International Journal Dental and Medical Sciences Research
Volume 4, Issue 1, Jan-Feb 2022 pp 555-561 www.ijdmsrjournal.com ISSN: 2582-6018

The Effect of Curcuminon Healing of Surgically Made Maxillary Mucosal Wounds in 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 purpose of present study is to evaluate the effect of curcumin on granulation tissue formation and angiogenesis and re-epithelialization stages of experimental healing process of maxillary oral mucosa in rabbits.
Materials and methods: The current experimental study was carried out
On twenty albino rabbits which were randomly selected and inhabited under same conditions involving ventilation, temperature and diet. Each rabbit was anesthetized separately and undergone surgical procedure in both sides of upper alveolar bone included longitudinal oral mucosal incision followed by circular bone defect creation, then placement of tiny gel foam piece and curcumin in left side, while right side bone hole was left empty to act as control. According to euthanizationtime, rabbits were divided into five groups and sacrificed at the3rd,7th,14th, 28th and 42 days post-operatively, histological assessment included evaluation of amount of granulation tissue formed and angiogenesis and re-epithelialization.
Results: Histological results revealed a statistically significant difference in amount of granulation tissue and neo-vascularization formed and re-epithelializationbetween control group and curcumin groups at certain time intervals within total period of experiment with favor to Curcumingroup.
Conclusion: The use of Curcumin exhibits a beneficial effect by increasing granulation tissue formation and subsequent angiogenesis and enhancement of re-epithelialization in healing process.
Keywords:healing, curcumin, oral mucosa.

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]

Wound healing is one of the most complicated processes in multicellular animals, comprising multiple phases such as hemostasis,inflammation, proliferation, and remodeling.[4]The oral mucosa is a well-adapted structure designed to protect the underlying tissues against mechanical damage andprevent microorganisms and their toxic materials entrance.However, this protective barrier may be disrupted by different causative agents which lead to wound formation. [5]Due to that wound healing is vital process and crucial step for survival finalizing in wound closure.Wound healing is a complicated and dynamic process of replacement of devitalized and missing cellular structures and tissue layers.[6]

The healing of wounds involves well-orchestrated series of events occurred as a carefully regulated, systemic cascade of overlapping processes which require coordinated completion of multiple different cellular activities.These cellular activities occur in correlation with appearance ofdifferent cell types in the wound during various stages of healing process. Healing process involves four overlapping but well-defined phases ofhemostasis,inflammation,proliferation, and remodelling and scar maturation.[7]

The proliferative phase characterized by fibroblast migration, extracellular matrix deposition and granulation tissue formation. With progression of the proliferative phase, newly formed granulation tissue is well established. Histologically granulation tissue has proliferating fibroblasts and loops of capillaries in a loose extracellular matrix. This phase is characterized by angiogenesis or neo-blood vessels formation from pre-existing vessels at the site of injury. Neovascularization represents an essential component in woundhealing process due to its principal impact on all healing stages, ensuring the sufficient nutritive perfusion of the wound, oxygen supplyand immune cells recruitment. [8]

Epithelialization of the wound represents the final stage of the proliferative phase.[7]Recently, the pericyte received more attention in wound healing issues.Its role in modulating angiogenesis and vascular development and stabilization of endothelium in newly formed blood vessels is well characterized. [8]

CD31 (PECAM-1 platelet/endothelial cell adhesion molecule-1) is a single chain trans-membrane protein that plays a role in adhesive interactions between adjacent endothelial cells as well as between leukocytes and endothelial cells , it is highly expressed at endothelial cell–cell junctions, it was originally described in the mid-1980s as the CD31 differentiation antigen expressed on the surface of many human cells .The immunohistochemicaldetection of this moleculehas been largely used in micro-vessel density assessment with reliable information.[9] [10]

Immunohistochemistry is a method for demonstration of distribution and localization of specific cellular components within cells and within their proper histological context (such as proteins molecules) in tissue sectionsusingantibody-antigeninteractions. Immunohistochemical staining is accomplished with antibodies that recognize the target antigen. Antibodies are highly specific,for that it will bind only to the antigen of interest in the tissue section. The antibody-antigen interaction is then visualized using either chromogenic or fluorescent detection. [11]

