International Journal Dental and Medical Sciences Research
Volume 4, Issue 1, Jan-Feb 2022 pp 562-566 www.ijdmsrjournal.com ISSN: 2582-6018

Assessment of Surgically Made Alveolar Bone Defects Healing in Response to Curcumin Powder: An Experimental Study on Rabbits

1, 2Ahmed Gh. Altaeeand Omer W. Majid
1BDS, M.Sc. Student, Nineveh Health Directorate, Ministry Of Health, Nineveh, Iraq.
2BDS , MSc , FICMS (Prof.) , Ministry Of Higher Education and Scientific Research , University of Mosul , College of Dentistry , Department of Oral and Maxillofacial Surgery, Nineveh, Iraq.

Submitted: 01-02-2022 Revised: 12-02-2022 Accepted: 16-02-2022

ABSTRACT

Objective: The aim of current study is to evaluate the effect of curcumin powder on healing process of surgically made maxillary alveolar bone defects and new bone tissue formation in rabbits.

Materials and methods: Twenty albino rabbits were randomly chosen to conduct the study, all selected rabbits were inhabited under the same circumstances involving ventilation, temperature and diet. Each rabbit received the anesthetic solution separately and underwent surgical procedure in both sides of maxillary alveolar bone which involved a longitudinal oral mucosal incision followed by circular bone defect creation and further placement of 25mg of curcumin mixed with 2 drop od distilled water and a tiny gel foam piece and in left side of the animal's jaw bone, while right side bone hole was left empty for controlling. According to euthanization date, rabbits were divided into five groups and euthanized at the 3rd, 7th, 14th ,28th and 42 days' post-surgery, histomorphometric assessment included evaluation of amount of granulation tissue formed and calculation of surface area of newly formed bone tissue in relation to microscopical field using special image analysis software.

Results: Histomorphometric analysis results revealed a statistically significant difference in amount of granulation tissue and new bone tissue surface area between control and curcumin groups at all-time intervals of the study of curcumin groups.

Conclusion: The use of Curcuminhas a beneficial effect on bone healing process with its enhancement effect on new bone tissue formation.

Keywords:healing, curcumin, bone.

I. INTRODUCTION

Curcumin is a plant species in the ginger family (Zingiberaceae) that has been utilized as a dietary spice and coloring ingredient in Indian and Chinese cuisines throughout history. [1] Curcumin has previously been found to improve epithelial regeneration, fibroblast proliferation, and vascular density when applied to wounds. [2] The results of in vitro experiments have also shown that curcumin can inhibit osteoblast proliferation and function.[3] Bone repair process is unique and highly organized, repaired bone can be replaced to its original form without scar formation, so the term 'bone heals by bone' is used to describe this bone characteristic. [4]

Bone healing is a series of complex biological events. It is a multistage wellorchestrated regenerative process initiated in response to injury and resulted in optimal repair and restoration of function. It involves intracellular and extracellular molecular signaling for induction and conduction of bone tissue formation. Many local and systemic regulatory factors including growth factors, hormones, cytokines, and extracellular matrix, interact with various cell types recruited at the injury site or from the circulation. All components involved in the injury site, involving the bone cortex, periosteum, bone marrow, and external soft tissues, participate in repair process at different extent, depending on multiple parameters present at the injured tissue such as growth factors, hormones, nutrients, pH, oxygen tension and others. [5, 6]

Alveolar bone is considered as a specialized bone tissue due to its distinctive features, it has continuous and rapid remodeling events in response to stimuli by force since it supporting teeth which rendering it to functional demands such as the forces derived from mastication andswallowing and further unceasing adaptation to such stimuli. Another particularity is the constant microbial challenge that encounter alveolar bone and make it prone to infectious processes and associated impaired bone healing sequel which is opposite to the other bone fracture sites which are usually considered a sterile milieu. While long bone healing occurs by endochondral

ossification, alveolar bone healing typically occurs without histological cartilage formation.[7]

