Received: 12 April 2021 | Accepted: 14 September 2021

DOI: 10.1111/iej.13633

ORIGINAL SCIENTIFIC ARTICLE

INTERNATIONAL ENDODONTIC JOURNAL | WILEY

Effect of red wine or its polyphenols on induced apical periodontitis in rats

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Funding information
This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo—FAPESP: 2017/27219-3. The authors thank the Multi-User Laboratory of FOA-UNESP and FINEP (FINEP/CT-INFRA—Agreement FINEP: 01.12.0530.00-PROINFRA 01/2011) for providing the high-resolution-computed microtomography system (SkyScan Model 1272) for the analysis of this study.

Abstract

Aim: To evaluate the effect of red wine consumption or its polyphenols on the inflammation/resorption processes associated with apical periodontitis in rats.

Methodology: Thirty-two three-month-old Wistar rats had apical periodontitis induced in four first molars and were then arranged into four groups: control (C)—rats with apical periodontitis; wine (W)—rats with apical periodontitis receiving 4.28 ml/kg of red wine; resveratrol+quercetin (R+Q)—rats with apical periodontitis receiving 4.28 ml/kg of a solution containing 1.00 mg/L of quercetin and 0.86 mg/L of resveratrol and alcohol (ALC)—rats with apical periodontitis receiving the alcoholic dose contained in the wine. The oral gavage treatments were administered daily, from day 0 to day 45. On the 15th day, apical periodontitis was induced, and on the 45th day, the animals were euthanized. Histological, immunohistochemical (RANKL, OPG, TRAP, IL-10, TNF-α and IL-1β) and micro-computed tomography for bone resorption analysis were performed in the jaws. The Kruskal–Wallis with Dunn's test was performed for nonparametric data, and the ANOVA with Tukey's test for parametric data, $p < .05$.

Results: The median score of the inflammatory process was significantly lower in the R+Q group (1) compared to the C (2) ($p = .0305$) and ALC (3) ($p = .0003$) groups, and not different from the W (1.5) group. The immunolabeling for OPG was significantly higher in the R+Q group ($p = .0054$) compared to all groups; the same was observed for IL-10 ($p = .0185$), different from groups C and ALC. The R+Q group had the lowest TRAP cell count ($p < .0001$), followed by the W group, both inferior to C and ALC groups. The lowest bone resorption value was in the R+Q group ($0.50mm^3 \pm 0.21mm^3$), significantly lower ($p = .0292$) than the C group ($0.88mm^3 \pm 0.10mm^3$). The W group ($0.60\ mm^3 \pm 0.25\ mm^3$) and R+Q group had less bone resorption compared to the ALC group ($0.97\ mm^3 \pm 0.22\ mm^3$), $p = .0297$ and $p = .0042$, respectively.

Conclusion: Red wine administration to rats for 15 days before induction of apical periodontitis decreased inflammation, TRAP marking and periapical bone resorption compared to alcohol. Resveratrol-quercetin administration reduced the

inflammatory process in apical periodontitis, periapical bone resorption, and altered the OPG, IL-10 and TRAP expression compared to C and ALC groups.

KEYWORDS
periapical periodontitis, polyphenols, quercetin, resveratrol, wine

INTRODUCTION

Red wine is a popular worldwide drink and is reported to be beneficial for the body when ingested in appropriate amounts (Artero et al., 2015). The wine is composed mainly of water, ethanol, glycerol, polysaccharides, various acids and phenolic compounds (Snopek et al., 2018). The beneficial properties of red wine such as the cardioprotective potential, the inhibition of the low-density lipoprotein (LDL) oxidation and prevention of endothelial dysfunction occur at the expense of the ingestion of wine polyphenols, especially resveratrol, anthocyanins and catechins, which are the most effective wine antioxidants (Haseeb et al., 2017). The phenolic compounds of wine can be divided into flavonoids and non-flavonoids, and the precise content of each one is dependent on elements, such as the grape variety, and manufacturing technique, but it is known that red wine contains 10-fold more phenolic compounds than white wine (Markoski et al., 2016). In addition to the polyphenols, the low alcohol content is reported to be responsible for the beneficial effect that red wine can exert (Golan et al., 2019). In general, the beneficial effect of regular and moderate wine consumption is obtained with approximately 150 ml/day for women and 300 ml/day for men, as defined by previous studies and by the Dietary Guidelines for Americans, 2020–2025 (Pavlidou et al., 2018; Rotondo et al., 2001; U.S. Department of Agriculture & U.S. Department of Health & Human Services, 2020).

Resveratrol (3,5,4′-trihydroxystilbene) is a nonflavonoid polyphenol present in red wine and in foods that are ingested commonly in the human diet, such as strawberries, blueberries, mulberries, grapes, grape juice, peanuts and dark chocolate (Galiniak et al., 2019). The compound started to gain importance in 1992 when it was postulated to explain some of the cardioprotective effects of red wine consumption, referred to as the "French Paradox", which described the inverse relationship between mortality from coronary heart disease and red wine consumption predominantly seen in France (Renaud & de Lorgeril, 1992). Since then, the substance has been extensively investigated in the health field, with benefits reported to be cancer prevention, neuroprotection, cardiovascular disease, ischaemic injuries, anti-ageing, enhance stress resistance and extend the lifespans of various organisms (Galiniak et al., 2019; Li et al., 2019; Rauf et al., 2018). Specifically in bone tissue, the non-flavonoid resveratrol acts by inducing SIRT1 and regulating RUNX2, to induce osteoblasts differentiation. It also suppresses NF-κB activation, leading to reduced differentiation and osteoclastic activity (Pandey et al., 2018; Shakibaei et al., 2011).

In addition to resveratrol, the flavonoid quercetin (3,3′,4′,5,7-pentahydroxyflavone) is also found in red wine, as well as in vegetables, fruits and teas, being one of the most prominent dietary antioxidants in health research (Li et al., 2016). Its properties have been gaining notoriety due to antioxidant, anti-inflammatory, anti-tumour, metabolic regulation and neuroprotective activities (Khan et al., 2019; Li et al., 2016; Reyes-Farias & Carrasco-Pozo, 2019; Wong et al., 2020). In bone tissue, quercetin has been reported to decrease osteoclastogenesis via inhibition of the activating receptor for nuclear factor-kB ligand (RANKL), involved in osteoclastic differentiation (Wong et al., 2020), in addition to its ability to directly induce apoptosis of mature osteoclasts (Wong et al., 2020). Quercetin is also believed to activate osteoblasts, either by activating the TGF beta-signalling pathway, p38 mitogen-activated protein kinases and WNT/β-Catenin pathways, thus regulating bone metabolism (Casado-Diaz et al., 2016; Pandey et al., 2018; Yamaguchi & Weitzmann, 2011). These studies demonstrate that quercetin has great potential to be used as a bone health supplement.

Studies specifically evaluating the influences of red wine on the inflammatory response and bone tissue are important, but scarce. These investigations are included in epidemiological studies on alcohol consumption, and only a few specify the type of alcoholic beverage. Red wine is reported to be more beneficially than other beverages, due to the rich phenolic components allied to a low alcohol content, which would facilitate the absorption of these phenolic compounds (Kutlesa & Budimir Mrsic, 2016). To date, only one study has evaluated the effect of red wine and its major components on periodontitis and revealed that animals exposed to red wine had a lower occurrence of spontaneous marginal periodontitis, and lower levels of TNF-α and C-reactive protein (Wagner et al., 2019).

Apical periodontitis results from the persistent bacterial contamination of the root canal system, characterized by an inflammation of periradicular tissues (Kakehashi et al., 1965). When the dental pulp becomes infected, bacteria and their byproducts evoke nonspecific inflammatory responses, as well as specific immunological

reactions, leading to the destruction of bone by osteoclasts and resorption cementum and dentine by multinucleated cells designated as odontoclasts (Galler et al., 2021). Red wine and the isolated polyphenols (resveratrol and quercetin) have been reported to alter the functioning of bone tissue and the immune system, both crucial elements for the development of apical periodontitis (Das & Das, 2007; Esteban-Fernández et al., 2017; Li et al., 2016; Pervaiz & Holme, 2009; Wong et al., 2020; Wong & Rabie, 2008a, 2008b).

This study aimed to evaluate the effect of red wine or polyphenols solution consumption on apical periodontitis induced in rats. Thus, the null hypothesis tested in this study was that exposure to wine or resveratrol associated with quercetin does not alter the lesion volume and severity of apical periodontitis.