II. MATERIALS AND METHODS

The study was accomplished at university of Mosul / College of Dentistry and approved by Research Ethics Committee board in Oral and Maxillofacial surgery department. Twenty Albino male rabbit's weighted 1.5 Kg ± 150gm and aged 3 – 6 months had been selected to conduct the study. All study rabbits were let to acclimate to facility and examined for general health condition by veterinary physician and were housed in the animal house in a standard environment receiving the same feeding protocol. The surgical procedure was undertaken under aseptic conditions, each animal was generally anesthetized with a mixed solution composed of 1ml ketamine hydrochloride and 0.5 ml of xylazine hydrochloride intramuscularly, 10 - 15 minutes later the animal reflexes were checked to ensure loss of consciousness, then the animal was laid down on his right side on the surgical board and covered with sterile towel exposing oral cavity only.A longitudinal incision was made in the maxillary oral mucosa at left side perpendicular on the alveolar ridge in the saddle area posterior to upper central incisors of the animal, with blunt flap dissection a full thickness mucoperiosteal flap was reflected which exposing alveolar bone and prepare it for drilling. A circular bone hole was created using trephine bur with straight surgical handpiece and slow motor dental engine under copious distilled water irrigation , then a tiny piece of gel foam was placed within the hole to act as a vehicle , then thebony defect received25mg of CUR powder with 2 drops of distilled water was placed within thebony defect followed by a small piece of gel foam delivery into the defectwith gentle adaptation to maintain the study material settled, then the softtissue edges were repositioned andsutured on the defect which filled withcurcuminusing 5/0 black silk suture and simple interrupted technique with wound toilet . The same procedure was undertaken on the right side also but the right bone hole received nothing to act as control. Post-operative animal care was introduced by a veterinary physician which had been including daily checkup for general and oral health condition and daily single dose of 50mg/kg oxytetracycline during the first three days aftersurgery. Rabbits were randomly divided into five groups according to the time interval of euthanization, they were sacrificed at the 3rd day , 7th , 14th 28th , 42day successively , each time interval group contained four rabbits which were represent both control and Curcumingroup as each rabbit was subjected to surgery at both jawsides .After each group rabbits had been euthanized , the oral mucosal tissue at the operated areaswere dissected with sufficient margins and directly preserved into 10% freshly prepared buffer formalin in glass containers for three days period for tissue fixation , then the specimens were sent for histopathological preparation and subsequent expertise examination and assessment .For histological assessment of re-epithelialization and amount of granulation tissue formed in oral mucosa specimens a semi-quantitative method was conducted to give a scoring system ranged 0 – 4 for re-epithelialization and 0 – 3 for granulation tissue.[12][13]While angiogenesis was evaluated semi-quantitatively with aid of immunohistochemistry technique involving detection of CD31 antigen expression intensity in oral mucosal specimens with scoring system ranged 0 – 3 .Statistical analysis was employed by use of SPSS software version 24. Data were expressed as mean scores ± standard deviation, and statistically analyzed using Mann-Whitney test at$P \leq 0.05$.

International Journal Dental and Medical Sciences Research
Volume 4, Issue 1, Jan-Feb 2022 pp 555-561 www.ijdmsrjournal.com ISSN: 2582-6018

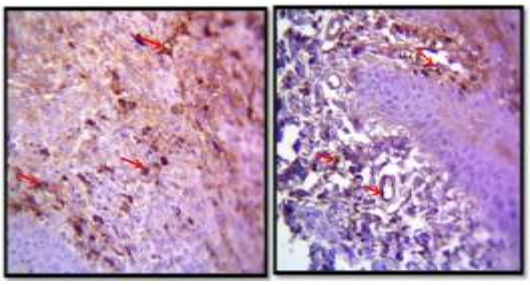

Figure (1): Oral mucosal specimens stained with Immunohistochemistry technique for CD31 epitope detection, red arrows refer to positively marked cells.

III. RESULTS

The results of histological evaluation and statistical analysis revealed that there was significant difference in amount of granulation tissue formed in oral mucosal specimens between control and curcumin groups at 2 and 4 weeks period, while there was no significant difference at 3 days, 1 and 6 weeks period with significant higher scores recorded forCurcumin group.Regarding angiogenesis evaluation of oral mucosal specimens, results revealed that there was significant difference in intensity of CD31 marker expression between control and Curcumin groups at 1 and 6 weeks period, while there was no significant difference at 3 days, 2 and 4 weeks period. On the other hand re-epithelialization showed a statistically significant differences at 1,2, and 4 weeks' time intervals with favor to Curcumingroup. The results are summarized in Table (1) and Figure (2),(3) and (4).

Table (1): Statistical Analysis of Oral Mucosal Healing assessment scores represented as mean ± standard deviation.

