

RESEARCH ARTICLE

Clinical, Histopathological, and Immunohistochemical Investigations of the Effects of Aloe Vera (*Aloe barbadensis* Miller) on Open Wound Healing in Rats

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ABSTRACT

The aim of this study was to investigate the efficacy of freshly prepared (from the freeze-dried powder form) solution of *Aloe barbadensis* Miller on experimentally induced excisional wounds in rats. Eighty female Sprague Dawley rats were divided into ten groups: control, Madecassol[®] pomade, Carravet[®] gel, and different concentrations of the solution form of *Aloe vera*. Bilateral excisional wounds were created on both the right and left sides of the dorsal midline. All drugs were applied topically for 21 days, wounds were observed daily, and biopsies were taken on days 7, 14, and 21. Inflammatory cell count, vascularization, VEGF, and TGF- β 1 levels were investigated. Biomechanical evaluations were also performed in a stretching device by using skin samples taken on day 21 after ending the study. Wounds in the 1% *Aloe vera* group closed earlier than in the control group. Wounds applied with *Aloe vera* had lower inflammatory cell counts and a higher degree of neovascularisation when compared with the wounds applied with Madecassol[®] pomade and Carravet[®] gel. TGF- β 1 levels decreased gradually only in the control group, while VEGF levels in the 2.5 and 10% *Aloe vera* groups were significantly higher than in the other groups on day 21. The 10% *Aloe vera* group had a higher tensile strength than other groups. As a conclusion, the solution form of *Aloe vera* is more effective than the gel form in wound healing. Notably, the 1% concentration of *Aloe vera* solution can be a potential wound healing agent.

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INTRODUCTION

Botanical and pharmacological studies obtained from natural sources and bringing new agents to the pharmaceutical industry are essential for human and animal health (Abou Auda, 2011). They have become complementary and even alternative agents to medicine. Natural compounds are considered more acceptable over time because they successfully prevent or treat various diseases such as diabetic wounds (Inpanya *et al.*, 2012), cancer (Majumder *et al.*, 2019), and bone diseases (Godoy *et al.*, 2018).

Wound healing occurs after the tissue is damaged and its integrity is destroyed. It is a complex course consisting of three intertwined steps (inflammation, granulation, and remodeling). For the physiological recovery to be completed, the whole process must proceed correctly (Jia

et al., 2008; Munger and Sheppard, 2011; Behm *et al.*, 2012;).

Various plants and their extracts such as *Aloe vera*, *Centella asiatica*, *Curcuma longa*, and *Hypericum perforatum* are often preferred for wound healing because of their high healing potential (Maver *et al.*, 2015; Pushparani *et al.*, 2018). *Aloe vera* is a traditional and popular phytotherapeutic agent used worldwide since ancient times (Kim *et al.*, 2019) and among the most researched plants in the class of natural compounds (Rahman *et al.*, 2017).

Aloe vera is a xerophytic, perennial, succulent plant from the *Liliaceae* family. Of the more than 300 *Aloe* species used in public and modern medicine, *Aloe barbadensis* Miller is the most popular form (Dal'Beló *et al.*, 2006). Its leaves have a yellow latex known as *Aloe* juice, which has a bitter taste. The leaf has a central pulp

consisting of parenchymal cells containing the gel (Steenkamp and Stewart, 2007). *Aloe vera* gel contains active ingredients that reportedly affect wound healing through such mechanisms as cell migration and proliferation, inflammation reduction and collagen maturation (Carlen *et al.*, 2013).

In previous studies, the beneficial effects of *Aloe vera* on different venues such as antitumoral, antibacterial, antidiabetic, antifungal, antiviral, antiseptic, immunomodulatory, laxative, and tissue repair were reported (Prakoso *et al.*, 2018; Majumder *et al.*, 2019; Chelu *et al.*, 2023; Kim *et al.*, 2023). Managing the wound healing is directly related to minimizing the patient's pain and making the area suitable for repair and remodeling as soon as possible. Although many studies have been carried out with phytotherapeutic agents on wound healing, there is a need for more comprehensive studies on plants with broad therapeutic effects, such as *Aloe vera* (Lee *et al.*, 2025). The present study aimed to evaluate the effects of various concentrations of *Aloe vera* solutions on the healing process of excisional wounds in rats from clinical, histopathological, immunohistochemical, and biomechanical aspects and to compare the results with the commercial gel form of *Aloe vera* (Carravet®), Madecassol® ointment (with cicatrizing effect obtained from *Centella asiatica* plant), and the control. The data thus obtained from this study are aimed to contribute to the issue of wound treatment in human and veterinary medicine.

