https://doi.org/10.48047/AFJBS.6.14.2024.6691-6703

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Aloe Vera Gel Accelerates Vasculogensis In Wounded Oral Mucosa, A Special Study Using CD34 Immunohistochemical (IHC) Stain

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Article History
Volume 6, Issue 14, Aug 2024
Received:03 July 2023
Accepted : 31 July 2023
Published :22 Aug 2024
doi:10.48047/AFJBS.6.14.2024.6691-6703

Abstract

Introduction: The healing of oral mucosa wounds is a dynamic process that involves a sequence of events orchestrated by intricate cellular interactions.

Objectives: The main objective of the study is to find the role of aloe vera gel that accelerates vasculogensis in wounded oral mucosa, a special study using CD34 Immunohistochemical (IHC) stain.

Material and methods: An experimental animal study was conducted at the Experimental Research Laboratory of Postgraduate Medical Institute Lahore to study the histological changes in tongue mucosa wounds at 5th and 7th day post wounding. The therapeutic reagent used in this study was *Aloe vera* (*Aloe barbadensis Miller*) gel. Day 5 & 7 pw were selected to see the prolonged effects of the drug on the healing tissue.

Results: Tissue samples from group A (control) had incomplete epithelium formation. It was partially covering the ulcer area and was immature with poorly defined epithelial cell layers. Granulation tissue had high macrophage count and predominant fibroblasts. Macrophages had a smaller nucleus covered by a larger rim of foamy cytoplasm. Fibroblasts were active, spindle shaped with dark purple nucleus and comparatively lighter staining cytoplasm. Few collagen fibrils were seen among the cells and were light pink in staining.

Conclusion: Results of the study clearly show that *Aloe vera* gel accelerates healing in tongue mucosa wounds in Wistar rats. It plays its role by bringing enhanced inflammation, epithelization, angiogenesis and fibrosis.

Introduction

The healing of oral mucosal wounds is a dynamic process that involves a sequence of events orchestrated by intricate cellular interactions. The successful regeneration of blood vessels is pivotal for ensuring an adequate supply of nutrients, oxygen, and immune cells to the healing site. Impaired vasculogenesis can lead to delayed wound healing, compromised tissue repair,

and increased susceptibility to infections [1]. Aloe vera, with its rich repository of bioactive compounds such as polysaccharides, vitamins, and minerals, has long been explored for its potential to enhance wound healing. However, the specific effects of Aloe vera on vasculogenesis in the context of wounded oral mucosa remain a realm of curiosity. This study aims to unravel this aspect by focusing on CD34 Immunohistochemical (IHC) staining, a robust technique that allows the visualization and quantification of endothelial cells in tissue sections [2].The potential of Aloe vera to expedite vasculogenesis could open new avenues for augmenting oral mucosal wound healing outcomes. If the results of this study substantiate the hypothesis that Aloe vera gel accelerates the formation of new blood vessels within the wounded oral mucosa, it could pave the way for novel therapeutic interventions. These interventions might harness the regenerative potential of Aloe vera to enhance the recovery of patients following oral surgeries, minimize postoperative complications, and improve overall patient outcomes. Wound healing is a series of events in which an injured tissue restores its normal architecture and function [3]. Whatever the wound type and organ is, an inflammatory cascade is seen immediately following injury. Initially a barrier is required that stop fluid loss. Next step is the control of the hazardous infection and entry of foreign organisms and materials that may worsen the inflammation. Further, there is regeneration of the tissue and recovery of blood and lymph vessels and finally the tissue is remodelled to attain normal structure [4]. Ulcerative lesions are very common in the oral cavity. There are many reasons why such oral wounds occur for example, stress, lack of proper diet, digestive problems, hereditary factors, hormonal imbalance, viral infections, immune deficiency, trauma during a surgical procedure or a dental appliance (denture induced stomatitis, crown etc), malocclusion, cheek bites, irritation by chemical, tobacco and alcohol, radiation therapy and medication. There is a general old term used for ulcers called “aphthae”. Various ulcerative lesions lead to poor quality of life due to severe pain and inability to eat [5].

