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The Effects of Curcumin Gel Against Tannerella Forsythia (Clinical and Microbiological study)

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Abstract

Aim of the study: To determine the effects of curcumin gel on Tannerella forsythia.
Material and method: Eleven individuals were chosen with probing pocket depths of more than or equal to 5 mm. Scaling and root planing (SRP) were done on entire mouth, then curcumin and placebo gel were applied. At baseline and third visit (after one month), bleeding on probing (BOP), probing pocket depth (PPD), and relative attachment levels (RAL) were measured with a collection of subgingival plaque. The real-time polymerase chain reaction method has been used for bacterial analysis.
Results: There was a substantial lessening in the bacterial outcomes, PPD and RAL when comparing the curcumin with the placebo group, while the difference was not significant with respect to BOP. On intra-group comparison, only the test group showed a significant decrease in bacterial count, BOP, and RAL, while both groups showed a significant reduction in PPD.
Conclusion: Curcumin gel has an antimicrobial impact on T.Forsythia and a significant effect on improving BOP, PPD, and RAL.

Keywords: Real-time PCR, periodontitis, Curcumin gel.

Introduction

Periodontitis is a progressive breakdown of the apparatus for supporting teeth. Its key characteristics are clinical attachment loss (CAL) as well as bone loss established by radiographic examination, the development of pocketing and bleeding at the gingiva [(1)].

Periodontitis is a serious general health issue as a result of its widespread incidence, and because it can result in tooth loss, it has a detrimental impact on the oral function and aesthetics. It contributes significant role in the development of edentulism and mastication disorder, has a detrimental influence on overall health, and leads to significant increases in the expense of dental care. [(2)].

Scaling and root planing (SRP) were and are the standard treatments for periodontitis. Mechanical therapy, on the other hand, may fail to eradicate pathogenic germs in inaccessible places, resulting in disease recurrence. The greater understanding of anaerobic bacteria as the primary cause of the periodontal disease has resulted in new therapeutic options based on systemic or local anti-microbial treatment [(3)].

The emergence of resistance of bacterial has raised the warnings against the unrestrained use of antibiotics when treating periodontitis. As a result, systemic antibiotics in periodontal disease must be limited to specific patients with specific periodontal diseases (stages III-IV, grade C, "active," "refractory," and "recurrent" disease), and they should be utilized rationally while complying to ideal procedures [(4)].

Curcumin (CU) is a diferuloyl methane found in plant extracts (turmeric or rhizome). Three main curcuminoids are found in CU longa (approximately 77 percent CU, 17 percent demethoxycurcumin, and 3 percent

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bisdemethoxycurcumin) [5]. Curcumin exhibits anti-oxidant, anti-carcinogenic, anti-viral, and anti-microbial activities. Curcumin comes in several different forms. Gel delivery systems offer advantages such as simplicity of administration, non-greasy texture, patient compliance, and improved drug release [6].

This study was designed to estimate the antibacterial efficiency of commercially available preparations of curcumin (Each gram of Curenext Oral Gel®, manufactured by Abbott Health Care in Mumbai, Maharashtra, India, contains 10.00 milligrams of curcuma longa extract (rhizome)) by microbiologic analysis using RT-PCR and its effect on periodontal clinical parameters (BOP, PPD, and RAL).

Material And Methods

Study population

Conducting of this research was at the department of periodontics /College of the Dentistry /University of Baghdad. All samples were taken between April and July of 2021. Individuals aged 28 to 45 years old voluntarily participated in the study after signing a consent form and completing a questionnaire that included their name, age, gender, and medical history and diagnosed as having periodontitis of stages II and III. Subjects who had used antibiotics or other medications in the previous three months, smokers, and pregnant or lactating women were excluded. The primary outcome in this study was the reduction in p.gingivalis and we determine the sample size by the use of https://gpower.software.informer.com/3.1/ Depending on the SD and mean of SD of p. gingivalis of test group [5].

