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The Impact of Aloe Vera Gel on Healing of Surgically Made Maxillary Mucosal Wounds in Rabbits

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Abstract

Objective: The purpose of present study is to evaluate the effect of Aloe Vera gel 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 Aloe Vera gel in left side, while right side bone hole was left empty to act as control. According to euthanization time, rabbits were divided into five groups and sacrificed at the 3rd, 7th, 14th,28th and 42 days post-wounding, histological and histomorphometrical 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-epithelialization between control and Aloe Vera groups at certain time intervals within total period of experiment with favor to Aloe Vera group.

Conclusion: The use of Aloe Vera gel exhibits a beneficial effect by increasing granulation tissue formation and subsequent angiogenesis and enhancement of re-epithelialization in healing process.

Introduction:

Aloe Vera is a cactus herb that has been used to treat wounds and burns since ancient times. Numerous medicinal products are produced from its mucilaginous tissue located in the center of the Aloe leaf called Aloe Vera gel which contains various bioactive compounds that having different pharmacological properties [(1)].

The oral mucosa is a well-adapted structure designed to protect the underlying tissues against mechanical damage and prevent microorganisms and their toxic materials entrance. However, this protective barrier may be disrupted by different causative agents which lead to wound formation [(2)]. 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 [(3)]. 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, including chemotaxis, cell division, neovascularization, synthesis of new extracellular matrix components. These cellular activities occur in correlation with appearance of different cell types in the wound during various stages of healing process. Healing process involves four overlapping but well-defined phases of hemostasis, inflammation, proliferation, and remodelling and scar maturation. Many important cells and growth factors involved within various stages of the healing process. The regulation of those events is multifactorial [(4)]. 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 wound healing process due to its principal impact on all healing stages, ensuring the sufficient nutritive perfusion of the wound, oxygen supply and immune cells recruitment [(2, 3)].

Epithelialization of the wound represents the final stage of the proliferative phase.

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. Pericytes provide adhesive substrates to initiate neutrophil crawling at the endothelium to migrate into the extravascular tissue [(5)].

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, where it acts as an adhesive stress-response protein to both maintain endothelial cell junctional integrity and speed restoration of the vascular permeability barrier following inflammatory or thrombotic challenge which providing protection of the vascular bed.The immunohistochemical detection of this molecule has been largely used in micro-vessel density assessment with reliable information [(6, 7)].

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 sections using antibody-antigen interactions. 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 [8].

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 under ethical No. Max.O.F.S/A.L.I/19 . Twenty Albino male rabbits 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 1 cm 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 1 drop (0.05 ml) of pure Aloe Vera gel and a tiny piece of gel foam was placed within the hole. Flap edges were repositioned and sutured on the defect which filled with Aloe Vera gel using 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 after surgery.

Rabbits were randomly divided into five groups according to the time interval of euthanization, they were sacrificed at the 3rd day, 7th , 14th 28th , 42 day successively, each time interval group contained four rabbits which were represent both control and Aloe Vera group as each rabbit was subjected to surgery at both jaw sides. After each group rabbits had been euthanized, the oral mucosal tissue at the operated areas were 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 scale was adopted to give a scoring system ranged 0 – 4 for re-epithelialization and 0 – 3 for granulation tissue, scoring systems are explained in Table (1) and (2) [10 ,9]. While angiogenesis was evaluated with aid of immunohistochemistry technique involving estimation of the intensity of immunoreaction expression against CD31 epitope in oral mucosal specimens with scoring system ranged 0 – 3. And histomorphometry was adopted also and involved counting of number of positively marked cells (i.e. immunoreactive cells against CD31 epitope) in the wound zone; which characterized by dark brown appearance under microscope as shown in Fig.(2); which expressed as percentage in relation to the total cells in the wound area, and had been scored for statistical purposes, scoring systems associated with immunohistochemistry assessment are demonstrated in Table (3) and (4), each single slide in each group had 4 readings of the above mentioned examined parameters, statistical analysis and comparisons between control and Aloe Vera group were done using Mann-Whitney test at $\rho \leq 0.05$ [11,12]. Statistical

analysis was employed by use of SPSS software version 24. Data were expressed as mean scores ± standard deviation.

