Received: 31 August 2018 | Revised: 17 May 2019 | Accepted: 17 June 2019

DOI: 10.1111/jre.12683

ORIGINAL ARTICLE

Journal of PERIODONTAL RESEARCH WILEY

# Impact of natural curcumin on the progression of experimental periodontitis in diabetic rats

Suzana P. Pimentel[1] | Marcio Z. Casati[1] | Fernanda V. Ribeiro[1] | Monica Grazieli Corrêa[1] | Felipe C. Franck[1] | Bruno B. Benatti[2] | Fabiano R. Cirano[1]

[1]Dental Research Division, School of Dentistry, Paulista University, São Paulo, Brazil

[2]School of Dentistry, Federal University of Maranhão, São Luís, Brazil

**Correspondence**
Fabiano R. Cirano, Depto. de Odontologia, Universidade Paulista – UNIP, Av. Dr. Bacelar, 1212, 4o andar, Vila Clementino, São Paulo, 04026-002 SP, Brazil.
Email: cirano.fabiano@gmail.com

**Funding information**
São Paulo Research Foundation, Grant/Award Number: 2014/09480-8 ; National Counsel of Technological and Scientific Development, Grant/Award Number: 308045/2014-9 and 441464/2014-9

## Abstract

**Objective:** To evaluate the role of natural curcumin (CURC) on experimental periodontitis (EP) in animals with diabetes mellitus (DM).

**Material and Methods:** One hundred rats were assigned to DM + placebo (PLA); DM + CURC; DM + insulin (INS); DM + CURC + INS; and Non-DM. Diabetes was induced by streptozotocin. After 3 days, they were initiated CURC and PLAC solutions and insulin administrations, daily for 30 days. This included a period of 19 days prior to EP induction (ligature at the first mandibular and the second maxillary molar) and then additional 11 days. Specimens from the mandible were processed for morphometric examination of bone level. Gingival tissues from mandibular molars were collected for quantification of IL-1β, IL-4, IL-6, IL-17, IFN-γ, and TNF-α using a Luminex/MAGpix assay. Gingivae from maxillary molars were subjected to RT-PCR for assessment of Runx2, RANKL, OPG, SIRT, Dkk1, and Sost levels.

**Results:** Lower linear bone loss was detected in ligated molars of DM + CURC + INS vs DM + PLAC and DM + INS groups ($P < 0.05$). In ligated sites from DM rats treated with CURC + INS, IL-6, IL-1β, INF-γ, and TNF-α levels were the lowest in comparison with PLAC and/or INS and CURC as monotherapies ($P < 0.05$). CURC, independently of INS, increased Runx2 and SIRT when compared to DM + PLAC ($P < 0.05$) in ligated sites, whereas only CURC + INS reduced the RANKL/OPG ratio when compared to DM + PLAC ($P < 0.05$).

**Conclusion:** Natural CURC, when associated with INS, reduces the DM-induced loss of supporting alveolar bone and promotes favorable modulation on osteo-immune-inflammatory mediators.

KEYWORDS

alveolar bone loss, anti-inflammatory agents, curcumin, diabetes mellitus, periodontitis, plants, medicinal

## 1 | INTRODUCTION

Periodontitis is a complex infectious inflammatory condition with multifactorial contributory factors that destroys tooth-supporting tissues leading to alveolar bone breakdown and tooth loss.[1] The understanding of the dynamic interactions between the various periodontal pathogens and the host-mediated inflammatory-immune responses has increased significantly, highlighting the importance of genetics, epigenetics, lifestyle, and environmental factors in the pathogenesis of periodontitis.[2-4]

In this context, studies have indicated that the diabetic/hyperglycemic state increases the risk for the development and progression of periodontitis and it is associated with future tooth loss, especially in poorly controlled glycemic status.[5-7] In line, earlier investigations revealed that one-third of individuals with diabetes mellitus (DM) presents severe forms of periodontal disease, and adults aged 45 years or older, with poorly controlled glycemic status, are three times more likely to have severe periodontitis than those without diabetes.[8,9]

The greater susceptibility of diabetic individuals to periodontal breakdown could be attributed to the modulation of several cyto/chemokines in favor of a pro-inflammatory host response profile.[5,10,11] In addition, more severe tissue destruction in periodontitis is related to oxidative stress mechanisms and excessive production of reactive oxygen species which also play an important role in the development of diabetic complications.[12] Thus, the knowledge of innovative strategies to treat periodontal disease is especially relevant in patients presenting increased susceptibility to periodontitis, as diabetics.

Curcumin (CURC), a bioactive component of turmeric and derived from the plant *Curcuma longa*, is a phytochemical substance with ability to modulate the immune system.[13] Several studies have ever demonstrated the potential therapeutic influence of natural CURC in attenuating the progression of experimental periodontitis (EP) by the modulation of immune-inflammatory biomarkers.[14-17] It has also been reported as an efficient antioxidant agent, protecting innumerous organs from oxidative stress-induced pathophysiology.[18] Because of its anti-inflammatory and antioxidant properties, natural or chemically modified curcumin has also been considered as a promising therapeutic option for diabetes and related complications,[19,20] as periodontitis.[21,22]

However, no study has investigated the impact of natural CURC, alone or in combination with insulin, on controlling the progression of EP in diabetic animals by assessing alveolar bone loss and local host modulation of osteo-immunoinflammatory and oxidative stress mediators. The hypothesis was that natural CURC could inhibit alveolar bone loss and favor the pattern of osteo-immunoinflammatory and antioxidant markers in EP, even in animals submitted to diabetes induction.

## 2 | MATERIAL AND METHODS

### 2.1 | Animals

The experimental procedure was accepted by the Paulista University Institutional Animal Care and Use Committee (226/14). The animal cohort was composed of 100 10-week-old male Wistar rats, weighing 300.12 ± 10.34 g at the beginning of the study, obtained from the Butantan Institute in São Paulo, Brazil. The rats were acclimatized for 15 days before use, and they were kept in temperature-controlled cages, exposed to a 24-hour light-dark cycle of equal time, and had free access to water and food ad libitum (Labina, Purina1) in the Bioterium of Paulista University.

### 2.2 | Study design and treatment groups

The study design is presented in Figure 1.

Animals were allocated to the following groups: non-diabetic placebo treatment (Non-DM; n = 20); induced DM and placebo treatment (DM + PLAC; n = 20); induced DM and curcumin treatment (DM + CURC; n = 20); induced DM and insulin treatment (DM + INS; n = 20); and induced DM and curcumin/insulin treatment (DM + CURC+INS; n = 20).

Diabetes was induced by intraperitoneal injection of streptozotocin[23] (STZ; 60 mg/kg; Sigma-Aldrich) dissolved in citrate buffer (0.01 mol/L, pH 4.5). The Non-DM animals received intraperitoneal injection of equal volume of 0.1 mol/L citrate buffer. After 3 days, blood samples were collected from the tail of the animals and placed on test strips for glucose analysis using a glucose meter (Accu-Check Active®; Roche Diagnóstica Brasil). Glucose levels above 300 mg/dL were considered to diabetes.

