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ORIGINAL ARTICLE

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Efficacy of quercetin nano-emulgel as adjuvant local delivery drug in non-surgical treatment of periodontitis

Eficácia do nano-emulgel de quercetina como terapia adjuvante de liberação local no tratamento não cirúrgico da periodontite

Khoshoe Al-mokhtar MOHAMMED[1], Alzahraa A. ALGHRIANY[1], Abdullah Ibrahim Abd Rabbouh ALI[2], Helal F. HETTA[3], Ibrahim M. MWAFEY[2]

1 - Assiut University, Faculty of Dentistry, Department of Oral Medicine, Periodontology, and Oral Diagnosis. Assiut, Egypt.
2 - Al-Azhar University, Assiut Branch, Faculty of Dental Medicine, Department of Oral Medicine, Periodontology, Oral Diagnosis and Dental Radiology. Assiut, Egypt.
3 - University of Tabuk, Faculty of Pharmacy, Department of Natural Products and Alternative Medicine, Division of Microbiology, Immunology and Biotechnology. Tabuk, Saudi Arabia.

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ABSTRACT

Objective: The objective of this study was to assess the impact of quercetin nano-emulgel on clinical and biochemical markers in patients with stage I and II periodontitis as an adjuvant to scaling and root planing (SRP). **Material and Methods:** Two groups were randomly assigned to 20 test sites from patients with stage I and II periodontitis: Group I, in which 10 sites got scaling and root planing, and Group II, in which 10 sites received scaling and root planing alongside quercetin nano-emulgel. Clinical parameters were recorded at baseline, 1 month, and 3 months; these included plaque index (PI), gingival index (GI), probing depth (PD), and clinical attachment level (CAL). Biochemical evaluations were conducted to measure the gingival crevicular fluid (GCF) interferon-gamma (IFN-γ) level and total antioxidant capacity at baseline, 1 month, and 3 months. **Results:** All clinical variables improved after treatment in both groups, with significant improvement in Group II, which was higher than that in Group I at different intervals. The GCF total antioxidant level revealed a significant rise, while the IFN-γ level showed a significant decrease throughout the study within both groups, with no significant difference between the 2 groups. **Conclusion:** Quercetin nano-emulgel showed promising results in improving clinical and biochemical parameters in treating periodontitis When used in conjunction with scaling and root planing. This encourages using quercetin nano-emulgel in clinical practice as an adjunctive treatment for stage I and II periodontitis.

KEYWORDS

Drug Delivery System; Emulgel; Nanomedicine; Periodontitis; Quercetin.

RESUMO

Objetivo: O objetivo foi avaliar o impacto do nanoemulgel de quercetina nos marcadores clínicos e bioquímicos de pacientes com periodontite estágio I e II como adjuvante à raspagem e alisamento radicular (RAR). **Material e Métodos:** Vinte sítios de pacientes com periodontite estágio I e II foram aleatoriamente distribuídos em dois grupos: Grupo I, no qual 10 sítios foram tratados com RAR, e Grupo II, no qual 10 sítios receberam RAR em associação com o nanoemulgel de quercetina. Os parâmetros clínicos foram registrados no início, após 1 mês e 3 meses, incluindo índice de placa (IP), índice gengival (IG), profundidade de sondagem (PS) e nível de inserção clínica (NIC). Avaliações bioquímicas foram realizadas para medir os níveis de interferon-gama (IFN-γ) e a capacidade antioxidante total no fluido gengival crevicular (FGC) nos mesmos períodos. **Resultados:** Todas as variáveis clínicas apresentaram melhora após o tratamento em ambos os grupos, com uma melhora significativamente maior no Grupo II em comparação ao Grupo I em diferentes momentos da avaliação. Os níveis de capacidade antioxidante

total no FGC aumentaram significativamente, enquanto os níveis de IFN-γ diminuíram ao longo do estudo em ambos os grupos, com mudanças mais expressivas no Grupo II em relação ao Grupo I. **Conclusão:** O nanoemulgel de quercetina demonstrou resultados promissores na melhoria dos parâmetros clínicos e bioquímicos no tratamento da periodontite quando utilizado em conjunto com a raspagem e alisamento radicular. Esses achados incentivam o uso do nanoemulgel de quercetina na prática clínica como tratamento adjuvante para periodontite estágio I e II.

PALAVRAS-CHAVE

Sistema de Liberação de Fármacos; Emulgel; Nanomedicina; Periodontite; Quercetina.

INTRODUCTION

Periodontitis is a multifactorial inflammatory condition affecting the teeth's supporting tissues that causes progressive deterioration of the periodontal ligament and alveolar bones, resulting in irreparable tooth loss if left untreated. It is defined as a complex disease in which the biofilm interacts with the host's immunoinflammatory response, resulting in changes in bone and connective tissue homeostasis. Numerous studies indicate that both the host's immune system and the commensal oral microbiota that colonizes the biofilm are responsible for the initiation and progression of this periodontal disease [1]. Furthermore, immune cells' reactive oxygen species (ROS) further aggravate gingival tissue injury [2].

Mechanical debridement, scaling and root planing (SRP), is the mainstay of periodontal treatment. To allow periodontal tissues to adhere to the treated root surface in the future, it aims to remove supragingival and subgingival biofilms and restore the biological compatibility of periodontally diseased root surfaces. Nevertheless, not all pathogenic microorganisms in the subgingival region can be eradicated by instrumentation [3]. Adjunctive pharmacological drugs can increase the effectiveness of mechanical therapy. However, systemic drugs have limitations due to their systemic effects, leading to ineffective medication concentrations at the site of action, which negatively impacts patient outcomes. Therefore, it was essential to design other localized delivery methods [4]. This necessity prompted researchers to investigate alternative localized drug delivery systems. Various in situ localized drug delivery systems have been evaluated with favorable outcomes for periodontitis [5]. However, in certain clinical circumstances, such as localized deep pockets or during supportive periodontal therapy, commercially available local antimicrobials might be useful. However, because of an unclear cost-benefit ratio, their clinical usefulness is limited [6].

