Subgingival irrigation with phytotherapics adjunct to scaling and root planing on the treatment of experimental periodontal disease in rats

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Abstract

Aim: To evaluate subgingival irrigation with *Matricaria recutita* (MAT) and *Plantago major* (PLA) adjunct to scaling and root planing (SRP) on treatment of experimental periodontitis (EP).

Design: EP was induced in 72 rats. After 7 days, animals were randomly distributed in groups: SRP – SRP and irrigation with saline; MAT - SRP and irrigation with MAT solution; and PLA - SRP and irrigation with PLA solution. Euthanasia was performed after 7, 15 and 30 days (n=8). It was evaluated colony-forming units (CFU), bone loss (BL), percentage of mature and immature collagen fibers, TRAP, RANKL and OPG ($p < 0.05$).

Results: Groups MAT and PLA had significantly lower number of CFU than Group SRP (15 days). Group PLA had significantly lower BL than Group MAT (7 days). Group MAT had significantly higher percentage of immature collagen fibers than groups SRP and PLA (15 days). Group PLA presented significantly higher OPG than Group SRP (7 days) and significantly lower RANKL than groups MAT and SRP (15 days).

Conclusions: Combine MAT or PLA with SRP to treat EP presented additional antimicrobial and anti-inflammatory effects when compared to SRP. However, PLA presented significantly higher collagen maturation and protective effect against bone resorption than MAT.

Keywords: Periodontitis; herbal medicine; Plantago; Chamomile; immunohistochemistry.

Introduction

Periodontal disease is a chronic inflammatory disease considered a public health problem because it affects most of the population and because it is diagnosed as the main reason for edentulism (Papapanou *et al.*, 2018; Pradeep *et al.*, 2013). Periodontal diseases have beginning and progress related to the interaction between an immune response to the host and colonization by periodontopathogenic microorganisms, such as *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia*. Also, it can be aggravated by environmental and behavioral factors (Nagasri *et al.*, 2015). They provoke local inflammatory responses but corroborate to trigger several systemic conditions in the body, such as arteriosclerosis and several other diseases (Kolte *et al.*, 2019).

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With purpose of achieve disease control, more conventional periodontal therapy (scaling and root planing or SRP), is the most common treatment used to reduce periodontopathogens and inflammation (Nagarakanti *et al.*, 2015; Bhatia *et al.*, 2014; Anuradha *et al.*, 2015). In some cases, it is necessary to associate an antimicrobial agent to completely eliminate microorganisms present in deep and/or strait pockets, and furcation regions (Anuradha *et al.*, 2015; Matesanz-Pérez *et al.*, 2013). To avoid the use and adverse effects of antibiotics, researchers seek alternative therapeutic resources (Nagasri *et al.*, 2015; Almeida *et al.*, 2019; Behal *et al.*, 2011; Shah *et al.*, 2016; Hugar *et al.*, 2016). Herbal medicines, used since ancient times, have antimicrobial, antioxidant, antiseptic, anti-inflammatory and anti-collagenase properties (Almeida *et al.*, 2019; Behal *et al.*, 2011; Shah *et al.*, 2016; Hugar *et al.*, 2016; Lins *et al.*, 2013; Moro *et al.*, 2018). Contribute to improving the population's access to prevent and treat formal economically viable diseases, in addition to having synergistic effects of its phytochemicals, a set of compounds composed of several molecules that are the target of studies of integrated performance of the organism, lower costs and easy access (Pai *et al*., 2019).

Some studies have pointed out several pharmacological effects of *Matricaria recutita L*. and *Plantago major L*. due to their phytochemical aspects (Nardini *et al*., 2019; Kumar *et al*., 2009). *Matricaria recutita L.*, family *Asteraceae*, popularly known as chamomile, is considered a medicinal plant by anti-inflammatory, antimicrobial, antioxidant, anxiolytic, antimutagenic, healing, antidiabetic, antiseptic, spasmolytic, antidiarrheal, neuroprotective and antiallergic effects (Kumar *et al*., 2009; Cárcamo *et al*., 2011). In its composition, there are volatile compounds, sesquiterpene lactones and phenolic compounds, such as flavonoids and coumarins (Lucena *et al*., 2009). The chemical constituents actively present chamomile extracts are isolated phenolic compounds that are not eligible or bioactive (Lucena *et al*., 2009). The main component of essential oil extracted from chamomile is the terpenoid α-bisabol (Anushree *et al*., 2015). Bioactivity of *Plantago major L*., from the *Plantaginaceae* family, popularly known as *Tanchagem major* or *Tansagem major*, is attributed to its chemical compounds such as flavonoids, alkaloids, phenolic compounds, caffeic acids, polysaccharides, terpenoids, lipids, iridoid glycosides, fatty acids and chemicals (Navarro *et al*., 1998). It has been widely used due to its gastroprotective, hepatoprotective, antiulcerative, anti-diabetic, anti-inflammatory, anti-inflammatory, anti-cancer, anti-nociceptive, antioxidant, anti-hypertensive, anti-microbial and anti-viral properties (Kumar *et al*., 2009).