Material and methods

An experimental animal study was conducted at the Experimental Research Laboratory of Postgraduate Medical Institute Lahore to study the histological changes in tongue mucosa wounds at 5^{th} and 7^{th} day post wounding. The therapeutic reagent used in this study was *Aloe vera* (*Aloe barbadensis Miller*) gel. Day 5 & 7 pw were selected to see the prolonged effects of the drug on the healing tissue. The study protocol was approved by the Advanced Studies and Research Board of University of Health Sciences, Lahore and Ethical Committee of

Postgraduate Medical Institute, Lahore. Study was conducted on 42 albino rats in animal house, Anatomy department, Postgraduate Medical Institute (PGMI), Lahore, Pakistan.

Inclusion Criteria:

1. Rats weighing 200-250 gm.
2. Rats with healthy oral mucosa.

Exclusion Criteria:

1. Diseased rats
2. Overweight rats
3. Underweight rats.

Procedure

Animals were examined thoroughly for any external deformity. The animals were kept at animal house of Post Graduate Medical Institute Lahore, in iron cages for a week of acclimatization in a climate-controlled environment. Animals were anaesthetized with IM ketamine with the dosage of 40mg/kg (Nisbet et al., 2010). 1 cc tuberculin syringe with 1 inch needle was used for this purpose. Each animal was placed on the wooden dissecting board turn by turn after anesthesia. A pair of tweezers was used to keep the mouth open by retracting floor of mouth and palate placing its ends between them. Another was used to pull the tongue out of the mouth. A 4mm punch biopsy was used to induce circular wounds on anterior 2/3rd of the dorsal surface of the tongue (Sasithanasate et al., 2008). Haemostasis was achieved by blotting and compressing the wound with the cotton swab. Procedure was carried out under aseptic conditions.

***Aloe vera* gel extract, animal grouping and dosage:**

I prepared *Aloe vera* (*Aloe barbadensis Miller*) gel extract (appendix II) and gave to the animals in two doses which were, 300 mg/kg and 500 mg/kg (Anshoo et al., 2005; Oyeyemi and Fayomi, 2011) (appendix II). The rats were divided randomly in to three groups, each having two subgroups as under:

Group A: Fourteen rats as control group, received 1ml distilled water orally with the help of insulin syringe and gastric tube as a single morning dose.

Group B: Fourteen rats as an experimental group, received *Aloe vera* gel extract (300mg/kg body weight/day) (Oyeyemi and Fayomi, 2011), dissolved in distilled water, orally with the help of insulin syringe and gastric tube, as a single morning dose.

Group C: Fourteen rats as an experimental group, received *Aloe vera* gel extract (500mg/kg body weight/day) (Anshoo et al., 2005), dissolved in distilled water, orally with the help of insulin syringe and gastric tube, as a single morning dose.

Dissection, tissue sampling, staining and microscopic assessment:

The anesthetized animal was placed on the dissecting board. The extremities were nailed to the board. The tongue was dissected separating the main part (body of the tongue) with surgical scissors and the base of the tongue was left attached. Specimen was preserved and the animal was sacrificed. Tissue samples were fixed, processed and stained. Slides stained with Hematoxylin & Eosin and IHC stains (CD34 immunohistochemical stain) were studied under light microscope. Immunohistochemistry or IHC refers to the process of detecting antigens (e.g. proteins) in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues. CD34 is a glycoprotein expressed on capillary endothelial cells.The cell counting was done at High Power Field (HPF) i.e. with magnification of X400. Different zones of the tissue were examined to see the cellular changes in the anatomy of wounded tongue mucosa for evaluation of healing status of the tissue in experimental as well as the control groups (Tarameshloo et al., 2012). Mean of three most cellular and vascular areas per high power field was calculated (Weidner et al., 1993). The Abramov's scoring system (modified Greenhalgh's scoring system) was used for scoring macrophage count, fibroblast count, angiogenesis and degree of epithelization as follows (Nisbet et al., 2010):

Macrophages:

- 0-25 macrophages = 1
- 26-50 macrophages = 2
- >50 macrophages = 3

Fibroblasts:

- None to minimal Fibroblasts = 0
- Few Fibroblasts = 1

- More Fibroblasts = 2
- Predominant Fibroblasts = 3

Blood vessels:

- No blood vessels = 0
- Up to 5 blood vessels = 1
- 6 to 10 blood vessels = 2
- More than 10 blood vessels = 3

Epithelization:

- None = 0
- Partial = 1
- Complete but immature and thin = 2
- Complete and mature = 3

Histological observations were made under light microscope after staining the tissues with H & E and IHC stains. Macrophage count, fibroblast count and degree of epithelization were examined by staining tissues with H & E. Angiogenesis was observed by using CD34 IHC stain.

