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Original Article

The effect of curcumin 1% methanolic extract on the expression of Matrix Metalloproteinase-1, Matrix Metalloproteinase-8, Matrix Metalloproteinase-13, neutrophil, macrophage, lymphocyte counts in *Porphyromonas gingivalis* induced periodontitis: a randomized controlled trial

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ABSTRACT

Objective: Hailed as one of the most fundamental and important treatment in management of periodontal disease, scalling and root planning has limitations regarding microorganism elimination. Meanwhile, turmeric has been proven to have a therapeutic effect on gingivitis and periodontitis. This study aimed to analyze the impact of curcumin 1% methanolic extract on the expression of Matrix Metalloproteinase-1, Matrix Metalloproteinase-8, Matrix Metalloproteinase -13, neutrophil, macrophage, and lymphocyte number in the gingiva of Wistar rats exposed to *Porphyromonas gingivalis bacteria* (*P. gingivalis*).

Materials and methods: The experimental animals used were Wistar rats, arbitrarilly split up into three experimental groups namely: normal, control (induced with periodontitis, received no treatment), and treatment (Periodontitis with 1% curcumin treatment). The samples were taken from the gingival tissue of the mandibular incisors on 1 and 7 days. Immunohistochemical and Hematoxylin Eosin staining were performed to measure the expression of Matrix Metalloproteinase-1, Matrix Metalloproteinase-8, Matrix Metalloproteinase-13, and the number of neutrophils, macrophages, and lymphocytes. Data were analyzed for mean standard deviation. The Oneway ANOVA was then performed to see whether there is a significant difference between experimental groups, the test then commenced with Tukey's Honestly Significant Difference test with a significance level (α=0.05).

Results: MMP-8, MMP-13, neutrophil, and lymphocyte numbers in the treatment groups were significantly lower with $P<0.05$ than in the control groups in the 1 and 7 days. Meanwhile, in MMP-1 and macrophage numbers the difference was deemed not significant when control and treatment groups are compared.

Conclusion: The administration of 1% curcumin can significantly reduce the expression of MMP-8, MMP-13, neutrophil and lymphocyte cell numbers, but there is no reducing the number of macrophage cells and MMP-1 expression in gingiva of Wistar rats exposed to *P. gingivalis* bacteria.

1. Introduction

Periodontitis is an inflammatory condition that causes degeneration of the periodontal tissue and affects the supporting structure of the teeth (Dubey and Mittal, 2020). Based on the GBD Study (2019), periodontitis is ranked 7th as utmost common disease across the world, with the incidence of 1.09 billion cases (Wiernik et al., 2024).

The presence of oral microbial biofilm, Porphyromonas gingivalis (P.

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gingivalis), could trigger inflammation of the periodontal tissue (Izui et al., 2021; Kugaji et al., 2019). In response to inflammation, PMN leukocytes, such as neutrophils, are released as the initial defense of periodontal tissue, followed by the predominance of lymphocyte cells. These lymphocytes play a crucial part in identifying the severity of inflammation due to bacterial infection (Muñoz-Carrillo et al., 2020). Macrophages are a crucial source of cytokines and inflammatory mediators that regulate bacterial diffusion in connective tissue. (Cafiero et al., 2021; Ramadan et al., 2020). Acute inflammation, characterized by an escalation of neutrophil migration through the junctional epithelium into the gingival crevice (Muñoz-Carrillo et al., 2020). As neutrophils migrate through the tissues, they release many destructive enzymes, such as matrix metalloproteinases (MMPs), which cause the periodontium's structural components to break down and collagen-depleted areas to develop (Newman et al., 2024).

MMPs break down extracellular matrix components, basement membrane, and protective protease inhibitors, substantially impacting cytokine alters, activation of osteoclast, tissue regeneration, also connective tissue attachment loss. The collagenase group of MMPs is the most frequently implicated in the pathologic condition of periodontitis. MMP-1, will destroy collagen, resulting in a decrease in collagen products. MMP-8 is produced by neutrophils and non-neutrophil-derived cells. MMP-13 is required to form a new bone matrix and can degrade fibrillar collagen, fibronectin, aggrecan, and gelatin in the extracellular matrix (Khuda et al., 2021). Previous studies have shown that MMP-1 and MMP-8 are also intensively affected in periodontal pathology (Luchian et al., 2022).