MATERIALS AND METHODS

Experimental animals: This study was approved by the Animal Experiments Local Ethics Committee of Bursa Uludag University (2018-15/03), and the management and disposal of the animals in the experiment complied with the Guidelines for the Care and Use of Laboratory Animals and the ARRIVE guidelines (Kilkenny *et al.*, 2010). Eighty healthy Sprague Dawley, 3-month-old female rats weighing 248.5 ± 51.88 g (mean weight \pm standard deviation) were provided by the Animal Experiment Centre of Bursa Uludag University.

Before starting experiments, all rats were acclimatized for seven days and kept in an environment (12 h dark/light cycle, temperature: $22 \pm 4^\circ\text{C}$, humidity: $55 \pm 5\%$) with free access to standard diet and water in standard-sized cages. The rats were randomly divided into ten groups (8 rats per group). Detailed information on the study groups and the administered drugs is given in Table 1. On days 0, 7, 14, and 21, 0.5 ml blood was collected from the lateral tail vein of each animal and used for complete blood count.

Preparation of the rats: The rats were fasted for 24 hours preoperatively. On day 0, all groups were anesthetized by an inhalation chamber of sevoflurane (4.0-5.0%, v/v). After induction, anesthesia was maintained with a facial mask by inhalation of sevoflurane (2.5-3.5%, v/v). A single dose of tylosin (8.8 mg/kg, intramuscularly) (Tavilin®, Vilsan Veterinary Drug Co., Ankara, Turkey) was administered for prevention, and carprofen (Rimadyl®, Pfizer Inc., Zaventem, Belgium) was injected (4mg/kg, subcutaneously) for analgesia to all animals once before the procedure.

In all rats, the hair on the dorsum (from the scapula to the ilium region) was clipped with a shaver. The skin surface for the excisional wound was cleaned with benzalkonium chloride (Zefiran Forte®, 1/1000, İlsan İlaç Drug Co., Kocaeli, Turkey) and povidone-iodine (Betadine®, 10%, Kansuk, Istanbul, Turkey). Each rat was positioned in sternal recumbency and surgically draped.

Excisional wound creation: One full-thickness wound (2x2cm) was created on each side, nearly 0.5 cm from the dorsal midline just caudal to the caudal border of the scapula. The skin, including panniculus carnosus, was excised with a no. 11 scalpel blade and scissors, and the hemorrhage was controlled by sterile surgical sponges. The same surgeon (HC) formed excisional wounds on rats and performed the procedures for each group on the same day.

Treatment protocol: Topical applications were applied daily to both wound areas of all rats at the indicated doses from day 0 to day 21 by the same person (HC). In Group 1, 0.5 ml of 0.9% NaCl solution was applied to wound areas from the beginning until the wound was closed entirely. In Group 2, Madecassol® ointment was used in an amount to cover the entire wound area and was absorbed by the tissues within 24 hours. In Group 3, 1 g of Carravet® gel was applied topically. In Groups 4-10, *Aloe vera* solutions (0.1, 0.25, 0.5, 1, 2.5, 5, and 10%) were prepared daily by dissolving *Aloe vera* powder (Aleocorp Inc., Tacoma, WA, USA) in 100 ml of 0.9% NaCl and were applied at an amount of 0.5ml to both wounds. The amount of the applied medicine was decreased in parallel to the decreasing wound size. No signs of pain were detected during the application or observation periods.

Wound healing evaluation

Observations during daily wound care: Each wound was monitored daily for the presence of any exudate and the progress of wound healing until the healing was completed. Before topical applications, any exudate or bleeding on the wound was cleaned with sterile gauze. No intervention was made until the crusts on the wounds fell off spontaneously. Any hair growing around the wound area was gently removed with a razor on days 14 and 21 without damaging the wound area. The day the first granulation tissue was observed, the day the wound was filled with granulation tissue to skin level, and the day of total epithelialization of the wound were recorded.