Statistical analysis

Data was analysed by using SPSS version 20. All the study variables were qualitative. Chi square and Fisher exact test was used to determine association of variables among groups. P-value ≤ 0.05 was taken as statistically significant association.

Results

Tissue samples from group A (control) had incomplete epithelium formation. It was partially covering the ulcer area and was immature with poorly defined epithelial cell layers (figure 1). Granulation tissue had high macrophage count and predominant fibroblasts. Macrophages had a smaller nucleus covered by a larger rim of foamy cytoplasm. Fibroblasts were active, spindle shaped with dark purple nucleus and comparatively lighter staining cytoplasm. Few collagen fibrils were seen among the cells and were light pink in staining (figure 2).

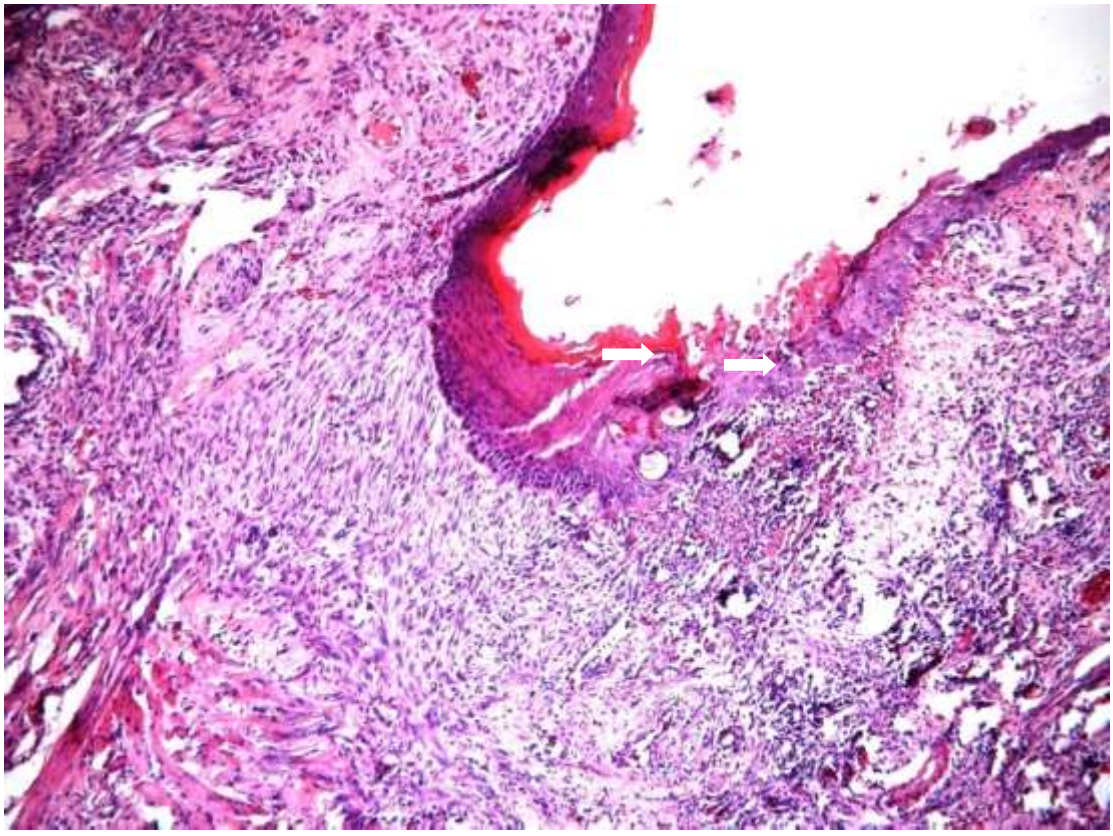

Figure 01: Photomicrograph of histological section of wounded tongue mucosa of group A. H & E X40