In this context, the academic community began concentrating on adjuvants to conventional periodontal therapy which are less likely to induce side effects and lack antibiotic resistance. Recent research has proven phytochemicals to be useful in the treatment of periodontitis [7,8]. Among the organic phenolic chemicals found in fruits and vegetables are flavonoids, which possess anti-inflammatory, antibacterial, and antioxidant qualities [9].

Quercetin is an example of these crucial flavonoids. It has been proven that quercetin possesses significant pharmacological characteristics as an antioxidant [10], anti-inflammatory [11], antimicrobial [12], antineoplastic [13], and antiallergic [14].

Due to its potent antibacterial action against periodontal microorganisms, quercetin can be used as an adjuvant local delivery medication in the non-surgical treatment of periodontitis [15]. Quercetin may impact mature plaque biofilm's morphology and metabolic activity [16]. Due to its anti-inflammatory properties, quercetin may reduce inflammation caused by lipopolysaccharide (LPS) by interfering with the nuclear factor kappa-B (NF-κB) signaling pathway and inhibiting the production of inflammatory cytokines such as IL-12, IFN-γ, IFN-α, IL-6, IL-8, cyclooxygenase-2, and prostaglandin E2 [17]. Quercetin inhibits the release of reactive oxygen species (ROS) by increasing the expression of antioxidant enzyme-related genes such as catalase (CAT), glutathione peroxidase 3 (GPx3), quinone oxidoreductase 1 (NQO-1), and heme oxygenase-1 (HO-1), as well as enhancing superoxide dismutase (SOD) activity [18]. Quercetin has the ability to increase collagen expression, enhance wound healing, and restore periodontal soft tissue integrity [19].

Furthermore, quercetin has a strong effect on promoting osteogenesis [20].

Even though quercetin has been shown in numerous studies to be a potential phytochemical for maintaining the integrity of periodontal tissue, its limited bioavailability, low water solubility, low absorption, high metabolic rate, and quick elimination from the body limit its practical application. As a result, scientists have focused on developing local delivery systems that avoid biopharmaceutical challenges and minimize systemic clearance, thus improving its therapeutic and prophylactic potential using various techniques [21,22].

The incorporation of oils and emulsifiers improves quercetin absorption. Therefore, nano-emulsion may be an appropriate drug delivery medium for loading quercetin [23]. That can be combined with the polymer solution to create an in-situ nano-emulgel that facilitates easy administration and sustained/controlled drug delivery, increasing patient compliance [24].

The key research question for this study was, "Does quercetin nano-emulgel, as an adjuvant local delivery drug in the phase I (non-surgical) treatment of periodontitis, improve clinical and biochemical parameters?"

MATERIAL AND METHODS

Study design and population

This randomized, controlled clinical and biochemical study was conducted from August 23, 2023, to February 2024. It involved 23 patients with stage I to stage II periodontitis, comprising 13 females and 10 males aged 20 to 45 years. Patients were chosen from individuals attending the Oral Medicine and Periodontology Departments' outpatient clinics at the Faculty of Dental Medicine, Al-Azhar University, Assiut Branch, and the Faculty of Dentistry, Assiut University. The diagnosis was made based on recording case history and conducting extraoral and intraoral clinical examinations, and patients selected for the study met the inclusion criteria.

Ethical considerations

This randomized controlled trial followed the ethical guidelines established in the Helsinki Declaration for medical research and was approved by the ethical committee of the Faculty of Dental Medicine, Al-Azhar University (Approval number: AUAREC202200001-6).

The clinical trial was registered under the number NCT05928546 on ClinicalTrials.gov [25].

Before participation, all patients were provided with comprehensive information regarding the nature of the trial, potential hazards, and benefits. They were fully informed and subsequently signed their informed consent forms.

Inclusion criteria

The inclusion criteria for this study were as follows:

- Patients in stages I and II of periodontitis (Stage I has no tooth loss, a clinical attachment level (CAL) of 1 to 2 mm, and a probing depth of ≤4 mm; Stage II has no tooth loss, a CAL of 3 to 4 mm, and a probing depth ≤ 5mm [26] (Figure 1a).
- Patients possessing more than 20 natural teeth with mesial and distal neighboring teeth.
- Patients who do not exhibit any systemic diseases that could impact their periodontal condition, as determined by the Cornell Medical Index and its modifications.

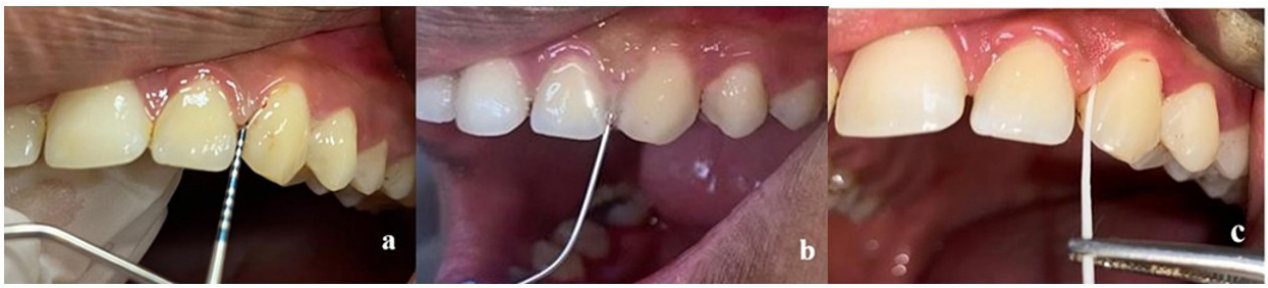

Figure 1 - (a) probing depth and attachment level measurement using a UNC15 periodontal probe; (b) quercetin nano-emulgel application; (c) GCF sample taking.