MATERIAL AND METHODS

Animals

Thirty-two three-month-old adult male Wistar rats (*Rattus norvegicus albinus*), weighing between 250 and 300 g, were used. The animals were maintained in a temperature-controlled environment (22℃ ± 1℃, 70% humidity) with a 12-h light-dark cycle and ad libitum access to water and food. All experimental protocols were approved by the Institutional Ethics Committee on Animal Use (00154-2019) of Universidade Estadual Paulista, São Paulo, Brazil. The general health condition was evaluated weekly. The animals were arranged randomly into four groups ($n = 8$): control (C)—rats with apical periodontitis; wine (W)—rats with apical periodontitis receiving wine; resveratrol+quercetin (R+Q)—rats with apical periodontitis receiving a solution with resveratrol and quercetin; and alcohol (ALC)—rats with apical periodontitis receiving an alcoholic solution. All the treatments were conducted through oral gavage for 45 days, starting 15 days before induction of apical periodontitis and extending for 30 more days after induction of the disease (Figure 1).

Sample size calculation

The sample size was estimated based on the parameters used in previous studies (Cintra et al., 2014). Using an alpha error of 0.05% and 95% power to recognize a significant difference, a minimum of seven animals per group was considered necessary. Taking into consideration the

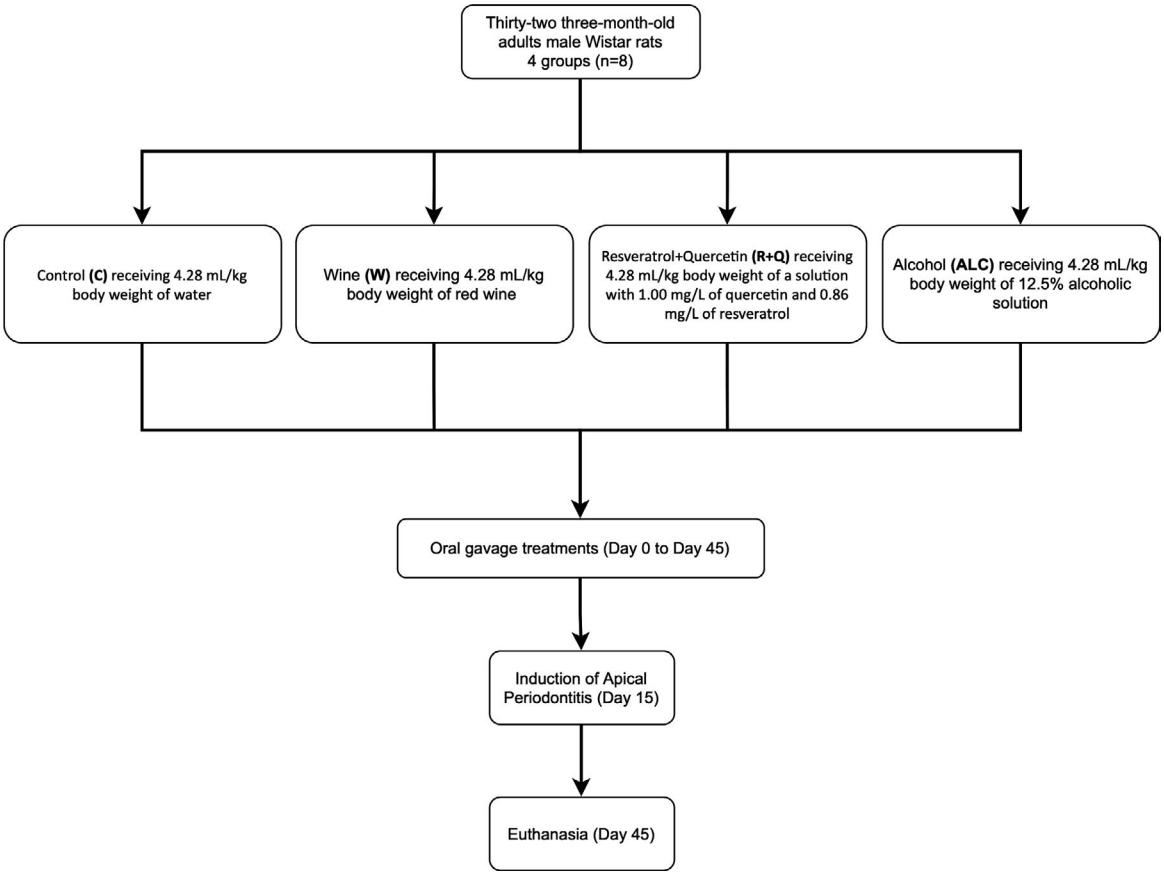

FIGURE 1 Flowchart showing the experimental stages and their order fulfilment.

possible complications that could occur during the study, one more animal was added to each group, resulting in eight rats per group, giving a total of 32 animals.

Quantification of the phenolic compounds present in the red wine

The total phenolic compounds present in the red wine Miolo Seleção® (Miolo Wine Group Vitivinicultura S.A) were determined using a high-performance liquid chromatographic/diode array detector (HPLC/DAD) coupled with electrospray ionization/mass spectrometry (ESI-MS). For quantification, the wine sample was diluted thrice with a solution of water acidified with 0.2% formic acid and filtered using a nylon filter with 0.45-μm porosity. The quantification of phenolic compounds was performed using a high-performance liquid chromatography (Shimadzu LC-20T; Shimadzu Corporation), equipped with a diode array detector, degassing system, autosampler and oven column. Chromatographic separation was performed on a reverse-phase column (C18, 150 mm × 4.6 mm, particle size 3.5 μm (Agilent Technology). The mobile phase consisted of water acidified with 0.2% formic acid (v/v) (mobile phase A) and acetonitrile (mobile phase B). For chromatographic separation, the gradient elution used was composed of 0 min 100% A, 10 min 88% A, 15 min 45% A, 17 min 65% A, 23 min 100% A and 30 min 100% A. The temperature of the column was maintained at 40℃, the sample injection volume was 10 μl, with a flow rate of 0.5 ml/min. The wavelengths used were 306 nm for t-resveratrol and 360 nm for quercetin. Quantification was performed by external calibration, with seven concentrations equidistant from the analytical standards (Lago-Vanzela et al., 2011a, 2011b; Nixdorf & Hermosin-Gutierrez, 2010). The concentrations of resveratrol (0.86 ± 0.02 mg/L) and quercetin (1.00 ± 0.02 mg/L) found in the red wine were used to prepare the solution administered to the R+Q group.

Oral gavage treatments

The animals from the C group received water at 4.28 ml/kg body weight through gavage to mimic the same procedure endured by the other animals. The animals from the W group received 4.28-ml/kg body weight of red wine (Miolo Seleção®, Wine Group Vitivinicultura S.A) (Schmatz et al., 2013). For the animals from the R+Q group, a solution with 1.00 mg/L of quercetin and 0.86 mg/L of resveratrol was prepared and administered in the same volume to red wine (4.28 ml/kg body weight). The animals from the ALC group received 4.28-ml/kg body weight of an alcoholic solution containing 12.5% alcohol by volume (the same amount of alcohol present in the wine administered to group W), prepared by diluting absolute ethyl alcohol in water (Dal-Fabbro, Marques-de-Almeida, Cosme-Silva, Ervolino, et al., 2019).

Induction of apical periodontitis

Fifteen days after the oral gavage has been started, the pulp tissue of the rats was exposed (Cosme-Silva et al., 2019). Briefly, rats were anaesthetized with 87 mg/kg ketamine (Francotar, Virbac do Brazil Ind e Com Ltda, Roseira) and 13 mg/kg xylazine (Rompum, Bayer AS) by intramuscular injection and placed on a jaw-retraction board. All four first molars of each rat had the dental pulps surgically exposed using a no. 1/4 dental round bur (Jet Carbide, Kavo Kerr Group) using an electric handpiece. The size of the exposure was approximately equivalent to the diameter of the bur. The access cavity was left open to the oral cavity after the removal of the pulp tissue using an endodontic file, allowing contamination of root canals with the oral commensal microorganisms. Rats were killed on day 30 after pulp exposure.

Sample collection

The animals were killed with an overdose of anaesthetic solution (Thiopentax, Cristalia Produtos Quimicos Farmaceuticos Ltda.). After, the right and left sides of the jaws were removed, they were stored in a 4% buffered formaldehyde solution, and subsequently sent for microtomographic and histological/immunohistological analysis, respectively.