White arrows = epithelium

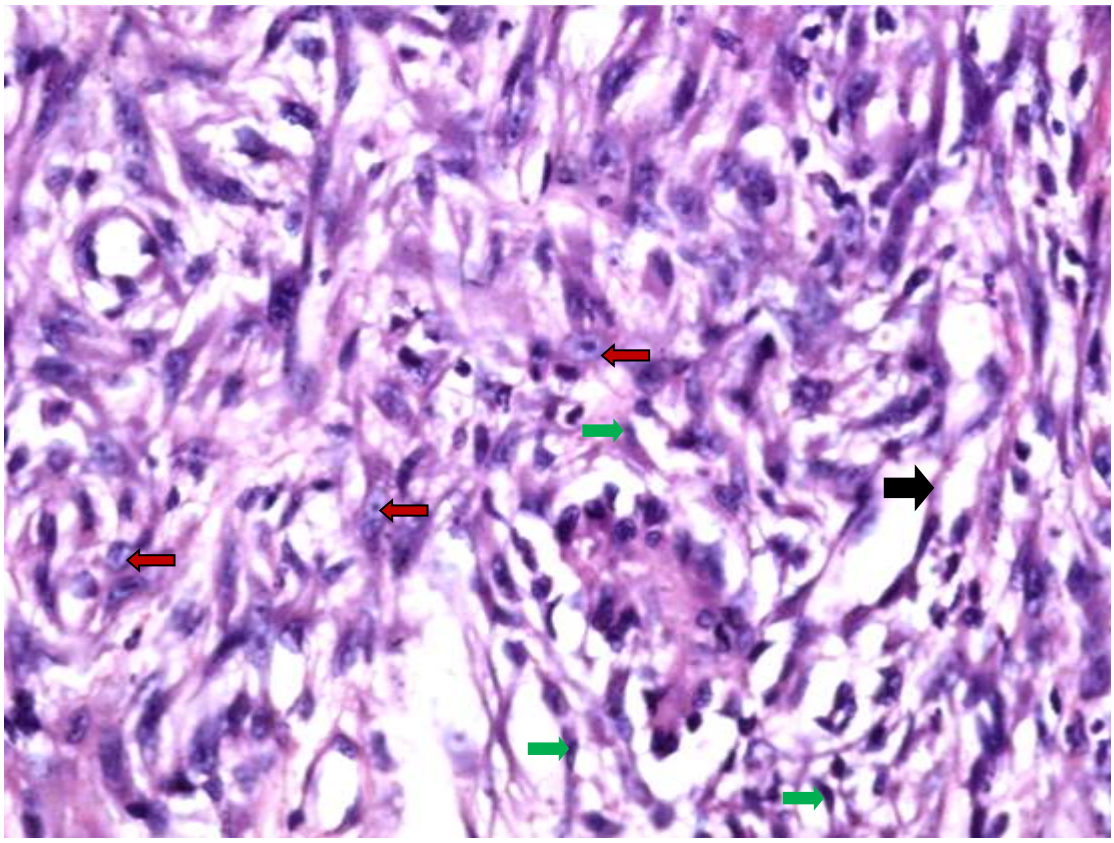

Figure 02: Photomicrograph of histological section of wounded tongue mucosa of group B. H & E X400

Red arrows = macrophages, Green arrows = fibroblasts, Black arrow = blood vessel

Epithelium was completely formed and mature but was thin in group B (experimental). The inflammation had started subsiding. Just like control group, still the predominant cells in granulation tissue were fibroblasts and macrophages, but comparatively lesser fibrosis was observed. The histological findings were different from the control group. Fibroblasts were maturing and had formed much collagen. Fibroblasts had started arranging themselves parallel to the collagen fibers (figure 3). Epithelization was complete with mature epithelium i.e. all layers of epithelium were well defined. The acute inflammatory phase was shifted to chronic in the tissues from groups C. Very few or no macrophages and maturing fibroblasts were present as compared with control with active fibroblasts (decreased fibrosis) (figure 4).