II. MATERIALS AND METHODS

The study was conducted at university of Mosul / College of Dentistry and approved by Research Ethics Committee board under ethical approval no. Max.O.F.S/A.L.I/19. Twenty Albino male rabbits with 1.3 – 1.5 Kg weight range and 4 – 6 months' age range were chosen for achievement of this study. All study rabbits were examined and observed for general health condition by veterinarian and inhabited in an animal house in a standard environment receiving the same feeding protocol. The surgical procedure was carried out under aseptic conditions, each rabbit was generally anesthetized with a solution of a mixture contained 1ml of ketamine hydrochloride (35mg/kg dose) and 0.5ml of Xylazine hydrochloride (5mg/kg dose) intramuscularly (9), 10 -15 minutes later ear pinch reflex was performed to ensure effectiveness of anesthesia, then the animal was laid down on his right side on the surgical board and covered with sterile towel exposing oral cavity only. Thereafter about 1 cm longitudinal incision was made in the maxillary oral mucosa perpendicular on the alveolar ridge in the left saddle area posterior to upper central incisors, followed by blunt tissue dissection for full thickness mucoperiosteal flap elevation and alveolar bone exposure. Then a circular bone defect of standard 4 mm diameter and 4.5 mm depth was made with aid of a trephine bur and straight surgical hand piece mounted on a slow motor dental engine under copious irrigation of distilled water then 25mg of curcumin powder with 2 drops of distilled water and a tiny piece of gel foam were placed within created bone defect successively. Flap edges were repositioned and sutured on the defect with 2-3 simple interrupted stitches accordingly using 5/0 black silk suture and wound toilet. The same procedure was carried out on the right side of the jaw bone also but the right bone hole received nothing and had been made for controlling. Postoperative animal care was done by a veterinarian which had been involving daily checkup for general and oral health condition of the rabbits and daily single dose of 50mg/kg Oxytetracycline during the first three days of surgery. Study rabbits were randomly divided into five groups according to the time interval of euthanization, they were euthanized at the 3rd day, 7th, 14th 28th, 42 days successively, each time interval group contained four rabbits which were act as control and curcumin group as each rabbit was subjected to surgery at both jaw bone sides. After each group rabbits euthanization, operated bone defects areas were dissected as blocks of bone tissue with sufficient margins and were directly preserved into 10% freshly prepared buffer formalin for three days' period for tissue fixation, then the specimens were sent for decalcification process and subsequent histological preparation and expertise examination and assessment in specialized laboratory. For histological assessment of amount of granulation tissue formed in bone specimens a semi-quantitative scale was adopted to give a scoring system ranged 0: for mild amount to 3: for profound amount of granulation tissue formed. [8] Whereas for histomorphometric surface area calculation of newly formed bone spicules in relation to microscopical field a computerized image analysis was used with specialized image analysis software designed to be integrated with digital camera (OMAX ToupView software and OMAX digital camera, showed in Figure (1), and Olympus® CX31 light microscope which equipped with it microscopic lenses calibration was achieved using,stage micrometer, surface area calculation of manually demarcated bone spicules was appeared on screen in square micrometers (μm^2) as seen in Figure (2). [9] Statistical analysis was done using SPSS software version 24. Data were expressed as means of newly formed bone tissue surface area in relation to 10X operating field surface area, and statistically analyzed by use of Mann-Whitney and Independent Samples t - tests at $\rho \leq 0.05$.

RESULTS

The results of histological evaluation and statistical analysis revealed a significant difference in surface area of newly formed bone tissue in alveolar bone specimens between control and curcumin groups at all-time intervals of the study, the resultant measurements of Curcumin group were bigger and highly significant as shown in Figures (3) and summarized in Table (1). However histological evaluation and statistical analysis also revealed a statistically significant difference in amount of granulation tissue formed in alveolar bone specimens between control and Curcumin groups at all-time intervals with favor to Curcumin groups with significant higher scores recorded as shown in Figure (4) and summarized in table (2).

International Journal Dental and Medical Sciences Research
Volume 4, Issue 1, Jan-Feb 2022 pp 562-566 www.ijdmsrjournal.com ISSN: 2582-6018

Figure (1): Special image analysis software and equipment.