Some studies have evaluated anti-inflammatory and antimicrobial effects of *Matricaria recutita* and *Plantago major* in Dentistry. *Matricaria recutita* demonstrated through randomized clinical trials to be as effective as chlorexidine to reduce plaque and bleeding indexes (Lins *et al*., 2013; Cárcamo *et al.*, 2011; Lucena *et al*, 2009). *Plantago major* have shown to be as effective as triclosan in toothpaste to reduce biofilms of oral microorganisms *in vitro* (Anushree *et al*., 2015) and its effectiveness can have residual effects in mouthrinse for long periods in a serial cases report (Navarro *et al*., 1998).

Further studies are needed to evaluate the effectiveness of herbal medicines in reducing microorganisms and inflammatory reactions as well as its indication to periodontal treatment. Therefore, the purpose of the present study was to evaluate the influence of subgingival irrigation with *Matricaria recutita* (MAT) and *Plantago major* (PLA) coadjutant to scaling and root planing (SRP) on the treatment of experimental periodontitis (EP) in rats.

Materials and Methods

Ethical assessment and experimental model

The research was carried out respecting the ethical principles of animal experimentation established by the Brazilian College of Animal Experimentation, and the ARRIVE guide (Animal Research: Reporting of in vivo Experiments). The experimental protocol was approved by the Ethics Committee on Animal Experimentation of the University of Western São Paulo - Unoeste (Protocol 4496). The animals were kept in shared ventilated cages with 3-4 animals/cage under a controlled environment with 12-hour cycles of light per day and temperature between 22-24ºC. Food and water were offered ad libitum.

It was used 72 male rats (*Rattus norvegicus, albinus*, Wistar), weighing 250 to 300 g. The animals were randomly assigned to 3 experimental groups: SRP – SRP and irrigation with saline; MAT - SRP and irrigation with MAT solution; and PLA - SRP and irrigation with PLA solution. Each experimental group was subdivided into 3 subgroups (n = 8) for euthanasia at 7, 15 or 30 postoperative days. Figure 1 shows experimental design of the study.

To perform all procedures, animals were anesthetized by intramuscular injection with ketamine (Dopalen, Agribands Purina do Brasil Ltda., Paulinia, SP, Brazil) (70 mg/kg) and xylazine (Coopazine, Coopers, São Paulo, São Paulo, Brazil) (6 mg/kg).

Acute experimental periodontitis (EP) induction in the mandibular left first molar of each rat (de Molon *et al*., 2018; Johnson, 1975) and scaling and root planing (SRP) protocol were performed as previously described (Prietto *et al*., 2020). Briefly, a cotton thread (Corrente Algodão no. 24, Coats Corrente, São Paulo, São Paulo, Brazil) was tied

around the tooth and kept for 7 days. If ligature was not in position after 7 days, the animal was excluded. After 7 days, ligature was removed and SRP was performed with a curette (1–2 Min. Five curette, Hu-Friedy, Chicago, IL). It was performed ten distal–medial and cervical–occlusal traction movements over the buccal and lingual surfaces, interproximal and furcation area.

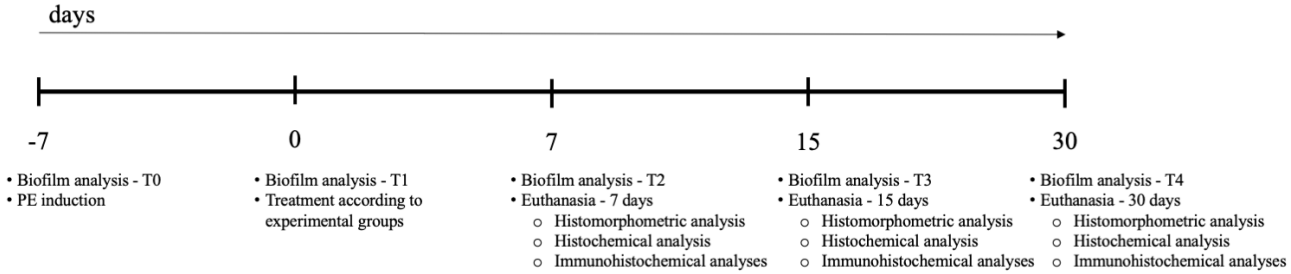

Figure 1. Scheme illustrating the experimental design of the study.

Subgingival irrigation

After SRP, animals from Group SRP received subgingival irrigation with 1 ml of saline solution. Animals from groups MAT and PLA received, respectively, subgingival irrigation with 1 ml of *Matricaria recutita* solution and *Plantago major* solution produced in pharmacy (Apothicário Farmácia de Manipulação, Araçatuba, SP, Brazil) by evaporation of ethanol/water. Solutions were inserted slowly into the periodontal pocket, using a 1 ml syringe and insulin needle without bevel.

Microbiological analysis

Biofilm samples were collected before (T0) and 7 days after (T1) the EP induction, and 7 (T2), 15 (T3) and 30 days (T4) after treatments, before the euthanasia. For this, sterile absorbent paper tips number 20 (Dentsply Maillefer, Ballaigues, Switzerland) were inserted into the periodontal pockets and kept for 1 minute. The tips with biofilm samples were transferred to microtubes containing 1 mL of Brain Heart Infusion (BHI). Microtubes were then vortexed for 10 seconds and the biofilm suspensions, serially diluted in saline solution. Afterwards, each dilution was plated in triplicate on BHI agar. The agar plates were aerobically incubated at 37°C and the number of colony-forming units (CFUs), counted after 48 h. Microbiological results were represented as Log10 CFU/mL.