Planimetry: Planimetry was done on days 0, 7, 14, and 21 on anesthetized animals (anesthesia as described above) by tracing the perimeter of the wound area onto a sterile piece of transparent acetate film with a special marking pen. The examiner (HC), wearing a 2.5x loupe and blinded to the group, traced the wound margin at the border between the normal skin and the wound. The outlined area was defined as the 'total wound area'. The examiner then traced the margin at the leading edge of the advancing epithelium. The area within the margin of the advancing epithelium was defined as the 'non-epithelialized wound area'. Wound tracings were scanned and transferred to a computer, and the area (mm²) and perimeter were calculated for each wound tracing using the Sigma Scan software (SPSS Inc., Chicago, IL, USA). The percentage of total wound healing

Table 1: Observations during daily wound care in animals until wound healing was completed.

Groups	Animals with punctate serosanguinous discharge (n and day)	Animals with serous discharge (n and day)	Punctuated blood focus (n and day)	Day of the first crust (n and day)	Other findings
Group 1 (Control)	2 (6), 2 (8), 1 (13)	-	-	1 (6)	After liquid application, the tissue's color changed to a brighter red with absorption.
Group 2 (Madecassol®)	3 (6), 2 (8), 2 (11)	-	-	1 (6)	Dark red-heterogeneous wound areas during the first 5 days.
Group 3 (CarraVet®)	-	1 (6), 1 (11)	-	1 (6)	The wound areas were observed as bright red on day 0.
Group 4 (0.1% <i>Aloe vera</i>)	-	1 (4), 2 (5)	1 (3)	-	No discharge after 24 hours.
Group 5 (0.25% <i>Aloe vera</i>)	-	1 (4), 2 (5), 1 (6), 1 (9)	-	-	No discharge after 24 hours.
Group 6 (0.5% <i>Aloe vera</i>)	-	1 (5)	1 (3)	-	No discharge after 24 hours.
Group 7 (1% <i>Aloe vera</i>)	-	1 (4), 1 (6), 1 (9)	-	-	No discharge after 24 hours.
Group 8 (2.5% <i>Aloe vera</i>)	-	1 (3), 1 (5, 8), 1 (6), 1 (6, 8, 11)	-	-	No discharge after 24 hours.
Group 9 (5% <i>Aloe vera</i>)	-	1 (4, 8, 11), 3 (8)	1 (8)	1 (5)	On day 8, a turbid, brown discharge was seen in the wound of subject 5.
Group 10 (10% <i>Aloe vera</i>)	-	1 (3), 2 (4), 1 (5), 1 (6), 1 (7), 2 (8)	1 (4)	-	Recurrent discharge after 24 hours in subjects 4 and 8 and after 48 hours in subject 6.

was calculated for wounds on the right side using a previously described two-step formula (Swaim *et al.*, 1993).

Step 1: Open wound day_n as a percentage of original = Open wound area day_n × 100 / Original wound area day₀

Step 2: % total wound healing day_n = 100 – Open wound day_n as percentage of original.

The unhealed wound area and the percentage of total wound healing were recorded on each measurement day and used for statistical analysis.

Histopathological evaluation: Three-millimeter diameter punch biopsy instruments (KAI Group, Japan) were used to take skin specimens from different corners of the left-side wound of each rat on days 7, 14, and 21 immediately after the planimetry was performed. While one specimen of the skin biopsies was reserved for ELISA, the other was fixed in 10% buffered formalin for 48 hours and subjected to routine tissue processing for histopathological and immunohistochemical examinations. An area just below the epidermis or crust formation was randomly selected to count the inflammatory cell infiltrate, and then three consecutive areas moving towards the deep dermis were evaluated. All four regions were examined under x400 magnification by the same researcher (ITC), and the semiquantitative scoring was established as: 0-25 inflammatory cells=1, 26-50 inflammatory cells=2, 51-75 inflammatory cells=3, and >75 inflammatory cells=4.

Immunohistochemical evaluation: Streptavidin-peroxidase-DAB immunohistochemical technique demonstrated vessels using the recombinant anti-CD31 (Ab182981, Abcam, Cambridge, England) as the primary antibody. A total number of vessels in four areas (as described above for the inflammatory cell counts) were counted blindly by the same researcher (ITC).