Then, it was initiated CURC, PLAC, and INS administration (Figure 1). A stock solution of curcumin (C-1386—Sigma-Aldrich Brasil Ltda) was prepared in 9% ethanol (Merck KGaA) and diluted in water to obtain the required concentration (100 mg/kg), as used in previous studies.[17] Placebo solution (NaCl saline 0.9%) was prepared by dilution in 9% ethanol (Merck KGaA). Both CURC and PLAC solutions (1 mL) were administered daily via gavage. Insulin was also daily administered by subcutaneous injection of neutral protamine harguerdon (NPH insulin, Biohulin NU-100 100 IU/mL; Eli Lilly and Company) diluted in 0.9% NaCl (5.5-3.5 mL at 6:00 PM and at 6 AM.[24] All therapies (CURC, PLAC, and INS) were administered for 30 days. This included a period of 19 days prior to initiation of EP and then for another 11 days following ligature placement (Figure 1).

### 2.3 | Rat periodontitis model

To induce EP, a first mandibular and a second maxillary molar in each animal were assigned to receive a cotton ligature no.10 (Coats Corrente) knotted subgingivally at the cementoenamel junction (CEJ).

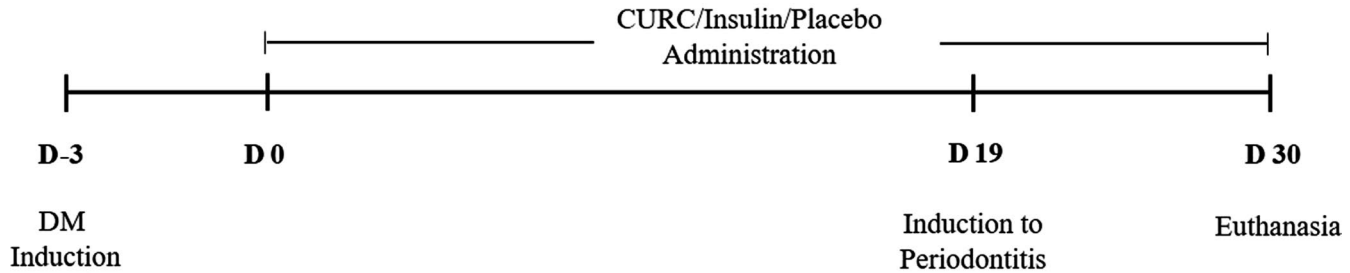


**FIGURE 1** Study design

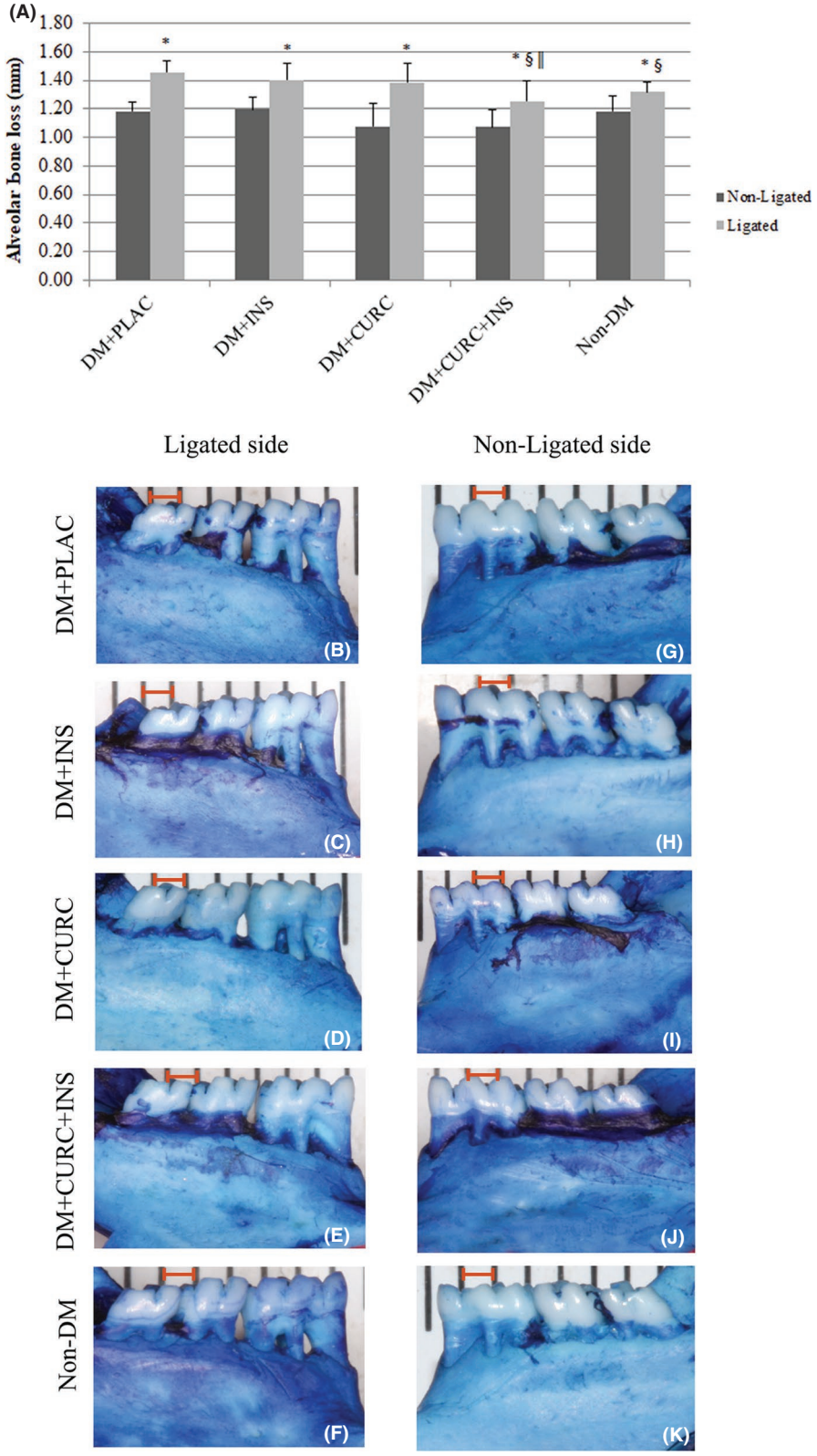


**FIGURE 2** A, Means and standard deviation of linear alveolar bone loss (mm) observed on molars for ligated and non-ligated teeth. *Significant intra-group differences (Student's *t* test; $P < 0.05$). §Significant inter-group differences when compared to DM + PLAC (ANOVA/Tukey; $P < 0.05$). ‖Significant inter-group differences when compared to DM + INS (ANOVA/Tukey; $P < 0.05$). B-K, Representative photographs illustrating the morphometric findings of all experimental groups at non-ligated and ligated teeth. Red lines represent a scale corresponding to 1 mm

The ligatures were kept in position in order to allow biofilm accumulation over 11 days thereby providing for initiation of EP (Figure 1), as previously described.[17] Contralateral teeth were left un-ligated so that they could be used as controls. Thirty days after the start of the study, the animals were euthanized by $CO_2$ inhalation (Figure 2). The mandibles were excised for morphometric analysis to assess alveolar bone loss. The gingival tissues surrounding the first mandibular molars were collected for quantification of immune-inflammatory mediators using a Luminex/MAGpix assay system. The gingival tissue of the second maxillary molars was subjected to analyses of gene expression focused on modulators of the osteo-immune-inflammatory cascade by way of quantitative real-time polymerase chain reaction (qRT-PCR).