Exclusion criteria

The exclusion criteria for this study included:

- long-term use of medicines including antibiotics and non-steroidal anti-inflammatory drugs that may have an effect on the condition or rate at which periodontal tissues heal.
- Expectant and nursing mothers.
- Individuals with a history of traumatic occlusion.
- Patients who have undergone mechanical debridement or periodontal surgery within the last three and six months, respectively, and those with teeth having both endo-perio lesions.

Sample size calculation

The sample size for this study was determined with $\alpha = 0.05$ and 95% power. A 1 mm value was used, with clinical attachment level (CAL) change as the primary outcome variable. Using the G*Power statistical power analysis program (version 3.1.9.4) and Sonar et al. [27] as a basis, a sample size of 10 (five per Group) was found to be sufficient to find a notable effect size (d) of 2.98, with an actual power (1-β error) of 0.95 (95%) and a significance level (α error) of 0.05 (5%) for the two-sided hypothesis test.

The sample size was increased by 100% to account for potential dropout, resulting in a total sample size of 20 (n = 10 in each Group). 23 patients received the treatment but 3 patients were lost in the follow up. Making the final sample size 20 (Figure 2).

Patient grouping and randomization

Using online software [28], patients were randomly assigned to two groups, with numbers concealed in locked envelopes.

The periodontally affected sites selected in this study were classified into two groups:

- Group I: Sites received non-surgical periodontal therapy (scaling and root planing).
- Group II: Sites received the same treatment followed by applying quercetin nano-emulgel local delivery.

The allocation ratio was 1:1 for both groups.

Allocation concealment mechanism

Allocation concealment was achieved by assigning sequential numbers to opaque sealed envelopes containing the interventions to be administered to the recruited individuals following the randomized numbers in the randomization list. After scaling and root planning, patients were allotted to either Group I or Group II. At this stage, a person other than the operator selected the number and opened the sealed envelope with the treatment assignment.

Blinding

Blinding was implemented for participants, the outcome assessor, and the biostatistician; however, blinding the operator was not feasible.

Preparation of quercetin nano-emulgel

For 30 minutes, 4 mg of quercetin (purchased from Loba India) was dissolved in 1.25 g of cinnamon oil, 9 g of surfactant (tween 80) (purchased from Loba India), and 2.5 g cosurfactant (ethylene glycol) (purchased from Loba India). Additionally, 10 g of aqueous phase poloxamer was dissolved in 37.5 g of distilled water. The samples were stored for 72 hours at 37 °C in a water bath shaker [24]. The total concentration of quercetin is 6.64% w/w. The particle size (mean size) and polydispersity index (PDI) of the generated cinnamon oil nano-emulsion were analyzed using dynamic light scattering after proper dilution with distilled water (Malvern Instruments, Malvern, Worcestershire, UK; Zetasizer, Nanoseries, Nano-zs). Standard operating procedures were employed to regulate every measurement and analysis configuration. The preparation was conducted by NanoGate (25 Ibrahim Abou Elnaga St., Ext. of Abbas El Akkad, Nasr city, 11765, Cairo, Egypt).

Zeta potential measurement:

After proper dilution of the samples with distilled water, charges on the produced Q@ cinnamon oil nano-emulsion were examined, and their zeta potential values were obtained using a Zeta-sizer under standard operating circumstances.

Size & shape:

