Evaluate the Anti-inflammatory Effects of Nanocinnamon Gel on Salivary Cytokines:Tumor Necrosis Factor-alpha (TNF-α) and Interleukine-6 (IL-6) in Chronic Gingivitis

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الخلاصة

الأهداف :تهدف الدراسـة الـى تقييم الأثـار المضـادة للالتهابـات لهـلام القرفـة النـانوي علـى تركيز كـل مـن عامل نخرالـورم - الفـا و انترلوكين -٦ في لعاب مرضى التهاب اللثة المزمن . **المواد وطرائق العمل** : تم تحضير هلام القرفة النانوي الفموي بتركيز (٢٪) مـن مسحوق القرفة النانوي. جرت الدراسة على التجارب السريرية العشوائية شملت (٤٥) مريض مصاب بألتهـاب اللثـة المـزمن تراوحـت أعمارهم بين (١٨-٥٠)سنة ,قسموا الى ثلاثة مجاميع كل مجموعة تضم خمسة عشر مريضا , المعالجة كانت تتضـمن عـلاج ميكـانيكي لتنظيف وتلميع الأسنان في العيادة ثم العلاج الدوائي ,فالمجموعة الاولى أستخدمت العلاج الميكانيكي فقط ,المجموعة الثانيـة استخدمت هلام الكلورو هيكسيدين الموضعي اما المجموعة الثالثة استخدمت هلام القرفة النانوي موضعيا, يستعمل العلاج مرتين فـي اليـوم ويبقـى لمدة عشر دقائق على الاقل لمدة ثلاث اسابيع ,ثم يتم جمع اللعاب غير المستحث بواسـطة السـلافيت لكـل فـرد علـى الأقـل ٢ سـاعة بعد تناول الطعام لأستبعاد تأثير تحفيز الغذاء على أفرازات اللعاب. تـم قيـاس كـل مـن انترلـوكين -٦ وعامـل نخرالـورم - الفـا فـي اللعاب بواسطة جهاز قياس المناعة المرتبط بالانزيم (ELISA).تم أستخدام أختبار كروسكل لتحليل البيانـات بـين المجـاميع وأختبـار فريـدمان للأزمنة خلال المجموعة الواحدة. **النتائج**:أظهرت النتائج الأحصائية لهـذه الدراسـة الـى عـدم وجـود دلالـة أحصـائية فـي معـدل تركيز الأنترلوكين -٦ في اللعاب بعد العلاج في المجاميع الثلاث بينما أظهرت النتائج أنخفاضـا معنويـا لمسـتوى عامـل نخرالـورم - الفـا فـي اللعاب لمجموعة القرفة النانوي بعد ٢١ يوم من فترة العلاج عند المقارنة مع المجموعة الأولى القياسية. **الأستنتاجات**: أن أستخدام جـل القرفة النانوي له تأثير مضاد لألتهاب اللثة وأختلاف تركيزكل من الأنترلوكين -٦ وعامل نخرالورم – الفـا فـي اللعـاب يمكـن أن يكـون مؤشر يساعد في تشخيص أمراض اللثة.

ABSTRACT

Aims: The study aimed to evaluate the anti-inflammatory effects of nanocinnamon gel on salivary concentrations of cytokines: interleukine-6(IL-6) and tumor necrosis factor-alpha (TNF-α) in chronic gingivitis.**Material and Methods**: prepared nanocinnamon gel 2% concentration from nanocinnamon powder. The study was carried out on the randomized clinical trial of chronic gingivitis comprises of 45 patients of age between (18-50)years old .They were divided into three groups, fifteen for each group , treatment involved mechanical therapy by scaling and polishing at dental clinic and drug treatment in which the first group applied to the mechanical treatment alone, , second group received chlorhexidine gel topically and third group received nanocinnamon gel topically , the treated groups used the gel twice daily at least ten minute for three weeks. Unstimulated saliva was collected for each individuals and at least two hours after any food intake to disregard the food stimulatory effect on salivary secretions. ELISA kits (Enzyme Linked Immunosorbent Assay) used to measure IL-6 and TNF-α in saliva . Data were analyzed using kruscal-wallias H test and Friedmans test analysis range test. **Results**: the results of present study showed that there were no significant differences in salivary IL-6 among groups after treatment however , there were significantly decreasing in salivary TNF-α of nanocinnamon group after 21 days of interval therapy in comparison to control group. **Conclusion** :The use of nanocinnamon gel have anti-inflammatory effect against gingivitis and the changes in the salivary concentrations of IL-6 and TNF-α can help in the diagnosis of periodontal disease

Key words: Cinnamon nanoparticles, chlorhexidine, gel, saliva, salivary biomarker, chronic gingivitis.