Histological/ immunohistochemical analysis

Fixed left-side semi-jaws were decalcified in 10% EDTA solution, embedded in paraffin, and sectioned at 6-μm thickness following a general histology protocol. Haematoxylin and eosin (H&E) staining were used for the analysis of the inflammatory profile and to check the condition of the periapical region of the first mandibular molar. Histopathological analysis was conducted following the guidelines of quality of inflammation and the cellularity pattern of dental and periodontal tissues to score the inflammatory infiltrate as follows: low (0 to few inflammatory cells, score =0), mild (<25 cells, score =1), moderate (25–125 cells, score =2) and severe (>125 cells, score =3) (Cintra et al., 2016). The immunohistochemical

analysis was performed using the immunoperoxidase technique as previously described (Dal-Fabbro et al., 2021). Histological sections were arranged into six batches, incubated with one of the following primary antibodies 1:100 diluted: Receptor Activator of Nuclear factor Kappa-B Ligand (RANKL) (Goat anti-RANKL—SC7627; Santa Cruz Biotechnology), Osteoprotegerin (OPG) (Rabbit anti-OPG—SC11383; Santa Cruz Biotechnology), Tumor Necrosis Factor-Alpha (TNF-α) (Goat anti-TNF-α SC1348, Santa Cruz Biotechnology), Interleukin 1-beta (IL-1β) (Rabbit—orb101745 Biorbyt), Interleukin 10 (IL-10) (Rabbit—orb221323, Biorbyt) and Tartrate-Resistant Acid Phosphatase (TRAP) (Goat anti-TRAP—SC30832; Santa Cruz Biotechnology). In sequence, biotinylated secondary universal antibodies were applied to the slices for 2 h followed by streptavidin–horseradish peroxidase conjugate for 2 h (Universal Dako-Labeled Streptavidin-Biotin Kit; Dako Laboratories). Finally, the slices received chromogen 3,3′-diaminobenzidine tetrahydrochloride (DAB chromogen Kit—Dako Laboratories). The negative controls contained sections following the same protocol described above, excluding the application of the primary antibody. Semiquantitative immunolabeling analyses of RANKL, OPG, TNF-α, IL-1β and IL-10 through the designation of the ensuing score system: 0 = absence of immunoreactive (IR) cells; 1 = low immunolabeling of both IR cells and extracellular matrix; approximately one-quarter of the field; 2 = moderate immunolabeling of both IR cells and extracellular matrix; approximately one half of the field and 3 = high immunolabeling of both IR cells and extracellular matrix, approximately three-quarters of the field (Azuma et al., 2017). The TRAP analysis was performed as follows: First, surrounding the entire perimeter of bone resorption resulting from apical periodontitis, followed by counting the positive multinucleated TRAP cells. Finally, the ratio between TRAP cells and perimeter size was obtained (Gomes-Filho et al., 2015). All the analyses were performed by a single-certified histologist using a light microscope (DM 4000 B; Leica) and a colour camera (DFC 500; Leica) blinded regarding the groups.

Micro-computed tomography

Fixed right side semi-jaws were scanned as previously described using a cone beam-type tomograph (SkyScan 1272; Bruker) using the following parameters: 50 kV, 800 μA, 1° rotation step, 7000 ms exposure, 3 frame averages, 11 a pixel image and 13 a pixel camera size (Cosme-Silva et al., 2020). In brief, the images were reconstructed using the software NRecon (SkyScan) and the alveolar bone volume loss was calculated using the CTAn software (SkyScan) and expressed in cubic millimetres. The region of interest is comprised of the empty space of the periapical resorption area, containing the distal root canal and apical foramen, starting from the first transversal cut at the beginning of the resorption of the periapical bone, continuing through the apex of the root, ending at the last slice, where the bone resorption was seen.

Statistical analysis

The data were analyzed using Prism 8 software (GraphPad). After the test of normality, the Kruskal–Wallis with Dunn's multiple comparisons test was performed for nonparametric data, and the one-way analysis of variance with Tukey's multiple comparisons test was performed for the parametric data. The level of significance was 5%.

RESULTS

Histological analysis

The inflammatory scores and the representative haematoxylin–eosin-stained sections are shown in Figures 2 and 3. All the animals submitted to pulp exposure had pulp necrosis with an inflammatory infiltrate in the periapical region consisting mainly of neutrophils and mononuclear cells, concomitantly with bone destruction, confirming the effectiveness of the 30 days pulp exposure method. The C group had moderate levels of inflammation, attributed a median of score 2, significantly greater than that presented by the R+Q group, which had a mild inflammatory reaction in the periapical region, with a median score of 1 ($p = .0305$). There was no significant difference when the W group (median of scores 1.5) was compared with the C group and neither with the R+Q group. Moreover, the ALC group had the highest median score for inflammation (3), significantly higher than the inflammatory process found in groups W ($p = .0037$) and R+Q ($p = .0003$).

Immunohistochemical analysis

The immunoreactivity pattern for IL-10, RANKL, OPG, TNF-α, IL-1β and TRAP are described below and in Figures 2 and 4. The R+Q group had moderate immunoreaction for IL-10 expression (median of score 2), which was significantly greater ($p = .0185$) than groups C and ALC (median of score 1). Although the W group had the same median as the R+Q group, the statistical analysis revealed no differences when compared to the other groups.

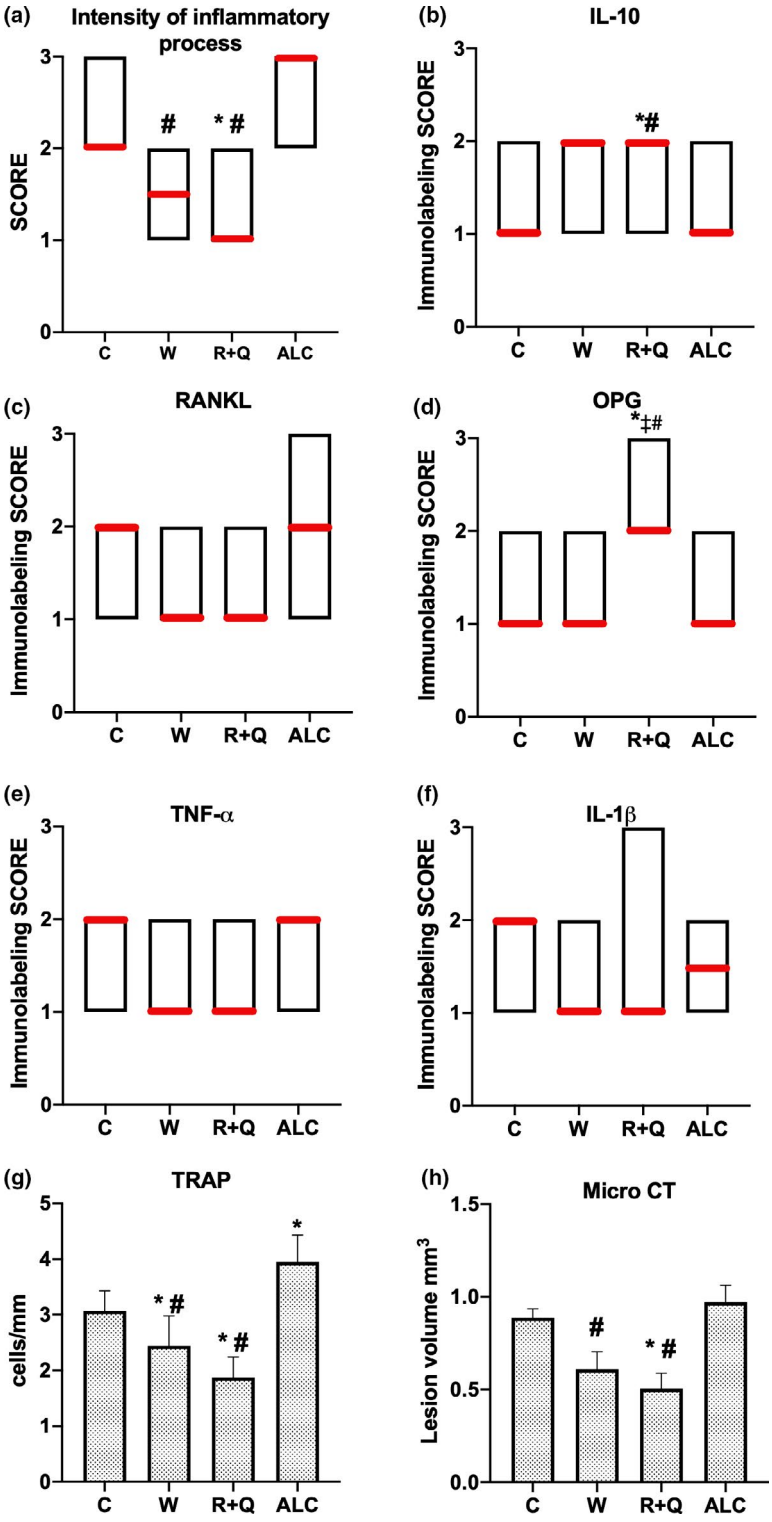

FIGURE 2 (a) The chart shows the scores for the intensity of the inflammatory process, the red line indicates the median of each group. (b) The chart shows the scores for the IL-10 immunolabeling, the red line indicates the median of each group. (c) The chart shows the scores for the RANKL immunolabeling, the red line indicates the median of each group. No significant differences were present between the groups. (d) The chart shows the scores for the OPG immunolabeling, the red line indicates the median of each group. (e) The chart shows the scores for the TNF-α immunolabeling, the red line indicates the median of each group. No significant differences were present between the groups. (f) The chart shows the scores for the IL-1β immunolabeling, the red line indicates the median of each group. No significant differences were present between the groups. (g) The bar graph for positive TRAP cells per millimetre perimeter of apical periodontitis shows the mean and the standard deviation for each group. (h) The bar graph for lesion volume shows the mean and the standard deviation for each group. Symbols: *$p < .05$ versus C; ‡$p < .05$ versus W; #$p < .05$ versus ALC.