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 Aloe Vera groups at 14- and 28-days period favoring Aloe Vera groups, while there was no significant difference at 3-, 7- and 42-days period despite of higher scores recorded for Aloe Vera groups. Fig. (1) demonstrates histological findings, while statistical analysis is illustrated in Fig.(3). Regarding angiogenesis evaluation of oral mucosal specimens, results revealed that there was significant difference in intensity of CD31 marker expression between control and Aloe Vera groups at 7 and 42 days periods, while there was no significant difference at 3, 14 and 28 days periods, whereas percentage of immunoreactive cells showed statistically significant differences at 7, 14, and 42 days periods, with favor to Aloe Vera group. Fig.(5) demonstrates related statistical analysis. On the other hand re-epithelialization showed a statistically significant differences at 7, 14, and 28 days periods of the study with favor to Aloe Vera group, histological findings are shown in Fig.(1) and statistical analysis is illustrated in Fig.(4). Statistical analysis of the all estimated findings is summarized in Table (5).

Discussion :

Aloe Vera is, probably, one of the most important medicinal plants used due to the beneficial effects related to its bioactive compounds, which involving bioactive polysaccharides, acemannan in particular, glucomannan and others and different phenolic compounds. Aloe Vera gel contains more than 75 biologically active constituents, those bioactive chemicals seem to be the key to explain most of the pharmacological properties of Aloe Vera plant and they act on synergistic effect basis [13,14].

Acemannan is regarded as the main functional component of Aloe Vera, it is a complex carbohydrate composed of a long chain of acetylated mannose, recent studies have demonstrated the acetyl groups importance and their connection with cell proliferation and vascular endothelial growth factor (VEGF) expression, studies revealed that acemannan stimulates wound healing and granulation tissue formation by its inducing effect of cell proliferation and stimulation of VEGF which is important in new blood vessels formation and endothelial cell proliferation induction and migration [15,16]. Direct binding of acemannan to growth factors and their stabilization may lead to prolong stimulation of granulation tissue formation and subsequent more tissue quantity which had been noticed in Aloe Vera gel treated wounds in 14 and 28 days period post-operative groups. Also acemannan may stimulate releasing of fibrogenic cytokines which is induced by macrophage activation by binding to mannose receptor and further recruitment of fibroblast cells and granulation tissue formation [15,17]. Glucomannan; another Aloe Vera polysaccharide; accelerates wound healing by its interaction with growth factor receptors of fibroblast which stimulate its activity and proliferation and increases collagen synthesis and extracellular matrix deposition [15]. VEGF is the single most significant mediator promoting wound angiogenesis, its releasing stimulates capillary growth for withstanding nutrients and oxygen requirements of the injury site, and providing inflammatory cells recruitment pathways. VEGF is released on a higher extent in response to Aloe Vera activated macrophages [18]. Immunohistochemistry aid approach for angiogenesis evaluation was conducted in this study suggesting use of CD31 epitope directed antiserum since CD31 would provide more consistent detection of small blood vessels and it has been reported to be more consistently expressed on developing capillaries [19]. As acemannan acts as prolonging factor for stimulation of granulation tissue formation as mentioned earlier, it is suggested that neo-vascularization process would be

prolonged too with more development of small blood vessels (i.e. capillaries ≤ 25 μ^2) which can be detected more accurately by positive marked cells counting, counting of cells provided more sensitive detection for delicate capillaries with tiny diameters which were stained to CD31 antigen detection but may be under estimated during evaluation of CD31 expression intensity, which explaining our study results as significant higher percentage of positively marked cells in Aloe Vera group compared to control group at 14 days period of experiment [(19)]. 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 [(20)]. Improvement effect of wound epithelialization of Aloe Vera 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 epithelial keratinocyte migration and proliferation, subsequent higher number of fibroblasts and keratinocytes leads to acceleration and enhancement in epithelialization process [(21)].

Conclusion:

It can be concluded that Aloe Vera gel 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.