Euthanasia and laboratorial processing

At 7, 15 or 30 days after treatments, animals were euthanized with an anesthesia (Thiopental, Cristália Produtos Químicos Farmacêuticos LTDA, Itapira, SP, Brasil) overdose (150 mg/kg) and laboratorial processing was performed. Histological sections of each experimental group and period were submitted to hematoxylin and eosin (H.E.) staining (histomorphometric analysis), to picrosirius red staining (collagen maturation analysis) or to indirect immunoperoxidase method to detect tartrate-resistant acid phosphatase (TRAP), ligand of the nuclear factor kappa B activator receptor (RANKL) and osteoprotegerin (OPG) (osteoclastogenesis analysis).

For Picrosirius red staining, the histological sections were deparaffinized, hydrated and immersed in a Sirius F3BA solution in aqueous picric acid for 1 hour. The colored sections were washed in two baths of 0.5% acetic acid solution for 1 minute. After dehydration, the sections protected with mounting medium and glass cover slip.

For immunoperoxidase method, the histological sections were submitted to the same reactions described by Santinoni *et al*. (2020).

Histomorphometric analysis

Images of the furcation region of histological sections stained with H.E. were captured with a digital camera connected to a microscope. The area of bone loss (BL) in the furcation region was determined by an examiner calibrated and blind to the treatments, using an image analysis program (Image J - National Institutes of Health, Washington, DC, USA (ImageJ 1.51p https://imagej.nih.gov/ij/download.html).

Histochemical analysis

Histological sections stained with Picrosirius red were analyzed under polarized light microscopy. Images of the furcation region of the histological sections stained with picrosirius red were captured with a digital camera connected to a polarized light microscope at 40x magnification. Using a color limit function of a software (Leica ICC50 HD, Wetzlar, Germany), it was selected furcation region which was the interest area. After, it was used the function "RGB Measure" that provide information about red (R), green (G) and blue (B) of the

circulated area. Values of red (R) were used to calculate percentage of mature collagen fibers in the furcation region and values of green (G) were used to calculate percentage of immature collagen fibers in the furcation region (Santinoni *et al*., 2020).

Immunohistochemical analyses

Number of TRAP-positive cells in the furcation region was quantified. Immunostaining for OPG and RANKL in the furcation region were semi-quantified through scores following the criteria of Santinoni *et al.* (2020). Briefly, absence of immunostaining (score 1), low pattern of immunostaining (score 2), moderate pattern of immunostaining (score 3) and high pattern of immunostaining (score 4).

Statistical analysis

All tests considered a significance level of 5%. Data were separately analyzed. Microbiological data were evaluated by one software (SigmaPlot, version 12.0; Systat Software Inc., San Jose, USA). Histomorphometric, histochemical and immunohistochemical data were analyzed in other software (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY, USA: IBM Corp.).

All data were submitted to normality verification by Shapiro-Wilk test. Subsequently, to verify the differences among groups, Student-Newman-Keuls test was performed for microbiological analysis; ANOVA followed by Tukey post-test for histomorphometric and histochemical analyses, and TRAP-positive cells; and Kurskal-Wallis for OPG and RANKL.

Results

Microbiological analysis

Biofilm samples at T0 presented significantly higher CFU than biofilm samples at T1. For T3, treatments with MAT and PLA resulted in CFU counts significantly lower than that noted for the Group SRP (Figure 2). However, significant differences among treatments were not observed for T2 and T4 (Figure 2).

Histological analysis

In Group SRP, it was observed an intense inflammatory infiltrate in the connective tissue in the furcation region at 7 days. At 15 and 30 days, it was observed a moderate inflammatory infiltrate. Bone loss occupied approximately half the furcation region.

In groups MAT and PLA, it was observed a moderate inflammatory infiltrate in the connective tissue in the furcation region at 7 days. At 15 and 30 days, it was observed low inflammatory infiltrate. Bone loss occupied approximately a quarter or less of the furcation region (lower than Group SRP).