With regard to OPG, all groups had mild immunoreaction (score 1), except for the R+Q group, which had a significantly greater ($p = .0054$) expression of this osteoclastogenesis inhibitory factor (score 2), when compared with the other three groups. Although the groups W and R+Q had fewer immunoreactive cells for RANKL, TNF-α and IL-1β, these differences were not significant ($p > .05$). The TRAP immunolabelling technique applied was specific to identify osteoclasts. The W and R+Q groups had a significantly lower ($p < .0001$) load of TRAP-positive multinucleated cells per mm of the perimeter in the periapical region, 2.44 ± 0.53 and 1.88 ± 0.36, respectively, when compared to groups C (3.07 ± 0.35) and ALC (3.95 ± 0.048). The ALC was significantly ($p < .05$) more TRAP inductive than the C group. No difference was found when comparing W and R+Q groups.

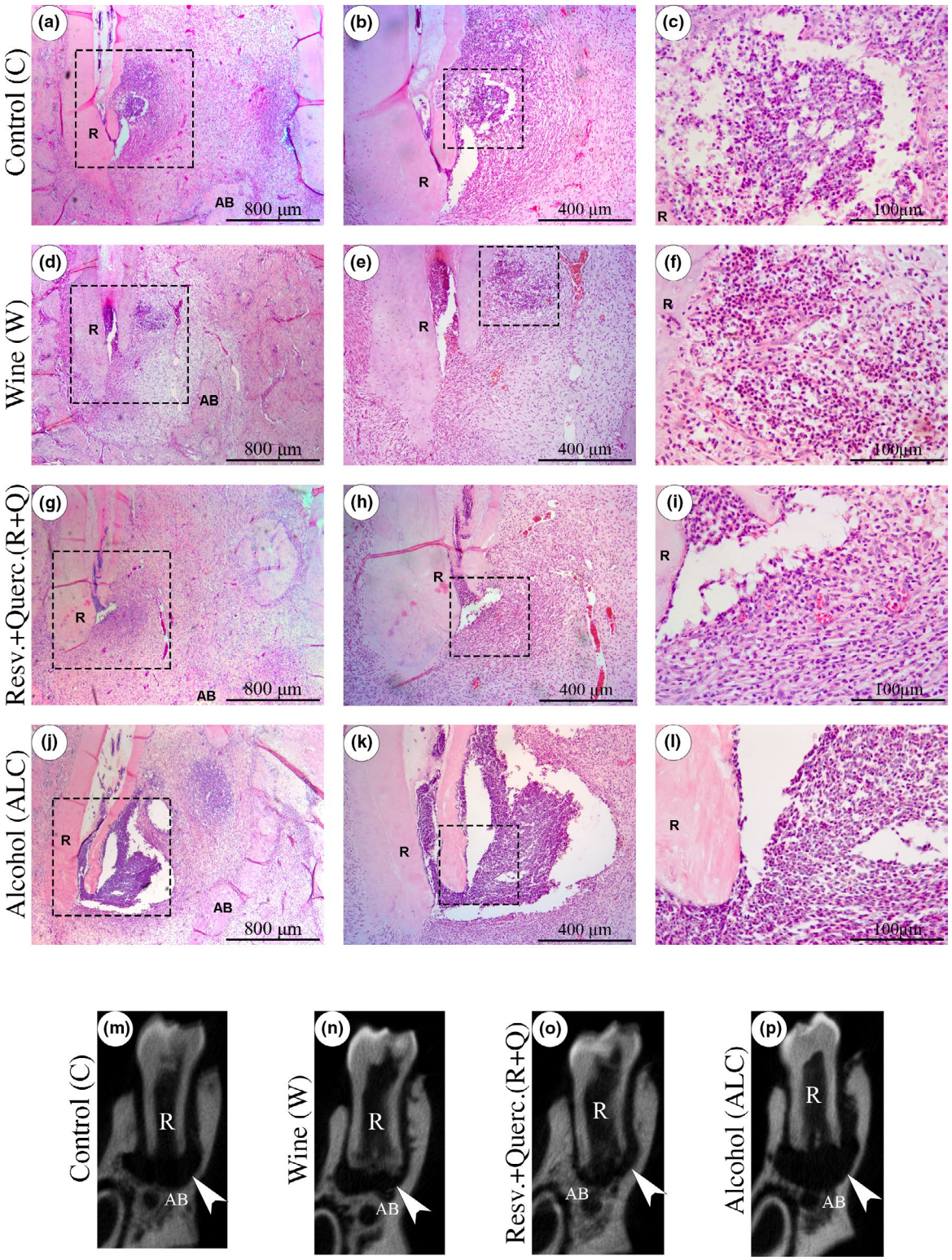

FIGURE 3 The photomicrographs show the histological aspects of the apical regions around the foraminal opening of the distal root (R) of the mandibular first molar (a–l). The moderate inflammatory process can be observed in the C group (a, b and c at 50×, 100× and 400× magnification, respectively). The mild to moderate inflammatory process can be observed in the W group (d, e and f at 50×, 100× and 400× magnification, respectively). Mild inflammatory process can be observed in the R+Q group (g, h and i at 50×, 100× and 400× magnification, respectively). Severe inflammatory infiltrate is observed in the ALC group (j, K and l at 50×, 100× and 400× magnification, respectively). Haematoxylin and eosin staining. Rectangles indicate the enlarged area in the next magnification. (a, d, g, j) Scale bars: 800 µm, (b, e, h, k) Scale bars: 400 µm, and (c, f, i, l) Scale bars: 100 µm. Micro-computed tomography images (µCT) of apical periodontitis in distal root (R) sections of the mandibular first molars (m, n, o, p). Diminished bone resorption on microtomography is noted in the R+Q group. White arrowheads point to the area of the apical periodontitis surrounded by radiopaque alveolar bone (AB).