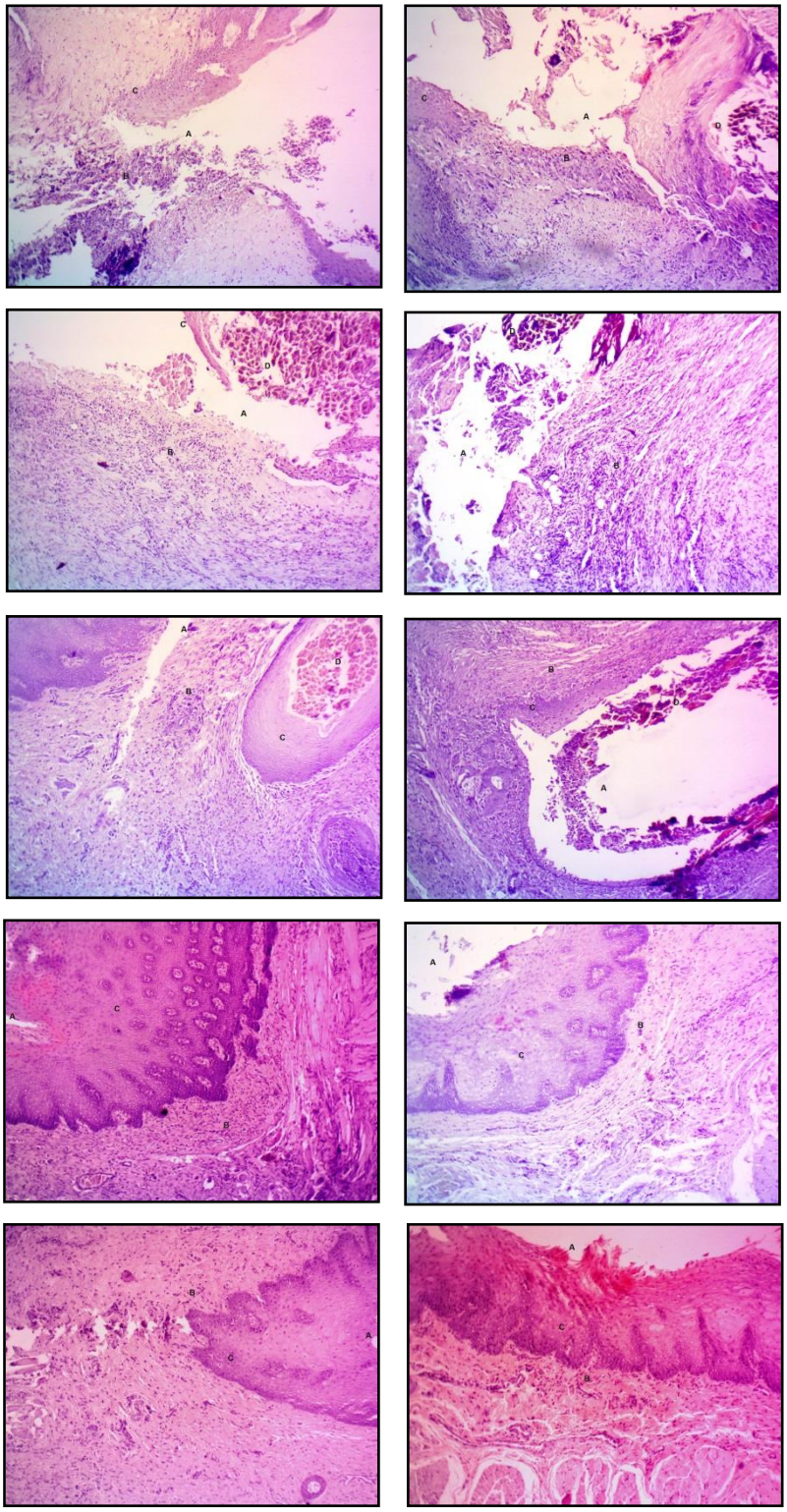


Fig.(1): Photomicrographs for histological findings of oral mucosa healing assessment during the periods of study arranged in ascending manner from 3 days period photomicrographs in top to 42 days period photomicrographs down, control slides are on the right whereas Aloe Vera slides are on the left. A= site of wound, B= granulation tissue, c= re-epithelialization, D=suture material and remnants of Aloe Vera gel, H&E stain, at 100X magnification power.