Group	3DAYs	1 WEEK	2 WEEKs	4 WEEKs	6 WEEKS
CD31 Expression					
Control	0.75±0.9 A	1±0.9 AAAA	2.75±0.9	2.75±0.9	1.75±0.9
Curcumin	0.75±0.9 A	1.75±0.9 B	2.75±0.9 A	2.75±0.9 A	3±0.9 B
Granulation Tissue Formation					
Control	0.75±0.6 A	1.75±0.6 AAAA	1.75±0.6	2±0.6	1±0.6
Curcumin	0.75±1 A	1.75±1 A	2.75±1 B	3±1 B	1±1 A
Re-epithelialization					
Control	0.25±1.4 A	1±1.4 AAAA	1.75±1.4	3±1.4	4±1.4
Curcumin	0.75±1.3 A	1.75±1.3 B	2.75±1.3 BB	3.75±1.3 A	4±1.3

* Mann-Whitney test at $p \leq 0.05$

International Journal Dental and Medical Sciences Research
Volume 4, Issue 1, Jan-Feb 2022 pp 555-561 www.ijdmsrjournal.com ISSN: 2582-6018

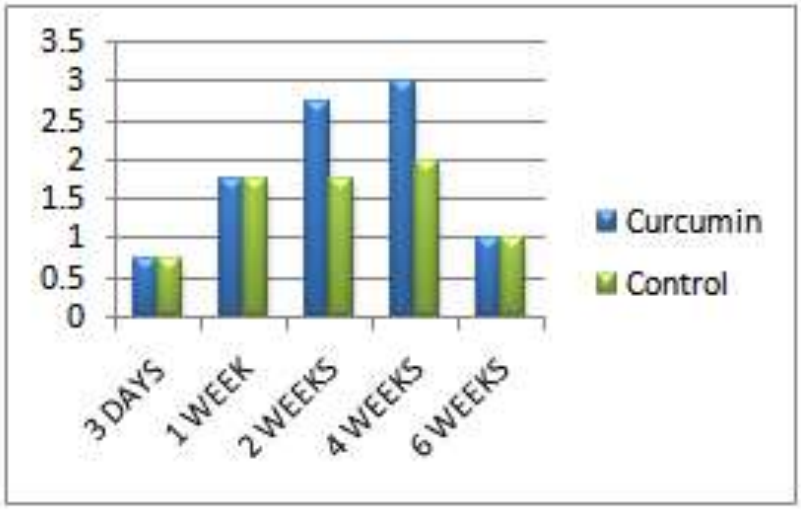

Figure (2) Diagrammatic Representation for Mean Values of Granulation tissue formation scores of oral mucosal Healing.

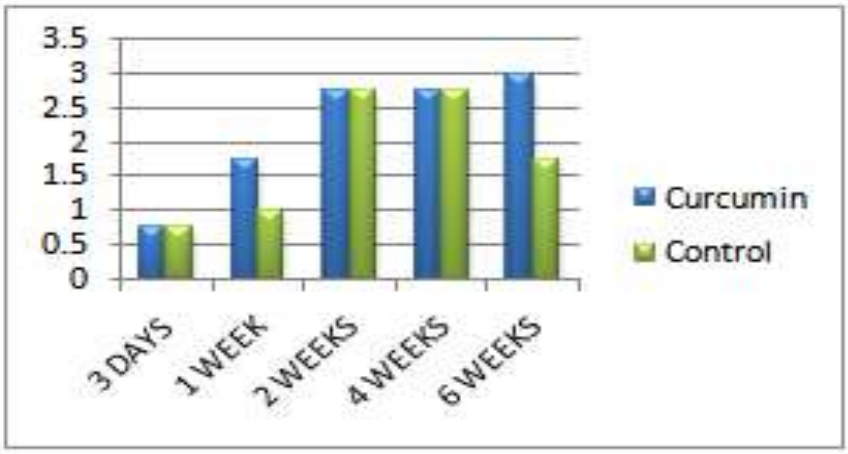

Figure (3) Diagrammatic Representation for Mean Values of CD31 Expression scores

International Journal Dental and Medical Sciences Research
Volume 4, Issue 1, Jan-Feb 2022 pp 555-561 www.ijdmsrjournal.com ISSN: 2582-6018