A JEOL JEM-2100 high-resolution transmission electron microscope operating at

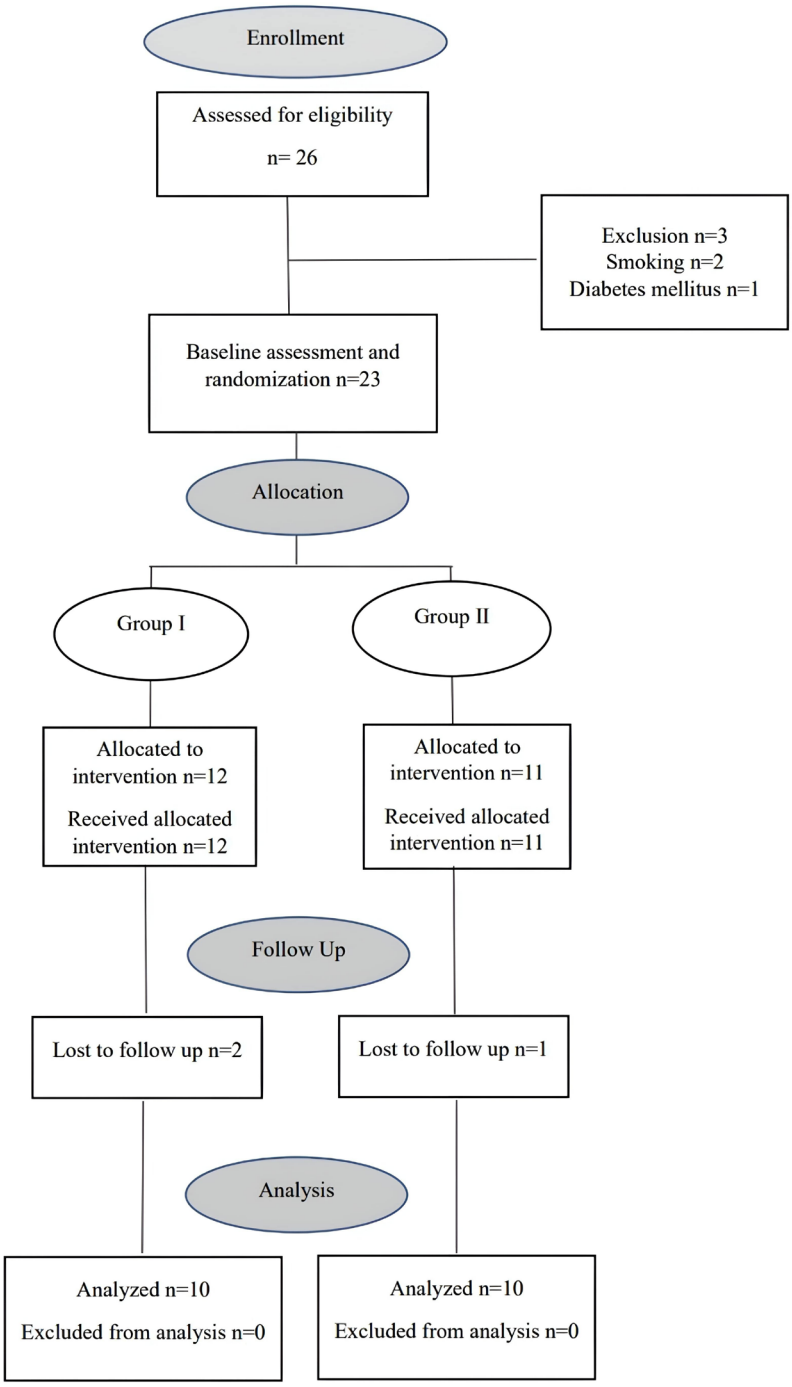

Figure 2 - Flow diagram.

an accelerating voltage of 200 kV was used to perform transmission electron microscopy (TEM), respectively (Figure 3).

Periodontal intervention

Phase I periodontal treatment was administered to each patient, involving non-surgical mechanical periodontal debridement (full-mouth scaling and root planing). This procedure was conducted without the use of adjunct disinfectants.

Intra-pocket application of quercetin nano-emulgel:

A cotton roll was used to isolate application sites after standard periodontal treatment. The gel was carefully applied sub-gingivally (about 2 to 4 ml depending on pocket depth) until it appeared from the gingival margin, using a needle inserted into the base of the periodontal pocket (Figure 1b).

Patients were asked not to eat, spit, or drink for one hour following the application.

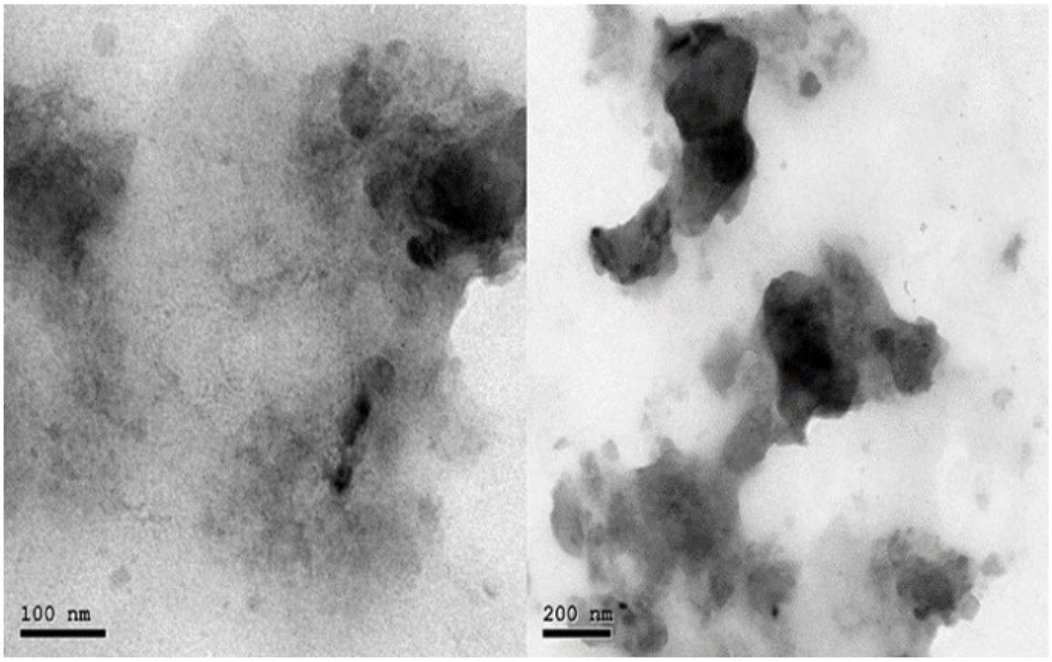

Figure 3 - TEM images of the prepared Q@cinnamon oil nano-emulsion.

Additionally, brushing and flossing were prohibited for four hours post-application. Patients were instructed on a plaque control regimen. The application was repeated after 2 weeks, resulting in the gel being applied twice, once on the day of non-surgical periodontal treatment and again after 2 weeks.

Periodontal assessment

Clinical assessment

All patients' periodontal condition was clinically evaluated at baseline, one month, and three months after treatment using the following parameters: modified plaque index (PI) [29], gingival index (GI) [30], probing depth (PD) [31], and clinical attachment level (CAL) [32].

