ABSTRACT

INTRODUCTION: Nowadays, the application of nanotechnology is recommended for potential treatments in dentistry. Curcumin nanoparticles (CURN) have been found to possess potential antioxidant and anti-inflammatory activities that can treat periodontitis which is considered as an inflammatory disorder that can spread down the gingiva causing alveolar bone destruction.

OBJECTIVES: To evaluate the biological effect of CURN on alveolar bone structure in rats with ligature induced periodontitis.

MATERIALS AND METHODS: Thirty-five healthy adult male albino rats were divided into 3 groups: Group A (control group), Group B (periodontitis group) and Group C (curcumin nanoparticles treated group). A silk ligature was used to induce periodontitis in group B and C. After two weeks, the ligature was removed. CURN was then administrated orally to rats of group C (15 mg/kg/day). The animals were euthanized by the end of the experimental periods, after 2 and 4 weeks from removal of ligature. Right side mandibles were dissected out and prepared for scanning electron microscope (SEM) and energy dispersive x-ray microanalysis (EDX).

RESULTS: Scanning electron microscopic results revealed restoration of the alveolar bone level around mandibular first molar with decrease in the porosity in the surface of the alveolar bone in group C treated with CURN rather than in periodontitis group B. These results were confirmed by EDX which showed more calcium and less phosphorous percentage in group C than in group B.

CONCLUSIONS: Curcumin nanoparticles have a positive biological effect on alveolar bone structure in rats with ligature induced periodontitis.

KEY WORDS: Curcumin nanoparticles, anti-inflammatory, periodontitis.

RUNNING TITLE: CURN effect on periodontitis induced alveolar bone damage.
The anti-inflammatory effects of CUR or CURN are due to reduction in the level of reactive oxygen species (ROS) that produced. These reactive oxygen species cause increase in the oxidant load together with reduction in the antioxidant capacity, leading to oxidative stress inside the affected tissues such as, the periodontal tissues involving the alveolar bone (8). Periodontitis is an inflammatory disorder elicited by dental plaque biofilm in the oral cavity causing progressive deterioration of the periodontium, involving gingiva, cementum, periodontal ligament (PDL) and alveolar bone (tooth socket) that surrounds the teeth. Patients having periodontitis suffer a range of symptoms, mainly gingival bleeding, periodontal pocketing, resorption of the alveolar bone and may cause tooth loss. As acknowledged, localized alveolar bone injury due to excessive activation of osteoclasts around tooth root has a high relation with periodontitis and acts as the most obvious histopathological marker of periodontitis (9).

Management of periodontitis aims at the restoration of damaged tissues, as well as, prevention of disease progression. It involves mechanical, surgical and non-surgical treatment besides the usage of systemic and topical antibiotics and anti-inflammatory drugs (10). Chemotherapeutic agents used for the management of periodontitis may have undesirable side effects, such as, nausea, vomiting, rash, diarrhea taste alterations (11). The utilization of natural products in the prevention and treatment of oral diseases has increased nowadays (12). Recently, a new treatment strategy has been advocated by using nanotechnology which was suggested by many researchers (13).

Since there are few literatures about effect of CURN on bone regeneration (6). Therefore, this study aimed to evaluate the effect of curcumin nanoparticles administration on the alveolar bone structure of rats with ligation induced periodontitis. Null hypothesis of this study was that there will be no difference in the healing outcome of the periodontitis among control, periodontitis and curcumin nanoparticles treated groups, otherwise the opposite would be proved.

MATERIAL AND METHODS

Study sample
Thirty-five healthy adult male albino rats 6 months old (200-250 grams in weight) were used in this study. Animals were obtained from the animal house of Medical Research Institute, Alexandria University. They were kept under the same environmental conditions in the experimental animal house.

Grouping (Randomization technique)
Rats were randomly assigned by (using computer generated random numbers) into three groups:
- **Group A**: Control group: (7 healthy rats).
- **Group B**: Periodontitis group: ligation induced periodontitis (14 rats +ve control).
- **Group C**: Curcumin nanoparticles treated group: ligation induced periodontitis treated with oral administration of curcumin nanoparticles (14 rats test group).

Induction of periodontitis (14)
In animals of groups B and C ligation placement under general anaesthesia using ketamine 50 mg/ml was applied. A 4-0 silk ligation was secured at the gingival sulcus level of the mandibular right first molars of all animals in both groups. Two weeks after the application, the silk ligation was removed.

