The antimicrobial mechanisms of *Boswellia serrata* extract in *P. gingivalis* infected gingival epithelial cells

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BACKGROUND

Periodontitis is a severe gum infection that damages the soft tissue and destroys the bone that supports teeth.[1,2] One of the major etiologic agents of periodontitis is *Porphyromonas gingivalis* (*P. gingivalis*).[1,2] If untreated, periodontitis can lead to tooth loss and has been linked to systemic disorders such as heart disease and cancer.[3] Frankincense is a resin obtained from the Boswellia tree and possesses potent anti-inflammatory properties, which have been shown to minimize the symptoms of inflammatory diseases, such as diabetes, rheumatoid arthritis, and microbial infections.[4] *Boswellia serrata* extract is reported to affect *P. gingivalis* growth, biofilm formation, showing an antimicrobial action in *P. gingivalis*-infected gingival epithelia cells (GEC). However, the microbicidal mechanism activated by *B. serrata* extract is not known.

Host cells employ several intracellular mechanisms to control bacterial infections. The phagocytosis is the main cellular response to bacteria, and it is related with endosomal pathway that involves engulfing bacteria into phagosomes, which fuse with lysosomes containing digestive enzymes, acidic pH, and antimicrobial peptides to degrade the bacteria. As part of this process, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are released into phagolysosome compartment to damage bacterial components and further degradation. Finally, infected host cells may undergo apoptosis or pyrolysis to contain and eliminate the bacteria.[5,6]

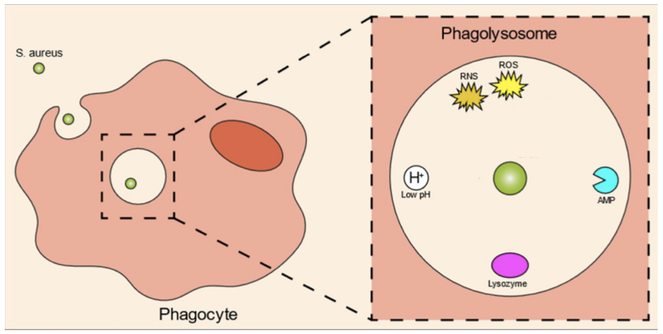

Figure 1. Phagolysosome Induction Following Phagocytosis of *S. aureus*. Once inside the phagolysosome, the bacteria get exposed to various antimicrobial molecules designed to kill the bacteria. These mechanisms include ROS and RNS, AMPs, lysozyme, and acidic pH. Abbreviations: ROS, reactive oxygen species; RNS, reactive nitrogen species; AMP, antimicrobial peptide

OBJECTIVES

The aim of this study is to elucidate the antimicrobial mechanism of *B. serrata* extract against *P. gingivalis* in the experimental periodontitis model.

METHODS

GECs were infected with *P. gingivalis* and treated with 16 µg/mL of *B. serrata* extract for 2 hours. To remove adherent and extracellular bacteria, the cells were washed with PBS and incubated with 300 µg/mL of gentamicin and 200 µg/mL of metronidazole for 1 hour. After 6 hours the nitric oxide (NO) production was measured using Griess reagent and the reactive oxygen species (ROS) production was measured with 5 µM of H2DCF-DA probe by spectrophotometer and fluorescence microscopy.

RESULTS

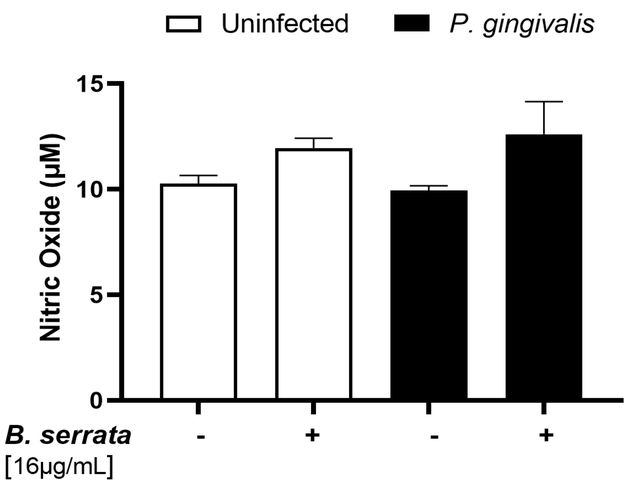

Figure 2. *B. serrata* extract promoted the production of nitric oxide induced by GECs. Gingival epithelial cells were split into four conditions. Cells were either infected with *P. gingivalis* (black bars) or remained uninfected (white bars). Cells were either treated with Frankinscence (**+**) or untreated (**-**). The presence of extract was correlated with an increase in Nitric Oxide in both infected and uninfected cells. NO production migth play a role in the antimicrobial properties of the *B. serrata* extract.