Biochemical assessment

Gingival crevicular fluid samples collection

Before GCF collection, sample sites were carefully dried and isolated from saliva contamination using cotton rolls. The supragingival plaque was removed without touching the marginal gingiva. Samples of GCF were collected from the site with the greatest probing depth (range 4-5mm) and CAL less than 5 mm. Sterilized paper point size #30 was used for GCF collection. This can be accomplished by carefully placing the paper point into the periodontal pocket until little resistance was noticed and holding it there for 30 seconds (Figure 1c). Any paper points stained with blood were discarded.

Following GCF collection, the samples were promptly placed in Eppendorf tubes filled with phosphate-buffered saline (PBS containing 137 mm NaCl, 10 mm Na_2HPO_4, and 2.7 mm KCl; pH 7.3). The tubes were then frozen at -80 °C [33]. Interferon-gamma (IFN-γ) and total antioxidant capacity concentrations were assessed using enzyme-linked immunosorbent assay (ELISA).

IFN-γ analysis

The laboratory procedure was conducted using the ELISA method. An ELISA kit from SinoGeneClon Biotech was utilized to detect the level of IFN-γ in pg/ml in the sample of GCF, following the manufacturer's directions.

Total antioxidant capacity analysis

The samples were assayed using the Bio-Diagnostic total antioxidant capacity kit (http://bio-diagnostic.com) to evaluate the antioxidant impact of quercetin. The instructions provided by the manufacturer were followed for utilizing the kit.

Outcomes

The primary outcome considered in this study was the CAL, while the PI, GI, PD, IFN-γ, and total antioxidant capacity analysis were defined as secondary outcomes.

Statistical analysis

The mean and standard deviation values were calculated for each Group in each test. The data's normality was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests, which revealed a non-parametric (non-normal) distribution. Continuous data were expressed as minimum and maximum, mean ± standard deviation (SD), or median (interquartile range).

The Mann-Whitney test was utilized to compare unrelated sample results between two groups. To compare more than two groups in related samples, the Friedman test was used, whereas the Wilcoxon signed-rank test was used to compare two groups in related samples. The Pearson test was employed to find correlation coefficients.

The significance level was set at $p < 0.05$ indicated by "*", with a very significance level set at $p < 0.01$ indicated by "**" and highly significant at $p < 0.001$ indicated by "***". Statistical analysis was performed using IBM® SPSS® statistics version 26 for Windows.

RESULTS

The mean gingival index (GI) score in Group I was 2 ± 0 at baseline, which significantly reduced to 1.4 ± 0.52 and 1.5 ± 0.71 after 1 month and 3 months of treatment, respectively ($p < 0.05$). In Group II, the mean GI score was 2 ± 0 at baseline, significantly reduced to 0.8 ± 0.42 and 0.6 ± 0.52 at 1 month and 3 months after treatment, respectively ($p < 0.01$).

There is a statistically significant difference between the two groups at 1 month ($p < 0.05$) and a very statistically significant difference at 3 months ($p < 0.01$) (Table I).

The mean plaque index (PI) score in Group I was 2.7 ± 0.67 at baseline, which very significantly reduced to 1.1 ± 0.57 after 1 month and significantly reduced to 1.2 ± 1.03 after 3 months of treatment ($p < 0.05$). In Group II, the mean plaque index score was 2.4 ± 0.52 at baseline, which very significantly reduced to 0.7 ± 0.48 and 0.4 ± 0.52 at 1 month and 3 months after treatment, respectively ($p < 0.01$).

Table I - Distribution of gingival index scores among the studied groups over time

Gingival index (GI)	Group I (without gel) n=10	Group II (with gel) n=10	p-value
Baseline			
Min. - Max.	2 - 2	2 - 2	
Mean ± SD	2 ± 0	2 ± 0	
Median (Q1-Q3)	2(2-2)	2(2-2)	1.000
1 month			
Min. - Max.	1 - 2	0 - 1	
Mean ± SD	1.4 ± 0.52	0.8 ± 0.42	
Median (Q1-Q3)	1(1-2)	1(0.75-1)	**0.015***
3 months			
Min. - Max.	0 - 2	0 - 1	
Mean ± SD	1.5 ± 0.71	0.6 ± 0.52	
Median (Q1-Q3)	2(1-2)	1(0-1)	**0.007****
p-value2	***0.016****	***<0.001****	
p1	***0.014****	***0.003****	
p2	*0.059*	***0.004****	
p3	*0.564*	*0.317*	

p-value: calculated by the Mann-Whitney test between the two groups for each period. p-value2: calculated by the Friedman test between baseline, 1 month, and 3 months for each Group. p1: calculated by the Wilcoxon test between baseline & and 1 month for each Group. p2: calculated by the Wilcoxon test between baseline & and 3 months for each Group. p3: calculated by the Wilcoxon test between 1 month & and 3 months for each Group.

Non-statistically significant differences were found when the comparison was carried out between groups (Table II).