## 2.4 | Linear measurement of alveolar bone loss

To the linear measurement of mandibular alveolar bone loss, photographs of the buccal aspects were taken, and linear alveolar bone loss was assessed on the buccal surface of the lower first molars by measuring the distance of the CEJ from the crest of alveolar bone at three equally distant sites, as previously described.[25] The average alveolar bone loss of each tooth was calculated. A single examiner (MGC), who was blinded to the experimental group identities, carried out morphometric measurements. The measurements were performed after intraexaminer calibration by evaluating 10 images not taken for this study. The examiner took the linear measurements of all photographs twice within 24 hours. The intraclass correlation was 97.1%.

## 2.5 | Immune-enzymatic assay

The samples derived from the first mandibular molar soft tissues were placed into sterile tubes containing 400μl phosphate-buffered saline (PBS) with 0.05% Tween-20 and stored at −20°C, as earlier described.[17] Then, the tissues were weighed, cut into small pieces (1-2 $mm^3$) using scissors, and solubilized in PBS to a final concentration of 100 mg tissue/mL. After extraction on a Vortex mixer for 10 minutes, each sample was centrifuged at 370 *g* for 5 minutes, and the supernatant was collected, divided into small portions, and stored at −70°C until use. The levels of interleukin (IL)-1β, IL-4, IL-6, IL-17, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α were determined with the MAGpix instrument (MiraiBio) using commercially available kits (CYTOMAG-80K - Millipore Corporation) and following the manufacturers' instructions. The samples were analyzed individually, and the levels were estimated using a 5-parameter polynomial curve (xponent software; Millipore Corporation).

The standard curve range used for IL-1β measurement was 2.4 ± 10.000 pg/mL; for IL-4 measurement, 4.9 ± 20.000 pg/mL; for IL-6, 7.3 ± 300.000 pg/mL; for TNF-α, 2.4-10.000 pg/mL; for IFN-γ, 14.6 ± 60.000 pg/mL; and for IL-17, 7.3 ± 30.000 pg/mL.

## 2.6 | Gene expression analysis

The gingival tissues from the second maxillary molars were stored in RNA later at −70°C for the evaluation of the mRNA levels for runt-related transcription factor-2 (Runx2), Receptor activator of NF-KB ligand (RANKL), osteoprotegerin (OPG), sirtuin (SIRT), Dickkopf Wnt signaling pathway inhibitor 1 (Dkk1), and sclerostin (Sost), by qRT-PCR, as previously define.[26] Total RNA from the samples was isolated by the TRIzol method (Turbo DNA-frees; Gibco BRL, Life Technologies). Total RNA was DNase treated (Ambion Inc), the RNA concentration was determined from the optical density using a micro-volume spectrophotometer (NanoDrop 1000; NanoDrop Technologies LLC), and 1 μg was employed for complementary DNA (cDNA) synthesis using

**TABLE 1** Primer sequences for each gene, amplification profiles, and the estimated length of qPCR product for each gene

| Gene | Sequence (5´-3´)<br>Sequence (3´-5´) | Length of qPCR product (bp) | Amplification profile [temperature (°C)/time (s)] |
|---|---|---|---|
| Runx2 | GCCACTTACCACAGAGC<br>GCGGGACACCTACTCTCATA | 157 | 95/10, 56/8, 72/7 |
| RANKL | AGCGCTTCTCAGGAGTT<br>TACCAAGAGGACAGACTGACTTTA | 156 | 95/5, 55/4, 72/6 |
| OPG | GCAGAGAAGCACCTAGC<br>GTCCGGTTTCTGGGTCA | 168 | 95/10, 56/8, 72/7 |
| SIRT | GGTCAGGCTGGTTTACAACG<br>GGCATAGACCCACACACATCT | 68 | 95/10, 61/8, 72/10 |
| Dkk1 | CGGGAATTACTGCAAAAACG<br>CAATGATGCCTTCCTCGATT | 83 | 95/10, 59/10, 72/10 |
| Sost | GAGAACAACCAGACCATGAACC<br>GCAGCTGTACTCGGACACG | 90 | 95/10, 59/10, 72/10 |
| GAPDH | TGAGTATGTCGTGGAGTCTACTG<br>TTGAGGGAGTTGTCATATTTCTCG | 159 | 95/10, 56/8, 72/7 |

*Note:* Receptor activator of the NF-KB ligand (RANKL), osteoprotegerin (OPG), Dickkopf 1 (Dkk1), Runt-Related Transcription Factor 2 (Runx2), Sclerostin (Sost), Sirtuin (SIRT), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

the First-Strand cDNA Synthesis Kit (Roche Diagnostic Co.). Primers were designed using probe-design software (Light-Cycler Roche probe design software; Diagnostics GmbH). The amplification profiles, primer sequences, and lengths of PCR products are described in Table 1. The qPCRs were performed in a real-time PCR device (LightCycler® 96 Instrument; Roche Diagnostics GmbH) using a Syber Green kit (FastStart DNA Masterplus Syber Green; Roche Diagnostics GmbH). The results were analyzed as relative amounts of the target gene using GAPDH as the inner reference gene by means of the relative quantification.

### 2.7 | Statistical analysis

Statistical analysis was performed using SAS software (Program Release 9.3). The data were examined for normality using the Kolmogorov-Smirnov test, and the results that presented normality were analyzed using parametric methods. To test the null hypothesis that curcumin had no influence on linear alveolar bone loss, inter-group analysis was performed through analysis of variance (ANOVA) and Tukey's test. A paired Student's *t* test was used for intra-group comparisons between ligated and un-ligated teeth. The differences in immune-enzymatic mediator's concentration were compared by ANOVA/Tukey and Student's *t* test for inter- and intra-group analyses, respectively. The differences in mRNA levels among groups were compared using the Kruskal-Wallis and Dunn tests or ANOVA and Tukey's test, whereas intra-group differences were identified by Student's *t* test. Differences in glucose levels were compared using Student's *t* test and Kruskal-Wallis/Dunn tests for intra- and inter-group analyses, respectively. The significance level established for all analyses was 5%.