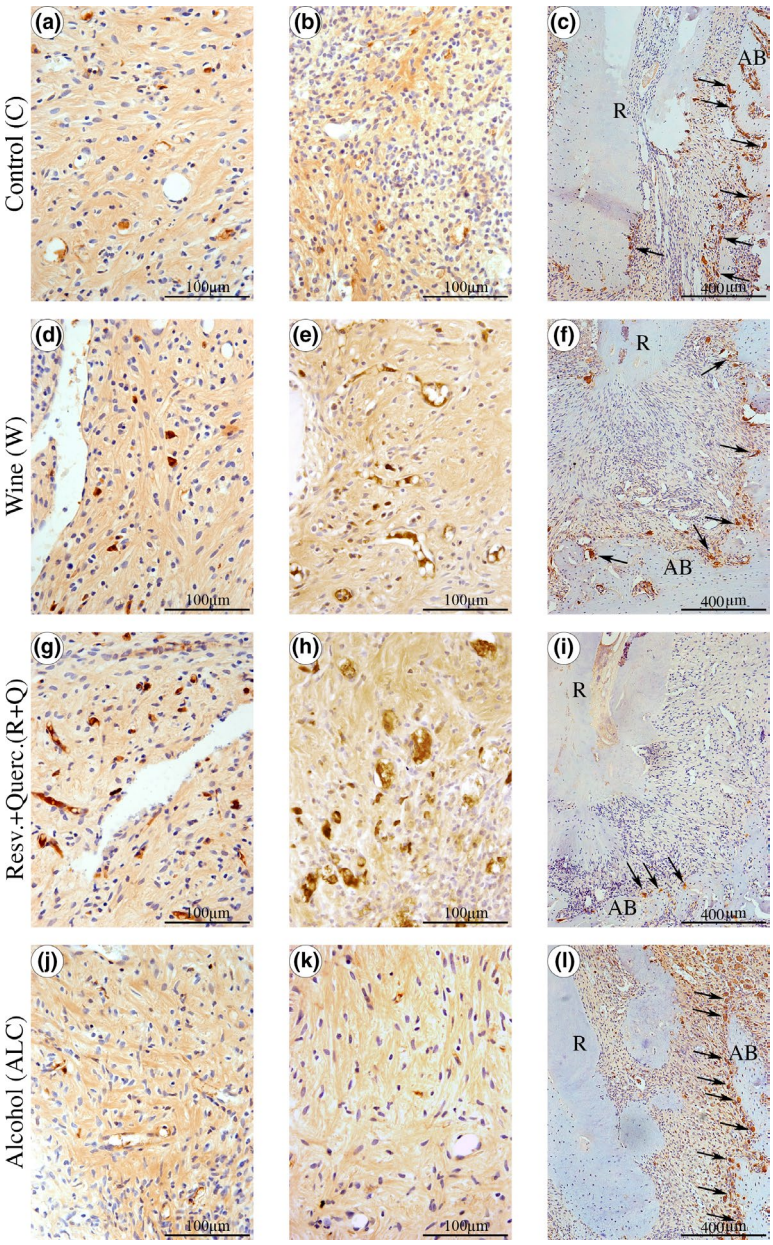

FIGURE 4 The photomicrographs show the immunostaining for OPG (a, d, g, j), IL-10 (b, e, h, k) and TRAP (c, f, i, l) in apical periodontitis around the foraminal opening of the distal root of the mandibular first molar in the C group (a, b, c), W group (d, e, f), R+Q group (g, h, i) and ALC group (j, k, l). Counterstaining: Harris haematoxylin. Original magnification for OPG and IL-10: 400× with scale bars: 100 µm, and 100× for TRAP with scale bars: 400 µm. Regarding OPG and IL-10, a large amount of immunoexpression of these proteins is noted in the R+Q group. For TRAP, black arrowheads point to osteoclastic cells reabsorbing the alveolar bone (AB) located below the opening of the apical foramen. A reduced TRAP immunolabeled cells are noted in the R+Q group.

Micro-computed tomography analysis

The micro-CT slices and data are shown in Figures 2 and 3. All animals with pulp exposures had increased periapical hypodense areas on microtomography by day 30, proving the development of apical periodontitis. The R+Q group had a bone resorption mean volume of 0.50 mm^3 ± 0.21 mm^3, significantly lower ($p = .0292$) than the bone resorption volume in groups C (0.88 mm^3 ± 0.10 mm^3) and ALC (0.97 mm^3 ± 0.22 mm^3) ($p = .0042$). The W group had a volume of bone resorption (0.60 mm^3 ± 0.25 mm^3), which was significantly lower ($p = .0297$) than that resorbed in the ALC group, however, without significant differences when compared to groups C and R+Q.

DISCUSSION

Red wine or its polyphenols compounds are consumed throughout the world, regardless of the source. The present study is the first to evaluate the effect of red wine consumption or the association between resveratrol and quercetin through oral gavage on the development of apical periodontitis in rats. The results revealed a beneficial effect of the consumption of the associated polyphenols on the magnitude of the inflammatory process and bone resorption associated with apical periodontitis. Red wine consumption also led to less osteoclastic marking than the control, rejecting the null hypothesis. Although light to moderate wine intake seems to have several beneficial effects, definitive recommendations on wine intake cannot

be made at this time (Minzer et al., 2020). Furthermore, excessive alcohol consumption is well known as a major public health concern (Ventura-Cots et al., 2019).

The administration of wine and its constituents via oral gavage in rats is widely used, as it allows the precise control of the amount to be ingested by the animal, which is not possible in the freely available water source (Correa et al., 2018; Venturini et al., 2010). Also, in human studies, the polyphenols investigated may come from countless types of food. It is difficult to determine wine consumption throughout an experiment on humans, as they are based on the collaboration of the patient regarding the protocol imposed for prospective studies and the accuracy of the answers provided in cases of retrospective studies. The 4.28 ml/kg of body weight dosage of red wine given to each animal in the present study was used based on the general recommendation of consumption of 300 ml/day of red wine for humans weighing 70 kg (Pavlidou et al., 2018; Rotondo et al., 2001). In order to have a homogeneous comparison, the wine used in the present investigation was first subjected to the quantification of the polyphenols of interest, through an established methodology (HPLC/DAD/ESI-MS) (Lago-Vanzela et al., 2011a, 2011b; Nixdorf & Hermosin-Gutierrez, 2010). This quantification allowed the group receiving the two associated polyphenols to receive the same amount that would be present in the wine administered to animals in group W.

The model used to induce apical periodontitis through the exposure of the pulp molars was chosen due to the similarity of periapical response to pulp exposure as seen in humans. That is, the bacterial infection from the oral environment infects the pulp tissue leading to pulp necrosis, resulting in apical periodontitis (Yamasaki et al., 1994). The 30th induction period is considered an adequate time to assess the extent of the inflammatory process and bone resorption resulting from the condition, as the plateau stage of development has been reached (Yamasaki et al., 1994).

The histological analysis revealed an inflammatory process associated with apical periodontitis of lesser extent and intensity in the R+Q group when compared to the other groups. This finding agrees with those already published in the literature demonstrating the anti-inflammatory activity of the two polyphenols. Resveratrol, for instance, protects from inflammation by acting at different phases of inflammation (Das & Das, 2007). This anti-inflammatory activity may occur through the inhibition of both cyclooxygenase 1 (COX-1) and cyclooxygenase 2 (COX-2) mediated pro-inflammatory signalling, suppression of pro-inflammatory mediator production, acting in nuclear transcription factor-kB (NFkB) in macrophage inhibition, suppressing interleukin-6 release and interleukin-8, which plays an important role in inflammation, as it recruits leucocytes to the lesion area (Das & Das, 2007). Quercetin downregulates nitric oxide synthase expression, inhibit matrix metalloproteinases, inhibits the production of inflammation-producing enzymes (COX) and lipoxygenase (LOX), blocks TNF-α and prevents it from directly activating extracellular signal-related kinase (ERK) and (NF-kB), which are potent inducers of inflammatory gene expression and protein secretion (Li et al., 2016).

The alcoholic solution in the 12.5% concentration administered alone was associated with greater inflammation; thus, excluding the hypothesis that low alcoholic levels could be one of the factors responsible for the beneficial effects of red wine consumption. This result agrees with those already published in the literature, which revealed a deleterious effect of 15% and 20% alcohol concentration on apical periodontitis (Dal-Fabbro, Marques-de-Almeida, Cosme-Silva, Capalbo, et al., 2019; Dal-Fabbro, Marques-de-Almeida, Cosme-Silva, Ervolino, et al., 2019). Although the red wine given in the present study has the same alcohol concentration, the inflammatory infiltrate was significantly lower when compared to these two groups. A possible explanation is that the non-alcoholic compounds present in the red wine, such as polyphenols, may have reversed the harmful effect evoked by the alcohol concentration present in the red wine (Esteban-Fernández et al., 2017).

Even though in the present study no significant reduction in IL-1β and TNF-α levels were associated with red wine consumption or polyphenols therapy, a propensity to a higher inhibition of these cytokines was observed in these two groups. However, numerous studies have confirmed that resveratrol and quercetin can suppresses the release of TNF-α (Chen et al., 2020; Lee & Moon, 2005; Lee et al., 2019; Takada et al., 2004; Wang et al., 2020; Yuan et al., 2018). TNF-α is a proinflammatory cytokine, released by macrophages and has a vital role in periodontitis mediated bone loss by inducing the expression of mediators that amplify or sustain the inflammatory response such as the production of prostaglandins and matrix metalloproteinases (Algate et al., 2016). Also, IL-1 and TNF-α act synergistically increasing bone resorption, and TNF plays a critical role in preparing the innate host response to defend against bacteria, except when overstimulated, which can cause significant collateral damage (Graves & Cochran, 2003). Moreover, the pro-inflammatory cytokine IL-1β, primarily secreted by macrophages, is a key regulator of host responses to microbial infection and is found frequently in elevated levels in persistent apical periodontitis (Yang et al., 2018). IL-1β is a potent stimulator of periodontal tissue breakdown, and its properties include the promotion of bone resorption and the production of tissue-degrading proteinases (Cheng et al., 2020). A possible explanation for the lack of significance in the reduction

found in the present study may be due to the dosage of resveratrol and quercetin administered, as they were lower than those applied by other studies that reported the effectiveness in reducing this interleukin (Napimoga et al., 2013; Ribeiro et al., 2017).

The supplementation with the mixture of resveratrol and quercetin caused an elevated and significantly greater release of IL-10. Red wine, in turn, also increased the production of IL-10, but without significance, probably due to the presence of alcohol in the drink, hindering the beneficial effects of polyphenols (Gavala et al., 2015). IL-10 is a pleiotropic cytokine with potent anti-inflammatory ability that suppresses both immunoproliferative and inflammatory responses, regulates B-cell proliferation and differentiation and downregulates a number of processes such as the release of proinflammatory cytokines and chemokines, such as IL-1, IL-6 and TNF-α, the production of nitric oxide, and collagenase (Akdis et al., 2016; Sun et al., 2019). Moreover, IL-10 affects osteoclast precursors, and inhibits osteoclast activation and has been regarded as a key regulator of bone homeostasis, in homeostatic and inflammatory conditions, as the lack of IL-10 in animals leads to the increased femur and alveolar bone loss (Cheng et al., 2020). Besides, Il-10 is recognized as an important suppressor for periodontal disease and apical periodontitis development *in vivo* (Sasaki et al., 2000, 2004). The molecular mechanism of bone loss prevention evoked by the IL-10 is based on the upregulation of OPG expression and downregulation expression of the RANKL (Cheng et al., 2020).