Administration of curcumin nanoparticles (15)
Curcumin nanoparticles was obtainable in powder form and it was dissolved in distilled water by using ultrasonicator and administrated orally using gastric gavage to Group C (15 mg/kg/day) after 2 weeks from time of induction of periodontitis. Concerning group B, distilled water only was orally administered using the same procedure.

Curcumin nanoparticle characterization (16)
Curcumin nanoparticles utilized in the current experiment were purchased from Nanotech Egypt Chemical Company. They were characterized by using the transmission electron microscope (TEM) {Electron Microscopy Unit, Faculty of Science, University of Alexandria}. A little drop of the CURN suspension was positioned onto the TEM grids, coated with a thin carbon film and let to evaporate. Then, electron micrographs of several locations on the grid were taken (17). The size and morphology of CURN in the current study revealed that the individual particles had a diameter < 40 nm. The shape of particles was mainly spherical and formed electron dense aggregates of atypical sizes, as presented by transmission electron micrographs (Fig. 1).

Euthanization time (18)
The end of the experimental period was at two intervals, after 2 and 4 weeks respectively from the beginning of detection of periodontitis (time of ligation removal). A total number of 14 rats were euthanized from both group B and C (7 from each group) at each interval by decapitation. Concerning the control group, 7 rats were euthanized at the end of experiment. The mandible of each rat was dissected out and the right molar segments were prepared for scanning electron microscope and energy dispersive x-ray microanalysis (EDX). Method of disposal of the rats was done by burning.

Scanning Electron Microscope (SEM) (19)
Specimens were examined by scanning electron microscope at the scanning electron microscope unit in the Faculty of Science Alexandria University to study the surface topography of alveolar bone in different groups. The samples were mounted using silver paint on the specimen holder then coated with gold to be equipped for scanning electron microscopic examination.

Energy Dispersive X-ray (EDX) (19)
Chemical characterization of the samples by analytical X-ray was used to compare the different percentages of calcium and phosphorus in the alveolar bone of the different groups.

Statistical analysis
The data obtained from EDX was collected and analyzed using ANOVA test to compare the overall difference between the three groups.

Research of animal ethics committee approval
The study was performed after gaining the approval of the Research of Animal Ethics Committee, Faculty of Dentistry, Alexandria University.

RESULTS

Scanning electron microscopic results
**Group A (control group)**
The surface topography of the buccal cortical plate of the alveolar bone revealed a generalized smooth and uniform
surface. In all the specimens examined the alveolar bone was intact with normal level around mandibular first molar. It also exhibited multiple nutritive canals with smooth regular borders (Fig. 2).

Figure (1): Transmission electron micrograph (TEM) of CURN suspension showing that most of the nanoparticles are spherical in shape with diameter < 40 nm. (X200)

Figure (2): SEM image of control group showing: intact alveolar bone with normal level around mandibular first molar (arrows). The surface of the buccal cortical plate of the alveolar bone appears smooth and uniform (X30). Inset: higher magnification revealed: smooth uniform surface topography of buccal cortical plate with regular border of the nutritive canal (arrow heads). (X500)

First observational period (2 weeks interval)

Group B (periodontitis group)
The surface topography of the buccal cortical plate at the area of the first molar revealed marked generalized roughness with irregular resorptive craters, porosities and prevailing deep areas of erosions and pits. Pronounced discontinuation of the alveolar bone surface architecture was observed with severe resorption in the level of alveolar bone around mandibular first molar extended to the root apical area (Fig. 3).

Group C (curcumin nanoparticles treated group)
The surface topography of the buccal cortical plate covering the root of the first molars exhibited moderate surface roughening and porosity in comparison to group B. Some homogenous and smooth bone surface areas free of defects were noticed alternating with roughened irregular areas that exhibited shallow depressions. Partial restoration in the level of alveolar bone was revealed around mandibular first molar (Fig. 4).

Second observational period (4 weeks interval)

Group B (periodontitis group)
The surface topography of the buccal cortical plate of the alveolar bone after 4 weeks of induced periodontitis revealed decrease in surface irregularities with moderate resorption and discontinuation of the bone surface. The architecture of bone surface exhibited less resorption in comparison to the same group at two weeks with less reduction in the level of alveolar bone around mandibular first molar. Irregular nutritive canals surrounded by roughly resorbed borders were also observed (Fig. 5).

