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ARQUIVOS EM ODONTOLOGIA

Antimicrobial effect of resveratrol on an etiological agent of human dental caries, Streptococcus mutans

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Aim: To evaluate the antimicrobial effect of resveratrol on the bacterium *S. mutans*.

Methods: In the execution of the study, samples were cultured on petri dishes plated with Mueller-Hinton Agar, containing sterile 6 mm diameter Blank discs (Cecton). The discs were soaked with 10 µl of negative control (phosphate-buffered saline - PBS), positive control (0.12% chlorhexidine), and different concentrations of resveratrol (100 µg/mL, 200 µg/mL, 400 µg/mL, and 800 µg/mL). The tests were performed in triplicate, totaling 18 analyses.

Results: After 24 hours of incubation, the halos were evaluated and measured using a digital caliper. The data collected were processed statistically using the Anova way program. The diameter measurement for the positive control halo was 2.84 cm; for the resveratrol at 800 µg/mL, it was 0.6 cm; at 400 µg/mL, it was 0.42 cm; and at 200 and 100 µg/mL, it was zero.

Conclusion: Resveratrol did not demonstrate efficiency against the bacteria when compared to the negative control (PBS). According to literature, resveratrol produces an antibacterial activity, although its optimal concentration for inhibitory effect is not yet well established[7].

Uniterms: microbiology; resveratrol; dental caries.

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INTRODUCTION

Dental caries is a disease that despite the advancement of preventive dentistry, remains a public health problem and brings a negative impact on the quality of life of affected individuals, mainly due to loss of function, worsened esthetics, the presence of pain, and high treatment costs[1,2]. It has a multifactorial etiology based on the interaction of factors related to the host, diet, and the presence of bacteria in the oral cavity[3]. The disease is characterized by a demineralization of hard dental tissues and subsequent degradation of the organic matrix and cavitation, triggered by changes in the dental biofilm microbiota due to an increase in acid-producing and acid-tolerant bacteria associated with high sucrose consumption and a decrease in the pH of the dental plaque[2].

Several microorganisms are involved in the process of caries development, and *Streptococcus mutans* is one of the bacteria with the highest cariogenic power. *S. mutans* is a gram-positive bacterium naturally found in the oral cavity, with a great capacity for acid production and adhesion to dental enamel, forming a biofilm[3].

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The proliferation of *S. mutans* and the consequent formation of the cariogenic biofilm are closely associated with the diet since excessive intake of sugars, especially sucrose, allows this bacterium to produce lactic acid responsible for demineralizing dentin enamel[3]. Additionally, this microorganism is also one of the main producers of exopolysaccharides, which are extracellular polymeric substances that provide stability and mechanical cohesion to the biofilm, allowing the creation of highly acidic microenvironments, which are critical for dental pathogenesis but that do not affect the reproduction of *S. mutans*, given that this bacterium has a high tolerance to low pH[4].

To control the proliferation of this bacterium and prevent dental caries, various herbal remedies are routinely studied. Among them, we have resveratrol, a polyphenolic phytoalexin of plant origin found in grapes, peanuts, medicinal plants, and processed products like wine[5]. This compound has a potent antioxidant, antitumor, and anti-inflammatory effect, and has been used to prevent and treat diseases such as cancer, diabetes, cardiovascular disorders, as well as degenerative, autoimmune, and metabolic diseases[6].

Some evidence suggests that resveratrol has potential antibacterial properties, including those that combat species of bacteria associated with oral infections[7]. Therefore, studying and verifying its action against *S. mutans*, and consequently dental caries, are of utmost importance, since these actions may lead to the development of an alternative product that is accessible to a major portion of the population and that can aid in the treatment of the disease. The present study aimed to evaluate the antimicrobial properties of resveratrol against *Streptococcus mutans*.

MATERIALS AND METHODS

Resveratrol

Resveratrol (Sigma-Aldrich, EUA) was dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich, EUA) until reaching a stock concentration of 80 mg/mL, and was then further diluted in the appropriate medium for the experimental concentrations. The maximum DMSO concentration used was up to 1% (800 µg/mL in the resveratrol group).