Although conventional SRP is hailed as the most fundamental and important treatment in eradication of periodontal disease, its limitation in eliminating the reservoir of microorganisms responsible for periodontal pathogens remains a drawback (Ivanaga et al., 2019). Traditional ingredients, like turmeric or Curcuma longa, have been considered extensively in treating inflammation of periodontal disease. It has been proven that turmeric has a therapeutic effect on dental and oral diseases, including gingivitis and periodontitis (Malekzadeh et al., 2021). Curcuma longa contains a curcuminoid component consisting of curcumin (*diferuloylmethane*), demethoxycurcumin, and bisdemethoxycurcumin. Curcumin, the most active polyphenolic component, has seen significant usage in traditional medicine, such as cell restoration, healing, and anticarcinogenic activity (Hassan et al., 2021). It has been proven that curcumin 1% administration could increase gingival epithelium reparation exposed by P. gingivalis LPS by suppressing NF-κB (Krismariono and Purwaningsih, 2022).

Therefore, this experiment is conducted to observe the efficacy of curcumin 1% methanolic extract on the expression of Matrix Metalloproteinase-1, Matrix Metalloproteinase-8, Matrix Metalloproteinase-13, neutrophil, macrophage, and lymphocyte counts in the gingiva of Wistar rat exposed to *P. gingivalis* bacteria.

2. Materials and methods

2.1. Study design

The experiment was submitted and authorized by The Ethical Committee of Dental Research Airlangga University(1328/HRECC. FODM/XII/2023) and being done at Biochemical, Histopathological Laboratorium Airlangga University. Wistar rats were adapted for one week and arbitrarily split up into 3 groups, namely the normal group (N), the control group (C), and the treatment group (T). The cages were labeled and then placed in a room with sufficient airflow, light, and dry conditions. Wistar rats were weighed to meet the sample criteria.

2.2. Preparation of porphyromonas gingivalis bacteria and application of treatment

P. gingivalis bacteria (ATCC 33277) were prepared for groups C and T. The rats were locally administered with 0.03 mL of *P. gingivalis* bacteria, containing ($1x10^9$) CFU, once every 2 days, and done in the gingival sulcus of mandibular first incisive of Wistar rats using an Eppendorf micropipette. This procedure was conducted to induce gingival inflammation and gingivitis. A curcumin concentrate was obtained by measuring 1 mg of curcumin powder. After that, corn oil was mixed with curcumin powder in a measuring cup until it reached 100 ml and formed a curcumin extract with a concentration of 1%. Furthermore, curcumin extract was applied topically to the gingival sulcus of incisor Wistar rats after exposure to *P. gingivalis* (Krismariono and Purwaningsih, 2022). 1% curcumin extract was administered twice daily for seven days. Subsequently, all animals were euthanized using Chloroform on day 1 also day 7 after treatment, then sample of gingival sulcus tissue from the proximal area of the mandibular incisors was obtained. The remains of the animal then receive burial in accordance with the ethics of animal experimental models.

2.3. Tissue analysis

After the tissue was processed, Hematoxylin Eosin (HE) staining and indirect immunohistochemistry (IHC) were done on the anterior part of the Wistar rat's mandible. Immunohistochemical staining was performed using primary anti-mouse monoclonal antibodies against Matrix Metalloproteinase-1, Matrix Metalloproteinase-8, Matrix Metalloproteinase-13, then continued using secondary antibodies, namely anti-mouse biotinylated.

The observations were performed using a 400x magnification ReHaze light microscope. The data collected by counting the amount of Matrix Metalloproteinase-1, Matrix Metalloproteinase-8, Matrix Metalloproteinase-13, neutrophil, macrophage, and lymphocyte cells were then evaluated for normality and homogeneity to see how the data was distributed in this study.

2.4. Statistical analysis

Data were statistically analyzed with a significance level ($\alpha = 0.05$) using IBM SPSS 26 (Chicago, IL, USA). The data is deemed to be normal after Shapiro-Wilk is done. Data homogeneity was tested using the Levene test, followed by Oneway ANOVA and Tukey's Honestly Significant Difference test. The difference is deemed to be significant statistically when $p < 0.05$.

3. Results

When we look at the result displayed on Table 1 and 2, it is known that according to each group and observation time (Control 1 and 7

Table 1
MMP-1 and MMP-13 expression analysis.

