Al-Rafidain Dental Journal

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Histomorphometric Assessment of Surgically Made Alveolar Bone Defects Healing in Response to Aloe Vera Gel: An Experimental Study on Rabbits

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Article information

Received: Juley 7, 2021
Accepted: August 11, 2021
Available online: March 10, 2023

Keywords
Aloe Vera
Bone regeneration
Acemannan
Histomorphometry

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Abstract

Aims: The aim of the current study is to evaluate the effect of Aloe Vera gel on the 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 a surgical procedure in both sides of the maxillary alveolar bone which involved a longitudinal oral mucosal incision followed by circular bone defect creation and further placement of Aloe Vera gel and a tiny gel foam piece in the left side of the animal's jaw bone, while right side bone hole was left empty for control. According to euthanization date, rabbits were divided into five groups and euthanized at the 3rd, 7th, 14th,28th, and 42 days post-surgery, the histomorphometric assessment included evaluation of the amount of granulation tissue formed and calculation of the surface area of newly formed bone tissue in relation to the microscopical field using special image analysis software. **Results:** Histomorphometric analysis results revealed a statistically significant difference in the amount of granulation tissue and new bone tissue surface area between control and Aloe Vera groups at all-time intervals of the study favoring Aloe Vera groups. **Conclusions:** Aloe Vera gel has a beneficial effect on bone healing process with its enhancement effect on new bone tissue formation.

الخلاصة

الأهداف تهدف هذه الدراسة الى تقييم تأثير مادة هلام الصبار في عملية شفاء العيوب العظمية في الأرانب. **المواد وطرائق العمل:** تم اختيار عشرون أرنباً أبيضاً بشكل عشوائي لإجراء هذه الدراسة وتم وضعهم في ظروف مناسبة متماثلة من حيث التغذية, درجة الحرارة والتهوية. تم خضوع كل ارنب الى تداخل جراحي في جهتي الفك العلوي بعد أن تم التخدير بشكل عام, كان التداخل الجراحي يشمل إجراء شق طولي في النسيج اللثوي ثم تكوين حفرة عظمية ذات ابعاد ثابتة في العظم السنخي, ثم يتم وضع مادة هلام الصبار والجل فوم فيها بشكل متتابع في الجهة اليسرى للعظم بينما تم ترك الحفرة في جهته اليمنى خالية ليتم اعتبارها كمجموعة ضابطة. تم تقسيم الارانب الى خمسة مجاميع بناءا على الفترات الزمنية المختلفة للتضحية بهم وهي 3 أيام, 7 أيام, 14 يوماً, 28 يوماً و42 يوماً لغرض قياس كمية النسيج الحُبيبي الالتئامي المتكون وقياس مساحة النسيج العظمي المتكون خلال عملية إعادة بناء العظم في منطقة العيب العظمي بعد أن تمت عمليات تحضير العينات نسيجياً. **النتائج:** كشفت النتائج النسيجية لهذه الدراسة عن زيادة ذات دلالة احصائية عالية في تكوين نسيج حُبيبي ونسيج عظمي التئامي في منطقة العيب العظمي المعالج بهلام الصبار مقارنة بالنسيجين الحُبيبي والعظمي الالتئامي المتكونين في العيب العظمي الضابط خلال جميع الفترات الزمنية للتجربة. **الاستنتاجات:** أن مادة هلام الصبار أظهرت تأثيراً ايجابياً ملحوظاً في عملية إعادة بناء العظم من حيث الزيادة الملحوظة في نمو النسيجين الحُبيبي والعظمي الالتئامي وتكوين نسيج عظمي جديد بنسبة اكثر.