## 3 | RESULTS

The animals also did not lose significant weight throughout the experimental period. Some animals were lost throughout the experimental period due to the conditions caused by the DM induction of diabetes. The number of animals at the end of the study was 18, 17, 15, 16, and 20 animals, for DM + PLAC, DM + CURC, DM + INS, DM + CURC + INS, and Non-DM groups, respectively.

### 3.1 | Glucose levels

Serum glucose levels were achieved 8 hours after insulin administration in the morning. All DM rats presented significantly higher serum glucose levels (above 300 mg/dL) at the beginning of the study when compared to Non-DM animals ($P < 0.05$; Table 2). Non-DM rats preserved glycemic levels within normal conditions throughout the study ($P > 0.05$; Table 2), whereas DM + PLAC group maintained elevated glucose levels at the end of experiment when compared to day 0 ($P > 0.05$; Table 2). DM + INS, DM + CURC, and DM + CURC + INS groups showed a significant reduction in glycemia at 30 days as compared to baseline ($P < 0.05$; Table 2). At the end of the study, DM + PLAC animals presented the highest glucose levels when compared to the other groups ($P < 0.05$; Table 2). Non-diabetic animals displayed lower glucose levels at 30 days when compared to DM + INS and DM + CURC groups ($P < 0.05$; Table 2), and no differences were detected when Non-DM animals were compared to those treated with the combination INS + CURC ($P > 0.05$; Table 2).

### 3.2 | Linear alveolar bone loss

The morphometric results related to linear measurements of alveolar bone loss are demonstrated in Figure 2. Significant differences in alveolar bone loss were demonstrated when compared bone levels between non-ligated and ligated teeth in all experimental groups ($P < 0.05$), supporting that bone loss was consistent with the development of EP. Significantly higher bone loss was demonstrated when comparing alveolar ligation-induced bone loss in the DM + PLAC group vs Non-DM and DM + CURC + INS groups ($P < 0.05$). Additionally, increased bone loss was observed in ligated molars of DM + INS rats when compared to those treated with the association CUR + INS ($P < 0.05$). When inter-group comparisons of alveolar bone loss were performed on non-ligated molars, there was no difference between groups ($P > 0.05$).

### 3.3 | Gingival tissue immune-inflammatory mediator's levels

Table 3 shows gingival tissue mediator levels of IL-1β, IL-6, IL-4, IL-17, IFN-γ, and TNF-α in sites induced and non-induced to periodontitis for all groups.

In the sites submitted to EP, inter-group analyses showed significantly higher levels of IL-4 in Non-DM animals when compared to the other groups ($P < 0.05$) and reduced amounts of IL-17 in Non-DM rats when compared to DM + PLAC group ($P < 0.05$), although a trend toward decreasing in IL-17 amounts had been observed in diabetic rats treated with INS, CURC, or association ($P > 0.05$). Both animals, not induced to diabetes and diabetics treated with

**TABLE 2** Glucose levels (mg/dL) measured before the start of the treatments (Day 0) and after 30 d (Euthanasia) of the experiment

| | DM + PLAC | DM + INS | DM + CURC | DM + CURC + INS | Non-DM |
|---|---|---|---|---|---|
| Day 0 | 440.53 ± 69.97 | 441.00 ± 83.47* | 426.92 ± 93.20* | 461.43 ± 69.68* | 158.85 ± 31.87† |
| Day 30 | 467.00 ± 62.20† | 272.55 ± 79.20‡ | 292.54 ± 116.16‡ | 248.79 ± 100.31 | 140.62 ± 35.87 |

*Intra-group differences statistically significant when compared to the baseline ($P < 0.05$; Kruskal-Wallis/Dunn).
†Inter-group difference statistically significant when compared to the other groups ($P < 0.05$; Kruskal-Wallis/Dunn).
‡Statistically significant inter-group difference when compared to the Non-DM group ($P < 0.05$; Kruskal-Wallis/Dunn).

CURC in association to INS, presented reductions in the levels of INF-γ when compared to the DM + PLAC group ($P < 0.05$) and in the concentrations of TNF-α when compared to the DM + PLAC and DM + CURC groups ($P < 0.05$). Moreover, the combined administration of INS and CURC reduced significantly the amounts of IL-6 as compared to the DM + PLAC group ($P < 0.05$) and the levels of IL-1β when compared to the other diabetic groups treated with placebo or with INS or CURC as monotherapies ($P < 0.05$). Considering the non-ligated sites, inter-group evaluation demonstrated that DM induction in animals treated with placebo or isolated therapies (INS or CURC) promoted higher IL-1β and TNF-α values when compared to Non-DM animals ($P < 0.05$). Moreover, lower levels of IL-6 and IFN-γ were detected in Non-DM and DM + CURC + INS rats when compared to the DM + PLAC group ($P < 0.05$). No IL-4 and IL-17 inter-group differences were detected non-ligated sites ($P > 0.05$).

Concerning the intra-group analyses, all groups revealed higher levels of IL-6 and IL-1β were in ligated than un-ligated sites ($P < 0.05$), except for CURC + INS-treated DM animals that did not reveal differences ($P > 0.05$).

### 3.4 | Gene expression levels

The gene expression inter-group analysis showed that Non-DM animals and those that received systemic intake of CURC, independently of INS association, presented significantly higher values of mRNA of Runx2 and SIRT when compared to DM rats treated with placebo ($P < 0.05$; Table 4) in sites with EP. Additionally, both Non-DM and DM + CURC + INS groups showed lower RANKL/OPG ratio when compared to diabetic rats that received placebo control ($P < 0.05$; Table 4). Intra-group comparisons indicated that DM + PLAC animals presented increased gene expression of SIRT at non-ligated sites when compared to teeth submitted to periodontitis ($P < 0.05$; Table 4).

## 4 | DISCUSSION

Diabetes mellitus and periodontitis are complex chronic diseases with an established higher risk for the development periodontitis and future tooth loss in the presence of DM.[5-7] CURC is a natural compound with promising effects in modulating the osteo-immune-inflammatory and antioxidant host response both in periodontally diseased sites[14-17,21,22] and in diabetes and related complications.[19,20] For the first time, this investigation assessed the natural curcumin's impact on the progression of EP in rats induced to DM. The results showed that the therapeutic use of natural CURC in association with INS reduced the alveolar bone loss in ligature-induced periodontitis, favorably modulating the host osteo-immune-inflammatory response.