The mean probing depth (PD) in Group I was 5 ± 0.82 at baseline, which very significantly lowered to 4 ± 0.94 after 1 month and significantly

Table II - Distribution of plaque index among the studied groups over time

Plaque index (PI)	Group I (without gel) n=10	Group II (with gel) n=10	p-value
Baseline			
Min. - Max.	1 - 3	2 - 3	
Mean ± SD	2.7 ± 0.67	2.4 ± 0.52	
Median (Q1-Q3)	3(2.75-3)	2(2-3)	0.136
1 month			
Min. - Max.	0 - 2	0 - 1	
Mean ± SD	1.1 ± 0.57	0.7 ± 0.48	
Median (Q1-Q3)	1(1-1.25)	1(0-1)	0.111
3 months			
Min. - Max.	0 - 3	0 - 1	
Mean ± SD	1.2 ± 1.03	0.4 ± 0.52	
Median (Q1-Q3)	1(0-2)	0(0-1)	0.062
p-value2	***0.001*****	***<0.001*****	
p1	***0.004*****	***0.004*****	
p2	***0.011****	***0.004*****	
p3	*0.705*	*0.257*	

p-value: calculated by the Mann-Whitney test between the two groups for each period. p-value2: calculated by the Friedman test between baseline, 1 month, and 3 months for each Group. p1: calculated by the Wilcoxon test between baseline & and 1 month for each Group. p2: calculated by the Wilcoxon test between baseline & and 3 months for each Group. p3: calculated by the Wilcoxon test between 1 month & and 3 months for each Group.

Table III - Distribution of probing depth among the studied groups over time

Probing depth (PD)	Group I (without gel) n=10	Group II (with gel) n=10	p-value
Baseline			
Min. - Max.	4 - 6	3 - 6	
Mean ± SD	5 ± 0.82	4.8 ± 0.92	
Median (Q1-Q3)	5(4-6)	5(4-5.25)	0.687
1 month			
Min. - Max.	3 - 5	2 - 4	
Mean ± SD	4 ± 0.94	3.2 ± 0.92	
Median (Q1-Q3)	4(3-5)	3.5(2-4)	0.098
3 months			
Min. - Max.	3 - 7	1 - 4	
Mean ± SD	4.3 ± 1.25	2.9 ± 0.74	
Median (Q1-Q3)	4(3-5)	3(3-3)	**0.005****
p-value2	***0.002*****	***<0.001*****	
p1	***0.004*****	***0.004*****	
p2	***0.035****	***0.004*****	
p3	*0.257*	*0.257*	

p-value: calculated by the Mann-Whitney test between the two groups for each period. p-value2: calculated by the Friedman test between baseline, 1 month, and 3 months for each Group. p1: calculated by the Wilcoxon test between baseline & and 1 month for each Group. p2: calculated by the Wilcoxon test between baseline & and 3 months for each Group. p3: calculated by the Wilcoxon test between 1 month & and 3 months for each Group.

reduced to 4.3 ± 1.25 after 3 months of treatment ($p < 0.05$). In Group II, the mean probing depth was 4.8 ± 0.92 at baseline, which significantly lowered to 3.2 ± 0.92 and 2.9 ± 0.74 at 1 month and 3 months following treatment, respectively ($p < 0.01$) (Table III).

When comparing between groups, very statistically significant differences were found at 3 months ($p < 0.01$). The reduction in PD between baseline and after 3 months was 14.0% in Group I and 39.6% in Group II (Table IV).

The mean clinical attachment level (CAL) in Group I was 2.7 ± 1.06 at baseline, which significantly reduced to 2.1 ± 0.88 at 1 month ($p < 0.05$) and very significantly reduced to 1.6 ± 1.07 after 3 months of treatment ($p < 0.01$). In Group II, the mean CAL was 2.5 ± 0.85 at baseline, which significantly reduced to 1.9 ± 0.99 at 1 month ($p < 0.05$) and very significantly reduced to 0.85 ± 1 at 3 months after treatment ($p < 0.01$) (Table V).

There were non-statistically significant differences between the two groups in CAL at baseline, 1 month, and 3 months. However, the reduction percentage between baseline and after 3 months was 40.7% in Group I and 66% in Group II, indicating that the amount of gain in clinical attachment after 3 months was 1.1 mm in Group I and 1.65 mm in Group II (Tables VI and VII).

Table IV - Reduction between baseline and after 3 months for each group according to probing depth (PD)

	Probing depth (PD)	
	Group I	Group II
	Mean	Mean
Baseline	5	4.8
3 months	4.3	2.9
Reduction (Between baseline and after 3 months follow up in each group)	**14.0%**	**39.6%**

Table V - Distribution of clinical attachment loss among the studied groups over time

Clinical attachment loss (CAL)	Group I (without gel) n=10	Group II (with gel) n=10	p-value
Baseline			
Min. - Max.	1 - 4	1 - 4	
Mean ± SD	2.7 ± 1.06	2.5 ± 0.85	
Median (Q1-Q3)	2.5(2-3)	2.5(2-3)	0.691
1 month			
Min. - Max.	1 - 3	1 - 4	
Mean ± SD	2.1 ± 0.88	1.9 ± 0.99	
Median (Q1-Q3)	2(1-3)	2(1-2.25)	0.524
3 months			
Min. - Max.	0 - 3	0 - 3	
Mean ± SD	1.6 ± 1.07	0.85 ± 1	
Median (Q1-Q3)	1(1-3)	0.75(0-1.25)	0.089
p-value2	***<0.001****	***<0.001****	
p1	***0.014****	***0.014****	
p2	***0.002****	***0.007****	
p3	***0.025****	***0.005****	

p-value: calculated by the Mann-Whitney test between the two groups for each period. p-value2: calculated by the Friedman test between baseline, 1 month, and 3 months for each Group. p1: calculated by the Wilcoxon test between baseline & and 1 month for each Group. p2: calculated by the Wilcoxon test between baseline & and 3 months for each Group. p3: calculated by the Wilcoxon test between 1 month & and 3 months for each Group.