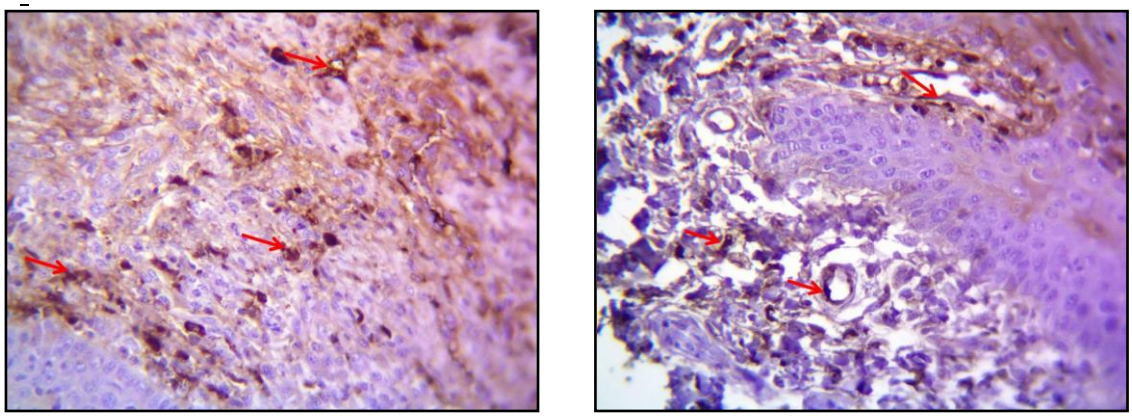


Fig.(2): Photomicrographs of oral mucosa specimens stained with Immunohistochemistry technique for CD31 epitope detection, red arrows refer to positively marked cells (immunoreactive cells against CD31) with characteristic dark brown color. Left picture is at 100X magnification power, whereas right one is at 400X magnification power.

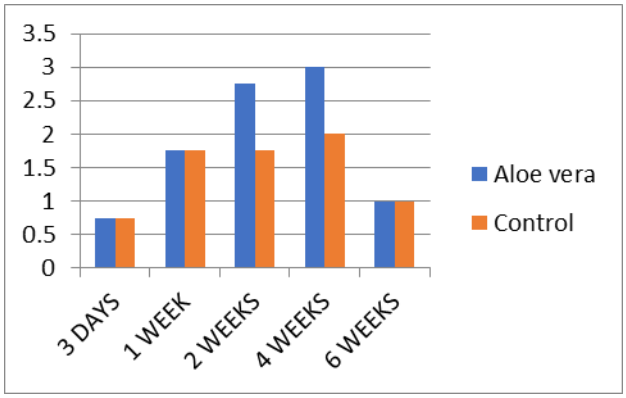


Fig.(3): Diagrammatic representation for mean values of granulation tissue formation scores of oral mucosal healing.

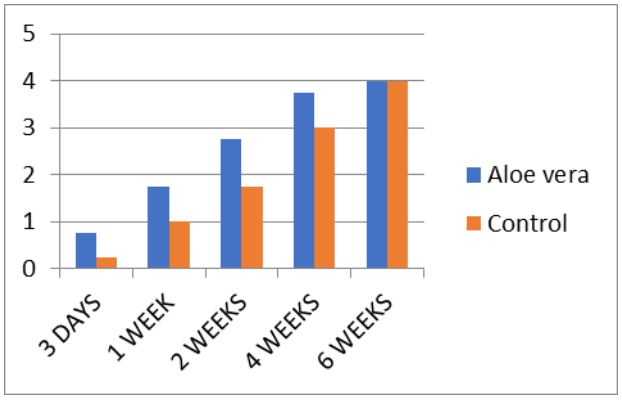
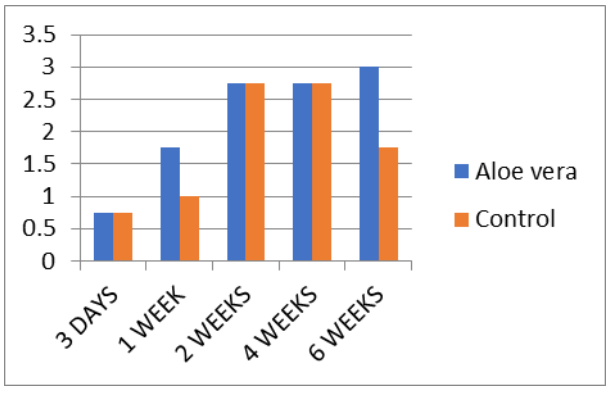


Fig.(4): Diagrammatic representation for mean values of re-epithelialization of oral mucosal healing.