The outcomes of the morphometric evaluations of the current study revealed that DM induction promoted higher periodontal disease progression and that diabetic rats treated with daily CURC + INS, as well as those Non-DM, presented lower linear bone loss values in periodontitis sites, suggesting that the combined use of CURC and INS, and not the substances used alone, may positively influence periodontal breakdown, even in the presence of DM. Although promising effects of natural CURC on periodontal tissues have already been revealed in earlier experimental[14,16,17,27] and also clinical studies,[28,29] only few information is available concerning the influence of a chemically modified CURC on periodontitis in the presence of a recognized at-risk condition as diabetes mellitus[21,22]

**TABLE 3** Mean (±SD) of immune-inflammatory mediators concentrations (pg/mL) measured by Luminex/MAGpix assay

| | IL-4 | IL-1β | IL-6 | IFN-γ | IL-17 | TNF-α |
|---|---|---|---|---|---|---|
| DM + PLAC | | | | | | |
| Ligated | 190.2 ± 132.0 B | 823.2 ± 875.6 A | 3230.5 ± 4385.6 A* | 703.4 ± 793.5 A | 208.2 ± 141.4 A | 63.1 ± 39.3 A |
| Non-ligated | 125.9 ± 60.3 A | 238.5 ± 154.4 A* | 1923.7 ± 1442.8 A | 158.5 ± 101.7 A | 158.5 ± 101.7 A | 67.2 ± 33.5 A |
| DM + INS | | | | | | |
| Ligated | 161.4 ± 223.1 B | 666.5 ± 561.2 AB | 1883.0 ± 3599.0 AB* | 299.9 ± 371.5 AB | 94.6 ± 31.7 AB | 48.3 ± 21.3 AB |
| Non-ligated | 117.94 ± 38.6 A | 213.6 ± 229.2 A* | 723.4 ± 231.3 AB | 161.8 ± 216.1AB | 161.8 ± 216.1 A | 34.9 ± 09.9 A |
| DM + CURC | | | | | | |
| Ligated | 116.6 ± 97.9 B | 619.9 ± 502.9 AB | 1804.9 ± 1268.9 AB* | 225.2 ± 208.4 AB | 148.6 ± 132.7 AB | 50.0 ± 27.2 A |
| Non-ligated | 146.2 ± 89.7 A | 260.3 ± 205.6 A* | 960.1 ± 621.0 AB | 190.7 ± 118.9 AB | 190.7 ± 118.9 A | 60.6 ± 25.9 A |
| DM + CURC+INS | | | | | | |
| Ligated | 90.4 ± 116.7 B | 87.1 ± 69.0 C | 636.8 ± 829.5 B | 122.3 ± 167.1 B | 82.0 ± 73.8 AB | 33.5 ± 15.9 B |
| Non-ligated | 87.6 ± 69.4 A | 59.1 ± 39.1 AB | 483.0 ± 618.4 B | 71.5 ± 46.0 B | 71.5 ± 46.0 A | 43.5 ± 23.4 AB |
| Non-DM | | | | | | |
| Ligated | 423.7 ± 212.1 A | 373.3 ± 121.1 BC | 1086.9 ± 694.4 AB* | 135.7 ± 212.9 B | 83.1 ± 56.4 B | 33.1 ± 23.9 B |
| Non-ligated | 184.6 ± 84.2 A | 37.46 ± 35.6 B* | 506.1 ± 411.1 B | 43.1 ± 53.1 B | 131.1 ± 47.4 A | 27.0 ± 08.1 B |

*Note:* Different letters indicate significant difference inter-group (ANOVA/Tukey; $P < 0.05$).
*Represents significant intra-group difference (Student's *t* test; $P < 0.05$).

**TABLE 4** Mean ± (SD) of relative levels of mRNA for all genes (mRNA gene/mRNA GAPDH)

| | *Gene/GAPDH* | | | | |
|---|---|---|---|---|---|
| | **Runx2 (×10$^{-3}$)** | **RANKL/OPG (×10$^{3}$)** | **SIRT** | **Dkk1(×10$^{2}$)** | **Sost (×10$^{2}$)** |
| DM + PLAC | | | | | |
| Ligated | 0.529 ± 0.575 | 0.249 ± 0.356 | 4.590 ± 5.712 | 4.953 ± 5.997 | 6.177 ± 6.273 |
| Non-ligated | 0.630 ± 0.579 | 0.041 ± 0.051 | 14.173 ± 9.115† | 2.013 ± 2.610 | 3.895 ± 4.793 |
| DM + INS | | | | | |
| Ligated | 1.589 ± 1.037 | 0.020 ± 0.028 | 15.617 ± 14.405 | 4.952 ± 2.320 | 6.008 ± 3.769 |
| Non-ligated | 2.238 ± 2.043 | 0.009 ± 0.009 | 22.769 ± 18.292 | 2.308 ± 1.086 | 4.642 ± 2.788 |
| DM + CURC | | | | | |
| Ligated | 2.255 ± 1.289* | 0.016 ± 0.014 | 22.533 ± 16.781* | 3.059 ± 3.379 | 4.181 ± 4.526 |
| Non-ligated | 2.067 ± 2.959 | 0.042 ± 0.090 | 29.071 ± 26.121 | 2.038 ± 2.228 | 2.187 ± 2.557 |
| DM + CURC + INS | | | | | |
| Ligated | 3.024 ± 3.244* | 0.005 ± 0.004* | 21.860 ± 16.617* | 2.301 ± 2.006 | 3.349 ± 5.124 |
| Non-ligated | 2.077 ± 1.340 | 0.003 ± 0.004 | 26.751 ± 12.292 | 2.277 ± 2.184 | 2.416 ± 2.901 |
| Non-DM | | | | | |
| Ligated | 2.829 ± 1.693* | 0.005 ± 0.006* | 24.822 ± 20.427* | 3.463 ± 2.086 | 2.766 ± 3.690 |
| Non-ligated | 1.977 ± 0.982 | 0.011 ± 0.014 | 30.132 ± 19.852 | 2.185 ± 1.493 | 1.135 ± 1.553 |

*Significant inter-group difference when compared to DM + PLAC (Kruskal-Wallis/Dunn; $P < 0.05$).
†Significant intra-group difference (Student *t* test; $P < 0.05$).

and there are no data revealing the impact of a natural CURC in the progression of EP associated with DM. Of importance, although some evidence has suggested that certain chemically modified CURC shows improved solubility and bioavailability in comparison with natural CURC,[30,31] advantages related to the natural form of CURC include the facility to obtain the substance and greater possibility of using this therapeutic agent for clinical application in the future. In addition, the use of the natural product (30 mg/kg/d, dose as employed in the current study) has already proved to be promising as a host modulatory agent in periodontal disease pathogenesis and to reduce the alveolar bone loss related to periodontitis.[16,17]

In fact, evidence from previous studies supports that the dysregulation of osteo-immune-inflammatory biomarkers, a known effect of diabetes, may be a contributing factor in the development of periodontal bone loss.[32] In this regard, we showed that sites with EP from the DM + PLAC group had reduced concentration of anti-inflammatory IL-4 in comparison with sites from Non-DM group and also presented the highest levels of pro-inflammatory IL-17, IL-1β, IL-6, IFN-γ, and TNF-α, supporting the notion that diabetes has a direct and negative impact on periodontitis. In line, a recent consensus of the European Federation of Periodontology and the International Diabetes Federation stated that elevations in IL-1β, TNF-α, and IL-6 may be reported as mechanistic links between periodontitis and diabetes.[33] Accordantly, Ribeiro et al[5] reported that diabetes mellitus up-regulates the levels of pro-inflammatory cytokines and down-regulates the production of IL-4 in sites with chronic periodontitis.