The mean IFN-γ level in Group I was 216.64 ± 164.73 at baseline, which significantly reduced to 92.6 ± 60.06 and 99.81 ± 57.56 after 1 month and 3 months of treatment, respectively ($p < 0.05$). In Group II, the mean IFN-γ level was 353.33 ± 175.71 at baseline, which significantly decreased to 109.64 ± 81.08 and 121.7 ± 99.11 after 1 month and 3 months of treatment,

Table VI - Reduction between baseline and after 3 months for each group according to clinical attachment loss (CAL)

	Clinical attachment loss (CAL)	
	Group I	Group II
	Mean	Mean
Baseline	2.7	2.5
3 months	1.6	0.85
Reduction (Between baseline and after 3 months follow up in each Group)	**40.7%**	**66%**

Table VII - The gain in clinical attachment (CA) between baseline, after 1 month and after 3 months for each group

	Clinical attachment loss (CAL)	
	Group I	Group II
	Mean	Mean
Baseline	2.7	2.5
1 month	2.1	1.9
3 months	1.6	0.85
CAL gain in 1 month (Mean of baseline – Mean of 1 month follow up)	**0.6**	**0.6**
CAL gain in 3 months (Mean of baseline – Mean of 3 months follow up)	**1.1**	**1.65**

Table VIII - Distribution of interferon gamma among the studied groups over time

Inter feron gamma	Group I (without gel) n=10	Group II (with gel) n=10	p-value
Baseline			
Min. - Max.	33.69 - 521.85	80.26 - 606.46	
Mean ± SD	216.64 ± 164.73	353.33 ± 175.71	
Median (Q1-Q3)	202.517(43.949-375.879)	340.179(244.819-536.526)	0.112
1 month			
Min. - Max.	23.42 - 186.38	19.62 - 245.06	
Mean ± SD	92.6 ± 60.06	109.64 ± 81.08	
Median (Q1-Q3)	90.936(35.146-147.257)	76.289(50.795-186.134)	0.821
3 months			
Min. - Max.	27.33 - 217.19	9.84 - 257.29	
Mean ± SD	99.81 ± 57.56	121.7 ± 99.11	
Median (Q1-Q3)	96.397(53.241-132.586)	113.758(26.868-218.777)	0.821
p-value2	***0.025****	***0.001****	
p1	***0.022****	***0.005****	
p2	*0.047**	***0.005****	
p3	*1.000*	*0.575*	

p-value: calculated by the Mann-Whitney test between the two groups for each period. p-value2: calculated by the Friedman test between baseline, 1 month, and 3 months for each Group. p1: calculated by the Wilcoxon test between baseline & and 1 month for each Group. p2: calculated by the Wilcoxon test between baseline & and 3 months for each Group. p3: calculated by the Wilcoxon test between 1 month & and 3 months for each Group.

Table IX - Distribution of total antioxidants among the studied groups over time

Total antioxidant	Group I (without gel) n=10	Group II (with gel) n=10	p-value
Baseline			
Min. - Max.	-0.77 - 1.3	-0.06 - 1.22	
Mean ± SD	0.61 ± 0.64	0.65 ± 0.41	
Median (Q1-Q3)	0.63(0.194-1.219)	0.813(0.354-0.919)	0.821
1 month			
Min. - Max.	0.39 - 1.31	0.5 - 1.4	
Mean ± SD	0.97 ± 0.39	1.04 ± 0.27	
Median (Q1-Q3)	1.189(0.44-1.233)	1.083(0.836-1.233)	0.940
3 months			
Min. - Max.	0.06 - 1.4	0.14 - 1.4	
Mean ± SD	0.78 ± 0.53	0.98 ± 0.48	
Median (Q1-Q3)	0.889(0.241-1.262)	1.154(0.457-1.387)	0.406
p-value2	***0.045****	***0.002****	
p1	***0.022****	***0.005****	
p2	*0.241*	***0.009****	
p3	*0.093*	*0.878*	

p-value: calculated by the Mann-Whitney test between the two groups for each period. p-value2: calculated by the Friedman test between baseline, 1 month, and 3 months for each Group. p1: calculated by the Wilcoxon test between baseline & and 1 month for each Group. p2: calculated by the Wilcoxon test between baseline & and 3 months for each Group. p3: calculated by the Wilcoxon test between 1 month & and 3 months for each Group.

respectively ($p < 0.01$). Non-statistically significant differences were found when the comparison was carried out between groups (Table VIII).

The mean total antioxidant capacity in Group I was 0.61 ± 0.64 at baseline, significantly increasing to 0.97 ± 0.39 after 1 month ($p < 0.05$) and 0.78 ± 0.53 after 3 months of treatment. In Group II, the mean total antioxidant capacity was 0.65 ± 0.41 at baseline, which very significantly increased to 1.04 ± 0.27 and 0.98 ± 0.48 after 1 month and 3 months of treatment, respectively ($p < 0.01$) (Table IX). When a comparison was conducted between groups, non-statistically significant differences were found. No harm or side effects were observed in this study.

DISCUSSION

From a pathological perspective, periodontitis can be described as the presence of inflammation associated with connective tissue attachment loss that leads to tooth-supporting tissue destruction [34]. It involves complex, dynamic interactions between specific bacterial pathogens, harmful host immune responses, and external factors such as smoking [35]. Periodontitis is primarily caused by bacterial biofilm on tooth surfaces, with factors like calculus, plaque, genetics, environmental, health, lifestyle choices, and social determinants also influencing its progression [36].

Improving the patient's gingival health and maintaining the residual periodontal tissues are the primary goals of periodontal therapy [37]. Eradication or inhibition of periodontopathic bacteria in the subgingival microbiota is crucial for periodontal repair [38]. Non-surgical periodontal therapy, including scaling and root planing, is foundational [35], but may not effectively remove plaque and calculus from inaccessible areas, including deep pockets and furcation areas, leading to high treatment failure rates. Additionally, it is impossible to eliminate all pathogenic bacteria through instrumentation within the subgingival region [39]. Medications can promote tissue regeneration by reducing inflammation and microbial burden. Effective pharmaceutical distribution can be achieved through systemic and local delivery, though systemic dosing can cause various adverse effects [40].