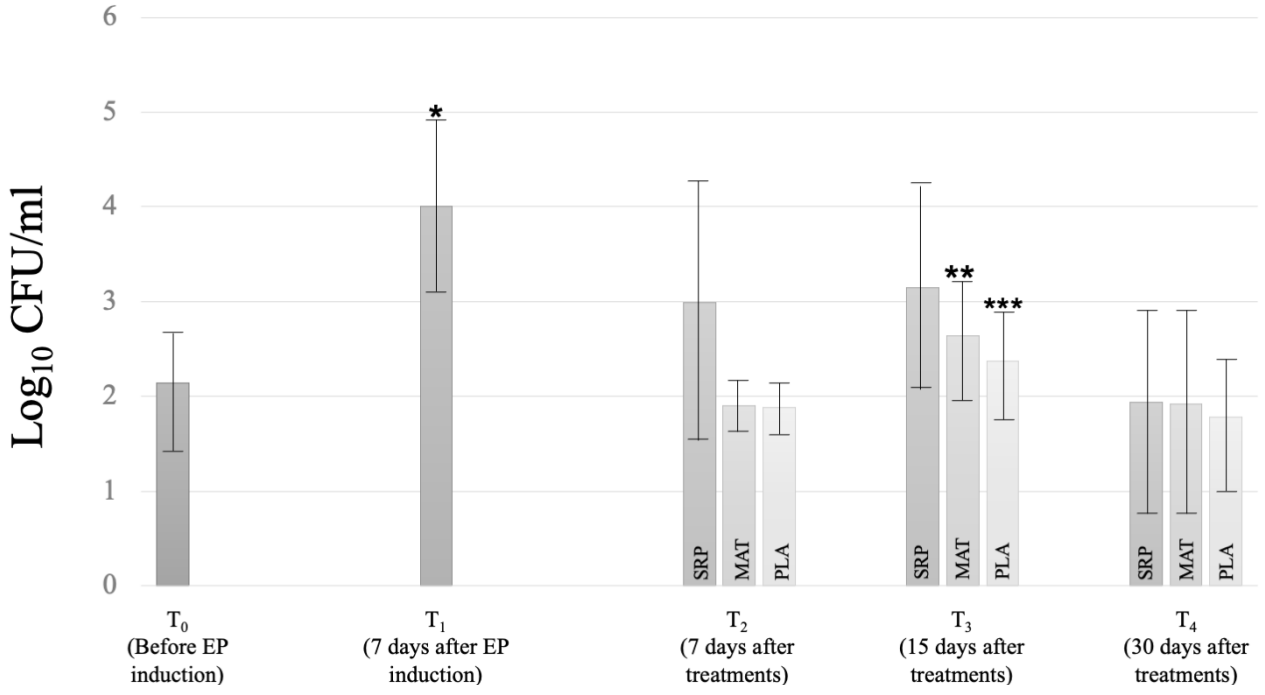

Figure 2. Mean values of the logarithm of colony-forming unit per mL (Log10 CFU/ml) obtained from the biofilm collected for each experimental group (7, 15 and 30 days) after treatments. Abbreviations and symbol: MAT, Matricaria recutita; PLA, Plantago major; SRP, scaling and root planing; CFU, colony-forming unit; *, significantly higher than T0; **, significantly lower than SRP group, within 15 days; ***, significantly lower than SRP group, within 15 days.

The inter-radicular septum was irregular for all experimental groups at 7 days. Table 1 shows parameters assessed in inflammatory infiltrate analysis of the mandibular first molar in all experimental groups. Parameters followed study by Zuza *et al*. (2018). Figure 3 show representative images of each experimental group and period.

Histometric analysis

Table 2 shows means and standard deviations of the percentage of BL in the furcation region in each experimental group and period, as well as the results of the intergroup comparisons. In the intragroup comparisons, no statistically significant differences were observed.

Table 1. Parameters assessed in inflammatory infiltrate analysis of the mandibular first molar in all experimental groups.

Parameters and scores	Percentage of animals								
	Parameters and scores								
	SRP			MAT			PLA		
	7d	15d	30d	7d	15d	30d	7d	15d	30d
Intensity of local inflammatory infiltrate									
(0) Absence of inflammation	28.57	0	0	33.33	37.5	66.67	42.86	42.86	33.33
(1) Small number of inflammatory cells	42.86	57.14	62.5	33.33	50	16.67	57.14	28.57	66.67
(2) Moderate number of inflammatory cells	28.57	42.86	37.5	33.33	12.5	16.67	0	28.57	0
(3) Large number of inflammatory cells	0	0	0	0	0	0	0	0	0
Extension of local inflammatory infiltrate									
(0) Absence of inflammation	28.57	0	0	33.33	37.5	66.67	42.86	42.86	33.33
(1) Extending to part of the connective tissue of the furcation area	71.43	100	100	66.67	62.5	33.33	57.14	57.14	66.67
(2) Extending to the whole connective tissue of the furcation area	0	0	0	0	0	0	0	0	0
(3) Extending to the whole connective tissue and to the bone tissue of the furcation area	0	0	0	0	0	0	0	0	0