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INTRODUCTION

Gingivitis is the inflammation of the gingival tissue that takes place in response to the germs that live in biofilms at the gingival boundary and in the sulcus.It is characterized by of erythema, edema, bleeding after probing and blunting of the interdental papillae[1]. Mechanical removal of the plaque usually results in the arrest of the inflammatory disease and in the case of gingivitis, returns of the gingival tissue to normal with no tissue destruction and loss of attachment of the junctional epithelium to the tooth [2].Saliva is a secretory product of the salivary glands producing stability in the oral cavity [3].Saliva maintains the proper environment of the oral cavity and a proper amount of saliva ensures the integrity of the oral mucosa and periodontal tissues[4] Nanoparticles (NPs)are tiny ingredients having size ranges from 1 to 100 nm and its retain unique chemical and physical properties due to their great surface area and nanoscale size [5] . Nanoparticles (NPs) are currently being investigated and used in many areas of medicine that allow for specific drug delivery [6] [8]. Herbs have been used for centuries to prevent and

control periodontal disease. Hence, the search for natural phytochemicals isolated from plants used in traditional medicine is reflected as good alternatives to synthetic chemicals [9]. Cinnamon has been used as a traditional remedy for treating toothache , infection in the mouth, ability to remove bad breath , promoting the regeneration of damaged tissues also it has been described as antimicrobial and anti-inflammatory properties [10]The distinctive smell and flavor of cinnamon comes from the essential oils contained in the bark, called cinnamaldehyde, which displays antibacterial ,antiseptic , analgesic and contains significant amounts of antioxidant polyphenols[7]. The idea of a biomarker emerged from understanding the need to be able to track health status, vulnerability to disease, progression, resolution and result of care with respect to a variety of specific medical conditions [12].Biomarkers were defined as "cellular, biochemical, molecular, or genetic changes whereby a normal, abnormal, or simply biological process can be identified or monitored"[3].Differential host responses are thought to contribute to different susceptibilities which play an important

role in determining inflammatory lesion progression [13][14].At the cellular level, exposure to bacterial products and lipopolysaccharides causes monocyte / macrophage activation that promotes cytokines and inflammatory mediators secretion, such as IL-6 and TNF- α, resulting in the release of matrix metalloproteinases (MMPs) [15] .These inflammatory cytokines and enzymes are detectable in oral fluids[16][17] . IL-6 acts as both a proinflammatory cytokine and an anti-inflammatory myokine which is secreted by T cells and macrophages to stimulate immune response during infection or other tissue damage leading to inflammation [11]. TNF-α is a key pro-inflammatory cytokine at several levels and is central to anti-microbial immunity. The latter which produces mainly by macrophages ,endothelial cells and fibroblasts[18]. The study aimed to evaluate the anti-inflammatory effects of nanocinnamon gel on salivary cytokines :TNF-α and IL-6 in chronic gingivitis patients.

MATERIALS AND METHODS

The protocol of our study was approved by the scientific committee of Nineveh Helth Directorate at Al-Noor Specialist Dental Center and in University of Mosul/ College of Dentistry/Department of Dental Basic Sciences (No. D.B.S./4/2432019-5) During the period between October 2019 and January 2020. participant provided vocal and written information explaining

the purpose of the research and signed an informed consent by person form before registration in the study. The preparation of nanocinnamon powder done by attrition method to obtain partials size between (1-100) nanometer. Preparation of natural product was conducted by taking 100 grams of cinnamon crust then it was cleaned ,wash with water and dried in the shade with the presence of air.The crust was broken into small pieces then was ground[19]. These preparation of nanocinnamon particles have been tested by Transmission electron microscopy (philips CM10,Holland origin) in Al-Nahrain University /College of Medicine in Baghdad) and the size was between (10-35) nm. According to the Shende,*et al.* 2018[20], nanocinnamon gel 2% for oral application was prepared . This randomized clinical trial was carried out on 45 patients, aged between (18-50) year with chronic gingivitis. The inclusion criteria obtained via a questionnaire included no mouth or systemic disease, non alcoholic ,non smoker ,non pregnant ,probing depth ≤ 3mm, good oral hygiene and non-sensitive to cinnamon. Patients were asked to cease from eating and drinking two hours before to saliva collection to obtain a relatively constant baseline. We took three groups, each group consist of 15 patients . Treatment involved mechanical therapy by scaling and polishing at dental clinic and chemical treatment in which the first