Bone is a dynamic complex tissue that undergoes renovation and repair constantly (or remodelling). The cells responsible for this process are osteoblasts, which secrete new bone, and osteoclasts that remove the existing tissue. Normally, the fine balance between these two cells is in harmony, with the result there is no change in bone mass. The control of bone metabolism is a key factor in reducing bone resorption in inflammatory diseases (Xiao et al., 2016). Therefore, the use of substances that are capable of interfering positively in inflammatory processes that lead to bone resorption should be considered.

Several signalling pathways maintain the activities of osteoblasts and osteoclasts. One of the most important and frequently targeted as a new treatment strategy in bone related-disease conditions is the RANK/RANKL/OPG system (Silva & Branco, 2011). The OPG/RANKL ratio is considered as important information to assess the cellular state of bone tissue, as the OPG is an osteoprotective protein, acting by binding to RANKL preventing it from binding to RANK and sequencing osteoclastic formation (Silva & Branco, 2011). In the present study, as the OPG-RANKL pathway was shifted toward OPG in group R+Q, due to a higher expression of this protein and no differences in RANKL, less formation of bone resorptive cells occurred, as previously reported (Ge et al., 2020; Ribeiro et al., 2017). These data are confirmed by the decreased number of positive TRAP multinucleated cells (osteoclasts) per millimetre of the perimeter of the apical lesion in the same group. Reduced periapical bone destruction was evidenced by the μCT analysis in the R+Q group, reinforcing the correlation between reduced TRAP-positive cells found on immunohistochemistry, displaying attenuated bone loss due to the lower osteoclast activating in the apical lesion when compared to the control. In addition to this pathway, others not evaluated in the present study, but already published in other areas, may be related to the diminished bone resorption volume by the phenolic group (He et al., 2010; Wattel et al., 2004).

Red wine consumption also led to fewer TRAP multinucleated cells when compared to the control, however, no significant difference was found in the lesion volume between these two groups. Nevertheless, a strong tendency towards a reduction in bone resorption analysed by μCT was observed in the red wine group. A longer observation period (e.g. 6 weeks) might have resulted in a significant reduction in bone resorption, as small changes in periapical bone remodelling still occur at this time point (Xu et al., 2019). The group receiving alcohol alone had the greatest number of TRAP-positive cells per millimetre of the lesion perimeter, confirming the deleterious effect of alcohol consumption on this marker in the rats experimental model (Dal-Fabbro, Marques-de-Almeida, Cosme-Silva, Capalbo, et al., 2019; Dal-Fabbro, Marques-de-Almeida, Cosme-Silva, Ervolino, et al., 2019). Interestingly, when comparing this group with the wine, the latter one had a significantly lower cell count, leading to the hypothesis that the polyphenols present in the drink counterbalanced the deleterious effect of alcohol.

In the periodontology field, a prospective cohort study reported that the intake of wine was inversely associated with clinical periodontal attachment loss in men (Kongstad et al., 2008). Another study in adults in southern Brazil reported evidence of a beneficial effect of wine on periodontal status (Susin et al., 2015). Previous investigations in ligature-induced periodontitis in animals demonstrated good properties regarding the use of the polyphenols present in red wine. First, continuous administration of resveratrol was reported to decrease periodontal breakdown induced experimentally in rats (Casati et al., 2013). Resveratrol administered via oral gavage to rats caused a significant reduction in inflammation-mediated destruction of periodontal soft tissues and bone (Correa et al., 2017). When given by subcutaneous injection in the same experimental model, it protected rats from periodontal tissue damage by inhibiting inflammatory responses and by

stimulating antioxidant defence systems (Bhattarai et al., 2016). The same results were observed when administered freely in drinking water (Tamaki et al., 2014). Moreover, resveratrol had a positive influence in decreasing periodontal breakdown during smoking in rats (Ribeiro et al., 2017). Similar to resveratrol, quercetin exhibited protective effects in bacterial-induced periodontitis, reducing alveolar bone loss by mechanisms involving the reduction of pro-inflammatory cytokine production and down-regulation of the osteoclastogenic cytokine RANKL (Napimoga et al., 2013). In addition, it reduced alveolar bone loss in ligature-induced periodontitis by increasing osteoblastic activity, decreasing osteoclastic activity, apoptosis and inflammation (Taskan & Gevrek, 2020). These data, concomitantly with the findings in the present study, highlight a promising approach to inhibit the development of bone loss during apical periodontitis development.

Considering the high prevalence of apical periodontitis throughout life, combined with frequent intake of red wine and the polyphenols through other sources, the present study offers some insights regarding the mechanisms of how these compounds may affect apical periodontitis. However, due to some limitations, such as the use of an animal model, the resveratrol and quercetin dosage, the ingestion frequency and time of administration treatment before the periapical injury induction, the results cannot be extrapolated to humans, and, therefore, more studies are encouraged considering these relevant parameters.

CONCLUSION

Red wine administration decreased apical periodontitis inflammation, TRAP marking and periapical bone resorption compared to alcohol. Resveratrol-quercetin administration reduced the inflammatory process in apical periodontitis, periapical bone resorption and altered the OPG, IL-10 and TRAP expression compared to C and ALC groups.

CONFLICT OF INTEREST

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

ETHICAL STATEMENT

Authors affirm that this is an original work, which has not been previously published elsewhere. Furthermore, the paper reflects the authors' research and analysis wholly and truthfully. All sources used are appropriately disclosed and cited. We also affirm that authors have been personally and actively involved in substantial word leading to the paper and will take public responsibility for its content.

AUTHOR CONTRIBUTIONS

All authors contributed to the development of this original research. In addition, all authors read, revised, and approved the manuscript. The authors Renan Dal Fabbro, João Eduardo Gomes Filho, Edilson Ervolino and Luciano Tavares Angelo Cintra also contributed to the original study design and performed the animal experiments. The authors Renan Dal Fabbro, Edilson Ervolino, Luciano Tavares Angelo Cintra e João Eduardo Gomes Filho contributed to the histological processing and histological analysis. All authors contributed to the interpretation of data and preparation of text and manuscript.

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REFERENCES

Akdis, M., Aab, A., Altunbulakli, C., Azkur, K., Costa, R.A., Crameri, R. et al. (2016) Interleukins (from IL-1 to IL-38), interferons, transforming growth factor β, and TNF-α: receptors, functions, and roles in diseases. *Journal of Allergy and Clinical Immunology*, 138, 984–1010.

Algate, K., Haynes, D.R., Bartold, P.M., Crotti, T.N. & Cantley, M.D. (2016) The effects of tumour necrosis factor-α on bone cells involved in periodontal alveolar bone loss: osteoclasts, osteoblasts and osteocytes. *Journal of Periodontal Research*, 51, 549–566.

Artero, A., Artero, A., Tarin, J.J. & Cano, A. (2015) The impact of moderate wine consumption on health. *Maturitas*, 80, 3–13.

Azuma, M.M., Gomes-Filho, J.E., Ervolino, E., Pipa, C.B., Cardoso, C.D.B.M., Andrada, A.C. et al. (2017) Omega 3 fatty acids reduce bone resorption while promoting bone generation in rat apical periodontitis. *Journal of Endodontics*, 43, 970–976.

Bhattarai, G., Poudel, S.B., Kook, S.H. & Lee, J.C. (2016) Resveratrol prevents alveolar bone loss in an experimental rat model of periodontitis. *Acta Biomaterialia*, 29, 398–408.

Casado-Diaz, A., Anter, J., Dorado, G. & Quesada-Gomez, J.M. (2016) Effects of quercetin, a natural phenolic compound, in the differentiation of human mesenchymal stem cells (MSC) into adipocytes and osteoblasts. *The Journal of Nutritional Biochemistry*, 32, 151–162.

Casati, M.Z., Algayer, C., Cardoso da Cruz, G., Ribeiro, F.V., Casarin, R.C.V., Pimentel, S.P. et al. (2013) Resveratrol decreases periodontal breakdown and modulates local levels of cytokines during periodontitis in rats. *Journal of Periodontology*, 84, e58–64.

Chen, T., Zhang, X., Zhu, G., Liu, H., Chen, J., Wang, Y. et al. (2020) Quercetin inhibits TNF-alpha induced HUVECs apoptosis and

inflammation via downregulating NF-kB and AP-1 signaling pathway in vitro. *Medicine (Baltimore)*, 99, e22241.