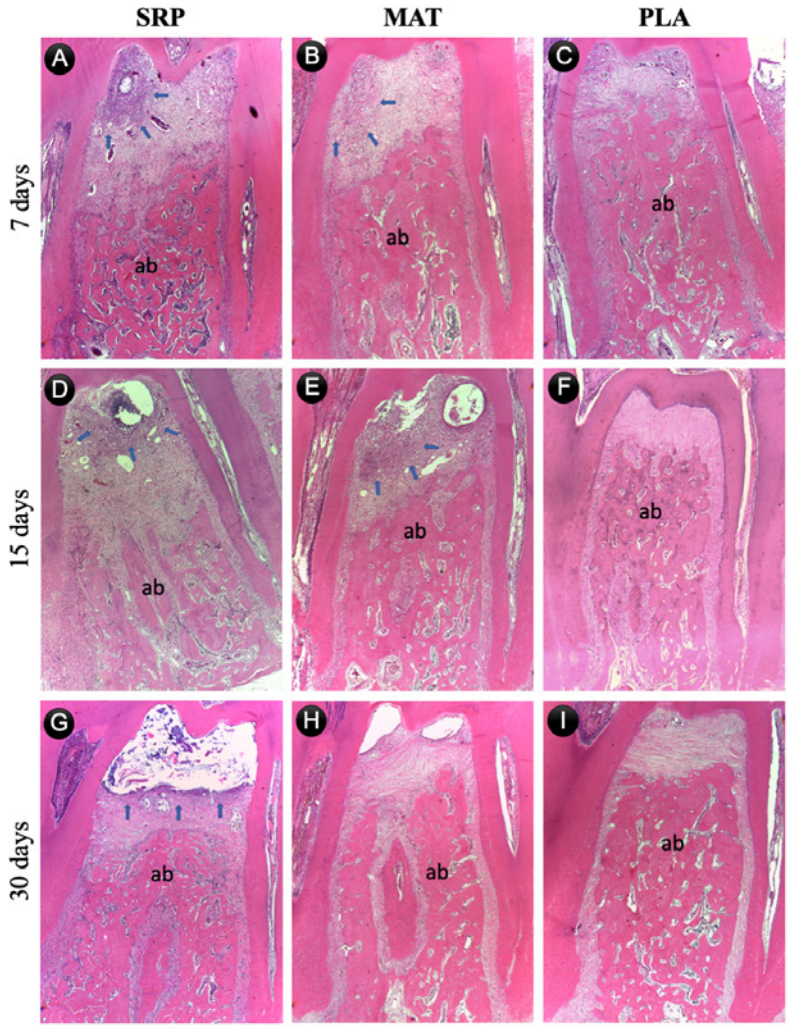

Figure 3. Photomicrographs showing BL area in the furcation region in groups SRP, MAT and PLA, respectively, 7 (A-C), 15 (D-F) and 30 (G-I) days after treatments. Blue arrows show intense inflammatory infiltrate in the connective tissue in groups SRP and MAT. It can be also noted extensive alveolar bone loss in group SRP greater than groups MAT and PLA. Group PLA did not present high concentration of inflammatory cells. Abbreviations: ab, alveolar bone; MAT, Matricaria recutita; PLA, Plantago major; SRP, scaling and root planing. Hematolixin and eosin staining; 40x.

Table 2. Means ± standard deviations (SD) of the percentage (%) of bone loss (BL) in the furcation region for each experimental group and period, and the result of the intergroup comparisons (p value).

	SRP			MAT			PLA		
	7d	***15d***	***30d***	***7d***	***15d***	***30d***	***7d***	***15d***	***30d***
Mean	8.78	9.84	10.06	14.08	11.16	9.21	6.62*	11.31	6.36
SD	5.76	5.03	5.91	7.73	5.42	4.85	3.32*	5.42	2.86

Intergroup comparisons:
*Significantly lower than Group MAT at 7 days (p=0.023).

Histochemical analysis (collagen maturation)

Figures 4A and 4B shows mean and standard deviation of percentage of immature and mature collagen fibers for each experimental group and period, as well as the result of intergroup comparisons. Figures 4C, 4D and 4E present photomicrographs of histological sections stained with Picrosirius red under polarized light in groups SRP, MAT and PLA at 15 days, respectively. In the intragroup comparisons, no statistically significant differences were observed.

Immunohistochemical analyses

It was not observed statistically significant differences among experimental groups and period regarding number of TRAP-positive cells. Figure 5A shows mean and standard deviation of number of TRAP-positive cells for each experimental group and period. Figures 5B-D show representative images of TRAP-immunolabeling.

Table 3 presents scores observed for immunostaining with both OPG and RANKL for each experimental group and period, as well as the results of intergroup comparisons. Figures 5E-G present, respectively, photomicrographs showing immunolabeling for RANKL in groups SRP, MAT and PLA at 15 days and Figures 5H-J present, respectively, photomicrographs showing immunolabeling for OPG in groups SRP, MAT and PLA at 7 days.

Discussion

The present study aimed to evaluate the influence of subgingival irrigation with two natural extracts associated with SRP in the treatment of experimental periodontitis and to compare the results with the conventional treatment of experimental periodontitis (SRP and irrigation with saline). This objective was based on the need for local application of products that have antimicrobial and / or anti-inflammatory action within the periodontal pockets as an adjunct to mechanical debridement, specifically in sites with periodontitis that did not regress after conventional treatment (Nagarakanti *et al*., 2015; Matesanz-Pérez *et al*., 2013; Tan *et al*., 2020). Among the advantages for the local use of chemical agents, including herbal products, the following can be evidenced: maximizing its effect in specific sites and the prevention of systemic toxicity and problems related to the patient's lack of commitment (Batista *et al*.,2014; Kartini *et al*., 2017; Gomes *et al*., 2018).

In the present study, groups treated with MAT and PLA showed less inflammatory infiltrate when compared to the control group in all experimental periods. In addition, these groups had less biofilm formation at 15 days postoperative and Group PLA presented significantly lower bone loss than Group MAT at 7 days. Thus, it can be inferred that MAT and PLA solutions have the potential to improve the results of conventional periodontal treatment.

Despite differences in experimental models, it can be inferred that results observed in Group MAT corroborate with other studies, which demonstrated that this plant has potential to improve periodontal healing through antimicrobial action and anti-inflammatory effect (Lins *et al*., 2013; Cárcamo *et al*., 2011; Lucena *et al*., 2009; Goes *et al*., 2016). These clinical studies compared the effectiveness of the mouthwash with chlorhexidine or MAT in patients with gingivitis. Results showed great reduction in plaque indexes with MAT that was so efficient as chlorhexidine. It is possible that benefits of MAT on periodontal healing are due to antioxidant activity of polyphenol and flavonoid content that down regulate both free-radical scavenging activity and expression of matrix metalloproteinases (Al-Dabbagh *et al*., 2019). In this context, it is also important consider that antioxidant activity of MAT demonstrated act in a dose dependent way (Al-Dabbagh *et al*., 2019). Here, we used MAT extract produced in pharmacy by evaporation of ethanol/water. Although the concentration was not evaluated here and it is a limitation of the present study, considering previous studies that performed same extraction method, it can be inferred we had an estimated concentration of 13.51% (Al-Dabbagh *et al*., 2019; Roby *et al*., 2013). However, the method to obtain the extract as well as the part of the plant may influence the result of substance used. Here, it was used all the plant in Group PLA. In in Group MAT, it was used only the flower, the same part used in the studies by Al-Dabbagh *et al*. (2019) and Roby *et al*. (2013).