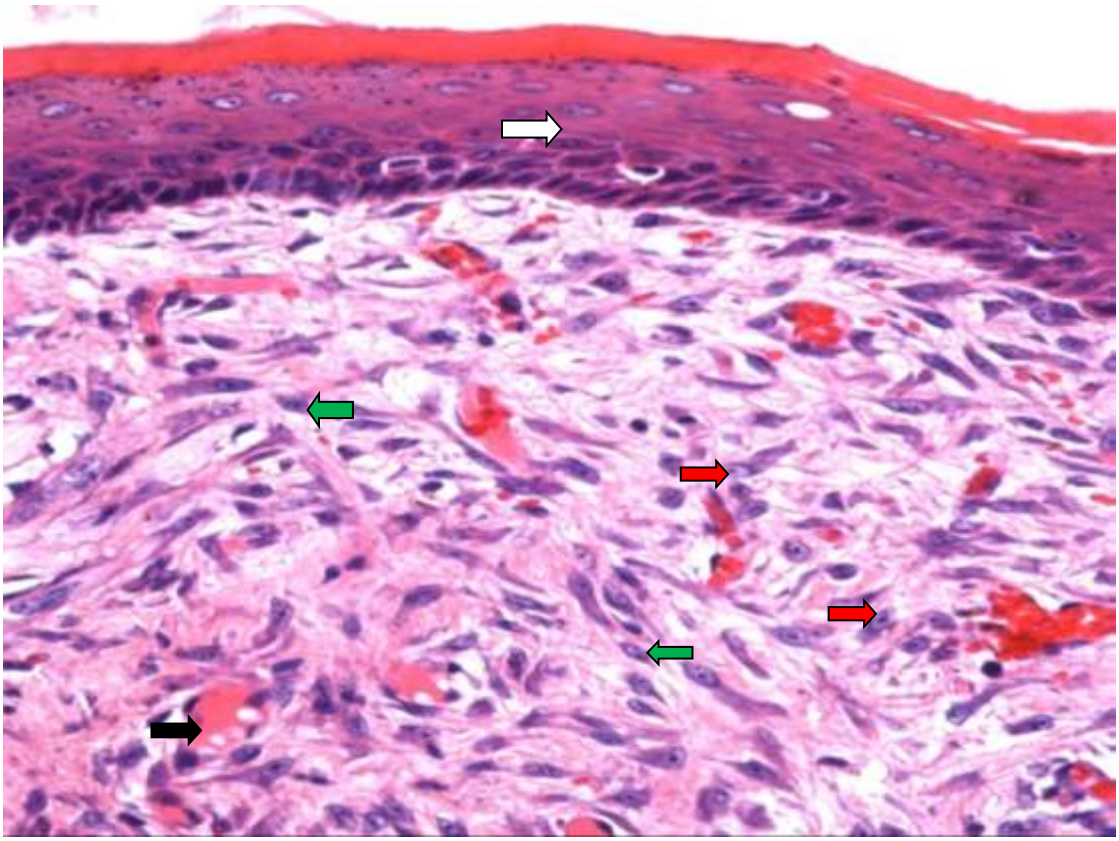

Figure 03: Photomicrograph of histological section of wounded tongue mucosa of group C. H & E X400