Cheng, R., Wu, Z., Li, M., Shao, M. & Hu, T. (2020) Interleukin-1β is a potential therapeutic target for periodontitis: a narrative review. *International Journal of Oral Science*, 12, 2.

Cintra, L.T.A., da Silva Facundo, A.C., Prieto, A.K.C., Sumida, D.H., Narciso, L.G., Mogami Bomfim, S.R. et al. (2014) Blood profile and histology in oral infections associated with diabetes. *Journal of Endodontics*, 40, 1139–1144.

Cintra, L.T.A., Samuel, R.O., Azuma, M.M., de Queiróz, A.O.S., Ervolino, E., Sumida, D.H. et al. (2016) Multiple apical periodontitis influences serum levels of cytokines and nitric oxide. *Journal of Endodontics*, 42, 747–751.

Corrêa, M.G., Pires, P.R., Ribeiro, F.V., Pimentel, S.Z., Casarin, R.C.V., Cirano, F.R. et al. (2017) Systemic treatment with resveratrol and/or curcumin reduces the progression of experimental periodontitis in rats. *Journal of Periodontal Research*, 52, 201–209.

Corrêa, M.G., Pires, P.R., Ribeiro, F.V., Pimentel, S.P., Cirano, F.R., Napimoga, M.H. et al. (2018) Systemic treatment with resveratrol reduces the progression of experimental periodontitis and arthritis in rats. *PLoS One*, 13, e0204414.

Cosme-Silva, L., Dal-Fabbro, R., Cintra, L.T.A., Santos, V.R., Duque, C., Ervolino, E. et al. (2019) Systemic administration of probiotics reduces the severity of apical periodontitis. *International Endodontic Journal*, 52, 1738–1749.

Cosme-Silva, L., Dal-Fabbro, R., Cintra, L.T.A., Ervolino, E., Plazza, F., Mogami Bomfim, S. et al. (2020) Reduced bone resorption and inflammation in apical periodontitis evoked by dietary supplementation with probiotics in rats. *International Endodontic Journal*, 53, 1084–1092.

Dal-Fabbro, R., Cosme-Silva, L., Capalbo, L.C., Chaves-Neto, A.H., Ervolino, E., Cintra, L.T.A. et al. (2021) Excessive caffeine intake increases bone resorption associated with periapical periodontitis in rats. *International Endodontic Journal*, 54, 1861–1870.

Dal-Fabbro, R., Marques-de-Almeida, M., Cosme-Silva, L., Capalbo, L.C., Ervolino, E., Cintra, L.T.A. et al. (2019) Effects of different alcohol concentrations on the development of apical periodontitis in rats. *Archives of Oral Biology*, 108, 104538.

Dal-Fabbro, R., Marques-de-Almeida, M., Cosme-Silva, L., Ervolino, E., Cintra, L.T.A. & Gomes-Filho, J.E. (2019) Chronic alcohol consumption increases inflammation and osteoclastogenesis in apical periodontitis. *International Endodontic Journal*, 52, 329–336.

Das, S. & Das, D.K. (2007) Anti-inflammatory responses of resveratrol. *Inflammation & Allergy – Drug Targets*, 6, 168–173.

Esteban-Fernández, A., Zorraquín-Peña, I., González de Llano, D., Bartolomé, B. & Moreno-Arribas, M.V. (2017) The role of wine and food polyphenols in oral health. *Trends in Food Science & Technology*, 69, 118–130.

Galiniak, S., Aebisher, D. & Bartusik-Aebisher, D. (2019) Health benefits of resveratrol administration. *Acta Biochimica Polonica*, 66, 13–21.

Galler, K.M., Weber, M., Korkmaz, Y., Widbiller, M. & Feuerer, M. (2021) Inflammatory response mechanisms of the dentine-pulp complex and the periapical tissues. *International Journal of Molecular Sciences*, 22,1480.

Gavala, A., Myrianthefs, P. & Venetsanou, K. (2015) Alcohol effects on TNF-a and IL-10 production in an ex-vivo model of whole blood stimulated by LPS. *Journal of Psychiatry*, 18,1–5.

Ge, Y.W., Feng, K., Liu, X.L. et al. (2020) Quercetin inhibits macrophage polarization through the p-38alpha/beta signalling pathway and regulates OPG/RANKL balance in a mouse skull model. *Journal of Cellular and Molecular Medicine*, 24, 3203–3216.

Golan, R., Gepner, Y. & Shai, I. (2019) Wine and health-new evidence. *European Journal of Clinical Nutrition*, 72, 55–59.

Gomes-Filho, J.E., Wayama, M.T., Dornelles, R.C.M., Ervolino, E., Coclete, G.A., Duarte, P.C.T. et al. (2015) Effect of raloxifene on periapical lesions in ovariectomized rats. *Journal of Endodontics*, 41, 671–675.

Graves, D.T. & Cochran, D. (2003) The contribution of interleukin-1 and tumor necrosis factor to periodontal tissue destruction. *Journal of Periodontology*, 74, 391–401.

Haseeb, S., Alexander, B. & Baranchuk, A. (2017) Wine and cardiovascular health: a comprehensive review. *Circulation*, 136, 1434–1448.

He, X., Andersson, G., Lindgren, U. & Li, Y. (2010) Resveratrol prevents RANKL-induced osteoclast differentiation of murine osteoclast progenitor RAW 264.7 cells through inhibition of ROS production. *Biochemical and Biophysical Research Communications*, 401, 356–362.

Kakehashi, S., Stanley, H.R. & Fitzgerald, R.J. (1965) The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. *Oral Surgery, Oral Medicine, Oral Pathology*, 20, 340–349.

Khan, H., Ullah, H., Aschner, M., Cheang, W.S. & Akkol, E.K. (2019) Neuroprotective effects of quercetin in Alzheimer's disease. *Biomolecules*, 10, 59.

Kongstad, J., Hvidtfeldt, U.A., Gronbaek, M., Jontell, M., Stoltze, K. & Holmstrup, P. (2008) Amount and type of alcohol and periodontitis in the Copenhagen City Heart Study. *Journal of Clinical Periodontology*, 35, 1032–1039.

Kutlesa, Z. & Budimir Mrsic, D. (2016) Wine and bone health: a review. *Journal of Bone and Mineral Metabolism*, 34, 11–22.

Lago-Vanzela, E.S., Da-Silva, R., Gomes, E., Garcia-Romero, E. & Hermosin-Gutierrez, I. (2011a) Phenolic composition of the Brazilian seedless table grape varieties BRS Clara and BRS Morena. *Journal of Agricultural and Food Chemistry*, 59, 8314–8323.

Lago-Vanzela, E.S., Da-Silva, R., Gomes, E., Garcia-Romero, E. & Hermosin-Gutierrez, I. (2011b) Phenolic composition of the edible parts (flesh and skin) of Bordo grape (*Vitis labrusca*) using HPLC-DAD-ESI-MS/MS. *Journal of Agricultural and Food Chemistry*, 59, 13136–13146.

Lee, B. & Moon, S.K. (2005) Resveratrol inhibits TNF-alpha-induced proliferation and matrix metalloproteinase expression in human vascular smooth muscle cells. *Journal of Nutrition*, 135, 2767–2773.

Lee, I.T., Lin, C.F., Huang, Y.L. et al. (2019) Protective mechanisms of resveratrol derivatives against TNF-alpha-induced inflammatory responses in rat mesangial cells. *Cytokine*, 113, 380–392.

Li, H., Xia, N., Hasselwander, S. & Daiber, A. (2019) Resveratrol and vascular function. *International Journal of Molecular Sciences*, 20, 2155.

Li, Y., Yao, J., Han, C., Yang, J., Chaudhry, M., Wang, S. et al. (2016) Quercetin, inflammation and immunity. *Nutrients*, 8, 167.

Markoski, M.M., Garavaglia, J., Oliveira, A., Olivaes, J. & Marcadenti, A. (2016) Molecular properties of red wine compounds and

cardiometabolic benefits. *Nutrition and Metabolic Insights*, 9, NMI.S32909.

Minzer, S., Estruch, R. & Casas, R. (2020) Wine intake in the framework of a Mediterranean diet and chronic non-communicable diseases: a short literature review of the last 5 years. *Molecules*, 25, 5045.

Napimoga, M.H., Clemente-Napimoga, J.T., Macedo, C.G., Freitas, F.F., Stipp, R.N., Pinho-Ribeiro, F.A. et al. (2013) Quercetin inhibits inflammatory bone resorption in a mouse periodontitis model. *Journal of Natural Products*, 76, 2316–2321.

Nixdorf, S.L. & Hermosin-Gutierrez, I. (2010) Brazilian red wines made from the hybrid grape cultivar Isabel: phenolic composition and antioxidant capacity. *Analytica Chimica Acta*, 659, 208–215.

