

Sustained Release of Liposomal Curcumin: Enhanced Periodontal Outcomes in Diabetic Patients

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Objective: To evaluate the effect of entrapment of curcumin within liposomal formulation and the sustained release attitude of the formulated liposomal gel on periodontal defects in diabetic patients in clinical and biochemical terms.

Methods: Thirty diabetic patients with periodontitis were randomly assigned to three equal groups and ten healthy participants were assigned as the control group. Group I was subjected to scaling and root planing (SRP) with application of sustained release liposomal curcumin gel. Group II was subjected to scaling and root planning with application of curcumin gel. Group III was subjected to scaling and root planning with application of placebo gel. Group IV (control group), no intervention was done. The following parameters were evaluated before treatment and after 6 and 12 weeks: plaque index (PI), gingival index (GI), probing depth (PD), clinical attachment level (CAL), tumour necrosis factor alpha (TNF- α), interleukin 1 beta (IL-1 β) and total antioxidant capacity (TAC).

Results: All study groups showed improvement in clinical and biochemical parameters that are statistically significant. Upon comparing the results of treatment modalities, the highest improvement was achieved in group I followed by group II then group III.

Conclusion: Sustained release liposomal curcumin gel enhanced the antioxidant capacity, decreased the inflammatory mediators and showed more improvement in clinical outcome for treatment of periodontitis in diabetic patients.

Keywords: curcumin, diabetes mellitus, liposomes gel, periodontitis, sustained release
Chin J Dent Res 2024;27(2):169–174; doi: 10.3290/j.cjdr.b5459607

Periodontitis is a multifactorial inflammatory illness characterized by gradual deterioration of the tooth supporting apparatus and linked to some bacterial species of dysbiotic plaque biofilms¹. Diabetes mellitus (DM) is a chronic, complex condition that necessitates ongoing treatment and multifaceted approaches to maintain glycemic control. Type 1 and type 2 DM are the two most common varieties. Type 1 diabetes is defined by insulin insufficiency produced by autoimmune destruction of

pancreatic B-cells, whereas type 2 diabetes is caused by insulin resistance^{1,2}. Periodontitis is considered as one of the complications in diabetic patients³. The periodontium is affected by diabetes in two ways: first, by modifying the host immunological, inflammatory, and wound-healing responses, encouraging the accumulation of advanced glycation end products; and second, by producing high amounts of pro-inflammatory cytokines.⁴

Curcumin is a polyphenolic dietary phytochemical, and the bioactive pigment present in the roots of *Curcuma longa* L. (turmeric)⁵. The major yellow bioactive component of turmeric, curcumin (diferuloylmethane), has been proven in literatures to have a wide range of biological actions such as anti-inflammatory, antioxidant antibacterial and anti-fungal properties.^{6,7} Natural or chemically modified curcumin has been proposed as a possible therapy alternative for diabetes and related problems such as periodontitis due to its anti-inflammatory and antioxidant effects.⁸⁻¹²

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However, suitable dosage form should be designed to guarantee easy administration, patient compliance, enhanced permeation through membranes and maximized therapeutic effect. Many reports have revealed the impact of nano-carriers on the improvement of targeting and cellular permeation of entrapped natural product.¹³⁻¹⁷

Liposomes are lipid-based nanostructures which have the advantages of being biocompatible, encapsulate both hydrophilic and lipophilic components within their aqueous core and lipid bilayer, respectively, enhance the stability of entrapped cargo and maximize cellular penetration¹³⁻¹⁷. The thick network of gel formulation retard the release of the entrapped components allowing sustained release and prolonged time of action.¹⁸

Hence, the present study was carried out as a clinical trial to evaluate the effect of sustained release gel of curcumin and liposomal curcumin on periodontal therapy in diabetic patient.

Materials and methods

This study was conducted on a total of 40 participant, 30 diabetic patients (type 2 DM) with periodontitis that were divided randomly to 3 groups each having 10 patients and 10 healthy participants (no systemic disease or periodontitis) as control group. The patients were selected from the outpatient's clinic of the Oral medicine, Oral diagnosis, and Periodontology Department, Faculty of Dentistry, Minia University.