Group	Mean value ± SD	p-value
MMP-1 day 1		0.00*
Control	59.08 ± 1.68	
Treatment	51.08 ± 1.03	
MMP-1 day 7		0.00*
Control	63.89 ± 1.76	
Treatment	52.02 ± 6.30	
MMP-13 day 1		0.00*
Control	7.7 ± 0.90	
Treatment	7.4 ± 2.16	
MMP-13 day 1		0.00*
Control	47.36 ± 2.16	
Treatment	27 ± 10.01	
MMP-8 day 1		0.00*
Control	40.49 ± 2.57	
Treatment	29.93 ± 3.27	
MMP-8 day 7		0.00*
Control	57.04 ± 2.68	
Treatment	24.05 ± 2.11	

Table 2
Neutrophil, macrophage, lymphocyte cells analysis.

Group	Mean value ± SD	p-value
Neutrophil day 1		0.00*
Control	146.72 ± 2.97	
Treatment	37.84 ± 1.45	
Neutrophil day 7		0.00*
Control	87.68 ± 4.56	
Treatment	26.12 ± 2.52	
Macrophage day 1		0.00*
Control	23.30 ± 1.30	
Treatment	25.80 ± 2.77	
Macrophage day 7		0.00*
Control	31.60 ± 4.61	
Treatment	28.60 ± 1.81	
Lymphocyte day 1		0.00*
Control	11.88 ± 3.36	
Treatment	9.56 ± 0.16	
Lymphocyte day 7		0.00*
Control	25.76 ± 0.16	
Treatment	9.32 ± 0.41	

days, treatment 1 and 7 days), there was a decrease in the mean expression of MMP-8 and neutrophils, and lymphocytes cells in all observation groups on day 7 compared to 1 day. The treatment groups produced lower MMP-8 expression and neutrophils, and lymphocytes cells than the control group in the 1 and 7 days (Figs. 1 and 2). The Oneway ANOVA show significance value of 0.000 (< 0.05) and based on that the difference between group is deemed to be significant. Next, the Tukey HSD test was carried out as a Post Hoc test as seen in Table 3.

According to Table 1 and 2, the result shows that according to each group and observation time (Control 1 and 7 days, treatment 1 and 7 days), there was an elevation of mean expression of MMP-1, MMP-13 and macrophage cells in all observation groups on day 7 compared to day 1. The treatment groups produced lower MMP-1, MMP-13, and macrophage cells than the control group in the 1 and 7 days (Figs. 1 and 2). The Oneway ANOVA show significance value of 0.000 (< 0.05) and based on that the difference between group is deemed to be significant. Next, the Tukey HSD test was carried out, as a Post Hoc test as seen in Table 3.

4. Discussion

This research was conducted to determine the effect of administering curcumin, which is believed to reduce the inflammatory process induced by *P. gingivalis*. The curcumin used in this study was 1% curcumin with corn oil as a carrier. It was based on previous research in which 1% curcumin was given to Wistar rats with gingivitis, with significant results in reducing the expression of TLR-4, NFκβ, and MMP-7 when compared with control group without curcumin extract. However, the presence of corn oil as a carrier did not affect alterations in the expression of inflammation variables (Krismariono and Purwaningsih, 2022).

The average expression of Matrix Metalloproteinase-1, Matrix Metalloproteinase-8, Matrix Metalloproteinase-13 increased in the control group on day-7 compared to day-1. This result corresponded with Khuda et al. research, in which showing increased MMP expression's correlation to the development of periodontitis, one of which can be caused by *P. gingivalis* (Khuda et al., 2021).

Neutrophils, macrophages, and lymphocytes were also examined in this study. *P. gingivalis* infiltrates the gingival sulcus, resulting in an inflammatory condition. The binding of LPS of *P. gingivalis* to the toll-like receptors (TLR4/2) results in the activation of NFκβ, leading to the secretion cytokines that induce inflammation. An elevation of cytokines release leads to the migration of phagocytes (Muñoz-Carrillo et al., 2020; Newman et al., 2024; Ramadan et al., 2020). These results in the increase of neutrophils and macrophages in the control groups.