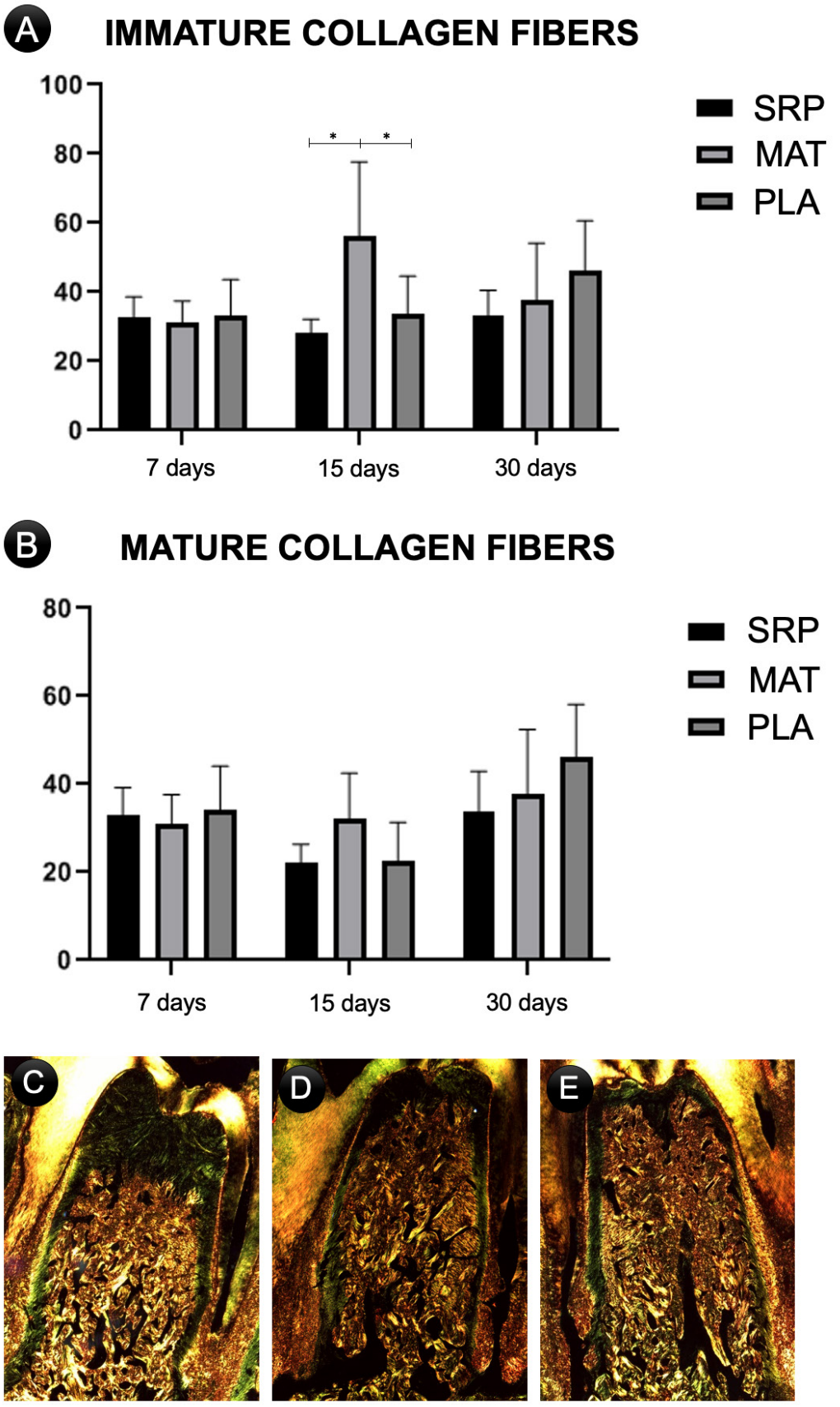

Figure 4. Graphs showing the percentage of immature (A) and mature (B) collagen fibers, and photomicrographs showing the maturation of collagen in groups SRP (C), MAT (D) and PLA (E) 15 days after treatments. Note that the Group MTA has greenish color compared with groups SRP and PLA that have a more yellow color. Abbreviations and symbol: ab, alveolar bone; MAT, Matricaria recutita; PLA, Plantago major; SRP, scaling and root planing; *, significantly higher than groups SRP and PLA at 15 days. Picrosirius red staining under polarized light; 40x.