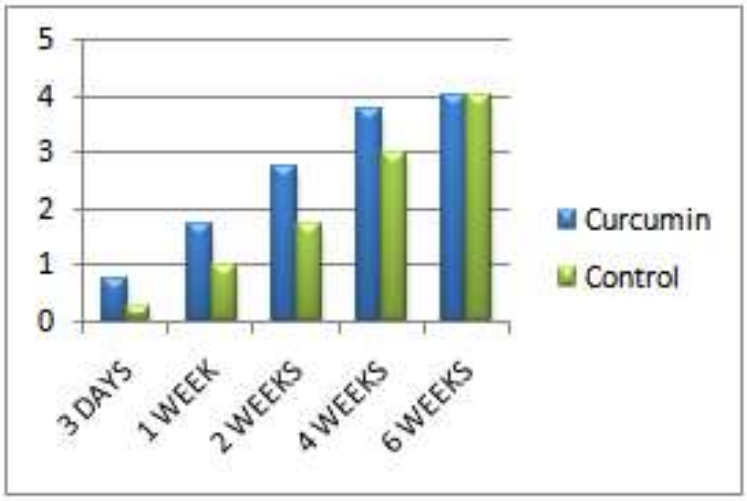

Figure (4) Diagrammatic Representation for Mean Values of Re-epithelialization of Oral Mucosal Healing.

IV. DISCUSSION

Turmeric is a spice made from the root of the Curcuma longa plant. It's a member of the Zingaberaceae ginger family. Turmeric has been used in Ayurveda for its therapeutic effects in a variety of therapies and via a variety of methods of administration, including topical, oral, and inhalation. Turmeric has been used as a herbal medication for years to cure a variety of diseases. Cur. (Chemical compound of turmeric) has been claimed to have anti-infective, anti-oxidant, anti-inflammatory, antimutagenic, anti-carcinogenic, and anti-coagulant capabilities, among other things. It works to speed up the wound healing process at various stages. Cur. can also help in granulation tissue formation, collagen deposition, tissue remodeling, and wound healing.[2] Cur. helps to regulate wound contraction faster by releasing growth factors that aid in the healing process [14] Cur has a dose-dependent effect on wound healing in vitro. At low concentrations, it can be stimulating, but at higher doses, it can be inhibiting. Curcumin was found to enhance fibrinolysis and cellular mobility in wound healing by changing the expression of urokinase plasminogen activator. [15]

The current study show that the curcumin powder has a beneficial effect on proliferative phase of healing process; through its promoting activity on the amount of granulation tissue formed. curcumin treated oral mucosal specimens showed granulation tissue formation means more than those of controls during the late time intervals of study and same means through the first two intervals of study which can be explained by delayed but persistent effect of curcumin on granulation tissue formation and the fact of rapid and highly active oral mucosa healing process rhythm. Delayed curcumin activity may be contributed to gel foam use as a vehicle for delivery of the powder to the surrounding tissues which settled within the bone hole and reducing the amount of the curcumin delivered to the oral mucosa. [16]The most prevalent connective tissue cell, fibroblasts, play an important role in wound healing. The majority of wounds heal without issues. Furthermore, numerous herbs exist that have the capacity to heal the wound without harming good tissue, minimize infection, and speed up wound healing. As a result, it promotes fibroblast migration, granulation tissue creation, collagen deposition, and overall re-epithelization, Improving wound contraction by increasing TGF- production and, as a result, fibroblast proliferation throughout the remodeling stage. Immunohistochemistry aid approach for angiogenesis evaluation was conducted in this study suggesting use of CD31 epitope directed antiserum because of that the literature indicates that CD31 would provide more consistent detection of small blood vessels and it has been reported to be more consistently expressed on developing capillaries.[17] Findings of the present study angiogenic evaluation of oral mucosa indicating a good effect of curcumin on neovascularization process indicated that systemic curcumin administration significantly accelerate the wound angiogenesis by the mechanism of upregulation of VEGF expression which confirmed by higher number of VEGF immunoreactive endothelial cells in the granulation tissue of curcumin treated

wounds. [18][19] It is advocated that new vascular formation would be sustained too with extra development of lesser blood vessels which can be noticed more perfectly by calculating of immunoreactive cells against anti-CD31 epitope as CD31 also can be expressed on the circulating platelets, neutrophils and monocytes surfaces, "counting of cells provided more precise and sensitive detection for delicate capillaries with tiny diameters which were stained to CD31 antigen detection but may be under assessed during evaluation of CD31 expression intensity semiquantitatively," which explaining our study results of higher means recorded for immunoreactive cells against anti-CD31 evaluation compared to CD31 expression and angiogenesis semi-quantitative evaluation.[20]Re-epithelialization represents the final step of the proliferative phase of healing process. It involves epithelial cells migration, proliferation and differentiation from the wound edges to cover the defect.(21) Improvement effect of wound epithelialization of curcumin has been proven , which mediated by promoting proliferation and migration of fibroblasts that play a central role in providing the extracellular matrix as a framework for keratinocyte migration and proliferation, subsequent higher number of fibroblasts and keratinocytes leads to acceleration and enhancement in epithelialization process .[21]

V. CONCLUSION

Within the current study limitations, it can be concluded that curcumin possesses a stimulatory effect on healing cells and their chemical mediators and granulation tissue formation process as well as neovascularization and re-epithelialization. Although the angiogenesis required for wound to heal could be controversial, a certain level of it is likely required for optimal healing.