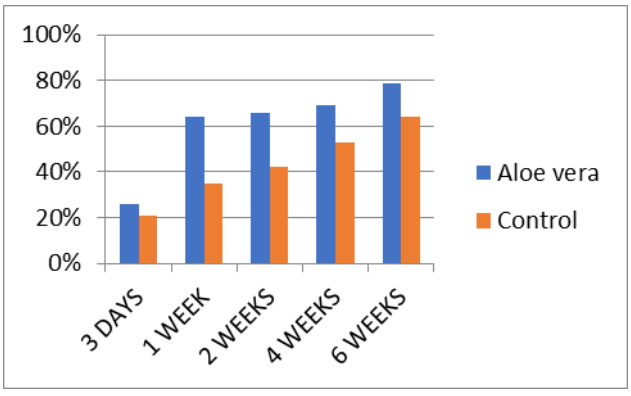


Fig.(5): Diagrammatic representation for Immunohistochemistry findings, mean values of CD31 expression scores on the left and mean values of positively marked cells percentage on the right.

Table (1): Re-epithelialization scoring criteria

Grade	Histopathological Response Observed
0	Re-epithelialization at the edge of the wound only.
1	Re-epithelialization covering less than half of the wound.
2	Re-epithelialization covering more than half of the wound.
3	Re-epithelialization covering the entire wound, with irregular epithelial thickness.
4	Mature re-epithelialization covering the entire wound, with normal epithelial thickness.

Table (2): Granulation tissue formation scoring criteria

Grade	Histopathological Response Observed
0 - Nil	No granulation tissue seen in the field of operation 10X.
1- Mild/Scanty	Scanty amount of granulation tissue formed, seen in less than half of the field 10X.
2- Moderate	Moderate amount of granulation tissue formed, could be seen in more than half of the field 10X.
3- Profound /Marked	Profound amount of granulation tissue formed, seen in more than 3/4 of the field 10X.

Table (3): CD31- immunoreaction expression scoring criteria

Grade	Histopathological Response Observed
0-Nil	No CD31-immunoreactive cells seen in the field of operation at 10X.
1-Mild	CD31-immunoreactive cells present in few numbers and seen in less than half of the field of operation at 10X.
2-Moderate	CD31-immunoreactive cells seen in more than half of the field of operation at 10X.
3-Marked	CD31-immunoreactive cells present in large numbers and seen in more than 3/4 of the field of operation at 10X.

Table (4): Scoring system for the estimated percentage of CD31-immunoreactive cells.

Score	Representing percentage of +ve cells	Score	Representing percentage of +ve cells
0	0%	**2**	34 – 66%
1	1 – 33%	**3**	67 – 100%

Table (5): Statistical analysis of oral mucosa healing assessment scores by use of Mann-Whitney test and represented as mean ± standard deviation at $\rho \leq 0.05$.

Parameter	Period / Group	3 DAYs Mean ± Sd	7 DAYs Mean ± Sd	14 DAYs Mean ± Sd	28 DAYs Mean ± Sd	42DAYs Mean ± Sd
CD31 Expression	Control Group	0.75 ± 0.9 A	1 ± 0.9 A	2.75 ± 0.9 A	2.75 ± 0.9 A	1.75 ± 0.9 A
	Aloe Vera Group	0.75 ± 0.9 A	1.75 ± 0.9 B	2.75 ± 0.9 A	2.75 ± 0.9 A	3 ± 0.9 B
Immunoreactive cells percentage	Control Group	21% 1 ± 0.5 A	35% 1.5 ± 0.5 A	42% 1.75 ± 0.5 A	53% 2 ± 0.5 A	64% 2.25 ± 0.5 A
	Aloe Vera Group	26% 1 ± 0.7 A	64% 2.25 ± 0.7 B	66% 2.5 ± 0.7 B	69% 2.5 ± 0.7 A	79% 3 ± 0.7 B
Granulation tissue	Control Group	0.75 ± 0.6 A	1.75 ± 0.6 A	1.75 ± 0.6 A	2 ± 0.6 A	1 ± 0.6 A
	Aloe Vera Group	0.75 ± 1 A	1.75 ± 1 A	2.75 ± 1 B	3 ± 1 B	1 ± 1 A
Re-epithelialization	Control Group	0.25 ± 1.4 A	1 ± 1.4 A	1.75 ± 1.4 A	3 ± 1.4 A	4 ± 1.4 A
	Aloe Vera Group	0.75 ± 1.3 A	1.75 ± 1.3 B	2.75 ± 1.3 B	3.75 ± 1.3 B	4 ± 1.3 A

Capital letters in Table (5) refer to comparison between groups at each period of study (comparison in columns) where changing in the capital letter means there was statistically significant difference at $p \leq 0.05$.

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