When the inflammatory response becomes chronic, the adaptive immune system's lymphocytes will be activated through a costimulatory signal from antigen-presenting cells (APCs). The binding of lymphocytes and APC would trigger an increase in lymphocyte production and migration to the site of injury, thus decreasing the endotoxin activity of *P. gingivalis* bacteria. These processes explain the increase in lymphocyte count in rats' periodontal tissue exposed to *P. gingivalis* (Muñoz-Carrillo et al., 2020; Ramadan et al., 2020).

The increase in cytokines that inducing inflammation comprised of IL-1β and TNF-α caused by *P. gingivalis* LPS and TLR-4 binding can affect the activity of other inflammatory cells, including the collagen enzyme MMPs (Izui et al., 2021). It is consistent with observations of increased Matrix Metalloproteinase-1,Matrix Metalloproteinase-8,Matrix Metalloproteinase-13 expression in the gingival tissue of Wistar rats exposed to *P. gingivalis* bacteria. The increase in MMP-1 expression in C

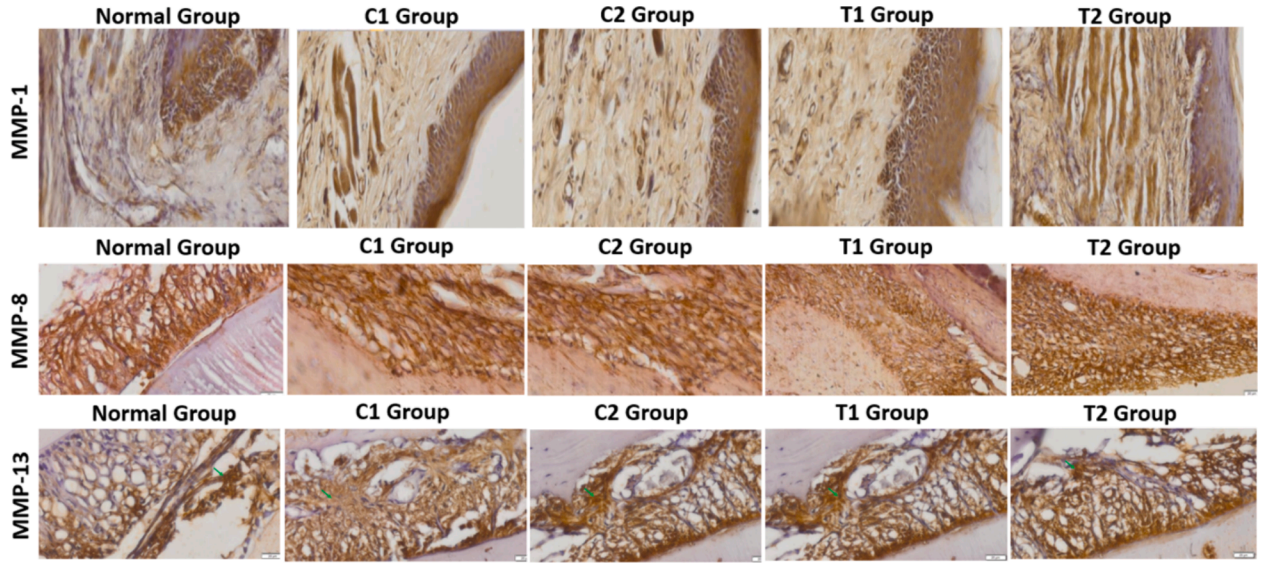

Fig. 1. HPA examination results for MMP-1, MMP-8, MMP-13 in the gingival epithelium of Wistar rats (using a microscope with 400x magnification) in Normal Group; *P.gingivalis* group on day 1 (C1 group); *P.gingivalis* group on day 7 (C2 group); *P.gingivalis*+Curcumin group on day 1 (T1 group); and *P.gingivalis*+Curcumin group on day 7 (T2 group)