Preparation of the placebo gel

To make the placebo gel, one gram of Carbopol 934 was liquified in 25 milliliters of distilled water. To raise the ph above 7, triethanolamine was added.

Study design

Eleven people were chosen to take part in this study (**Study No.322621**). Curcumin's antibacterial activity against the periodontal pathogen (T. Forsythia) was evaluated using a randomized split-mouth, double-blinded method. From each patient two sites had been taken; the involved pockets must be ≥ 5mm. Periodontal clinical parameters were assessed, and subgingival plaque samples were taken at the baseline and third visits.

During the first visit, assessment of BOP and PPD, full mouth supragingival scaling, collection of subgingival plaque sample, and an impression taken for the construction of the acrylic stent for RAL were done, figure **1**.

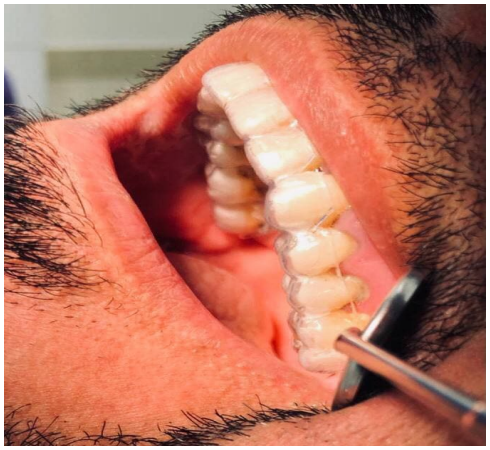

Figure 1: Acrylic stent

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The second visit included scaling and root planing, random administration of the curcumin, and placebo gel in the pockets by coin flip.

After 4 weeks, the third visit comprised assessing of BOP, PPD, and RAL and obtaining a subgingival plaque sample from the same pockets.

Collection of subgingival plaque sample

The sample was obtained with fine sterile Gracey curettes after the sampling area was separated with cotton rolls to prevent saliva contamination of the samples. Subgingival sampling was accomplished with one vertical stroke as soon as the curette encountered tissue resistance at the pocket and promptly placed in an Eppendorf tube containing DNA Rehydration solution by aggressively swirling the tip of the instrument in the solution. The sample was then kept in the refrigerator at (-20 C) until DNA extraction using ABIOpureTM Total DNA kit, figure **2**.

Figure 2: ABIOpureTM Total DNA kit

Quantification of *T.forsythia* Using Quantitative Real-Time PCR (qPCR)

1. DNA Extraction from Plaque Samples

The following steps were taken to isolate genomic DNA from bacterial growth using the ABIOpure Extraction protocol.

• For pellet calls, 1ml of overnight culture at 13000 rpm for 2-minutes. After that, the supernatant is then eliminated.

• The cell pellet was thoroughly resuspended in 200l of Buffer CL.

• For cell lysis and protein digestion, 20µl of Proteinase-K was put into 200µl of Buffer CL and cell pellet; after that, it was mixed forcefully by vortex and processed to 56˚C for 30-minutes followed by 30min at 70˚C for further lysis.

• Following incubation, 200µl of Buffer BL was put into the tube, then mixed forcefully by vortex and processed at 70˚C about 30-minutes.

• 200µl of absolute-ethanol was put into the tube, which was well combined using a pulse vortex.

• All mixtures were carefully transferring each combination to the minicolumn, centrifuged for one minute at a speed of 6,000 x g above (>8,000 rpm), and another exchange of the collecting tube.

• After mixing 600l of Buffer BW to the minicolumn, it was centrifuged about 1-minute at 6,000 x g above (>8,000 rpm) and the collecting tube was exchang with a newer one.

• 700 µl was taken from TW Buffer.1 minute centrifugation at 6,000 x g higher (>8,000 rpm). The pass-through was removed, and

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the minicolumn was exchanged in the new collection tube.

• The minicolumn was centrifuged at maximum speed (>13,000 x g) about 1 minute to remove any remaining wash buffer, then deposited in a new 1.5 ml tube.