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INTRODUCTION

Aloe Vera is a succulent perennial, drought-resisting plant, its therapeutic potential is well known and it had been used since ancient times by many cultures. A variety of beneficial effects of Aloe Vera have been reported including immunomodulatory, wound and burn healing, hypoglycemic, antioxidant, antifungal, and anti-inflammatory properties. It is a shrubby plant with green leaves shaped in a rosette pattern at the stem having a fleshy texture with serrated edges. Various parts of the plant contain approximately 200 active compounds including amino acids, polysaccharides, enzymes, vitamins, minerals, saponins, anthraquinones, lignin and salicylic acid. [1,2] Aloe is a rich source of polysaccharides and has many carbohydrate constituents, acemannan and mannose-6- phosphate are regarded as a major constituent of those Aloe carbohydrates. Acetylated mannan has a range of interesting biological activities, studies showed it has immunomodulatory effect activating macrophages, enhancement effect for cytokine release, and stimulatory effect on bone regeneration. While mannose-6-phosphate was found to be wound healing promoter dose-dependently. A greater wound healing effects were resulted by mannose-6-phosphate linkage to protein producing more potent healer a mucopolysaccharide. [3,4]

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. [5] Bone healing is a series of complex biological events. It is a multistage well-orchestrated regenerative process initiated in response to injury, resulting 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. [6,7]

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 and swallowing 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. [8]

MATERIALS AND METHODS

The study was conducted at the 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 a 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 1 drop (0.05 ml) of pure Aloe Vera gel 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. Post-operative 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 3^{rd} day, 7^{th}, 14^{th} 28^{th}, 42 days successively, each time interval group contained four rabbits which were act as control and Aloe Vera 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. [10] 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). [11] Statistical analysis was done using SPSS software version 24. Data were expressed as means and percentage 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 $\rho \leq 0.05$.

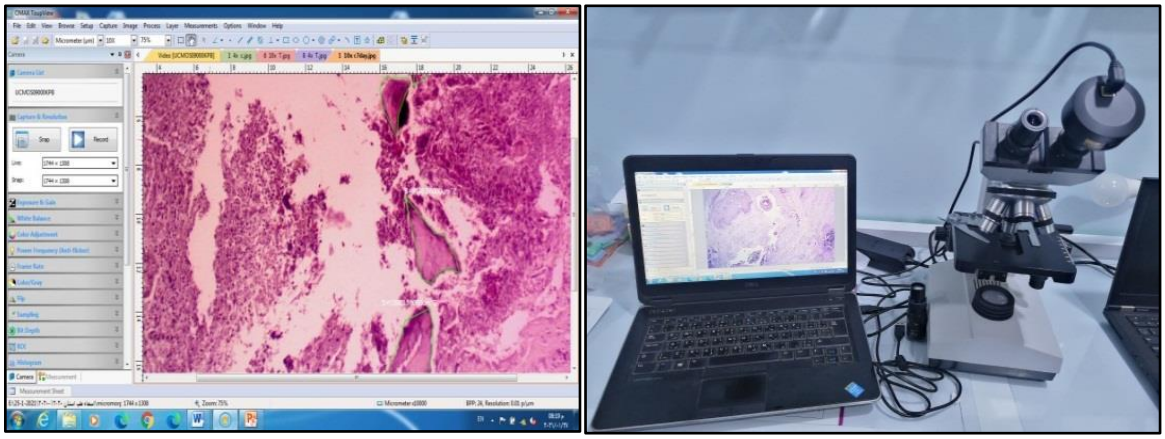

Figure (1): Special image analysis software and equipment.

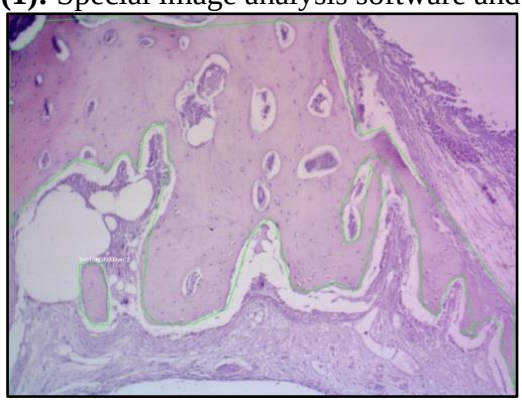

Figure (2): Demonstration for surface area of newly formed bone calculation technique using special image analysis software at 10X magnification power microscopical field.