group prescribed control group ,the second prescribed chlorhexidine group and the third nanocinnamon group . Each individual in group 2 and 3 after brushing were instructed to apply chlorhexidine gel 0.2% and nanocinnamon gel 2% topically respectively on the inflamed gingiva for at least 10 minutes twice a day for three weeks and thereafter rinsed with water to clear any residual medication .

Saliva collection:

Non-stimulated mixed saliva is currently assessed at different time intervals (Baseline, 7 days and 21 days) due to its simple method of achievement and the ease of its attainment without the need for specialized equipment [4]Furthermore, it shows a greater diagnostic value when compared to stimulated saliva; unstimulated saliva was collected for each individuals and at least 2 hours after any food intake to exclude the food stimulatory effect on salivary secretions. The individual asked to wash his mouth three times with 30ml distilled water to ensure complete removal of any remnant food or debris, then the individual seated in relaxed position and asked to accumulate saliva in his mouth by using salivette(Every salivette consists of a test tube with a smaller jar inserted into it and a cotton wedge similar to that used by a dentist, collect all saliva samples with these tubes and take away the lid from the tube containing the cotton wedge, remove the cotton wedge and gently chew until the cotton is saturated,

then bring the cotton back into the tube and firmly put the lid back on. (Make sure the cotton is placed in the bottle in the upper part of the test tube) ,then salivary sample placed in a centrifuge at 3000 rpm for 10 minute in centrifuge[21].

According to Aurer *et al*, the clear fluid was placed in sterile eppendroff tube and stored at deep freeze $(-20)^{o}c$, to be softened for analysis of anti-inflammatory mediators IL-6 and TNF- α . [22]

Statistical analysis

Statistical package for social sciences(SPSS) program was used to analyze the obtained data by using kruscal-wallis H test for independent sample and Friedman̓s test analysis for related samples

RESULTS

Measurement of Human IL-6 and TNF-α concentration in Saliva:

TNF-α and IL-6 in saliva were measured by Enzyme Linked Immunosorbent Assay (ELISA) device, a Sandwich-ELISA kit. IL-6 was measured by using Salimetrix salivary IL-6 (ELISA) kit (USA) ,while TNF-α was measured by using MyBioSource.com human TNF-α (ELISA) kit, these were based on Standard Sandwich-ELISA Assay Technology. According to the manufacturer instructions, the results were expressed as picogram per milliliter (pg/ml) for each mediator All analyzes were conducted in duplicate within 3 months of receiving the sample.

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All runs have included criteria, and all tests are recorded within the linearity of assays. The standard curve can be plotted as the relative optical density of the standard solution (X).The saliva TNF- α concentration of the sample can be interpolated from the standard curve of TNF- α Figure (1) while the saliva IL-6 concentration of the sample can be interpolated from the standard curve of IL-6 Figure(2) .
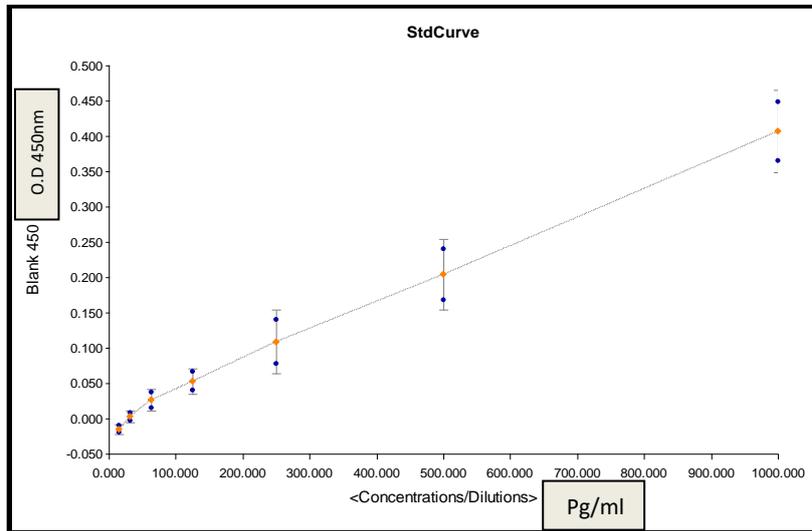
X: concentration of sample TNF-α **Y**:absorbance value of sample TNF-α determined by ELISA analysis.