• At room temperature, 100 of Buffer AE was mixed and processed about 1-minute, then processed at 5,000 rpm for 5minutes.

2. Reaction Setup and Thermal Cycling Protocol

❖ **Design of Primers**

Primers for the species-specific region on the 16S rRNA were created to detect and quantify *T. forsythia* bacteria. The primers used to detect the gene of bacteria 16S rRNA were created by the application using "Primer Quest of Integrated DNA Biotechnology", table **1**.

Table 1: Primers of T. forsythia

Primer Name	Seq.	Annealing Temp. (°C)
T.forsythia-F	5'-AGCGATGGTAGCAATACCTGTC-3'	**55**
T.forsythia-R	5'-TTCGCCGGGTTATCCCTC-3'	

F: Forward primer; R: Reverse primer

❖ **Preparation of Stock Primer**

Macrogen Company provided these primers in tube of lyophilized form. As a stock solution, the lyophilized primer was mixed with nuclease-free water (NFW) to make a 100pmol/l solution. To get a 10pmol/l primer that works, a functional primer of 10µl of solution of primer stock (kept in a fridge at -20 C) was mixed with 90µl of NFW. Magnetic Induction. Cycler (Mic) Real Time-PCR (RT-qPCR) amplifications were carried out in 10µl volumes containing 5µ l GoTaq-Green 2X; 1µl for each primer about 10 µM; 0.25 of MgCl2; 1.25µ l NFW; and 1.5µl of DNA. The RT-qPCR was cycled by "denaturing at 95°C about 5-minutes followed by 40 cycles of denaturation at 95°C about 20-seconds; annealing at 55°C about 20-seconds; then extension at 72°C about 20-seconds".

3. Absolute measurement by the use of the standard curve

In the qPCR experiment, the traditional curvature approach involves a dilution series with template replica numbers. The standard curve is obtained by a linear regression of data concentration (replica µl-1) vs. CT, which is then used to figure out the sample's template concentration (replica µl-1).

Five of about (0.2 ml) tubes were applied, and 90 µl of NFW was mixed into each one. Consequently, added 10 µl from the sample of $11*10^{10}$ replica μl^{-1} to the 1st tube , and 10 µl from the 1st tube to the 2nd one and so on. Figure 3, shows that the curve's reaction started with 11*109 replica µl-1 and ended with 11*107 replica µl-1. This was used to figure out how much target DNA was in the samples collected.

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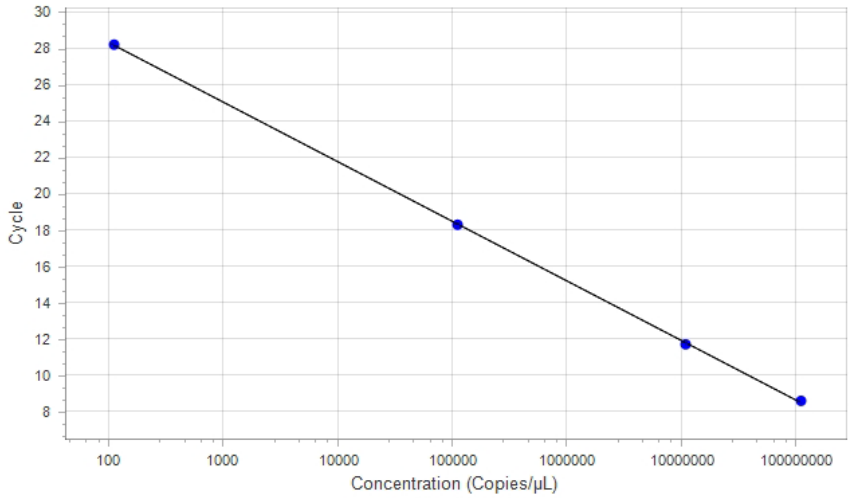

Figure 3: *T.Forsythia* Standard Curve

Statistical Analysis

It was done by a statistical package for social sciences (SPSS). The Shapiro-Wilk test was used to check for normality between study groups. The data were non-parametric and not normally distributed. The Wilcoxon sum rank-test and Wilcoxon sign rank-test were used to show the difference in rank between two independent groups with equal sample size and two related points respectively. The connection between bacterial results and clinical periodontal parameters was assessed using the spearman correlation test. The Chi-square test was used to evaluate the association of distribution between two qualitative variables. Mc Nemar's test was used for demonstrating the change in the qualitative variable when it is a dichotomous type. Cohen D (effect size) test was used in behavioral science research.