Ethical regulations

The complete treatment plan was explained to all patients including detailed steps, risks, and expected results, and their full signed consent was obtained before entry into the study. The study complied with the rules set by the International Conference on Harmonization Good Clinical Practice Guidelines, and the Declaration of Helsinki. The study was approved by the research ethics committee of the Faculty of Dentistry, Minia University (No. 663 in 2022).

Patient selection

Selected patients of both sexes were from 35-60 years with confirmed diabetes type 2, according to WHO guidelines in 1999 and its update in 2009 and all patients were screened for glycosylated hemoglobin (HbA1c) to confirm the diagnosis (6.5% or more)^{19,20}, and stage II periodontitis. Ten healthy participants for control group. All

subjects had not received any type of periodontal therapy, prescribed antibiotics, or anti-inflammatory medication within the preceding 6 months. Pregnant, lactating females and smokers were excluded from the study.

Preparation of sustained release curcumin gel (Cu-gel) and curcumin liposomal gel (Cuc-Lip gel)

Cuc-Lip were prepared using a new modified injection method.²¹ Briefly, lipid s75 (50 mmol), cholesterol (20% w/v) and curcumin (25 mg) were dissolved in the least volume of absolute ethanol then the solution was transferred to a spraying apparatus. In a closed system, the solution of lipids and curcumin was sprayed (200 ul per five seconds) on the surface of 1 ml of an aqueous media of distilled water containing sucrose (9% w/v) stirred at 1500 rpm at 80°C. Excess ethanol was evaporated with stirring and liposomes were formed spontaneously after further evaporation of the residual ethanol. Prepared liposomes were kept at 4°C overnight to allow annealing of the lipid bilayer.²² The liposomal suspension was then available for further processes of evaluation and in-vitro characterization. Particle size, poly dispersity index (PDI), and transmission electron microscopy (TEM). Curcumin liposomal gel (Cuc-Lip gel) was prepared using carboxy methyl cellulose (CMC). CMC solution (1 mg/mL) was injected on the surface of prepared Cuc-Lip under magnetic stirring at room temperature for 30 min. The suspension was sonicated in an ice bath.²³ Curcumin gel (Cu-gel) and curcumin liposomal gel were prepared by the injection of CMC solution (1 mg/mL) on the surface of curcumin suspension (25 mg/ml) and Cuc-Lip (25 mg/ml), respectively, with continuous stirring at room temperature. Liposomal size and size distribution were determined using laser light diffraction techniques. Briefly, the liposomal preparation was diluted using purified deionized water and was analyzed at 25°C using Mastersizer (3000E Malvern Instruments, UK). This procedure was done in triplicate for each preparation and the average values were used.²⁴

Treatment protocol

Each patient was given detailed instruction on self-performed oral hygiene measures using toothbrush and interdental brush or floss. Patients then were recalled after 7 days for baseline measurements of clinical parameters and baseline gingival cervicular fluid (GCF) sample collection.

The 30 patients were grouped into three 3 equal groups (I, II and III) and subjected to non-surgical periodontal therapy in the form of full mouth supra and sub



Fig 1 Application of curcumin gel.



Fig 2 GCF sampling.

gingival scaling and root planning using hand instruments with supragingival debridement followed by universal curette for proper subgingival debridement. The treatment was completed in three sessions over a period of 2 weeks. Ten healthy subjects were chosen as volunteers for negative control group (IV).

After one week of SRP, Group I received curcumin liposomal gel (Cuc-Lip gel), Group II received curcumin gel (Cu-gel) and Group III received placebo gel. Group IV (negative control group) no intervention was done.

The four groups were instructed to follow maintenance program consists of brushing 2 times daily using tooth paste with soft toothbrush, Interdental cleaning twice daily using dental floss or an interdental brush.