The pocket's anatomy makes it an ideal target for local delivery systems. Although

released in small amounts, gingival fluid aids in drug distribution in the pocket's limited locations [41]. Local drug delivery improves pharmaceutical outcomes by delivering drugs to targeted areas in higher concentrations, lowering side effects, and providing advantages such as solubility, action duration, and targeting, all while promoting tissue regeneration [40].

Quercetin, known for its antimicrobial properties, reduces inflammation, lowers ROS levels, and decreases bone loss [42]. Unfortunately, its low aqueous solubility and instability in physiological media hinder its bioavailability, permeability and absorption [43]. Scientists have attempted to improve quercetin bioavailability by various means [21]. Nanotechnological drug delivery systems have the advantage of making good contact with the mucosal areas of the periodontium, staying in the targeted tissue for a long time, and increasing epithelial transport of poorly absorbed drugs [44]. Nano-emulgel allows for convenient administration and sustained/controlled drug delivery [24]. The nano-emulgel is a colloidal system as the emulsion part protects pharmaceuticals from enzymatic destruction and hydrolysis, while the gel part enhances their viscosity, spreadability, retention time, and thermodynamic stability [45].

This study attempts to explore the efficacy of quercetin nano-emulgel as an adjuvant local delivery drug in the Phase I treatment of periodontitis. Between baseline and three months, the clinical evaluation revealed a significant decrease in plaque index in both groups. These findings may be attributed to all patients maintaining and reinforcing oral hygiene during the study's observation period. However, Group II showed better results at 1 and 3 months, possibly due to quercetin's anti-biofilm properties [16,46].

The study found a significant decrease in gingival index in both groups, with statistically significant differences between the two groups at one month and three months. These results are consistent with prior studies that have reported quercetin's capacity to inhibit the activation of NF-κB and induce molecules that extensively recruit leukocytes resulting in improved clinical parameters [27,47].

The study reported a significant lessen in probing pocket depth in both groups, with no significant differences at baseline or 1 month. However, Group II showed a very significant difference at 3 months. Quercetin's anti-inflammatory properties can reduce inflammation caused by lipopolysaccharide (LPS) by hindering the synthesis of inflammatory cytokines for instance prostaglandin E, IL-12, INF-α, IL-6, and IL-8 [17]. Moreover, quercetin improves wound healing, increases collagen expression, and preserves the integrity of periodontal soft tissues [19].

The study discovered significant CAL reductions at various intervals in both groups. Despite no significant differences between the groups during the trial period, Group II showed greater gains at the 3-month follow-up than Group I. This is attributable to quercetin's strong osteogenesis-promoting effects. Quercetin downregulates osteoclasts and reduces stimulating cytokines such as IL-1, TNF-α, and IL-17 [48].

Biochemically, the study found a significant drop in IFN-γ levels in both groups at baseline, 1 month, and 3 months. However, the two groups had no significant differences at these time points.

The results for Group I are consistent with previous research indicating that SRP improved periodontal health by significantly lowering IFN-γ levels [49-53]. In contrast, several investigations found that GCF IFN-γ levels remained unaltered after treatment. The low frequency and concentration of this cytokine's sensitivity to the immunoassay may contribute to this observation [54-57].

IFN-γ levels drop dramatically in Group II after 1 and 3 months of treatment with quercetin. In addition to SRP, quercetin suppresses IFN-γ production following T-cell receptor stimulation [58,59]. However, some studies suggest quercetin increases gene expression and Th-1-derived interferon-γ production while decreasing Th-2-derived interleukin-4 (IL-4) in normal peripheral blood mononuclear cells (PBMCs) [60,61]. Conversely, Rogerio et al. found that quercetin does not significantly affect IFN-γ levels [62]. The differences in quercetin concentrations utilized in various studies could explain this discrepancy [63].

The study revealed a significant increase in total antioxidant capacity in both groups at different intervals, consistent with previous studies indicating similar improvements [64-67]. Group II showed better results, though without

statistical significance, compared to Group I at the 1- and 3-month follow-up. This could be due to the antioxidant effect of quercetin [18].

The relatively small sample size and patient diversity in maintaining oral hygiene during the clinical trial are significant limitations of this study. Another limitation is that the control group did not receive a placebo.

CONCLUSION

This study's use of quercetin nano-emulgel as an adjunct to scaling and root planing for treating periodontitis stages I and II showed improvement in reducing clinical and biochemical parameters, particularly in gingival index and probing depth. This treatment approach holds promise for widespread use as an adjunct.

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Author's Contributions

KAM: Conceptualization, Writing – Original Draft Preparation, Project administration, Writing – Review & Editing. AAA: Methodology, Validation, Formal Analysis. AIARA: Investigation, Resources, Data Curation. HFH: Formal analysis, Investigation. IMM: Visualization, Supervision.

Conflict of Interest

The authors have no proprietary, financial, or other personal interest in any product, service, and/or company presented in this article.

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Regulatory Statement

This study was conducted following the Helsinki Declaration's ethical principles for medical research and was approved by the Faculty of Dental Medicine, Al-Azhar University's ethical committee (AUAREC202200001-6).

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Khoshoe Al-mokhtar Mohammed
(Corresponding address)
Assiut University, Faculty of Dentistry, Assiut, Egypt.
Email: KhoshoeAl-Mokhtar@dent.aun.edu.eg

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