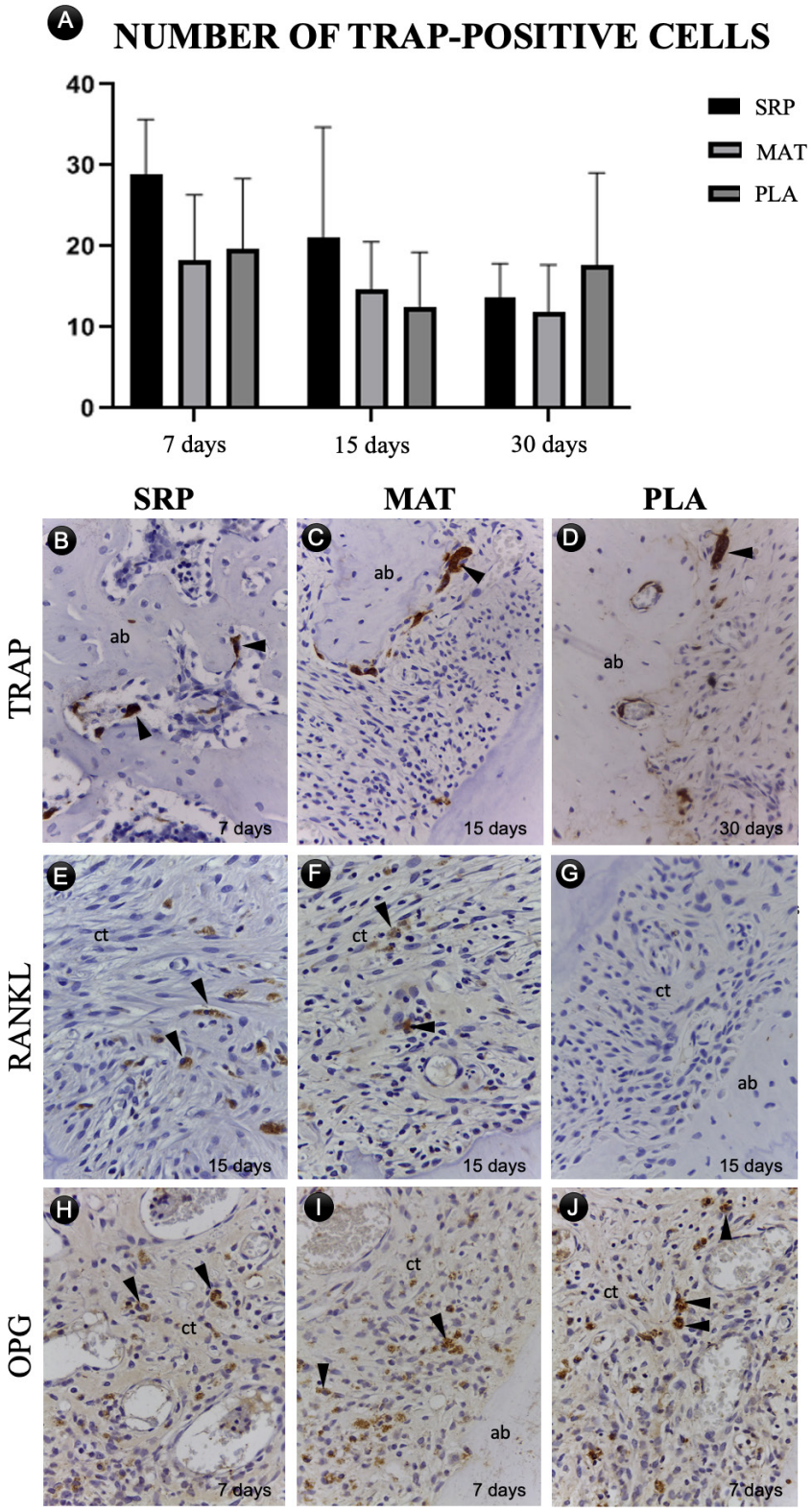

Figure 5. Graph (A) showing number of TRAP-positive cells in the experimental groups and period, in different analyzed periods. Photomicrographs showing immunolabeling for TRAP in groups SRP, MAT and PLA, respectively, 7 (B), 15 (C) and 30 (D) days after treatments presenting same immunolabeling pattern among groups. Photomicrographs showing RANKL-immunolabeling in groups SRP (E), MAT (F) and PLA (G) 15 days after treatments and photomicrographs showing OPG-immunolabeling in groups SRP (H), MAT (I) and PLA (J) 7 days after treatments. Groups SRP and MAT present similar pattern of RANKL-immunolabeling while Group PLA did not present immunolabeling in this histological section. Regarding OPG, Group PLA presents immunolabeling pattern higher than groups SRP and MAT. Abbreviations: ab, alveolar bone; ct, connective tissue; MAT, Matricaria recutita; PLA, Plantago major; SRP, scaling and root planing. Counterstaining with hematolixin; 400x.

Table 3. Scores observed for immunostaining with OPG and RANKL for each experimental group and period, in the different periods.

Marker	Score	SRP			MAT			PLA		
		7d	*15d*	*30d*	*7d*	*15d*	*30d*	*7d*	*15d*	*30d*
OPG	1	0/7	0/7	0/7	0/7	0/7	0/6	0/7	0/7	1/7
	2	2/7	3/7	2/7	3/7	2/7	3/6	0/7	3/7	3/7
	3	0/7	2/7	4/7	0/7	1/7	3/6	0/7	1/7	2/7
	4	5/7	2/7	1/7	4/7	4/7	0/6	7/7	3/7	1/7
	Mean	***2.40***	***1.86***	***1.86***	***2.14***	***2.29***	***1.50***	***3.00****	***2.00***	***1.43***
RANKL	1	1/7	1/6	1/6	2/6	0/7	1/6	4/7	6/7	3/7
	2	3/7	4/6	5/6	2/6	6/7	4/6	3/7	1/7	3/7
	3	2/7	1/6	1/6	1/6	1/7	1/6	0/7	0/7	1/7
	4	1/7	0/6	0/6	1/6	0/7	0/6	0/7	0/7	0/7
	Mean	***1.42***	***0.63***	***0.63***	***1.16***	***1.14***	***1.00***	***0.42***	***0.28*****	***0.71***

Intergroup comparisons:
**Significantly higher than Group SRP at 7 days (p=0.040).
***Significantly lower than groups MAT (p= 0.011) and SRP (p=0.045) at 15 days.