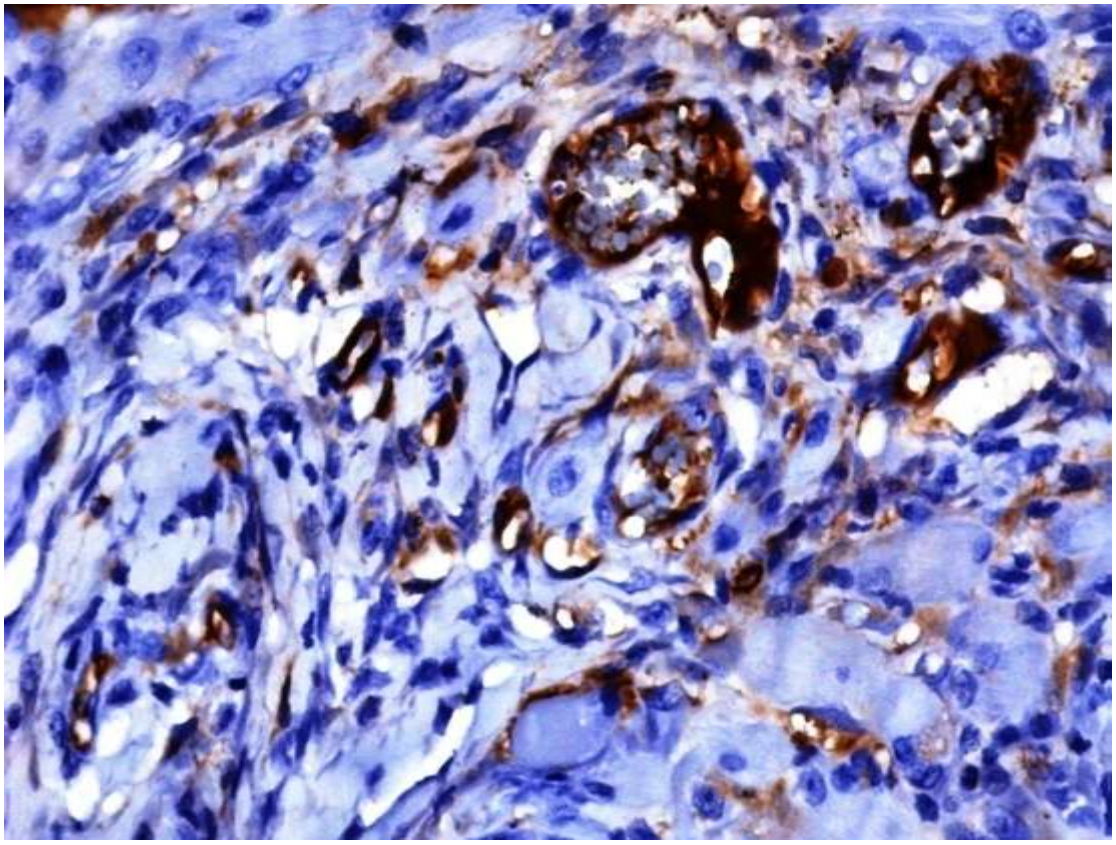

Figure 04: Photomicrograph of histological section of wounded tongue mucosa of group A in CD34

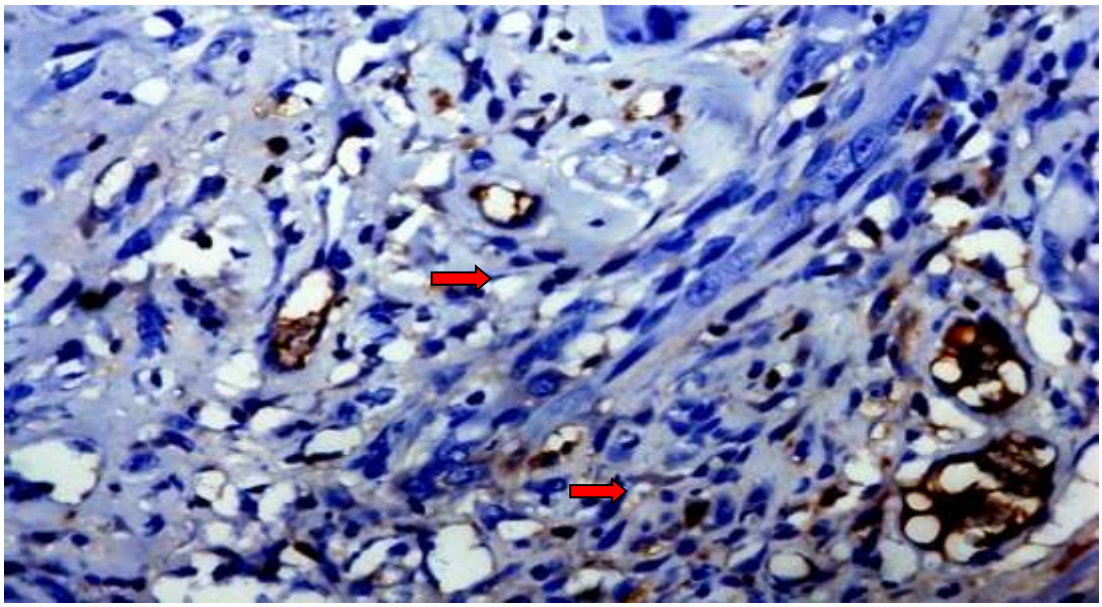

Figure 05: Photomicrograph of histological section of wounded tongue mucosa of group B in CD34 immunohistochemical stain.

The tissues from control group (A) animals showed vasculogenesis but the vessels were fewer in number than in experimental groups. Small capillaries were found among the inflammatory cells.