Application of the gel

After the isolation using cotton rolls, the gel was applied by a curved blunt-ended plastic tip syringe. The needle was carefully inserted in the periodontal pocket and the gel was injected to fill the selected pocket till the gingival margin (Fig 1). Patients were instructed to follow oral hygiene measures during the study period. They were also asked not to use the toothbrush at selected site after gel application for 24 hours and not to chew hard or sticky foods at the gel placement sites.

Assessment of periodontal parameters

The 3 study groups were evaluated clinically regularly at baseline, 6 weeks and 12 weeks post-operative. Every patient was assessed by the following clinical parameters include PI, GI, PD and CAL. Control group was assessed at baseline only.

GCF sampling

GCF samples were collected at baseline, 6 weeks and 12 weeks from study groups and at baseline from con-

trol group to determine total antioxidant capacity (TAC), IL-1 β and TNF- α using the absorbent filter paper from the deepest probing site. The selected tooth sites were isolated with sterile cotton rolls, the teeth were gently air-dried. The paper strips were placed carefully inside the gingival crevice for 30 seconds and then placed in Eppendorf tubes containing phosphate - buffered saline and stored at -20°C until further use (Fig 2).

Biochemical assessment

GCF samples were assessed using the total antioxidant capacity ELISA human kit (TAC ELISA kit) and The Picokine™ Human IL-1 β and TNF- α ELISA Kit (Boster, Pleasanton, California, USA) for the accurate detection of human total antioxidant capacity, IL1- β and TNF- α respectively.

Statistical analysis

Data was entered and analyzed by statistical program (SPSS version 20), displayed as mean and standard deviation. Comparison was done by one-way analysis of variance (ANOVA) test, considered significant at P < 0.05.

Results

Preparation of CU-Lip gel

The spraying method successfully produced nanosized uniform curcumin liposomal formulation (Fig 3), with a very unnoticeable aggregation of unencapsulated curcumin during preparation, and neat macroscopic appearance of the prepared liposomes. The prepared formulation (Cuc-Lip gel) exhibited particle size of 84.5 ± 5.6 nm. The prepared liposomes were homogeneously distributed with PDI 0.4.

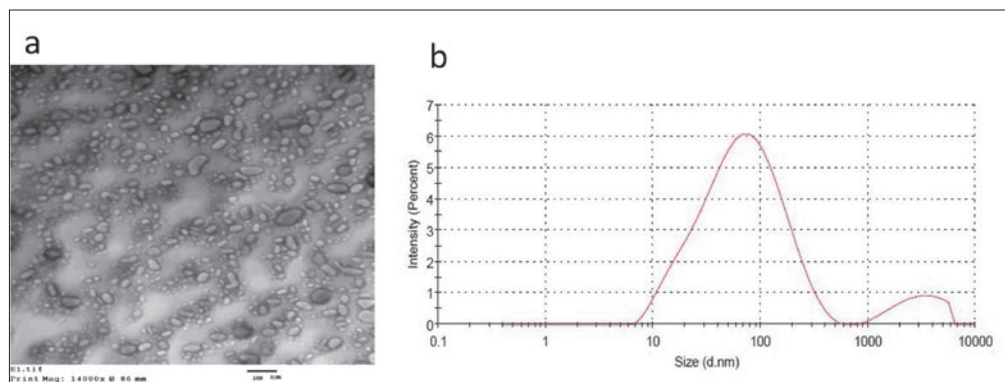


Fig 3 TEM and size distribution of Cuc-lip gel.

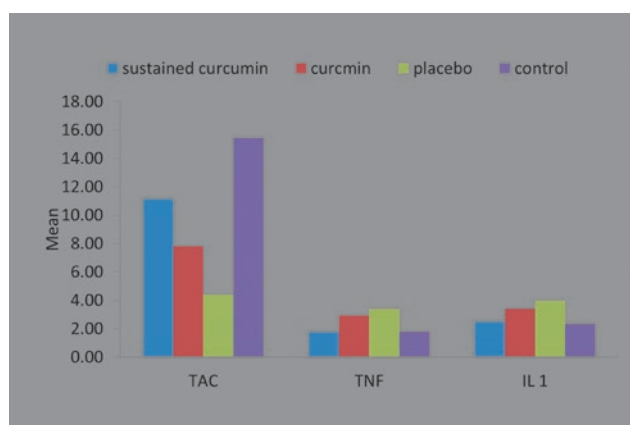


Fig 4 comparison of biochemical parameters of different groups 12 weeks post-treatment.