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 Aloe Vera groups at all-time intervals of the study except for three days' time interval in which there was no new bone tissue formation in both control and Aloe Vera groups, the resultant measurements of Aloe Vera group were bigger and highly significant as shown in Figures (3) and (5) 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 Aloe Vera groups at all-time intervals with favor to Aloe Vera groups with significant higher scores recorded as shown in Figure (4) and summarized in Table (2)

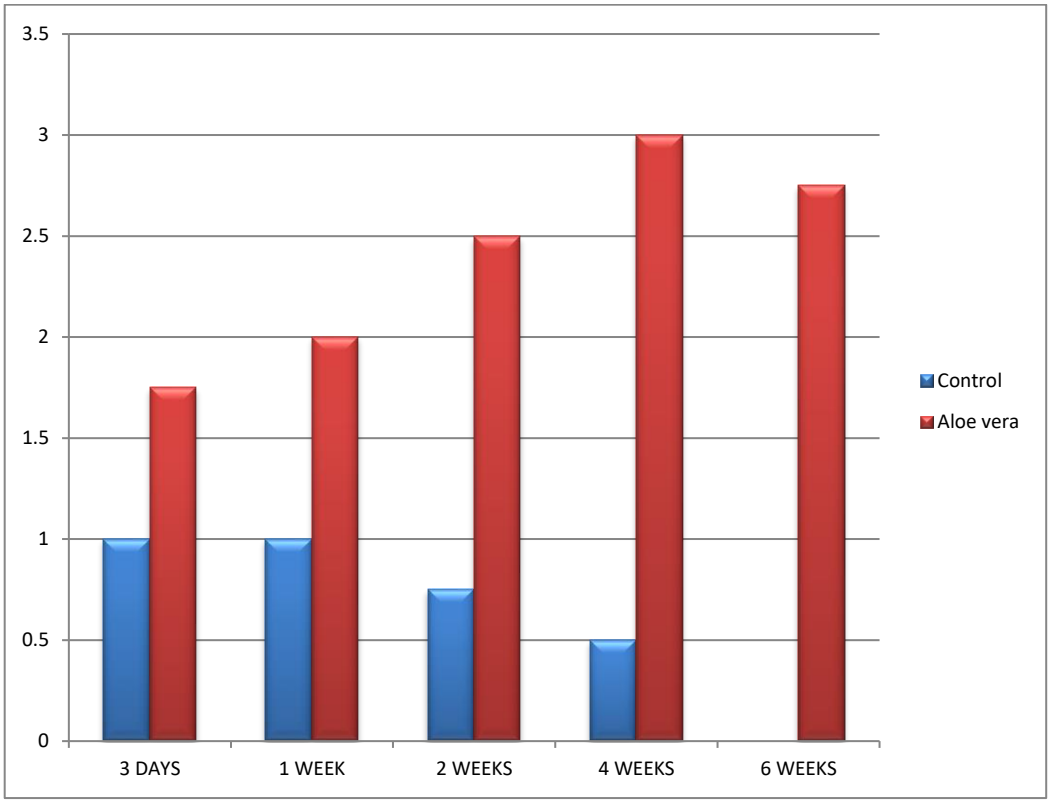

Figure (3): Diagrammatic Representation for Mean Values of New Bone Formation Measurements of Alveolar Bone.