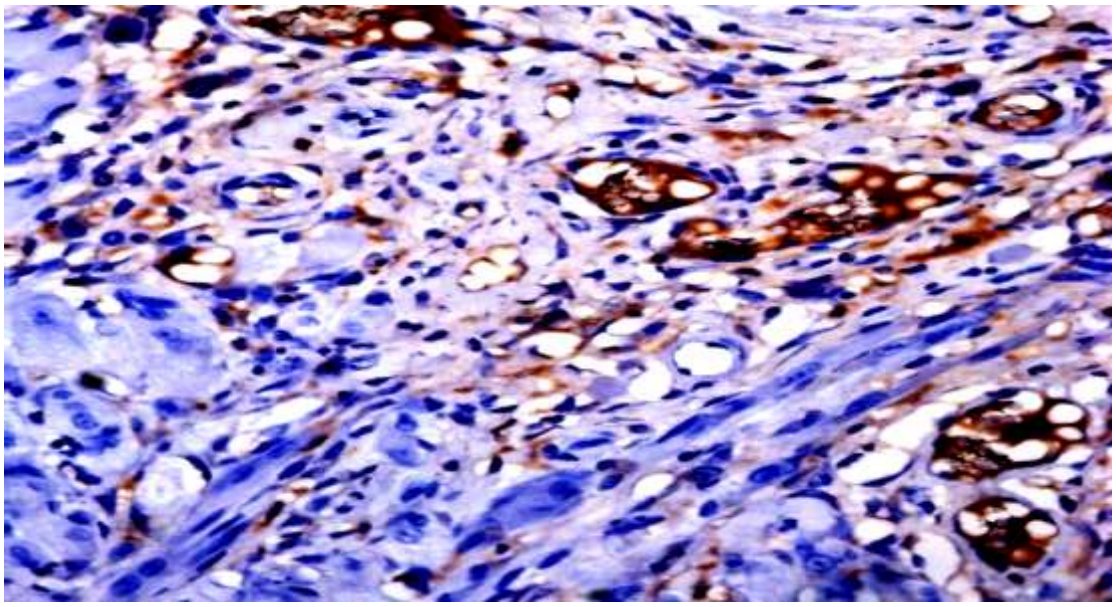

Figure 06: Photomicrograph of histological section of wounded tongue mucosa of group C in CD34 immunohistochemical stain.

Discussion

Aloe vera has been used for centuries for curing wounds and illnesses. It helps to heal skin burns and allergies. Surgical incisional wounds of skin show better healing when the gel extracted from the plant is applied topically [7]. Excisional wounds of skin also show early contraction, re-epithelization and wound closure. Apart from skin, role of the herb in the treatment of mucosal ulcers cannot be denied. Gastric ulcer can be treated with the extracts from *Aloe vera*. It shows anti ulcers activity against non-steroidal anti-inflammatory drugs. There are very few studies on oral mucosa regarding healing effects of *Aloe vera*. Topical application of Acemannan extract of *Aloe vera* gel heals gingival and palatal mucosa wounds of the rats. Mostly, the studies on oral mucosa ulcers are clinical [8]. There is no study yet regarding histological changes induced by *Aloe vera* gel in healing tongue mucosa. In this contest this is the only study in which *Aloe vera* gel was administered orally and the histological changes in tongue mucosa wounds of rats were observed. The most important outcome of the present study is that *Aloe vera* accelerates healing in tongue mucosa wounds in Wistar rats [9].

The observed increase in blood vessel density aligns with the known angiogenic properties of Aloe vera. Aloe vera contains various bioactive compounds, including polysaccharides and growth factors, which have been shown to stimulate endothelial cell proliferation and migration. These processes are essential for the formation of new blood vessels and play a crucial role in facilitating oxygen and nutrient supply to the wounded tissue, thus promoting a conducive environment for tissue repair [10]. The positive correlation between blood vessel density and the duration of Aloe vera gel application further supports the dose-dependent relationship between Aloe vera exposure and vasculogenesis. This suggests that a longer application period could potentially lead to more pronounced neovascularization, emphasizing the importance of consistent and prolonged treatment for optimal results. The clinical implications of these findings are noteworthy. Aloe vera gel, with its demonstrated ability to expedite vasculogenesis, could offer a valuable adjunct to oral surgical procedures. By enhancing the blood supply to the wound site, Aloe vera gel may contribute to quicker tissue regeneration, reduced inflammation, and minimized complications [11]. This could be particularly advantageous in cases of oral surgery, trauma, or conditions involving compromised oral mucosa. Re-epithelization is the process of restoration of epithelium by keratinocytes after injury and is an important step in oral mucosa healing. Several factors may hinder the normal healing process of oral wounds by disruption of newly formed epithelial barrier and delay it as oral mucosa is subject to various types of stresses in daily routine life like mastication, swallowing and speech [12].