Assessment of periodontal and biochemical parameters

The mean PI, GI, PD, CAL, IL-1 β , TNF- α and TAC at baseline, 6 weeks and 12 weeks after treatment are presented in Table 1.

Results of all treatments used in this study showed that all study groups showed statistically significant improvement after 12 weeks of treatment regarding PI, GI, PD, CAL, IL-1 β , TNF- α and TAC. Upon comparison of all groups, the highest improvement in clinical and biochemical parameters was in group I (sustained liposomal curcumin gel), followed by group II (curcumin gel) and least improvement was in group III (placebo).

Discussion

Periodontitis and diabetes mellitus have a bidirectional link that exists irrespective of associated risk factors, and the two diseases affect each other. Oxidative stress processes are increasingly implicated in the pathobiology of periodontitis and diabetes mellitus.²⁵

Antioxidants have been shown in epidemiological research to limit the effects of reactive oxygen species activity and reduce illness incidence, making natural-based antioxidants one of the most useful therapeutic agents for reducing oxidative stress-related disorders. Considering that total antioxidant level influences metabolic regulation and tissue damage, antioxidant supplementation as an adjunct to SPR in type 2 diabetic patients may be beneficial¹¹. Therefore, the current study was conducted to evaluate the potential of curcumin as a potent antioxidant and anti-inflammatory agent in periodontitis in diabetic patients.

Curcumin stimulates the activity of antioxidant enzymes resulting in high antioxidant activity. Curcumin's ability to scavenge free radicals is another mechanism maintained in the antioxidant activity of the drug. A previous study has reported the impact of reduction of levels of free radicles in human by increasing the activity of antioxidant enzymes.¹¹

This study evaluated the effect of curcumin on the improvement of clinical and biochemical parameters in diabetic patients with periodontitis. Moreover, the impact of encapsulating curcumin within liposomal gel of sustained release behavior with scaling and root planning was evaluated.

Bibi et al¹² reported that the typical operating method for GCF specimens includes using perio-paper as the best transportation source. This method has a number of advantages, including ease of use and ability to collect data from discrete spots.⁴ As a result, it was chosen for this study.

All groups demonstrated statistically significant improvements in clinical and biochemical parameters. The improvement in group III is likely to be due to correct scaling, root planning, and reinforcement of oral hygiene measurements during the initial therapy and follow-up periods. The further clinical and biochemical improvement in group II could be attributed to the antioxidant and anti-inflammatory effect of curcumin gel.

Table 1 Comparison of clinical and biomechanical parameters for all groups.