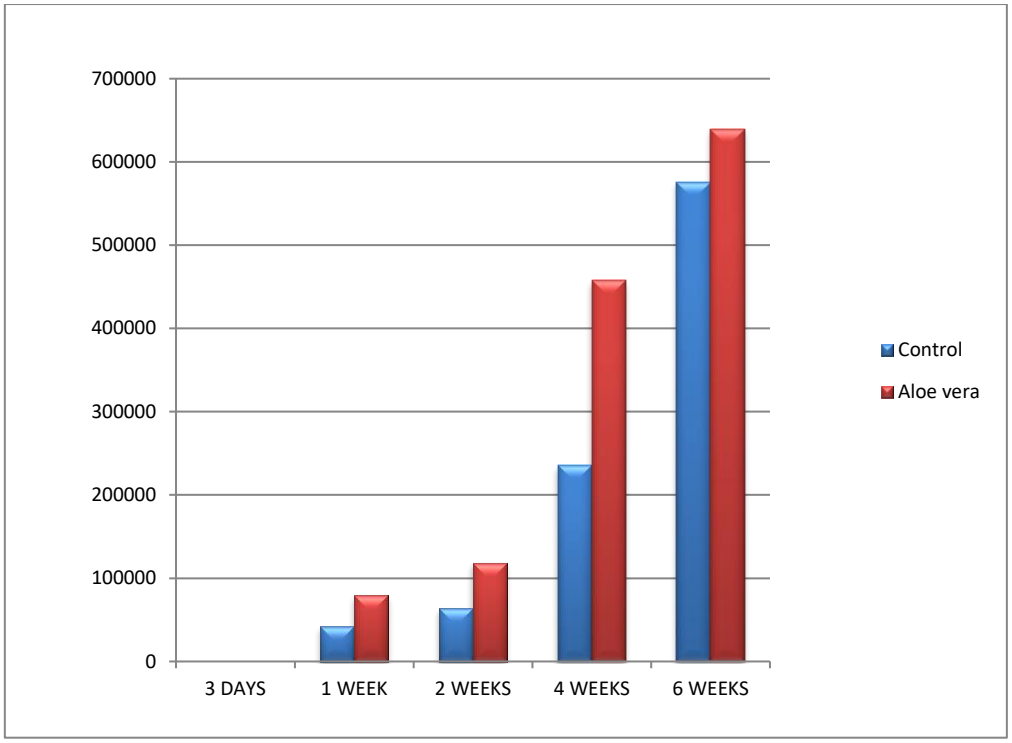

Figure (4): Diagrammatic Representation for Mean Values of granulation tissue formed in Alveolar Bone.