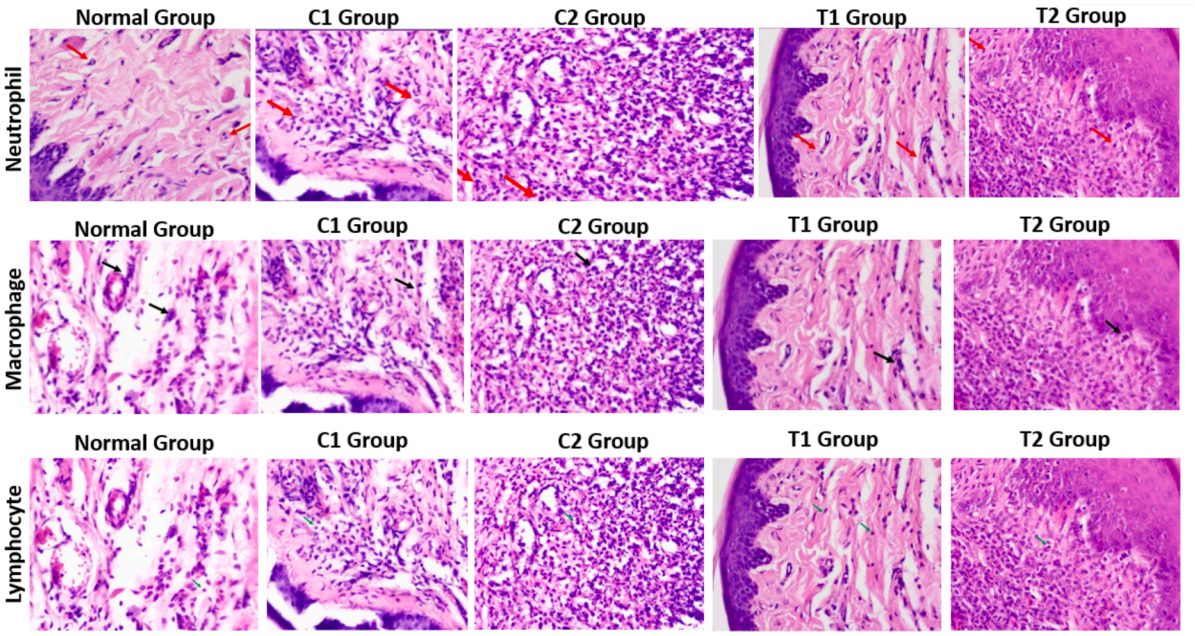

Fig. 2. HPA examination results for neutrophil (red arrows), macrophage (black arrows), and lymphocyte (green arrows) in the gingival epithelium of Wistar rats (using a microscope with 400x magnification) in Normal Group; *P.gingivalis* group on day 1 (C1 group); *P.gingivalis* group on day 7 (C2 group); *P.gingivalis*+Curcumin group on day 1 (T1 group); and *P.gingivalis*+Curcumin group on day 7 (T2 group)

(control) group was associated with elevation of macrophages level because MMP-1 is usually expressed by the macrophages, fibroblasts, and dendritic cells. Neutrophils mainly express MMP-8. Thus, increased neutrophil count in the control group was related to increased MMP-8 expression. Meanwhile, the increase in MMP-13 expression in the control group was related to an increase in lymphocytes that would act together with the gingival resident cells of Wistar rats to increase MMP-13 expression in response to the invasion of *P. gingivalis* bacteria (Khuda et al., 2021; Luchian et al., 2022).

The significant decrease in MMP-8 expression after curcumin administration on day 1 to day 7 is consistent with the previous study, which states that curcumin can reduce acetaminophen-induced hepatotoxicity by lowering MMP-8 expression (Rani et al., 2023). This finding aligns with the study that said that curcumin can inhibit the expression of MMP-8 (Heydari et al., 2023). There's also a decrease in MMP-13 expression. This aligns with the research conducted by Brochard et al., which found that curcumin has anti-inflammatory effects in human osteoarthritis synovial cells, thus decreasing MMP-13 expression (Brochard et al., 2021). Curcumin has also been shown inhibiting Matrix Metalloproteinases by blocking the activation of the NFκB signaling pathway. This was achieved by repressing the phosphorylation of Iκβa and inhibiting of p65 translocation into the nucleus (Swallow et al., 2024).

The anti-inflammatory effect of curcumin effects by inhibiting NFκB directly. The administration of 1% curcumin can increase the repair of gingival epithelium exposed to LPS of *P. gingivalis* by decreasing NFκβ expression, which is a target molecule whose activity can be inhibited by curcumin to repair gingival epithelial damage (Krismariono and Purwaningsih, 2022). Following curcumin administration, NFκβ expression decreases, which subsequently causes a decrease in expressed genes such as Matrix Metalloproteinase-8 and Matrix Metalloproteinase-13 (Augustina et al., 2019). In this study, the decrease in Matrix Metalloproteinase-8 and Matrix Metalloproteinase-13 activity for the *P. gingivalis* with curcumin group could be influenced by the activity of the cell membrane receptor, which also began to decrease in observations after one week.