Results

Findings in table 2 demonstrated that there was non-significant difference in T. Forsythia between groups before gel administration (p=0.365). While the difference was significant between groups after gel application (p=0.001) with a medium effect size (0.735). In terms of T. Forsythia reduction in each study group in the control group, there was a decreasing in T. Forsythia count but it was not significant (p=0.929), whereas it was significant in the curcumin group (p=0.003) with a medium effect size (0.626).

Table 2: Descriptive and Analytic Statistics of Mean rank Value of T. Forsythia between study groups

Groups		**Before**	**After**
Placebo gel	Mean rank[1]	10.18	16.27
	Mean rank[2]	6.8	5.33
Wilcoxon sign rank test		0.089	
P value		0.929 ^	
Curcumin gel	Mean rank[1]	12.82	6.73
	Mean rank[2]	8	0
Wilcoxon sign rank test		2.934	
P value		0.003*(ES=0.626)	
Wilcoxon sum rank test		0.952	3.448
P value		0.365^	0.001* (ES=0.735)

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*=significant at p<0.05, ^=not significant at p>0.05.,1=between groups, 2=within group(change), ES=effect size

Results in table 3 showed that before gel application, both groups had a tendency for BOP in each site examined (11 sites in each group), but after gel application, curcumin gel reduced BOP more than placebo gel with the percentage of sites without bleeding (63.64 versus 45.45), but the difference was not significant (p=0.392). Regarding the change of BOP in each study group, both groups have reduced the BOP but with no significant change in the placebo group (p=0.063) and a significant change in the Curcumin group (p=0.016) with a large effect size (0.966). Findings in table 4 demonstrated there was non-significant difference in PPD between groups before gel application (p=1), while there was a significant difference between them after gel application (p=0.000003) with a large effect size (0.917). In terms of PPD change in each study group, both groups had a significant reduction of PPD with slightly more reduction in the curcumin group (p=0.001) than the placebo group (p=0.003) and the effect size of the curcumin group was (ES=0.707) and the placebo group was (ES=0.638).

Table 3: Distribution of BOP between study groups

Time			Groups						
			Placebo			Curcumin gel			
			N.	%		N.	%		Chi-square P value
Before		With bleeding	11	100.00		11	100.00		----
After		With	6	54.55		4	36.36		0.392^
		Free	5	45.45		7	63.64		
MC Nemar's test			0.063			0.016*(ES=0.966)			

*=significant at p<0.05, ^=not significant at p>0.05, ES=effect size

Table 4: Descriptive and statistical test of Mean rank Value of PPD between study groups

Groups		Before	After
Placebo gel	Mean rank[1]	12	17
	Mean rank[2]	8	2.6
Wilcoxon sign rank test		2.994	
P value		0.003*(ES=0.638)	
Curcumin gel	Mean rank[1]	11.5	6
	Mean rank[2]	7	0
Wilcoxon sign rank test		3.317	
P value		0.001*(ES=0.707)	
Wilcoxon sum rank test		0	4.301
P value		1^	0.000003* (ES=0.917)

*=significant at p<0.05, ^=not significant at p>0.05.,1=between groups, 2=within group (change), ES=effect size

Table 5 presented that before gel application, there was no significant difference in RAL between groups(p=0.898). While after gel application, there was a significant difference between them (p=0.000020) with a large effect size (0.827). Regarding the change of RAL in each study group, both groups have reduced RAL but it was not significant in the placebo group (p=0.083) and significant in the curcumin

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group (p=0.003) with a medium effect size (0.633).