Concerning the therapeutic role of natural CURC on the modulation of these mediators in sites with EP, our data showed that only the combined use of CURC and INS was efficient to reduce the levels of INF-γ when compared to the DM + PLAC group and the concentrations of TNF-α when compared to the DM + PLAC and DM + CURC groups, highlighting the relevance of the associated approach to achieve significant benefits in the local host modulation of these biomarkers. Moreover, the combined administration of INS and CURC reduced significantly the amounts of IL-6 as compared to the DM + PLAC group, also decreasing the levels of IL-1β when compared to the other diabetic groups treated with placebo or with INS or CURC as monotherapies. Previous studies in non-diabetic rats have ever demonstrated that CURC was efficient to reduce the levels of pro-inflammatory cytokines, such as TNF-α, IL-6, and IL-1β, to essentially normal levels in the gingival tissues with EP.[14,15,34] However, no data are available concerning the impact of natural CURC in the host response related to periodontitis in the presence of diabetes. In this regard, recently, Elburki et al[21,22] studies evaluated the use of a chemically modified CURC (oral gavage of 30 mg/kg, during 3 weeks) to manage EP in a rat model of diabetes. According to the authors, gingival tissue with periodontitis from animals treated with a chemically modified CURC revealed reduction of the pathologically excessive levels of matrix metalloproteinase (MMP)-2, MMP-8, and MMP-9 and also decreased concentrations of TNF-α, IL-6, and IL-1β0.[21,22] Nevertheless, these studies[21,22] did not compare the effectiveness of CURC alone with the adjunctive role of CURC in combination with INS, as performed in the current investigation, making difficult comparisons. Taken together, our immune-enzymatic assay supports that the decrease in key immune-inflammatory mediators (INF-γ, TNF-α, IL-1β, and IL-6) related to a pro-inflammatory and osteoclastic profile can provide some pathways to explain the most

efficient impact of the associated use of CURC and INS in controlling the alveolar bone loss in periodontally diseased sites in the presence of diabetes. It is relevant to emphasize that the immune-enzymatic assay used in this study may present substantial variations on the levels of the mediators analyzed, as earlier reported in previous clinical[35-37] and experimental researches,[34,38-40] indicating that variations are expected when using these assays, as observed in the current data.

In the present study, both the immune-inflammatory outcomes and those observed in terms of linear bone loss at ligated sites of DM + CURC + INS group may also be supported by the qPCR data that demonstrated that RANKL/OPG mRNA ratio was down-regulated after using this combined approach when compared to diabetic rats that received placebo control. In line with our data, Zhou et al[15] evaluating the role of CURC on EP in non-diabetic situations confirmed that CURC may decrease alveolar bone loss via suppressing the expression of RANKL/RANK/OPG. Previously, in another experimental investigation, Chen et al[41] have ever revealed that CURC alleviates glucocorticoid-induced osteoporosis, among other pathways, through the up-regulation of the ratio of OPG to RANKL.

In addition, gene expression analysis of this study also showed in periodontally diseased sites that CURC therapy associated with INS or even alone, when compared to DM rats treated with placebo, promoted up-regulation of Runx2, which is essential for skeletal development.[42] Earlier in vitro investigations also demonstrated that CURC increased the expression of Runx2.[43,44] In accordance, in a study evaluating the protective effects of CURC on the bones of rats with dexamethasone-induced osteoporosis, Chen et al[41] reported that the bone-protective effects of CURC were mediated through the regulation of Runx2. Our gene expression outcomes also revealed that the use of CURC (alone or combined to INS), when compared to placebo therapy, encouraged up-regulation of SIRT, found persistently in reduced levels in DM patients[45] and associated with regulation of mitochondrial antioxidant defense.[39] Gounden et al[47] shown that SIRT, in fact, is important to increase antioxidant defense and to promote resistance to oxidative stress-induced damage under hyperglycemic conditions. In agreement with our findings, a recent in vitro study revealed that SIRT was significantly increased after the therapy with CURC under hyperglycemic conditions.[48] Interestingly, Ding et al,[49] using mouse pre-osteoblastic MC3T3-E1 cells, showed that SIRT is required for osteogenic differentiation, supporting the relevant biological role of this marker in conditions involving bone tissue breakdown, as revealed in the present study.

In this study, the experimental model for DM was accomplished by the administration of streptozotocin, which has a cytotoxic consequence on pancreatic β-cells, being that these cells are the main cells of the pancreas responsible for the production of insulin in the body. Thus, hyperglycemia is induced, producing an insulinopenic of DM type 1, since reduced residual insulin secretion is maintained.[23,50-52] It is important to highlight that, in the present study, CURC used alone was able to achieve reduction of blood glucose levels to near normal, as well as INS therapy or CURC + INS association, differently of outcomes observed by Elburki et al[21,22] investigations. In a line with our data, earlier in vivo and in vitro findings have ever reported that CURC is able to provide anti-diabetic effects by exerting anti-hyperglycemic properties, improving β-cell function and insulin secretion, recruiting glucose transporters to the cell surface and avoiding islet cell death.[18,53] Noteworthy, glucose homeostasis is not always achieved in experimental models following CURC treatment since the doses and vehicle employed and administration period are key characteristics that significantly impact on glycemic status.[54,55] In clinical studies, the therapeutic action of CURC on glucose levels is contradictory, presenting both successful[56] and no effective results[57] and additional data are required to support the predictability of CURC as an anti-diabetic agent.[58]

In conclusion, the findings of this study suggest that natural CURC, when associated with INS, may prevent periodontal breakdown in the presence of diabetes, supporting its employment as a therapeutic strategy to the treatment of periodontitis at this risk condition. A beneficial modulation on osteo-immune-inflammatory mediators and improve on oxidative stress in periodontitis sites clarify, at least in part, a probable pathway of curcumin action during periodontitis at diabetic circumstances. Additional studies, including supplementary biomarkers of osteo-immune-inflammatory and oxidative stress systems, are essential to clarify the molecular mechanisms by which natural CURC exerts its hopeful impact on periodontitis in the presence of DM.

## ACKNOWLEDGEMENTS

This study was supported by São Paulo Research Foundation (FAPESP), São Paulo, SP, Brazil—Processes 2014/09480-8 and National Counsel of Technological and Scientific Development (CNPq), Brasília, DF, Brazil—Processes 441464/2014-9 and 308045/2014-9.