Clinical parameter		Sustained curcumin	Curcumin	Placebo	Control	P value
PI	0	1.90 ± 0.21	1.94 ± 0.16	1.90 ± 0.21	0.01 ± 0.02	< 0.001*
	6 wk	0.65 ± 0.33	0.83 ± 0.25	0.80 ± 0.25	0.01 ± 0.02	0.337
	12 wk	0.05 ± 0.08	0.06 ± 0.16	0.37 ± 0.46	0.01 ± 0.02	0.034*
GI	0	1.90 ± 0.31	1.95 ± 0.15	1.95 ± 0.15	0.00 ± 0.00	< 0.001*
	6 wk	0.46 ± 0.35	0.60 ± 0.21	0.85 ± 0.24	0.00 ± 0.00	0.013*
	12 wk	0.25 ± 0.05	0.60 ± 0.15	0.50 ± 0.31	0.00 ± 0.00	< 0.001*
PD	0	2.93 ± 0.30	3.73 ± 0.66	3.65 ± 0.82	0.90 ± 0.07	< 0.001*
	6 wk	2.61 ± 0.20	2.93 ± 0.52	2.98 ± 0.65	0.90 ± 0.07	0.219
	12 wk	1.87 ± 0.34	2.44 ± 0.55	2.88 ± 0.55	0.90 ± 0.07	< 0.001*
CAL	0	0.98 ± 0.27	1.14 ± 0.50	1.01 ± 0.58	0.00 ± 0.00	< 0.001*
	6 wk	0.33 ± 0.20	0.53 ± 0.30	0.50 ± 0.29	0.00 ± 0.00	0.232
	12 wk	0.09 ± 0.15	0.26 ± 0.22	0.33 ± 0.39	0.00 ± 0.00	0.170
TAC	0	5.44 ± 0.71	3.62 ± 1.00	2.37 ± 1.31	15.45 ± 1.48	< 0.001*
	6 wk	7.79 ± 0.53	5.48 ± 1.04	3.66 ± 1.22	15.45 ± 1.48	< 0.001*
	12 wk	11.15 ± 0.75	7.82 ± 0.76	4.39 ± 1.03	15.45 ± 1.48	< 0.001*
IL-1β	0	4.33 ± 0.15	6.49 ± 1.98	8.20 ± 0.21	2.35 ± 0.10	< 0.001*
	6 wk	3.52 ± 0.10	5.35 ± 0.19	6.18 ± 0.28	2.35 ± 0.10	< 0.001*
	12 wk	2.52 ± 0.09	3.42 ± 0.13	3.97 ± 0.16	2.35 ± 0.10	< 0.001*
TNF-α	0	3.46 ± 0.29	5.55 ± 0.10	6.62 ± 0.19	1.79 ± 0.11	< 0.001*
	6 wk	2.51 ± 0.20	4.81 ± 0.07	5.22 ± 0.10	1.79 ± 0.11	< 0.001*
	12 wk	1.78 ± 0.18	2.92 ± 0.11	3.39 ± 0.08	1.79 ± 0.11	< 0.001*

*Statistically significant.

Those findings come in accordance with Mohammad et al²⁶ who revealed that curcumin gel had a significant effect on the reduction of IL-1β, TNF-α, copper, and clinical parameters and increase of zinc and magnesium levels.

The obvious significant enhancement in all clinical and biochemical parameters in Group I, receiving the sustained release liposomal gel, compared to the group receiving curcumin gel (group II) can be attributed to the impact of liposomal formulation in entrapping both the hydrophilic and the lipophilic components of the curcumin within the formed vesicles. Moreover, the small particle size and the homogenous distribution of the formulated vesicles enhance the cellular penetration of the nano-structured vesicles along with the entrapped cargo.²⁷ In addition, the formulation of curcumin liposomes within a sustained release gel formulation permits the prolonged action of the administered dose of curcumin.²⁸ This is significantly apparent in the remarkable reduction in IL-1β and TNF-α and substantial enhancement of PI, GI, CAL and TAC after 3 weeks of application of the curcumin liposomal gel (Group I) compared to application of curcumin gel (group II).

This study also revealed the antioxidant and anti-inflammatory effect of curcumin in treatment of peri-

odontitis in diabetic patient thus support its employment as a therapeutic strategy in treatment of periodontitis in diabetic circumstances. More studies with larger numbers of subjects are recommended to ensure the results of IL-1β and TNF-α since the base line of those tests are significantly different.

Conclusion

Sustained liposomal curcumin gel enhanced the antioxidant capacity, decreased the inflammatory mediators (IL-1β, TNF-α) and showed more improvement in clinical outcome in treatment of periodontitis diabetic patients by entrapped curcumin.

Acknowledgements

The authors would like to thank Deraya University for supporting the work.

Conflicts of interest

The authors declare no conflicts of interest related to this study.

Author contribution

Both authors contributed to methodology, interpretation of data, editing and revising the manuscript.

(Received Mar 15, 2023; accepted Sep 04, 2023)

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