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]. Wang PH, Huang BS, Horng HC, Yeh CC, Chen YJ. (2018). Wound healing. J Chinese Med Assoc: 81:94–101.
[5]. Deyhimi P., Khademi H., Birang R., and Akhoondzadeh M. Histological Evaluation of Wound Healing Process after Photodynamic Therapy of Rat Oral Mucosal Ulcer. J Dent Shiraz Univ Med Sci.2016. 17(1): 43-48.
[6]. Sorg H, Tilkorn DJ, Hager S, Hauser J, and Mirastschijski U. Skin Wound Healing: An Update on the Current Knowledge and Concepts.EurSurg Res. 2017. 58 (1-2):81-94.
[7]. Enoch S., and Leaper D.J.,Basic Science of Wound Healing. Surgery. 2008. 26(2): 31 - 37.
[8]. Bodnar R.J., Satish L., Yates C.C.,and Wells A. Pericytes: a newly recognized player in wound healing. Wound Repair and Regeneration J.2016. 24: 204–214.
[9]. Lertkiatmongkol P., Liao D., Mei H., Hu Y., and Newman P.J. Endothelial functions of platelet/endothelial celladhesion molecule-1 (CD31). Current opinion in hematology. 2016. 23 (3): 253 –259.
[10]. Pisacane A.M.,Picciottob F., and Risio M. CD31 and CD34 expression as immunohistochemical markers of endothelial transdifferentiation in human cutaneous melanoma. Cellular Oncology J. 2007. 29: 59–66.
[11]. Creager M.D. , Choi J., Hutcheson J.D., and Aikawa E. (2017). Immunohistochemistry. E. Aikawa. Comprehensive Biomaterials. II, Volume 3. Harvard Medical School,Boston,USA. 277-290.
[12]. Sinha U.K., Gallagher L.A. Effects of steel scalpel, ultrasonic scalpel, CO2 laser, and monopolar and bipolar electrosurgery on wound healing in guinea pig oral mucosa. Laryngoscope. 2003. 113: 228–236.
[13]. Gupta A., and Kumar P. Assessment of the histological state of the healing wound. PlastAesthet Res. 2015. 2: 239-242.
[14]. Tejada, Silvia, et al. "Wound healing effects of curcumin. (2016). A short review." Current Pharmaceutical Biotechnology, Vol. 17, No. 11, 2016, pp. 1002-1007.
[15]. MarjanFarshadi. (2017). Turmeric and the wound healing – beyond tradition: 2nd Euro-Global Congress on Melanoma and Skin Diseases London, UK.
[16]. Jones, K.B., and Klein, O.D. (2013). Oral epithelial stem cells in tissue maintenance and disease: the first steps in a long journey.

International Journal of Oral Science: 121–129.

[17]. Akhoondinasab M.R. , Akhoondinasab M. , and Saberi M. Comparison of Healing Effect of Aloe Vera Extract and Silver Sulfadiazine in Burn Injuries in Experimental Rat Model . World J PlastSurg. 2014. 3(1): 29-34.

[18]. Kant V, Gopal A, Kumar D, Pathak NN, Ram M, Jangir BL, Tandan SK, Kumar D.J. (2015). Curcumin-induced angiogenesis hastens wound healing in diabetic rats. Surg Res. 193(2):978-88.

[19]. Tremolada G, Del Turco C, Lattanzio R, et al. (2012). The role of angiogenesis in the development of proliferative diabetic retinopathy: impact of intravitrealantiVEGF treatment. Exp Diabetes Res.:728325.

[20]. Krishnamoorthy, B., Critchley, W.R., Bishop, P., Gieschen-Krische, M., Venkateswaran, R., Caress, A.C., Fildes, J.E., and Yonan, N. (2016). Validation of the Endothelial Markers CD31 and CD34 in Immunohistochemistry of the long Saphenous Vein for Coronary Artery By-pass Surgery. Madridge J Cardiol. 1(1): 8–11.

[21]. HidayatA.T.,Arifin M.T. ,Nur M. , Muniroh M. , and Susilaningsih N. Ozonated Aloe veraOil Effective Increased the Number of Fibroblasts and Collagen Thickening in the Healing Response of Full-Thickness Skin Defects . International Journal of Inflammation. 2021. 1 – 8.