Bacterial Strain and Growth Conditions

The bacterial strain *Streptococcus mutans* ATCC 25175 was obtained from the Microbiology Laboratory at the Institute of Biological Sciences, Federal University of Minas Gerais. The strain was anaerobically cultured on petri dishes plated with Mueller-Hinton Agar (Difco) by incubation for 48 hours at 37°C. Subsequently, bacterial growth was microscopically confirmed.

Microbiological Evaluation of Resveratrol

Petri dishes were used for bacterial cultivation, and sterile 6 mm diameter Blank discs (Cecton) were placed on the inoculated medium. Using sterile forceps (Kolplast, Brazil), sterile filter paper discs (cecton) were placed on the surface of the medium, soaked with 10 µl of the negative control (phosphate-buffered saline - PBS), the same quantity for the positive control (0.12% chlorhexidine), and different concentrations of resveratrol - Sigma-Aldrich, EUA (100 µg, 200 µg, 400 µg, and 800 µg). For better comprehension, the tests were performed in triplicate, totaling 18 analyses.

Measurements were taken at intervals of 5 hours, including times 0, 5, 10, 15, 20, and 24 hours, to generate the resveratrol inhibition curve. After 24 hours of incubation, the halos were evaluated again using a digital caliper to determine the effect of the compound.

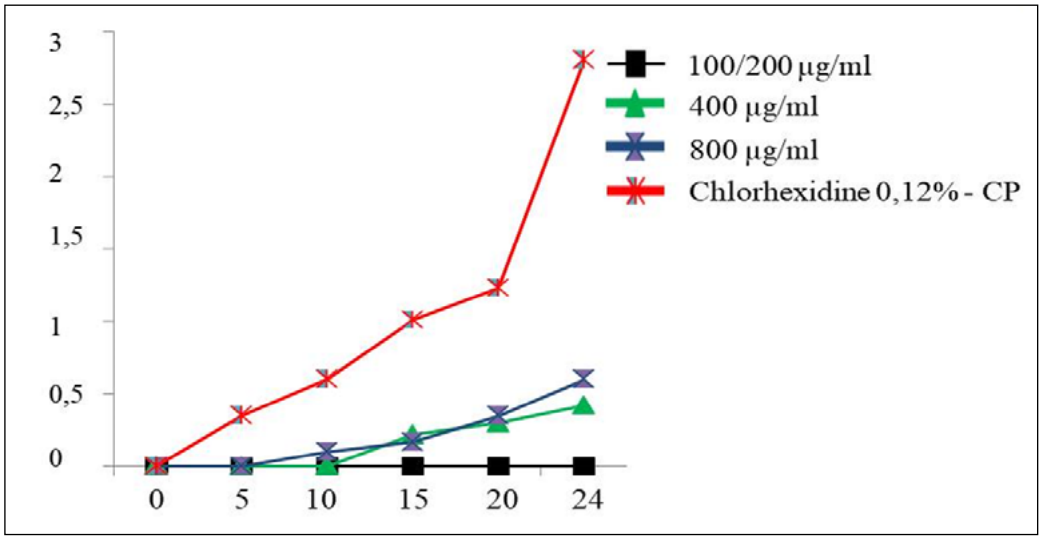
Statistical Analysis

After data collection, the data were processed using GraphPad Prism. The results were statistically analyzed using one-way analysis of variance (ANOVA) and applied in Anova using a post-hoc test. P-values < 0.05 were considered statistically significant.

RESULTS

When evaluating the effects of resveratrol at different concentrations on the inhibition rate of *S. mutans* over 24 hours, it was observed that higher concentrations of resveratrol resulted in a more rapid inhibitory effect on the bacteria, similar to the positive control (Fig. 1).

Figure 1. Inhibition curve of *S. mutans* at different concentrations of resveratrol.