Pandey, M.K., Gupta, S.C., Karelia, D., Gilhooley, P.J., Shakibaei, M. & Aggarwal, B.B. (2018) Dietary nutraceuticals as backbone for bone health. *Biotechnology Advances*, 36, 1633–1648.

Pavlidou, E., Mantzorou, M., Fasoulas, A., Tryfonos, C., Petridis, D. & Giaginis, C. (2018) Wine: an aspiring agent in promoting longevity and preventing chronic diseases. *Diseases (Basel, Switzerland)*, 6, 73.

Pervaiz, S. & Holme, A.L. (2009) Resveratrol: its biologic targets and functional activity. *Antioxidants & Redox Signaling*, 11, 2851–2897.

Rauf, A., Imran, M., Butt, M.S., Nadeem, M., Peters, D.G. & Mubarak, M.S. (2018) Resveratrol as an anti-cancer agent: a review. *Critical Reviews in Food Science and Nutrition*, 58, 1428–1447.

Renaud, S. & de Lorgeril, M. (1992) Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet*, 339, 1523–1526.

Reyes-Farias, M. & Carrasco-Pozo, C. (2019) The anti-cancer effect of quercetin: molecular implications in cancer metabolism. *International Journal of Molecular Sciences*, 20, 3177.

Ribeiro, F.V., Pino, D.S., Franck, F.C., Benatti, B.B., Tenenbaum, H., Davies, J.E. et al. (2017) Resveratrol inhibits periodontitis-related bone loss in rats subjected to cigarette smoke inhalation. *Journal of Periodontology*, 88, 788–798.

Rotondo, S., Di Castelnuovo, A. & de Gaetano, G. (2001) The relationship between wine consumption and cardiovascular risk: from epidemiological evidence to biological plausibility. *Italian Heart Journal*, 2, 1–8.

Sasaki, H., Hou, L., Belani, A., Wang, C.-Y., Uchiyama, T., Müller, R. et al. (2000) IL-10, but not IL-4, suppresses infection-stimulated bone resorption in vivo. *The Journal of Immunology*, 165, 3626–3630.

Sasaki, H., Okamatsu, Y., Kawai, T., Kent, R., Taubman, M. & Stashenko, P. (2004) The interleukin-10 knockout mouse is highly susceptible to Porphyromonas gingivalis-induced alveolar bone loss. *Journal of Periodontal Research*, 39, 432–441.

Schmatz, R., Mann, T.R., Spanevello, R., Machado, M.M., Zanini, D., Pimentel, V.C. et al. (2013) Moderate red wine and grape juice consumption modulates the hydrolysis of the adenine nucleotides and decreases platelet aggregation in streptozotocin-induced diabetic rats. *Cell Biochemistry and Biophysics*, 65, 129–143.

Shakibaei, M., Buhrmann, C. & Mobasheri, A. (2011) Resveratrol-mediated SIRT-1 interactions with p300 modulate receptor activator of NF-kappaB ligand (RANKL) activation of NF-kappaB signaling and inhibit osteoclastogenesis in bone-derived cells. *Journal of Biological Chemistry*, 286, 11492–11505.

Silva, I. & Branco, J.C. (2011) Rank/Rankl/opg: literature review. *Acta Reumatologica Portuguesa*, 36, 209–218.

Snopek, L., Mlcek, J., Sochorova, L., Baron, M., Hlavacova, I., Jurikova, T. et al. (2018) Contribution of red wine consumption to human health protection. *Molecules*, 23, 1684.

Sun, Y.I., Ma, J., Li, D., Li, P., Zhou, X., Li, Y.U. et al. (2019) Interleukin-10 inhibits interleukin-1β production and inflammasome activation of microglia in epileptic seizures. *Journal of Neuroinflammation*, 16, 66.

Susin, C., Wagner, M.C., Haas, A.N., Oppermann, R.V. & Albandar, J.M. (2015) The association between alcohol consumption and periodontitis in southern Brazilian adults. *Journal of Periodontal Research*, 50, 622–628.

Takada, Y., Bhardwaj, A., Potdar, P. & Aggarwal, B.B. (2004) Nonsteroidal anti-inflammatory agents differ in their ability to suppress NF-kappaB activation, inhibition of expression of cyclooxygenase-2 and cyclin D1, and abrogation of tumor cell proliferation. *Oncogene*, 23, 9247–9258.

Tamaki, N., Cristina Orihuela-Campos, R., Inagaki, Y., Fukui, M., Nagata, T. & Ito, H.O. (2014) Resveratrol improves oxidative stress and prevents the progression of periodontitis via the activation of the Sirt1/AMPK and the Nrf2/antioxidant defense pathways in a rat periodontitis model. *Free Radical Biology and Medicine*, 75, 222–229.

Taskan, M.M. & Gevrek, F. (2020) Quercetin decreased alveolar bone loss and apoptosis in experimentally induced periodontitis model in wistar rats. *Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry*, 19, 436–448.

U.S. Department of Agriculture and U.S. Department of Health and Human Services. (2020) *Dietary guidelines for Americans, 2020–2025*, 9th edition. Washington, DC.

Ventura-Cots, M., Ballester-Ferré, M.P., Ravi, S. & Bataller, R. (2019) Public health policies and alcohol-related liver disease. *Journal of Hepatology Reports*, 1, 403–413.

Venturini, C.D., Merlo, S., Souto, A.A., Fernandes Mda, C., Gomez, R. & Rhoden, C.R. (2010) Resveratrol and red wine function as antioxidants in the nervous system without cellular proliferative effects during experimental diabetes. *Oxidative Medicine and Cellular Longevity*, 3, 434–441.

Wagner, M.C., Cavagni, J., Gaio, E.J. et al. (2019) Effect of red wine and its major components on periodontitis and systemic inflammation in rats. *Journal of the International Academy of Periodontology*, 21, 139–147.

Wang, M., Weng, X., Chen, H., Chen, Z. & Liu, X. (2020) Resveratrol inhibits TNF-alpha-induced inflammation to protect against renal ischemia/reperfusion injury in diabetic rats. *Acta Cirurgica Brasileira*, 35, e202000506.

Wattel, A., Kamel, S., Prouillet, C. et al. (2004) Flavonoid quercetin decreases osteoclastic differentiation induced by RANKL via a mechanism involving NF kappa B and AP-1. *Journal of Cellular Biochemistry*, 92, 285–295.

Wong, R.W. & Rabie, A.B. (2008a) Effect of quercetin on bone formation. *Journal of Orthopaedic Research*, 26, 1061–1066.

Wong, R.W. & Rabie, A.B. (2008b) Effect of quercetin on preosteoblasts and bone defects. *Open Orthopaedics Journal*, 2, 27–32.

Wong, S.K., Chin, K.Y. & Ima-Nirwana, S. (2020) Quercetin as an agent for protecting the bone: a review of the current evidence. *International Journal of Molecular Sciences*, 21, 6448.

Xiao, W., Wang, Y., Pacios, S., Li, S. & Graves, D.T. (2016) Cellular and molecular aspects of bone remodeling. *Frontiers of Oral Biology*, 18, 9–16.

Xu, R., Guo, D., Zhou, X., Sun, J., Zhou, Y., Fan, Y.I. et al. (2019) Disturbed bone remodelling activity varies in different stages of experimental, gradually progressive apical periodontitis in rats. *International Journal of Oral Science*, 11, 27.

Yamaguchi, M. & Weitzmann, M.N. (2011) Quercetin, a potent suppressor of NF-kappaB and Smad activation in osteoblasts. *International Journal of Molecular Medicine*, 28, 521–525.

Yamasaki, M., Kumazawa, M., Kohsaka, T., Nakamura, H. & Kameyama, Y. (1994) Pulpal and periapical tissue reactions after experimental pulpal exposure in rats. *Journal of Endodontics*, 20, 13–17.

Yang, N.-Y., Zhou, Y., Zhao, H.-Y., Liu, X.-Y., Sun, Z. & Shang, J.-J. (2018) Increased interleukin 1α and interleukin 1β expression is involved in the progression of periapical lesions in primary teeth. *BMC Oral Health*, 18, 124.

Yuan, Z., Min, J., Zhao, Y., Cheng, Q., Wang, K., Lin, S. et al. (2018) Quercetin rescued TNF-alpha-induced impairments in bone marrow-derived mesenchymal stem cell osteogenesis and improved osteoporosis in rats. *American Journal of Translational Research*, 10, 4313–4321.

How to cite this article: Dal-Fabbro, R., Cosme-Silva, L., Rezende Silva Martins de Oliveira, F., Capalbo, L.C., Plazza, F.A., Ervolino, E., et al. (2021) Effect of red wine or its polyphenols on induced apical periodontitis in rats. *International Endodontic Journal*, 54, 2276–2289. https://doi.org/10.1111/iej.13633