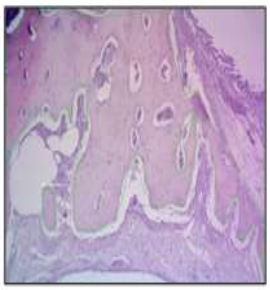

Figure (2): Demonstration for surface area of newly formed bone calculation technique using special image analysis software at 10X magnification power microscopical field.

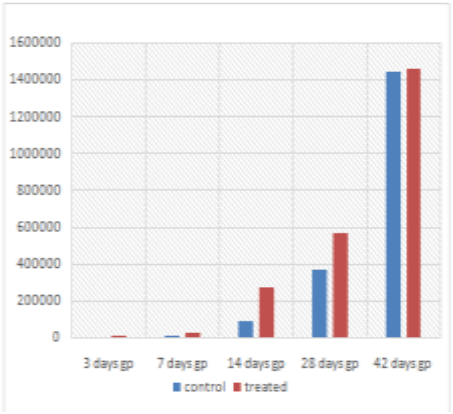

Fig. (3): Diagrammatic representation for mean value of new bone formation measurements of alveolar bone.

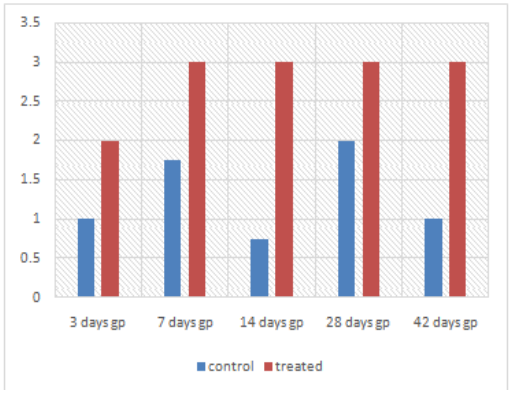

Fig. (4): Diagrammatic representation for mean values of granulation tissue formed in alveolar bone.

Table (1): Statistical analysis of newly formed bone surface area represented as a mean and analyzed with Independent Samples t-test at $p \leq 0.05$.

Time Intervals	**Means of new bone surface area of control groups in µm²**	**Means of new bone surface area of Curcumin groups in µm²**	**p -Value**
3 Days	0	11585.6	0.01*
7 Days	13149.4	33642.64	0.001**

International Journal Dental and Medical Sciences Research
Volume 4, Issue 1, Jan-Feb 2022 pp 562-566 www.ijdmsrjournal.com ISSN: 2582-6018

14 Days	96568.6	265904.2	0.002*
28 Days	366116.6	550878.8	0.001**
42 Days	1422925.8	1437737.66	0.01*

* The means are significantly different at $p \leq 0.05$.
** The means are highly significantly different at $p \leq 0.01$.

Table (2): Statistical analysis of newly formed granulation tissue represented as mean of scores and analyzed with Mann-Whitney test at $p \leq 0.05$.

Time Intervals	Means of granulation tissue scores of control groups	Means of granulation tissue scores of Curcumin groups	p-Value
3 Days	1	2	0.04*
7 Days	1.75	3	0.008**
14 Days	0.75	3	0.017*
28 Days	2	3	0.013*
42 Days	1	3	0.01*

*The means are significantly different at $p \leq 0.05$.
**The means are highly significantly different at $p \leq 0.01$.

DISCUSSION

Several studies have reported the effect of curcumin on bone formation which exhibit osteoinductive characteristics. [10] The present study outcomes suggested that the curcumin has a good effect on the healing of bone and regeneration development via its improvement effect on granulation tissue and new bone tissue formed, decreasing inflammatory infiltration, and acceleration of newly bone creation process. Bone defects treated with curcumin showed less inflammatory reaction and greater quantities of granulation tissue formed with subsequent marked increase in new bone tissue formation quantity throughout all time intervals of the study with greater means of surface area estimated in relation to microscopical histological field. The amount of bone formation is started from the first interval at three days group more than control group. This positive effect of curcumin on bone regeneration is in accordance with Cirano et al., (2018) who stated that the use of curcumin powder stimulates bone repair via increasing osteogenesis and calcification rate, and accelerating new bone formation process which they attribute to anti- inflammatory, antioxidant and regenerative power of such extract due to its active phytochemical components. This study investigated the optimal concentration of

curcumin on enhancing osteogenesis. Bone formation was evaluated by histological staining. The findings revealed that Cur. Suprpressed apoptosis and enhanced proliferation and osteogenesis-related gene expressions of osteoblasts under. The histological sections displayed reduced bone destruction and increased the growth rate of trabecular bone and the bone density of treated groups with curcumin, compared to control groups. These results showed that curcumin could reverse the harmful effects of osteocalast.