Bacterial growth was significantly inhibited with treatment at higher concentrations of resveratrol, 400 µg/mL and 800 µg/mL (Table 1), as compared to the negative control. However, the effect was considered to be minimal when compared to the positive control (0.12% chlorhexidine). Moreover, no difference was observed when tested at concentrations of resveratrol below 400 µg/mL.

Table 1. Antibacterial activity of resveratrol at different concentrations against *S. mutans.*

Treatment	Mean halo diameter (cm)	Standard deviation
Positive control (chlorhexidine) 0,12%)	2,8[a]	0,11
Resveratrol 800	0,60[b]	0,11
Resveratrol 400 µg/ml	0,42[b]	0,16
Resveratrol 200 µg/ml	0,00[c]	0,00
Resveratrol 100 µg/ml	0,00[c]	0,00
Negative control (PBS)	0,00[c]	0,00

Different letters indicate statistically significant differences.

DISCUSSION

The findings of the present study revealed a concentration dependent response of resveratrol, as evidenced by its inhibitory effect at higher concentrations of 400 µg/mL and 800 µg/mL. However, when comparing the results with the negative control, a substantial inhibition was observed, but the efficacy was considered to be minimal when compared to the positive control.

Previous studies have demonstrated that susceptibility to resveratrol may vary among bacteria, and this agent may exhibit different antibacterial properties and mechanisms of action depending on the microorganism, potentially exerting a bacteriostatic or bactericidal effect[8,9,10,11].

In this regard, it is still challenging to draw definitive conclusions about the difference in susceptibility between Gram-positive and Gram-negative bacteria to resveratrol. A study showed that susceptibility to phenolic compounds depended on the bacterial species[12]. Bacterial control demonstrated that Gram staining is not necessarily correlated with antimicrobial efficacy[13].

Our findings do not clearly show the positive effect of resveratrol in controlling *S. mutans*. However, previous studies may well support this antibacterial action, considering that some studies using resveratrol to control gram-positive bacteria, as well as the strain used in our study. The results show that resveratrol inhibits the bacteria at concentrations of 100 µg/ mL[14].

Other studies have demonstrated the effect at concentrations of 100–200 µg/mL[14-17] and even at lower concentrations, from 50 to 100 µg/mL. This can be attributed to the absence of a hydrophilic outer membrane in Gram-positive bacteria[18]. Furthermore, resveratrol acts as an anti-inflammatory mediator by restricting the activity of the nuclear factor kappa β (NF-κβ), the activity of procyclooxygenase-2, and the production of prostaglandins[19].

The antibacterial action of resveratrol is directly related to its concentration, with a response against *S. mutans* observed at concentrations between 100-200 µg/mL[20]. By contrast, another study[19] demonstrated the efficacy of resveratrol in inhibiting microbial growth at concentrations above 400 µg/mL, with the best result achieved at 800 µg/mL, though without statistical difference, thus corroborating the results found in this study[21,22].

The antimicrobial action of resveratrol can be interpreted as a combination of several mechanisms, including a decrease in pH; an inhibition of F-ATPase, water production, and biofilm formation; and genetic alteration. These mechanisms, together, likely contribute to a decrease in bacterial acid production and a decline in their enzymatic activity, thus producing a cariostatic effect[8]. Resveratrol also acts on immune cells, pro-inflammatory cells, and genetic expression. Its antioxidant action and potential to inhibit enzymes contribute to its anti-inflammatory properties[23].

CONCLUSION

In light of the above, the effect of resveratrol seems to have potential in controlling S. mutans bacteria; however, its ideal concentration for this inhibitory effect is not yet well established. Therefore, more studies with different methods need to be conducted in order to obtain a better understanding of the results.

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Ludmilla Regina Souza: Validação, Análise Formal, Investigação, Recursos, Curadoria de Dados, Redação - Preparação do Rascunho Original, Redação - Revisão e Edição.
Otávio Cardoso Filho: Conceituação, Metodologia, Software, Validação, Análise Formal, Investigação, Recursos, Curadoria de Dados, Redação - Preparação do Rascunho Original, Redação - Revisão e Edição, Visualização, Supervisão, Administração do Projeto

CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

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