Figure (1) Standard curve of saliva TNF- α by ELISA analysis
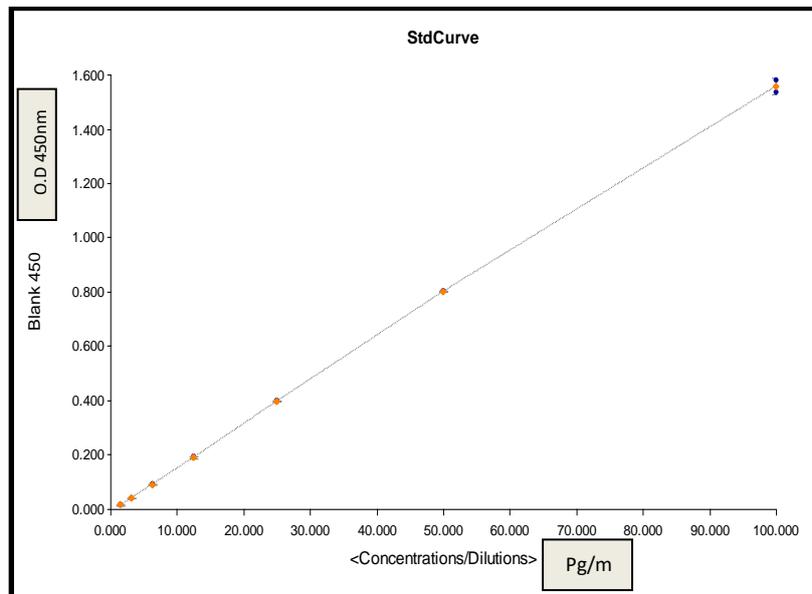
X: concentration of sample IL-6 **Y**:absorbance value of sample IL-6 determined by ELISA analysis

Figure (2): Standard curve of saliva IL-6 by ELISA analysis

Evaluation of Salivary IL-6 concentration

In the present study ,the results of saliva IL-6 concentration in all groups.

were statistically non-significant difference, as illustrated in Table (1), Table (2) and Figure (3).

Table (1): Comparison of Salivary IL-6 level among three groups at indicated time interval

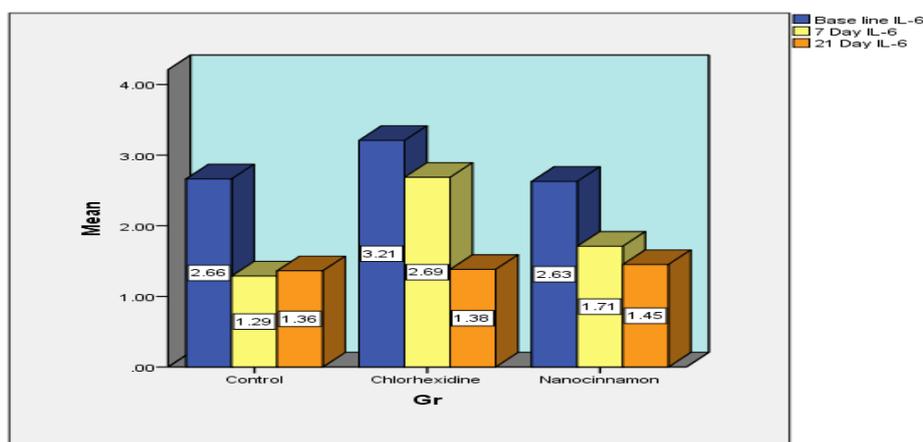| Time	Parameter			Kruskal-wallis test (independent samples)	P-value
	Control	Chlorhexidine	Nanocinnamon		
Base line IL-6	The distribution is the same across categories of Group			0.554	0.758
7 DAY IL-6				0.080	0.981
21 D Y IL-6				0.395	0.821