Group C (curcumin nanoparticles treated group)
The surface topography of the buccal cortical plate of the alveolar bone in the region of the first molar exhibited generalized uniform, smooth and homogenous pattern free of defects restoring its normal architecture as the control group A. In comparison to group B, the alveolar bone surface revealed marked masking of almost all resorptive changes related to periodontitis group B at this interval. Marked restoration in the level of the alveolar bone around mandibular first molar was revealed. Intact well-defined nutritive canals with regularly borders were also noticed (Fig. 6).

Figure (3): SEM image of periodontitis group (2 weeks after removal of ligature) showing: severe resorption in the level of the alveolar bone around mandibular first molar (arrows). The buccal cortical plate showed generalized roughness of the surface with severe porosity (X30). Inset: higher magnification revealed marked roughness, generalized pattern of bone surface porosity and loss of surface architecture in the buccal cortical plate. Note: various areas of deep erosions (arrow heads) (X500)

Figure (4): SEM image of curcumin nanoparticles treated group after 2 weeks showing: partial restoration in the level of alveolar bone around mandibular first molar (arrows). The surface topography of the buccal cortical plate exhibited a pattern of moderate surface roughening and porosity (X30). Inset: Higher magnification showed alternating areas of relatively smooth (white arrowhead) and rough areas (black arrowhead). (X500)
Abu Ayana et al.

**Figure (5):** SEM image of periodontitis group after 4 weeks showing: reduction in the level of alveolar bone around mandibular first molar (arrows). The surface topography exhibited generalized surface roughening. (X30). Inset: higher magnification revealed the buccal cortical plate with areas of severe resorption and porosity, associated with irregular bordered nutritive canal (arrowheads). (X500)

**Figure (6):** SEM image of curcumin nanoparticles treated group for 4 weeks showing: marked restoration of the level of the alveolar bone around mandibular first molar (arrows). The surface topography of the buccal cortical plate relatively restored its normal architecture. (X30). Inset: higher magnification revealed intact, smooth surface topography of buccal cortical with regular border nutritive canals (arrowheads). (X500).

**Results of EDX microanalysis**

**Energy Dispersive X-Ray Analysis (EDXA)**

The results of EDX regarding percentage of calcium in both intervals (after 2 and 4 weeks) showed the control group with the highest values followed by group C (periodontitis treated group by CURN). However, periodontitis group B showed the lowest values (Table 1).

**Table (1):** Comparison between different studied groups regarding calcium level at different periods of follow up.

<table>
<thead>
<tr>
<th></th>
<th>Control gp</th>
<th>Periodontitis gp</th>
<th>Curcumin nanoparticle treated gp</th>
<th>ANOVA test</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2 weeks</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>69.1-73.61</td>
<td>50.7-53.5</td>
<td>62.15-65.8</td>
<td>21.03</td>
<td>0.001</td>
<td>0.0023</td>
<td>0.005</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>70.88</td>
<td>51.823</td>
<td>63.251</td>
<td>0.623</td>
<td>0.000*</td>
<td>0.0023*</td>
<td>0.005*</td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td>1.60</td>
<td>1.157</td>
<td>0.623</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

At two weeks interval, the difference between group A and group B, as well as, in comparison to group C was statistically significant. Moreover, the difference between group B and group C was statistically significant. On the other hand, at four weeks, the difference between the control group A and group C was not statistically significant but it was significant in comparison to group B. A comparison between the three studied groups concerning percentage of phosphorus at the same two intervals revealed that in both intervals, the periodontitis group B had the highest values followed by group C treated by curcumin nanoparticles, while the control group A showed the lowest values (Table 2).

**Table (2):** Comparison between different studied groups regarding phosphorus level at different periods of follow up.

<table>
<thead>
<tr>
<th></th>
<th>Control gp</th>
<th>Periodontitis gp</th>
<th>Curcumin nanoparticle treated gp</th>
<th>ANOVA test</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2 weeks</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>25.4-31.2</td>
<td>46.5-49.3</td>
<td>36.2-37.85</td>
<td>16.05</td>
<td>0.001*</td>
<td>0.005</td>
<td>0.013</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>28.43</td>
<td>48.177</td>
<td>36.749</td>
<td>0.623</td>
<td>0.005*</td>
<td>0.013*</td>
<td></td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td>2.12</td>
<td>1.157</td>
<td>11.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

At two weeks interval, the difference between the control group A and group B was statistically significant, it was also significant in comparison to group C. In addition, the difference between the periodontitis group B and group C treated with CURN was significant too. On the contrary, at four weeks, the difference between the control group A and group C treated by curcumin nanoparticles was not statistically significant. However, the difference between the group B and group C was statistically significant.

