutans* в группах, получавших 5% и 10% нанопрополиса, было значительно ниже, чем в контрольной группе ($p$<0.05). Праймер, содержащий 1%, 5% и 10% (все ($p$<0.05) нанопрополиса), значительно снижал *L. acidophilus* через 24 часа. Кроме того, на 4-й день среднее количество колоний *L. acidophilus* в 5% и 10 % групп нанопрополиса были значительно ниже, чем в контрольной группе ($p$<0.05).Через 24 часа и 4 дня среднее количество колоний *S. sanguinis* в группе 1%, 5% и 10% нанопрополиса было значимо ниже, чем в контрольной группе ($p$<0.05).В присутствии всех концентраций нанопрополиса на 7-й день достоверной разницы не наблюдалось ($p$>0.05).

**Заключение:** Ортодонтический праймер, содержащий нанопрополис, значительно уменьшил количество колоний кариесогенных бактерий в модели на крысах.

## Ключевые слова

кариесогенные бактерии, нанопрополис, композит, ортодонтический клей, праймер, крыса