## ORCID

*Fernanda V. Ribeiro* https://orcid.org/0000-0002-2697-620X
*Monica Grazieli Corrêa* https://orcid.org/0000-0003-1895-3283
*Bruno B. Benatti* https://orcid.org/0000-0002-2080-2217
*Fabiano R. Cirano* https://orcid.org/0000-0002-7331-4612

## REFERENCES

1. Nilsson H, Berglund JS, Renvert S. Periodontitis, tooth loss and cognitive functions among older adults. *Clin Oral Investig*. 2018;22:2103-2109.
2. Pimentel SP, Kolbe MF, Pereira RS, et al. Papillon-Lefèvre syndrome in 2 siblings: case report after 11-year follow-up. *Pediatr Dent*. 2012;34:e231-e236.
3. Genco RJ, Borgnakke WS. Risk factors for periodontal disease. *Periodontol 2000*. 2013;62(1):59-94.
4. Meyle J, Chapple I. Molecular aspects of the pathogenesis of periodontitis. *Periodontol 2000*. 2015;69(1):7-17.

5. Ribeiro FV, de Mendonça AC, Santos VR, Bastos MF, Figueiredo LC, Duarte PM. Cytokines and bone-related factors in systemically healthy patients with chronic periodontitis and patients with type 2 diabetes and chronic periodontitis. *J Periodontol*. 2011;82:1187-1196.
6. Eke PI, Wei L, Thornton-Evans GO, et al. Risk indicators for periodontitis in US adults: NHANES 2009 to 2012. *J Periodontol*. 2016;87:1174-1185.
7. Ziukaite L, Slot DE, Van der Weijden FA. Prevalence of diabetes mellitus in people clinically diagnosed with periodontitis: a systematic review and meta-analysis of epidemiologic studies. *J Clin Periodontol*. 2018;45:650-662.
8. Taylor GW. Periodontal treatment and its effects on glycemic control: a review of the literature. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 1999;87:311-316.
9. Saremi A, Nelson RG, Tulloch-Reid M, et al. Periodontal disease and mortality in type 2 diabetes. *Diabetes Care*. 2005;28:27-32.
10. Javed F, Al-Askar M, Al-Hezaimi K. Cytokine profile in the gingival crevicular fluid of periodontitis patients with and without type 2 diabetes: a literature review. *J Periodontol*. 2012;83:156-161.
11. Mohamed HG, Idris SB, Ahmed MF, et al. Influence of type 2 diabetes on local production of inflammatory molecules in adults with and without chronic periodontitis: a cross-sectional study. *BMC Oral Health*. 2015;27:15-86.
12. Patil VS, Patil VP, Gokhale N, Acharya A, Kangokar P. Chronic periodontitis in type 2 diabetes mellitus: oxidative stress as a common factor in periodontal tissue injury. *J Clin Diagn Res*. 2016;10:BC12-6.
13. Jagetia GC, Aggarwal BB. "Spicing up" of the immune system by curcumin. *J Clin Immunol*. 2007;27:19-35.
14. Guimarães MR, Coimbra LS, de Aquino SG, Spolidorio LC, Kirkwood KL, Rossa C Jr. Potent anti-inflammatory effects of systemically administered curcumin modulate periodontal disease in vivo. *J Periodontal Res*. 2011;46:269-279.
15. Zhou T, Chen D, Li Q, Sun X, Song Y, Wang C. Curcumin inhibits inflammatory response and bone loss during experimental periodontitis in rats. *Acta Odontol Scand*. 2013;71:349-356.
16. Bakır B, Yetkin Ay Z, Büyükbayram Hİ, et al. Effect of curcumin on systemic T helper 17 cell response; gingival expressions of interleukin-17 and retinoic acid receptor-related orphan receptor γt; and alveolar bone loss in experimental periodontitis. *J Periodontol*. 2016;87:e183-e191.
17. Corrêa MG, Pires PR, Ribeiro FV, et al. Systemic treatment with resveratrol and/or curcumin reduces the progression of experimental periodontitis in rats. *J Periodontal Res*. 2017;52:201-209.
18. Rashid K, Sil PC. Curcumin enhances recovery of pancreatic islets from cellular stress induced inflammation and apoptosis in diabetic rats. *Toxicol Appl Pharmacol*. 2015;282:297-310.
19. Chougala MB, Bhaskar JJ, Rajan MG, Salimath PV. Effect of curcumin and quercetin on lysosomal enzyme activities in streptozotocin-induced diabetic rats. *Clin Nutr*. 2012;31:749-755.
20. Zhang DW, Fu M, Gao SH, Liu JL. Curcumin and diabetes: a systematic review. *Evid Based Complement Altern Med*. 2013;2013:636053.
21. Elburki MS, Moore DD, Terezakis NG, et al. A novel chemically modified curcumin reduces inflammation-mediated connective tissue breakdown in a rat model of diabetes: periodontal and systemic effects. *J Periodontal Res*. 2017;52:186-200.
22. Elburki MS, Rossa C, Guimarães-Stabili MR, et al. A chemically modified curcumin (CMC 2.24) inhibits nuclear factor κB activation and inflammatory bone loss in murine models of LPS-induced experimental periodontitis and diabetes-associated natural periodontitis. *Inflammation*. 2017;40:1436-1449.
23. Corrêa MG, Gomes Campos ML, Marques MR, Casati MZ, Nociti Jr FH, Sallum EA. Histometric analysis of the effect of enamel matrix derivative on the healing of periodontal defects in rats with diabetes. *J Periodontol*. 2013;84:1309-13118.
24. de Molon RS, Morais-Camilo JA, Verzola MH, Faeda RS, Pepato MT, Marcantonio Jr E. Impact of diabetes mellitus and metabolic control on bone healing around osseointegrated implants: removal torque and histomorphometric analysis in rats. *Clin Oral Implants Res*. 2013;24:831-837.
25. Pimentel SP, Barrella GE, Casarin RC, et al. Protective effect of topical cordia verbenacea in a rat periodontitis model: immune-inflammatory, antibacterial and morphometric assays. *BMC Complement Altern Med*. 2012;21:12-24.
26. Conte A, Ghiraldini B, Casarin RC, et al. Impact of type 2 diabetes on the gene expression of bone-related factors at sites receiving dental implants. *Int J Oral Maxillofac Surg*. 2015;44:1302-1308.
27. Theodoro LH, Ferro-Alves ML, Longo M, et al. Curcumin photodynamic effect in the treatment of the induced periodontitis in rats. *J Periodontol*. 2016;87:e183-e191.
28. Nagasri M, Madhulatha M, Musalaiah SV, Kumar PA, Krishna CH, Kumar PM. Efficacy of curcumin as an adjunct to scaling and root planning in chronic periodontitis patients: a clinical and microbiological study. *J Pharm Bioallied Sci*. 2016;8:S48-S52.
29. Elavarasu S, Suthanthiran T, Thangavelu A, Alex S, Palanisamy VK, Kumar TS. Evaluation of superoxide dismutase levels in local drug delivery system containing 0.2% curcumin strip as an adjunct to scaling and root planing in chronic periodontitis: a clinical and biochemical study. *J Pharm Bioallied Sci*. 2016;8:S48-S52.
30. Zhang Y, Gu Y, Lee HM, et al. Design, synthesis and biological activity of new polyenolic inhibitors of matrix metalloproteinases: a focus on chemically-modified curcumins. *Curr Med Chem*. 2012;19:4348-4358.
31. Zhang Y, Golub LM, Johnson F, Wishnia A. pKa, zinc- and serum albumin-binding of curcumin and two novel biologically-active chemically-modified curcumins. *Curr Med Chem*. 2012;19:4367-4475.
32. Liu R, Bal HS, Desta T, et al. Diabetes enhances periodontal bone loss through enhanced resorption and diminished bone formation. *J Dent Res*. 2006;85:510-514.
33. Sanz M, Ceriello A, Buysschaert M, et al. Scientific evidence on the links between periodontal diseases and diabetes: consensus report and guidelines of the joint workshop on periodontal diseases and diabetes by the International Diabetes Federation and the European Federation of Periodontology. *J Clin Periodontol*. 2018;45:138-149.
34. Elburki MS, Rossa C, Guimaraes MR, et al. A novel chemically modified curcumin reduces severity of experimental periodontal disease in rats: initial observations. *Mediators Inflamm*. 2014;2014:959471.
35. de Oliveira RR, Schwartz-Filho HO, Novaes AB, et al. Antimicrobial photodynamic therapy in the non-surgical treatment of aggressive periodontitis: cytokine profile in gingival crevicular fluid, preliminary results. *J Periodontol*. 2009;80:98-105.
36. Luchesi VH, Pimentel SP, Kolbe MF, et al. Photodynamic therapy in the treatment of class II furcation: a randomized controlled clinical trial. *J Clin Periodontol*. 2013;40:781-788.
37. Ribeiro FV, Casati MZ, Casarin RC, et al. Impact of a triclosan-containing toothpaste during the progression of experimental peri-implant mucositis: clinical parameters and local pattern of osteo-immunoinflammatory mediators in peri-implant fluid. *J Periodontol*. 2018;89:203-212.
38. Ribeiro FV, Pino DS, Franck FC, et al. Resveratrol Inhibits periodontitis-related bone loss in rats subjected to cigarette smoke inhalation. *J Periodontol*. 2017;88:788-798.
39. Corrêa MG, Pires PR, Ribeiro FV, et al. Systemic treatment with resveratrol reduces the progression of experimental periodontitis and arthritis in rats. *PLoS One*. 2018;13:e0204414.
40. Collins KH, Herzog W, Reimer RA, Reno CR, Heard BJ, Hart DA. Diet-induced obesity leads to pro-inflammatory alterations to the vitreous humour of the eye in a rat model. *Inflamm Res*. 2018;67:139-146.