The process of bone healing is affected by various factors with bone cell (osteoblast) activity and nutrient supply being two important factors. [11, 12].To further evaluate the effect of curcumin on fracture healing, the osteoblasts were observed histologically. Improved fracture healing is related to a sufficient number of osteoblasts and osteoblast activity Osteoblasts are cells that can be transported to the bone surface through the microvasculature, resulting in the production of bone matrix, with the transformation of osteoblasts to osteocytes. [13].the results of this study showed that the number of osteoblasts in the curcumin-treated group was greater than that of the control group.

CONCLUSION

A conclusion could be stated emphasizing on the positive effect of Curcumin ppowder on bone healing and regeneration, which supported by the study findings that revealed production of higher rate of granulation tissue quantity and newly formed bone tissue.

REFERENCES

[1]. Chattopadhyay I, Biswas K, Bandyopadhyay U, Banerjee R. (2004) Turmeric and curcumin: biological actions and medicinal applications. Current Science, 87, 44–53.
[2]. Akbik, Dania, et al. (2014). Curcumin as a wound healing agent. Life sciences, Vol. 116, No. 1, pp. 1-7.
[3]. Chen HT, Tsou HK, Chang CH, and Tang CH: Hepatocyte growth factor increases osteopontin expression in human osteoblasts through PI3K, Akt, c-Src, and AP-1 signaling pathway. PLoS One. (2012); 7
[4]. Greenbaum MA, Kanat IO. Current concepts in bone healing: Review of the literature. J Am Podiatr Med Assoc. 1993, 83(3):123-129.
[5]. Dimitriou R, Tsiridis E, Giannoudis PV. Current concepts of molecular aspects of bone healing. Injury, Int. J. Care Injured. 2005; 36(12): 1392–1404.
[6]. Burr DB, Allen MR. Basic and Applied Bone Biology. 2nd ed. Academic Press, Elsevier Inc. San Diego, USA. 2019; p: 235–237.
[7]. Vieira AE, Repeke CE, Ferreira Junior S, Colavite PM, Biguetti CC, Oliveira RC, Assis GF, Taga R, Trombone AF, Garlet GP. Intramembranous Bone Healing Process Subsequent to Tooth Extraction in Mice: Micro-Computed Tomography, Histomorphometric and Molecular Characterization. PLoS One Journal. 2015; 10(5): 1–22.
[8]. Gupta A, Kumar P. Assessment of the histological state of the healing wound. Plast Aesthet Res. 2015, 2(2015): 239-242.
[9]. Han, Z, Bhavsar, M, Leppik, L, Oliveira, KMC, Barker, JH. Histological Scoring Method to Assess Bone Healing in Critical Size Bone Defect Models. Tissue Engineering and Regenerative Medicine International Society. 2018; 24(5): 272–279.
[10]. Cirano, F.R.; Pimentel, S.P.; Casati, M.Z.; Corrêa, M.G.; Pino, D.S.; Messora, M.R.; Silva, P.H.F.; Ribeiro, F.V. (2018). Effect of curcumin on bone tissue in the diabetic rat: Repair of peri-implant and critical-sized defects. Int. J. Oral. Maxillofac. Surg. 1495–1503.
[11]. Camassa JA, Diogo CC, Sousa CP, et al. (2010). Bone turnover markers in sheep and goat: A review of the scientific literature. An Acad Bras Cienc. 89:231–45.
[12]. Khosla S, Westendorf JJ, Modder UI. (2010). concise review: Insights from normal bone remodeling and stem cell-based therapies for bone repair. Stem Cells: p2124–28.
[13]. Buza JR, Einhorn T. (2016). Bone healing in 2016. Clin Cases Miner Bone Metab: 13:101–5.