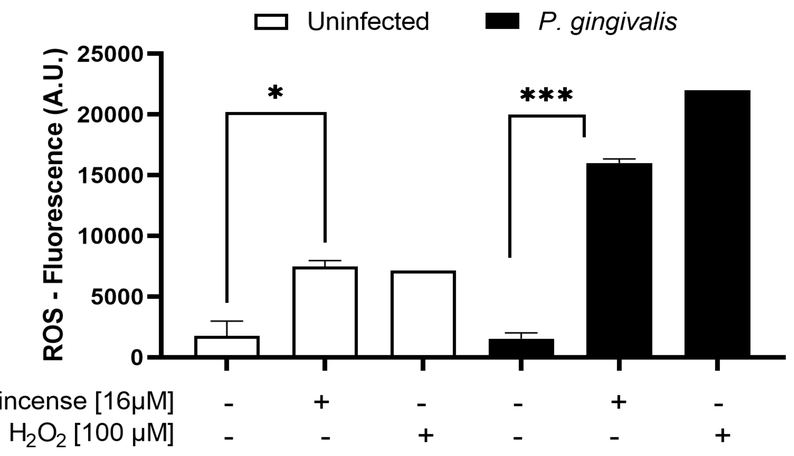

Figure 3. *B. serrata* extract induced the production of Reactive Oxygen Species (ROS) in GEC. Gingival epithelial cells were split into four conditions. Cells were either infected with *P. gingivalis* (black bars) or remained uninfected (white bars). Cells were treated or not with Frankinscence. Hydrogen peroxide was included as a positive control. The presence of Frankinscence correlated with a significant increase in ROS production. This effect was much more apparent in the infected cells. The presence of the extract promoted a large increase in ROS production, suggesting it plays a significant role in the antimicrobial effects. *= p<0.5; *** = p< 0.005

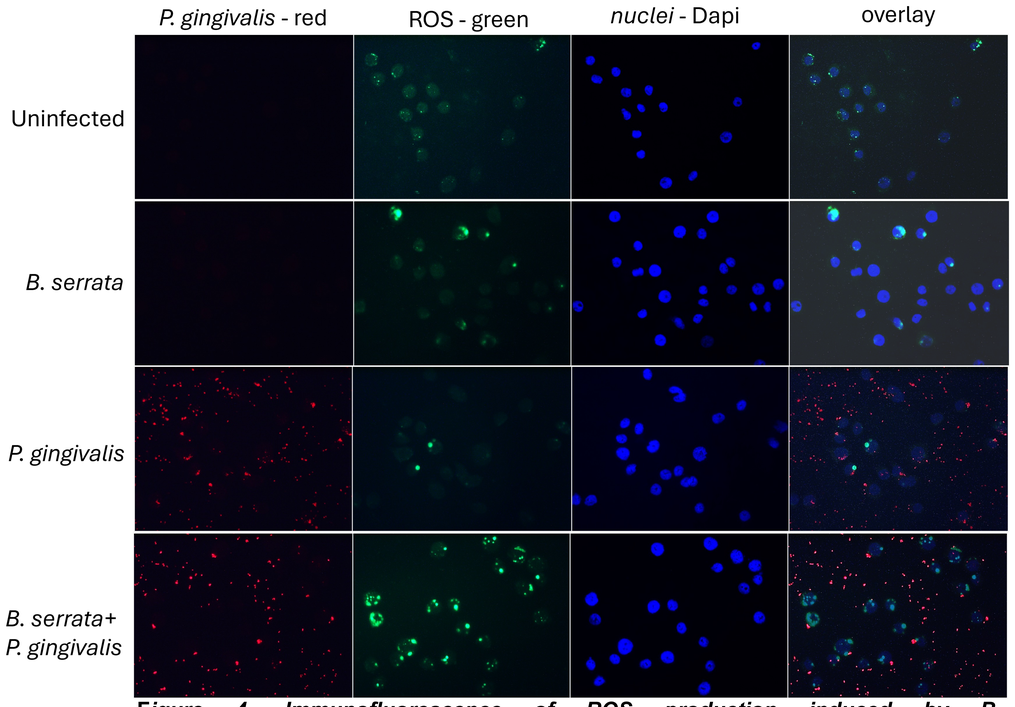

Figure 4. Immunofluorescence of ROS production induced by B. serrata extract. Gingival epithelial cells **(nuclei in blue)** were split into an infected group with *P. gingivalis* **(red)** or uninfected group. ROS expression **(green)** was then observed in each condition. The basal production of ROS was observed in the uninfected condition (column 2, row 1). the increase in basal ROS induced by *B. serrata* treatment *(*Column 2, row 2). The presence of *P. gingivalis* alone (Column 4, row 3) illustrated limited ROS production by cells. The presence of both *P. gingivalis* and *B. serrata* extract (Frankinscence) displayed more prevalent ROS production (column 4, row 4).

CONCLUSION

Our results suggest that the antimicrobial properties of *B. serrata* may involve the antimicrobial effects of ROS and NO production.

ACKNOWLEDGEMENTS

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Periodontitis is a severe gum infection that damages the soft tissue and destroys the bone that supports teeth. One of the major etiologic agents of periodontitis is Porphyromonas gingivalis (P. g). If untreated, periodontitis can lead to tooth loss and has been linked to systemic disorders such as heart disease and cancer. Frankincense is a resin obtained from the Boswellia tree and possesses potent anti-inflammatory properties, which have been shown to minimize the symptoms of inflammatory disease, such diabetes, rheumatoid arthritis, and microbial infections. Boswellia serrata extract was proposed to control the P. g growth in GEC, however the mechanism activated by B. serrata extract is not clear. The objective of this study is to elucidate the antimicrobial mechanism of B. serrata against P. gingivalis in the experimental periodontitis model. To address that, the GECs were infected with P. g and treated with 16 µg/mL of B. serrata extract for 2 hours. The cells were washed and incubated with 300 µg/mL of gentamicin and 200 µg/mL of metronidazole for 1 hour. After 6 hours the nitric oxide (NO) production was measured using Griess reagent and the reactive oxygen species (ROS) production was measured with 5µM of H2DCF-DA probe by spectrophotometer and fluorescence microscopy. Our results suggest that B. serrata induced a small amount of nitric oxide and promoted a reactive oxygen burst in the GECs during P. g infection. Our results suggest that the antimicrobial properties of B. serrata involve mainly the promotion of ROS and NO production through the endocytic pathway.
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