Table 5: Descriptive and statistical test of Mean rank Value of RAL between study groups

Groups		Before	After
Placebo gel	Mean rank[1]	11.32	9.18
	Mean rank[2]	6.7	2.1
Wilcoxon sign rank test		1.732	
P value		0.083^	
Curcumin gel	Mean rank[1]	11.68	6.32
	Mean rank[2]	6	0
Wilcoxon sign rank test		2.971	
P value		0.003*(ES=0.633)	
Wilcoxon sum rank test		0.140	3.881
P value		0.898^	0.000020* (ES=0.827)

*=significant at p<0.05, ^=not significant at p>0.05.,1=between groups, 2=within group(change), ES=effect size

Discussion

The test group showed a significant difference in microbiological outcomes when compared to the control group. The anti-bacterial and anti-plaque properties of curcumin may explain the considerable bacterial reduction observed in the test group [(7)]. Curcumin reduces the activity of trypsin-like enzymes of bacteria that cause periodontal diseases, such as T. forsythia, Treponema denticola, and Porphyromonas gingivalis [(8)]. The findings of the intragroup comparison revealed a substantial decrease in microbial count in the test group, while it was not significant in the control group, this could be due to the difficulty of performing extensive mechanical treatment of sites with deep periodontal pockets and pathogenic microorganisms found within the gingival and periodontal tissues, as well as other areas inaccessible to periodontal instruments [(3)]. These results were agreeing with a study done by Nagasri et al. [(7)] it was discovered that following the application of SRP and curcumin gels, the microbiologic parameters at test sites were significantly reduced.

On inter group comparison, the reduction of BOP was better in the test than in the control group but the difference was not significant between study groups. This is due to the elimination of plaque and irritant factors by SRP results in a reduction of a BOP. This finding was in disagreement with studies done by Bhatia et al., Vijayapremakumar et al., and Pandy et al. [(5,9,10)] which showed a significant difference in the BOP between groups.

On intragroup comparison, the reduction was significant of BOP in the test group may be due to the anti-inflammatory and antioxidant properties of curcumin via reducing the inflammatory mediators produced by the arachidonic acid pathway inhibits NF kβ activation and down-regulates the pro-inflammatory enzyme cyclooxygenase-2. This induces shrinking by decreasing inflammatory edema and connective tissue vascular engorgement [(11)] and by promoting the migration of different cells, comprising fibroblasts, into the wound, leading to a decrease in vascularization due to connective tissue fibrosis [(12)].

This result was in agreement with a study conducted by Behal et al. [8] it was shown a significant reduction of BOP in the test group.

A result of PPD and RAL presented a significant difference between study groups. It could be attributed to that the curcumin-treated areas had higher transforming growth

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factor-1 levels, faster reepithelialization, better neovascularization, decreased inflammatory cell infiltrate, higher collagen composition and fibroblastic cell, and stronger repair [7]. These results were in agreement with studies by Bhatia et al. and Vijayapremakumer et al. [5,9] they demonstrated that the difference was significant in the PPD and CAL between test and control study groups.

The intra-group comparison showed that the change in PPD in both study groups had a significant reduction and it was greater in the curcumin group than in the placebo group. These results were in agreement with the study done by Behal et al. [8] presented that there was a significant decrease in the PPD in both study groups, but the greater decrease was in the experimental group.

While intra-group comparison of RAL showed there was no significant gain in the placebo group and significant gain in the curcumin group. This result was in disagreement with other studies conducted by Behal et al. and Nagasri et al. [7,8] which found that both groups showed a significant gain in CAL.

Conclusion

This study concluded that:

1. Curcumin gel (Curenext®) had antibacterial activity against T. Forsythia by monitoring the effect using RT-PCR.
2. Quantitative RT-PCR is an efficient and sensitive method that allows for studying specific organisms.
    1. As PPD and RAL were both significantly decreased in the test group (SRP and curcumin gel), this may suggest that curcumin enhanced periodontal treatment.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Data Availability Statement

Data are available from the authors upon reasonable request.

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