Results observed with PLA also corroborate previous studies demonstrated potential of its antimicrobial and anti-inflammatory effects to be used in Dentistry and Periodontics. Anushree *et al*. (2015) carried out an *in vitro* study to compare the antimicrobial effect of toothpaste containing triclosan or PLA and concluded that this herbal agent can be as effective as conventional antimicrobial agents already used. Navarro *et al*. (1998) revealed the effectiveness in reducing the biofilm indices of patients treated with PLA mouthwash and a residual effect of this plant even after 42 days (Navarro *et al*., 1998). This characteristic is important to prevent recolonization (Goes *et al*., 2016). PLA may have remained for a longer time than MAT in periodontal tissues, leading to better healing results. This characteristic of remaining for a long period in periodontal tissues is one of the required characteristics of an antimicrobial agents used for the treatment and prevention of periodontal diseases, known as substantivity (Matesanz-Pérez *et al*., 2013). In addition to substantivity, PLA showed other desirable effects of antimicrobial agents, like significant reduction of bacterial biofilm and inflammation (Matesanz-Pérez *et al*., 2013). Also, it has been demonstrated presence of oligosaccharides in PLA that have beneficial effects on human health (Lukova *et al*., 2017; Adom *et al*., 2017; Parhizgar *et al*., 2018).

The present study is the first to evaluate histochemically and immunohistochemically the effects of subgingival irrigation with MAT and PLA associated with SRP to treat EP in rats. Considering that the group treated with MAT showed a percentage of immature collagen fibers significantly higher than the groups PLA and SRP, it can be suggested that the PLA showed a better result on periodontal healing than MAT. Both histomorphometric and immunohistochemical results of the present study corroborate and reinforce this hypothesis. Group PLA presented significantly lower BL than Group MAT at 7 days. Also, Group PLA presented significantly higher immunoexpression of OPG than Group SRP at 7 days and significantly lower expression of RANKL than groups MAT and SRP at 15 days. OPG has a protective effect on bone tissue and RANKL stimulates osteoclastogenesis and bone resorption (Boyce *et al*., 2008; Souza *et al*., 2013; Harada *et al*., 2011; Takahashi *et al*., 2011).

Presence of a specific component in the PLA composition may explain differences in the results obtained by each treatment in the present study. PLA present caffeic acid as one of its components (Navarro *et al*., 1998) that have been associated with oxidative stress reduction and inflammation dampen (Stähli *et al*., 2019; Li *et al*., 2017). *In vitro* studies with other plants that contains caffeic acid showed it can reduce P*orphyromonas gingivalis* and *Prevotella Intermedia* lipopolysaccharide pro-inflammatory action, and catalase antioxidant enzyme gene expression through reduction of intracellular reactive oxygen species levels and the expression of genes encoding-producing enzymes (Le Sage *et al*., 2017; Choi *et al*., 2015). With similar methodology used in the present study, Yiğit *et al*. (2017) evaluated the effect of caffeic acid on alveolar bone loss, serum cytokines (interleukin (IL)-1β, IL-6, tumor necrosis factor-α and IL-10) and gingival apoptosis, as well as the levels of antioxidants. They also evaluated low dose doxycycline combined or not with caffeic acid. Group treated with caffeic acid presented lowest alveolar bone loss, inflammatory infiltration and expression of serum cytokines among the experimental groups. The authors concluded that caffeic acid has more anti-inflammatory, antioxidant and anti-apoptotic effects than antibiotic evaluated.

In addition to MAT and PLA, other herbal products have also been evaluated as an adjunct treatment to conventional treatment of EP. Almeida *et al*. (2019) used assessed the effect of green tea extract on periodontal healing. They also performed histological analysis and immunohistochemical reactions for the detection of inflammatory proteins and osteoclasts in the furcation region. Promising results showed that the groups treated with green tea showed less inflammation, fewer osteoclasts and less BL, compared with control groups where only SRP was performed.

Few studies are found in the literature about the use of herbal products in periodontal treatment. Moro *et al*. (2018) carried out a recent systematic review to assess the effect of the application of adjuvant herbal agents to SRP on clinical parameters of patients with periodontitis compared with SRP alone. The results showed that the local combination of phytotherapics with SRP can promote additional benefits in reducing the probing depth and clinical attachment level. However, more studies are needed to better evaluate its application.

Conclusions

Within the limits of this study, it can be concluded that combine MAT or PLA with SRP to treat experimental periodontitis presented additional antimicrobial and anti-inflammatory effects when compared to SRP alone. However, PLA presented significantly higher collagen maturation and protective effect against bone resorption than MAT. Therefore, PLA presented better results in the initial stages of periodontal healing.

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