Figure (3) : comparison of salivary IL-6 mean rank at the indicated time intervals for nanocinnamon , chlorhexidine and control group

Table (2): Comparison the mean of salivary IL-6 level for the related samples at indicated time interval

Parameter	Time	Mean Rank	Friedman's test (Related samples)	P-value
Control	Base line IL-6	2.62	3.33	1.89
	7 DAY IL-6	1.38		
	21 D Y IL-6	2.00		
Chlorhexidine	Base line IL-6	2.58	3.55	1.70
	7 DAY IL-6	1.83		
	21 D Y IL-6	1.58		
Nanocinnamon	Base line IL-6	2.38	3.40	1.83
	7 DAY IL-6	1.96		
	21 D Y IL-6	1.67		

Salivary TNF-α concentration

The results showed that the level   of salivary TNF-α   concentration were non-

significant at baseline in three groups ,whereas the mean of salivary TNF-α was decrease significantly in nanocinnamon group after 21 days of treatment in compared to control group. As well as there was non significant differen in mean of salivary TNF-α concentration in chlorhexidine group after treatment,as illustrated in Table (3), Table (4), Table (5), Figure (4).

Table (3): Comparison of Salivary TNF-α level among three groups at indicated time interval

Time	Parameter			Kruskal-wallis test (independent samples)	P-value
	Control	Chlorhexidine	Nanocinnamon		
Base line IL-6	The distribution is the same across categories of Gr.			0.494	0.781
7 DAY IL-6				0.597	0.742
21 D Y IL-6				6.451	0.040

Table (4) :Comparison of Salivary TNF-α level between two groups by kruscal test.

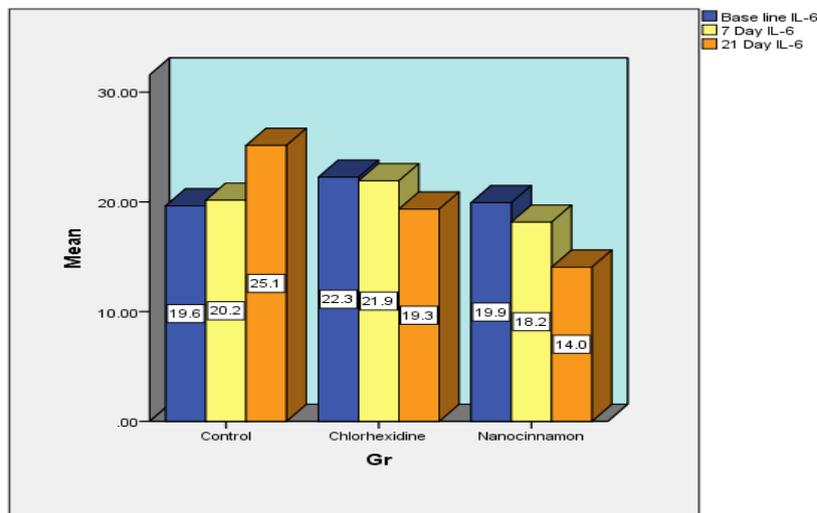| Comparison	Kruskal-wallis test (independent samples)	P-value
Control- Chlorhexidine	2.583	1.000
Control- Nanocinnamon	7.738	0.040
Chlorhexidine –Nanocinnamon	5.155	0.298

Figure (4): Comparison the mean rank of salivary TNF-α concentration at the indicated time intervals among nanocinnamon , chlorhexidine and control group .

Table (5): Comparison the mean rank of salivary TNF-α concentration for the related samples among three groups at the indicated time interval.