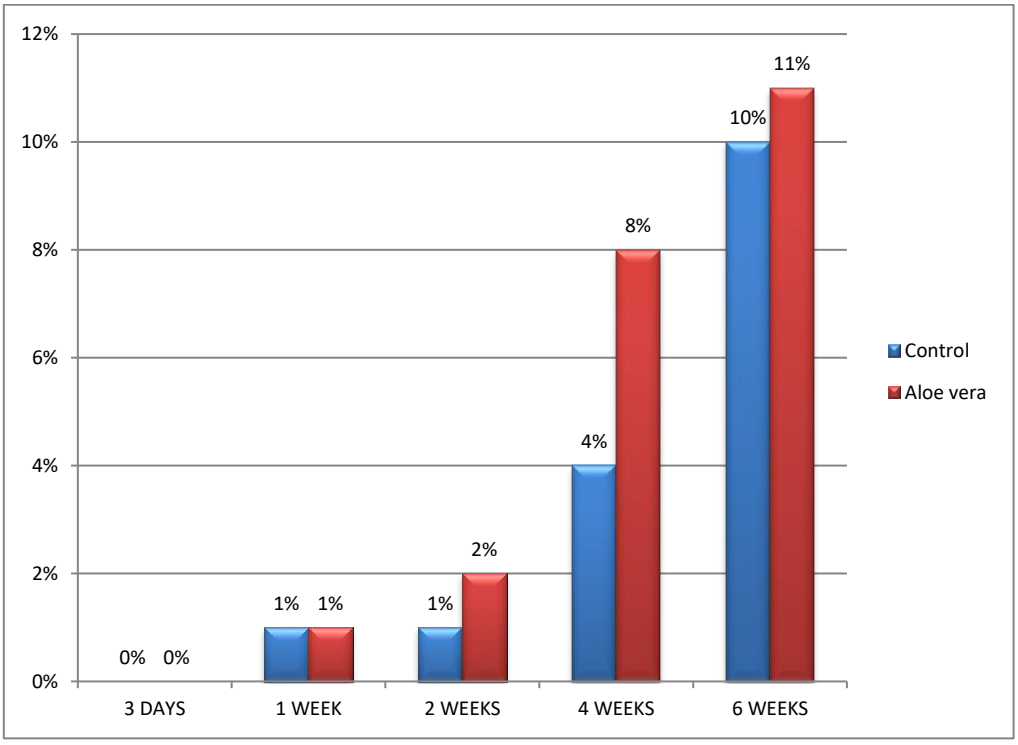

Figure (5): Diagrammatic Representation for Percentage of newly formed bone tissue of Alveolar Bone.

Table (1): Statistical analysis of newly formed bone surface area represented as mean and percentage of 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&its %	Means of new bone surface area of Aloe Vera groups in µm² &its %	***p-Value***
3 Days	0 0%	0 0%	– —
7 Days	41828.9 1%	79176.3 1%	0.001**
14 Days	63307.53 1%	118425.6 2%	0.002*
28 Days	235660.9 4%	457794.2 8%	0.001**
42 Days	575815.2 10%	639568.6 11%	0.01*

* The means are significantly different at $p \leq 0.05$.
** The means are highly significantly different at $p \leq 0.01$.
% = Percentage of new bone surface area in relation to the total 10X microscopical field surface area.

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 Aloe Vera groups	***p-Value***
3 Days	1	1.75	0.04*
7 Days	1	2	0.008**
14 Days	0.75	2.5	0.017*
28 Days	0.5	3	0.013*
42 Days	0	2.75	0.01*

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

DISCUSSION

The current study results revealed that the Aloe Vera gel has a positive impact on bone healing process by enhancement of amount of granulation tissue formed and improvement in new bone tissue formation quantity with increased percentage reaching to 2 folds more than percentage of new bone in control group at 28 days' time interval. Aloe Vera gel obtained from the mucilaginous gel located in the center of Aloe leaf, this gel is composed of a variety of chemical substances which exhibit a biological activity on tissues healing including bone, this effect sometimes attributed to synergistic effect among the different and multiple bioactive components within the gel.[12] Acemannan is a bioactive polysaccharide found in Aloe Vera gel in large proportion and involved in macrophage and fibroblast cells activation

and bone tissue healing and regeneration, studies found that acemannan induces macrophage and fibroblast proliferation by binding to cell receptors and stimulates fibrogenic cytokines releasing, more activated fibroblast cells lead to higher amounts of collagen fibers and extracellular matrix rates and granulation tissue formation noticed in Aloe Vera gel treated groups samples. [13] Another Aloe Vera gel bioactive component is mannose-6-phosphate, it binds to insulin-like growth factor and delivering it to cells which increases fibroblast activity and the wound's granulation tissue. [4] Researches stated that acemannan play a key role in recruitment of bone marrow stromal cells BMSCs which are considered to be mesenchymal stem cells or osteoprogenitor cells, BMSCs can migrate and differentiate into osteoblasts under appropriate circumstances, osteoblasts are the cells responsible for bone matrix laydown and new bone production. [14,15] Acemannan accelerates bone formation rate by its inducing effect on BMSCs proliferation and differentiation and expressions of VEGF, BMP-2, OPN, BSP (which are osteoblastic differentiation markers) and mineralization. [13] VEGF on the other hand induces both new capillary formation and osteoblast differentiation. Mineralization is enhanced in Aloe Vera gel treated group due to the mineral contents of the gel. [16]

CONCLUSION

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

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