**DISCUSSION**

Nanotechnology as a promising technology, plays an increasingly important role in the field of dentistry especially in periodontal management (13). Application of nanomaterials with excellent physical, chemical and...
biological properties play an important role in stimulating cell growth and periodontal regeneration (20).

Curcumin is a natural plant used mainly as a spice in diet. It is composed mainly of three curcuminoids including curcumin, demethoxycurcumin and bisdemethoxycurcumin. It exerts an effective anti-inflammatory, anti-cancer, antimicrobial and most importantly antioxidant properties (21). However, due to its low bioavailability, water insolubility, and a short plasma half-life, curcumin has a limited clinical use (22). Therefore, to overcome problems of curcumin, the researchers synthesized curcumin nanoparticles which are more effective than curcumin due to its better pharmacological properties (6).

The present experiment assessed the efficacy of this curcumin nanoparticles, when administrated orally (for 2 and 4 weeks) on the alveolar bone loss in rat model with ligature induced periodontitis using SEM and EDX.

Curcumin nanoparticles powder dissolved in distilled water was used in this research because of the improvement of its physicochemical properties that occur by reduction of its particle size and creation of an amorphous state with high-energy which boosted drug solubility in water and its release (23). Moreover, CURN were administrated orally with a dose (15 mg/kg/day). This dose has been shown to be efficient to ameliorate the periodontal tissue and restore alveolar bone when used for the treatment of experimental arsenic intoxication (24).

Induction of periodontitis in the current study was performed by using 4-0 silk ligature for 2 weeks. This is in accordance to Moraes et al (2020) (25) and other researchers (26,27) who used silk ligatures to induce experimental periodontitis because it is a simple, effective, cheap and an available method. In addition, Kurt et al (14) proved that tying silk ligatures around the experimental rats’ mandibular first molars for 2 weeks was capable to create an experimental periodontitis model.

The two and four weeks interval from time of ligature removal were estimated for this experiment. These intervals were in accordance with Zambrano et al (7) who demonstrated that locally administrated curcumin nanoparticles for four weeks in the lipopolysaccharides-induced model of experimental periodontitis inhibited inflammatory alveolar bone resorption.

The results of this study were not in favor of the null hypothesis, where CURN had a positive effect on reconstruction of the alveolar bone loss when compared to the untreated group.

The SEM results of the control group in the current experiment revealed generalized smooth and uniform surface topography of the buccal cortical plate of the alveolar bone.

Moreover, normal level of intact alveolar bone was observed around mandibular first molar of the control group with multiple nutritive canals exhibiting smooth regular borders. These observations were in accordance with De Souza et al (28) who confirmed presence of uniform intact alveolar bone in the control group.

In the present study, the SEM topography of periodontitis group B at two weeks interval after removal of ligature revealed pronounced generalized roughness of the surface of the buccal cortical plate with severe porosity and deep areas of erosions. On the other hand, at 4 weeks interval there was a decrease in surface roughness and irregularities of the buccal cortical plate but to a lesser extent in comparison to the same group at two weeks. Roughly borders nutritive canals were also detected denoting resorption process.

These results were in agreement with Li et al (29) who ensured that there is correlation between alveolar bone damage and periodontitis. In addition, Silva et al (30) proved that there was an increase in the alveolar bone loss in animals with periodontal disease due to stimulation of the release of inflammatory cytokines. Moreover, this bone loss was due to increased expression of the osteoclastogenic mediator RANKL and reduced expression of the osteogenesis related factor RUNX2, as well as, the anti-osteoclastogenic factor OPG (31).

Marked resorption in the level of the alveolar bone around mandibular first molar was observed in periodontitis group B after two weeks interval with less reduction observed in the same group after four weeks. These changes between two and four weeks were due to beginning of the signs of repair, removal of the cause and the time lapse between the two intervals. These results were in accordance with Vargas-Sanchez who found progressive bone resorption after fifteen days with no further progression after thirty days (32).