Parameter	Time	Mean Rank	Friedman's test (Related samples)	P-value
Control	Base line IL-6	1.50		
	7 DAY IL-6	1.83	4.33	0.115
	21 D Y IL-6	2.67		
Chlorhexidine	Base line IL-6	2.17		
	7 DAY IL-6	2.33	2.33	0.311
	21 D Y IL-6	1.50		
Nanocinnamon	Base line IL-6	2.43	5.42	0.066

DISCUSSION

The occurrence and progression of the periodontal disease depend on the periodontal microflora and the host's multi-faceted response, and cytokines and chemokines mediate these interactions [1]. Cytokines play an important role in the host response to the periodontal film [16], also they are essential to the pathogenesis of periodontal disease and may be used as diagnostic markers.[17]

In our study, we established an elevation in salivary level of cytokines, IL- 6 & TNF-α at baseline in all groups which correlated with multiple inflammatory disorders as in chronic gingivitis which in agreement with Jaedicke et al, and Jeffry et al, who found that diagnosis of periodontal disease and follow up treatment will progress dramatically soon with the development of disease specific salivary biomarkers, while difficulties remain, the use of saliva to assess periodontal health tends to help diagnose periodontal diseases and predict periodontal diseases. Treatment outcomes[23][43].

Other studies have focused on TNF-α analysis, providing various results,some of which reports difficulties in establishing TNF- α in saliva [22]. As well as other reports showing no statistical difference in TNF-alpha levels in patients diagnosed with periodontitis and the control group [24][25]. However, Frodge et al.,2008 showed that not only TNF-α levels to be significantly increased in patients with periodontal disease compared to the control group, but also a correlation of this cytokine level with the number of sites with bleeding on probing, loss of attachment and the number of periodontal pockets.[26]

In present study ,the level of TNF-α was statistically decreased in saliva after three weeks of treatment in nanocinnamon group compared to control group . This result in agreement with Sexton et al, who showed that TNF-α decrease significantly after therapy [27]. Joshi et al, demonstrated that the use of ethanol extract of cinnamomum Zeylanicum suppression of intracellular TNF-α release

into murine neutrophils and pleural fluid leukocytes[28].It was informed that the extract of cinnamomum zeylanicum inhibited TNF- α and probably accounted for its anti-inflammatory effect [29]. Addition to that, the results of our study was consistants with other studies who showed that statistically significant reduction of TNF- α after treatment of periodontal disease by herbal. [30][31][32]

Interleukin-6 is produced and raised in many inflammatory diseases. However the results of the present study showed no significant changes between and within the participaated groups. These findings were in agreement with results of previous studies who showed that there were no significant changes of salivary interleukin-6 concentrations post treatment of periodontal disease[33][43] Ebersole et al, [25] was showed the level of interleukin-6 concentration measured in saliva are little in both periodontal health and disease, while various studies like Rathnayake et al,[35]have been reported that salivary interleukin-6 concentration was significantly higher in periodontitis than in healthy individuals, other study was create no differences between the periodontal disease and healthy individuals [36]

These results indicated that shifting in the balance between the activities of pro-inflammatory and antiinflammatory cytokines during the periodontal inflammation could affect the intensity and duration of inflammation. The severity, length and resolution of the inflammation depend on the balancing of the production of proinflammatory and anti-inflammatory cytokines during periodontal infection [37].In addition to that , the range of cytokines concentration in saliva was often quite variable ,this variability in many studies may reflect the complex multifactorial nature of the disease and differences in sampling techniques and assay used for analysis. [33][38].

The mechanism of cinnamon as antiinflammatory agent represented by the fact that cinnamon can suppress nuclear factor kappa B (NF-k) and inhibited cytokines (IL-2, IL-4, and IFNγ) release from concanavalin-stimulated lymphocytes in vitro, as well as the redox sensitive, pro-inflammatory nuclear transcription factor NF-kappaB plays a key role in inflammation.[39][41] Hong et al, showed that cinnamon water extract (CWE) inhibited the expression of TNF-α in vivo and in vitro model, so in this research, the polyphenol-rich CWE fraction strongly inhibited the disruption of inhibitory protein kinase(IÿBα) and mitogen-activated protein (MAP) kinase phosphorylation induced by lipopolysaccharide in macrophages.[42] Other mechanisms of cinnamon proved the inhibition of angiogenesis by cinnamon extract through blocking the endothelial growth factor 2 (VEGF2) signaling and reducing the proliferation of endothelialcells, migration and tube forming [43].

CONCLUSION

Salivary concentrations of IL-6 and TNF-α can distinguish health from gingivitis and periodontitis. In the present study cytokines(IL-6 and TNF-α) were reduced after treatment with nanocinnamon gel in patients with gingivitis. The results suggest the need for clinical studies that confirm the antiinflammatory effects of nanocinnamon gel in the treatment of periodontal disease.

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