41. Chen Z, Xue J, Shen T, Mu S, Fu Q. Curcumin alleviates glucocorticoid-induced osteoporosis through the regulation of the Wnt signaling pathway. *Int J Mol Med*. 2016;37:329-338.
42. Otto F, Thornell AP, Crompton T, et al. Cbfa1, a candidate gene for cleidocranial dysplasia syndrome, is essential for osteoblast differentiation and bone development. *Cell*. 1997;89:765-771.
43. Gu Q, Cai Y, Huang C, Shi Q, Yang H. Curcumin increases rat mesenchymal stem cell osteoblast differentiation but inhibits adipocyte differentiation. *Pharmacogn Mag*. 2012;8:202-208.
44. Son HE, Kim EJ, Jang WG. Curcumin induces osteoblast differentiation through mild-endoplasmic reticulum stress-mediated such as BMP2 on osteoblast cells. *Life Sci*. 2018;193:34-39.
45. Milne JC, Lambert PD, Schenk S, et al. Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes. *Nature*. 2017;450:712-716.
46. de Kreutzenberg SV, Ceolotto G, Papparella I, et al. Downregulation of the longevity-associated protein sirtuin 1 in insulin resistance and metabolic syndrome: potential biochemical mechanisms. *Diabetes*. 2010;59:1006-1015.
47. Gounden S, Phulukdaree A, Moodley D, Chuturgoon A. Increased SIRT3 expression and antioxidant defense under hyperglycemic conditions in HepG2 cells. *Metab Syndr Relat Disord*. 2015;13:255-263.
48. Gounden S, Chuturgoon A. Curcumin upregulates antioxidant defense, lon protease, and heat-shock protein 70 under hyperglycemic conditions in human hepatoma cells. *J Med Food*. 2017;20:465-473.
49. Ding Y, Yang H, Wang Y, Chen J, Ji Z, Sun H. Sirtuin 3 is required for osteogenic differentiation through maintenance of PGC-1α-SOD2-mediated regulation of mitochondrial function. *Int J Biol Sci*. 2017;13:254-264.
50. Oelze M, Knorr M, Schuhmacher S, et al. Vascular dysfunction in streptozotocin-induced experimental diabetes strictly depends on insulin deficiency. *J Vasc Res*. 2011;48:275-284.
51. King AJ. The use of animal models in diabetes research. *Br J Pharmacol*. 2012;166:877-894.
52. Zhang H, Gan LU, Zhu X, et al. Moderate-intensity 4mT static magnetic fields prevent bone architectural deterioration and strength reduction by stimulating bone formation in streptozotocin-treated diabetic rats. *Bone*. 2018;107:36-44.
53. Seo KI, Choi MS, Jung UJ, et al. Effect of curcumin supplementation on blood glucose, plasma insulin, and glucose homeostasis related enzyme activities in diabetic db/db mice. *Mol Nutr Food Res*. 2008;52:995-1004.
54. Jang E-M, Choi M-S, Jung UJ, et al. Beneficial effects of curcumin on hyperlipidemia and insulin resistance in high-fat-fed hamsters. *Metabolism*. 2008;57:1576-1583.
55. Öner-İyidoğan Y, Koçak H, Seyidhanoğlu M, et al. Curcumin prevents liver fat accumulation and serum fetuin-increase in rats fed a high-fat diet. *J Physiol Biochem*. 2013;69:677-686.
56. Na LX, Li Y, Pan HZ, et al. Curcuminoids exert glucose lowering effect in type 2 diabetes by decreasing serum free fattyacids: a double-blind, placebo-controlled trial. *Mol Nutr Food Res*. 2013;57:1569-1577.
57. Chuengsamarn S, Rattanamongkolgul S, Phonrat B, Tungtrongchitr R, Jirawatnotai S. Reduction of atherogenic risk in patients with type 2 diabetes by curcuminoid extract: randomized controlled trial. *J Nutr Biochem*. 2014;25:144-150.
58. Mohammadi A, Sahebkar A, Iranshahi M, et al. Effects of supplementation with curcuminoids on dyslipidemia in obese patients: a randomized crossover trial. *Phytother Res*. 2013;27:374-379.

**How to cite this article:** Pimentel SP, Casati MZ, Ribeiro FV, et al. Impact of natural curcumin on the progression of experimental periodontitis in diabetic rats. *J Periodont Res*. 2020;55:41–50. https://doi.org/10.1111/jre.12683