Concerning Group C (curcumin nanoparticles periodontitis treated group) the SEM results after 2 weeks interval revealed a decrease in surface roughness of the buccal cortical plate with moderate porosities in comparison to group B. Whereas after four weeks interval, the SEM results in group C revealed a generalized uniform, smooth and homogenous pattern of the surface topography of the buccal cortical plate of the alveolar bone resembling the control group. These results were in accordance with Mau et al (33) who found that the alveolar bone loss in the curcumin treated group was significantly reduced compared to the teeth in the ligation group seen by scanning micro-CT analysis.

Slight restoration in the level of alveolar bone around mandibular first molar was also noticed in group C after 2 weeks interval in the present study as compared to periodontitis group. Whereas, pronounced restoration of the level of the alveolar bone in group C after four weeks interval was presented. The alveolar bone surface revealed marked masking of almost all resorptive changes related to group B with intact well-defined nutritive canals due to remineralization and restoration that occurred.

A systematic meta-analysis of preclinical in vivo studies in 2020 by Borges et al., (4) demonstrated that curcumin significantly decreases loss of the alveolar bone in models of experimental periodontitis in rats with the best results obtained from curcumin which undergo to some chemical modifications.

Moreover, Zambrano et al (7) confirmed that the local application of curcumin nanoparticles (CURN) in the LPS-induced experimental periodontitis model inhibits inflammatory bone resorption associated with a diminish in the osteoclast cells numbers. Furthermore, Peng et al (34) found that CURN is beneficial in treating periprosthetic joint infections by reducing ROS production and cytokine expression.

Energy Dispersive X-Ray was used in this study as it is an efficient method for detection of different percentages of calcium and phosphorous in the bone (35). In the present study,
the results of the EDX confirmed the SEM observations. Concerning the control group, the calcium level presented the highest values in comparison to periodontitis and curcumin nanoparticle treated groups. Whereas, phosphorous exhibited the lowest values compared to the other two groups. In a similar study, Farag et al (36) found that the EDX analysis on the alveolar bone after administration of nucleotide analogues revealed that the highest percentage of Ca was associated with the control group.

Calcium and phosphorous are considered the main components of the mineralized bone matrix. Calcium is the most plentiful cation in the body with about 99% in the mineral bone phase and the remaining 1% exists within the intracellular and extracellular fluids. It is necessary for maintaining the bone mass essential to support the skeleton. Likewise, about 85% of phosphorous in the body presents in the mineralized bone. It is a crucial structural component of cell membranes and nucleic acids and is involved in bone mineralization. These elements interact in several essential processes in the body because of physicochemical and hormonal factors (37).

Regarding the EDX results of the periodontitis group B, it revealed a marked decrease in calcium percentage in relation to phosphorous after two-weeks interval followed by four-weeks interval which exhibited lesser decrease in calcium. These results were in agreement with Kourkoumelis et al (38) who stated that “There is a strong relationship between lowered Ca/P ratio and induced bone loss, as Ca/P ratios of cortical bone are significantly reduced in all osteoporotic cases compared to normal bone seen in controls”. Moreover, the Ca level in femoral sections of osteoporotic animals was reported by Fei et al to be lowered than that in the control group due to partially release of calcium into the blood circulation during the process of bone resorption (39).

Energy Dispersive X-Ray results of the group C (CURN treated periodontitis group) in the present study revealed an increase in the calcium level with relative regaining of the normal ratio between Ca and P in relation to group (B) periodontitis group. Tzaphlidou et al (40) suggested that bone density, in addition to bone quality, played a major role in bone strength and had strong relation with the Ca/P ratio. Therefore, Ca/P ratio was not distributed regularly within areas exhibit bone loss. Whereas, high Ca/P ratio were observed when treatment was established followed by bone restoration. Appropriate balance between calcium and phosphorous levels is critical for sustaining bone mineral homeostasis and is considered a key for maintenance of bone health (38).

Finally, all the previous SEM and EDX findings in the present study of the deteriorated bone tissue of periodontitis group, revealed remarkable changes from those of CURN treated and control groups. The detected results of the current experiment guaranteed the beneficial effect of CURN administration on the re-establishment of the alveolar bone structure in rats with induced periodontitis.

CONCLUSION

Curcumin nanoparticles exerts positive influence on alveolar bone structure in rats with induced periodontitis. The efficacy of CURN therapy in periodontitis is possibly attributed to its antioxidant and anti-inflammatory effect.

Conflict of interest

We declare that we have no conflicts of interest.

Funding

The authors received no specific funding for this work.

REFERENCES