The results also showed a decrease in neutrophil and lymphocyte cells. This finding is consistent with previous research, which stated that administering curcumin to zymosan-induced arthritis rats inhibited the inflammatory response of the neutrophils during the first six hours of administration (Iweala et al., 2023). The number of lymphocyte cells also decreased from day-1 to day-7, in the control and treatment group. This aligns with the previous studies that have shown curcumin administration reduces inflammatory cells, such as neutrophils and lymphocytes (Benameur et al., 2023).

It is known that administering 1% curcumin twice a day daily to the gingival sulcus of rats exposed to *P. gingivalis* bacteria can decrease the number of variables studied. This aligns with previous studies stating that curcumin has antibacterial and anti-inflammatory properties. The antibacterial effect of curcumin against oral pathogens such as *P. gingivalis* has also been widely proven (Muñoz-Carrillo et al., 2020). Curcumin in the cell can affect TLR-2 signaling towards NFκβ activation, where the presence of curcumin can inhibit NFκβ activation in activating pro-inflammatory cytokines to produce inflammatory mediators. Curcumin can reduce the expression of anti-inflammatory cytokines (Peng et al., 2021). The decrease in the number of pro-inflammatory cytokines indicates that there has been a decrease in inflammatory activity, allowing signals to inflammatory cells as a form of advanced immune response to stop. In addition, a decrease in NFκB activity will increase the secretion of cytokines inducing inflammation process, namely Interleukin-10 and Tumor Growth Factor-β (Zhang et al., 2021).

The results of observations of MMP-1 expression and the number of microscopical macrophage cells counts on days 1 and 7 increase. This was attributed to the extended onset of curcumin and the brief duration of its effects. As a result, when treated with curcumin 20-30 minutes after exposure to *P. gingivalis* bacteria, the anti-inflammatory effect of curcumin has not yet worked, but the bacteria have invaded the host, and acute inflammation has occurred. During instances of acute inflammation, macrophages are deployed to combat pathogens and secrete both anti-inflammatory and pro-inflammatory cytokines. This may potentially explain the rise in macrophage cell counts. Macrophages can release matrix metalloproteinases (MMPs) in response to inflammation, leading to an increase in MMP levels. Hence, further research is necessary to examine the efficacy of curcumin, as the limited duration of the study may not accurately represent the lasting impacts of curcumin treatment. Furthermore, the concentration of curcumin used

Table 3
Results of post hoc test analysis.

MMP-1 Expression Analysis			
Time of Observation	**Groups**		**Significance**
Day-1	C1	T1	.013**
	T1	C1	.013**
Day-7	C2	T2	.272*
	T2	C2	.272*
MMP-8 Expression Analysis			
Time of Observation	**Groups**		**Significance**
Day-1	C1	T1	.000**
	T1	C1	.000**
Day-7	C2	T2	.000**
	T2	C2	.000**
MMP-13 Expression Analysis			
Time of Observation	**Group**		**Significance**
Day-1	C1	T1	.941*
	T1	C1	.941*
Day-7	C2	T2	.013**
	T2	C2	.013**
Neutrophil Cell Count Analysis			
Time of Observation	**Group**		**Significance**
Day-1	C1	T1	.000**
	T1	C1	.000**
Day-7	C2	T2	.000**
	T2	C2	.000**
Macrophage Cell Count Analysis			
Time of Observation	**Group**		**Significance**
Day-1	C1	T1	.224*
	T1	C1	.224*
Day-7	C2	T2	.428*
	T2	C2	.428*
Lymphocyte Cell Count Analysis			
Time of Observation	**Group**		**Significance**
Day-1	C1	T1	.191*
	T1	C1	.191*
Day-7	C2	T2	.000**
	T2	C2	.000**

Information:
*) significance $p>0.05$
**) significance $p<0.05$

in this study does not account for potential dose-response variations, and the limited outcome measures may not provide an adequate assessment of its therapeutic efficacy.

5. Conclusion

Based on this study, it can be inferred that the administration of 1% curcumin can reduce the expression of MMP-8, MMP-13, neutrophil and lymphocytes cells in the gingiva of Wistar rats exposed to P. gingivalis bacteria. However, it does not affect the expression of MMP-1 and the number of macrophage cells.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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