Conclusion

Results of the study clearly show that *Aloe vera* gel accelerates healing in tongue mucosa wounds in Wistar rats. It plays its role by bringing enhanced inflammation, epithelization, angiogenesis and fibrosis. Aloe vera gel, with its angiogenic properties, could serve as an effective adjunct to expedite tissue repair, reduce complications, and enhance overall patient outcomes. This potential benefit extends to various oral surgical procedures, traumatic injuries, and oral mucosal pathologies.

Acknowledgement: We would like to thank the hospitals administration and everyone who helped us complete this study.

Disclaimer: Nil

Conflict of Interest: There is no conflict of interest.

Funding Disclosure: Nil

Authors Contribution

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Final Approval of version: **Madiha Rasheed1**

References

1. Devis, R.H., Leitner, M.G., Russo, J.S. and Byrne, M.E., 1989. Wound healing, oral and topical activity of Aloe vera. *J Am Podiatr Med Assoc,* .**79**(11): 559-562.
2. Eshghi, F., Hosseinimehr, S.J., Rahmani, N., Khademloo, M., Norozi, M.S. and Hojati, O., 2010. Effects of *Aloe vera* cream on posthemorrhoidectomy pain and wound healing: Results of a randomized, blind, placebo control study. *J Altern Complement Med*, **16**(6): 647-650.
3. Mackay,D. and Miller, L.A., 2003. Nutritional Support for Wound Healing. *Altern Med Rev*, 8(4):359-377.
4. Mescher, L.A. 2010. Janquera's Basic Histology Text and Atlas. 12th ed. New York, McGraw-Hill Companies.
5. Moghaddasi, S. and Verma, S.K., 2011. *Aloe vera* their chemical and applications; A review. *Int J Biol Med*, **2**(1): 466-471.
6. Takzare, N., Hosseini, M.J., Hassanzadeh,G., Murtazavi, H., Takzare, A. and Habibi P., 2009. Influence of *Aloe vera* gel on dermal wound healing process in rats. *Toxicol Mech Methods*, **19**(1): 73-77.
7. Tal, W. and Dist, P., 2010. In vitro study of cytoprotection by Aloe vera leaf gel against APAP (AAP) mediated oxidative stress in rat liver slices. *Int.J.Adv.Biotechnol.Res*, **1**(2): 73-86.

8. Yousuf, S., Agunu, A. and Diana, M., 2004. The effect of *Aloe vera, A.berger* (Liliaceae) on gastric acid secretion and acute gastric mucosalinjury in rats. *J. of Ethno pharmacy*, **9.**
9. Fernanda A.S.M., Passarini J.R., Maecelo M.E., Josue S.M., Cristina C.F. and Glaucia M.T.S., 2009. Effect of application of Aloe vera (L.) and microcurrent on healing of wounds surgically induced in Wistar rats. *Acta Cirurgica Brasileirca*, **24** (2): 150-155.
10. Fernandes, K.P.S., Bussadori, S.K., Marques, M.M., SumiE, N., Wadt, Y., Bach E and Martins M.D., 2010. Healing and cytotoxic effect of *Psidium guajava* (*Mytraceae*) leaf extracts. *Braz J Oral Sci*, **9**(4): 449-454.
11. Jettanacheawchankit, S., Sasithanasate, S., Sangvanich, P., Banlunara, W. and Thunyakitpisal, P., 2009. Acemannan stimulates gingival fibroblast proliferation; expression of keratinocyte growth factor-1, Vascular endothelial growth factor, and Type 1 collagen; and wound healing. *J Pharmacol Sci*, **19**: 525-531.
12. Langmead, L., Makins, R.J. and Rampto, D.S., 2003. Anti-inflammatory effects of aloe vera gel in human colorectal mucosa in vitro. *Aliment Pharmacol Ther*, **19**(2004): 521–527.