Serological evaluation: All animals were euthanized on day 21 by exsanguination under anesthesia, blood samples were collected, and serum was obtained after

centrifugation. Commercially available rat VEGF (EK0540, Boster, CA, USA) and rat TGF-β1 (EK0514, Boster) PicoKine ELISA kits were used to measure the amount of the relevant proteins in serum.

Biomechanical evaluation: Biomechanical examinations were performed at the Physics Laboratory of Bursa Uludag University, Textile Engineering Department, immediately after the euthanasia process on day 21. Skin samples were taken by measuring 6x2cm areas (including the healed area in the center), and excised skin samples were placed in sterile physiological saline. During measurements, all samples were kept in sterile gauze moistened with sterile physiological saline.

The universal tensile testing machine (Shimadzu AGX plus®, Beijing, China) was used for biomechanical evaluation. The cranial and caudal ends of the 6x2cm skin samples were immobilized with the clamps, so the scar tissue line remained in the middle. The tension rate created by the clamps moving away from each other was set to a constant speed of 20mm/min. The strength of the skin samples taken from the healed wound areas was determined in Newton (N), and the elongation rates were determined and recorded as percentage values. All data were transferred to a computer and analyzed via the Trapezium program. The results of only one animal from Group 2 were not included in the analysis because the sample was torn during the process.

Statistical analysis: The mean values were compared among all groups using the Kruskal-Wallis test. In case of significant differences among the groups, pairwise comparisons were made with the Dunn-Bonferroni multiple comparison test. Since analyses were performed with non-parametric tests, descriptive statistics were given as median (min-max) values. P<0.05 was taken as the statistical significance level. Data were analyzed using the SPSS v22.0 (SPSS Inc., Chicago, IL, USA) program.

RESULTS

The hemograms of blood samples taken on days 0, 7, 14, and 21 were within normal limits (data not shown).

Observations during daily wound care: Right-sided wounds in all rats were monitored and photographed for the progress of wound healing (Table 1). Appearances of the wounds in control, Madecassol®, Carravet®, and *Aloe vera* solution groups on days 7, 14, and 21 are shown in Fig. 1.

Comparisons of the first day for the appearance of granulation tissue, complete filling, and total closure times with granulation tissue are summarized in Table 2. The time for the first appearance of granulation tissue was later in the 0.1% *Aloe vera* group (Group 4) compared to the Madecassol® and 0.25% *Aloe vera* groups (Groups 2 and 5, respectively) ($P < 0.05$). Times of the complete filling with granulation tissue were significantly shorter in the 1, 2.5, 5, and 10% *Aloe vera* groups (Groups 7-10, respectively) compared to the control, Madecassol®, and Carravet® groups (Groups 1-3, respectively) ($P < 0.05$). The time of the complete closure of wounds was significantly shorter in the 1% *Aloe vera* group than in the control group ($P < 0.05$).

Table 2: Comparison of the time (days) of the first appearance of granulation tissue, time of complete filling with granulation tissue, and time of the total closure of wounds

Group	Time of the first appearance of granulation tissue*	Time of the complete filling with granulation tissue*	Time of the total closure of wounds*
Group 1 (Control)	3.0 (2.0-3.0)	17.0 (14.0-21.0)	22.5 (17.0-28.0)
Group 2 (Madecassol®)	1.5 (1.0-6.0)	16.0 (14.0-23.0)	22.0 (18.0-28.0)
Group 3 (Carravet®)	3.0 (3.0-4.0)	12.0 (11.0-13.0)	19.0 (15.0-23.0)
Group 4 (0.1% <i>Aloe vera</i>)	4.0 (3.0-4.0)	12.0 (10.0-13.0)	19.5 (18.0-23.0)
Group 5 (0.25% <i>Aloe vera</i>)	2.0 (1.0-4.0)	17.0 (14.0-18.0)	22.0 (19.0-22.0)
Group 6 (0.5% <i>Aloe vera</i>)	3.0 (2.0-4.0)	12.0 (11.0-13.0)	19.5 (14.0-24.0)
Group 7 (1% <i>Aloe vera</i>)	3.0 (2.0-4.0)	11.0 (10.0-12.0)	17.5 (14.0-20.0)
Group 8 (2.5% <i>Aloe vera</i>)	3.0 (2.0-3.0)	11.0 (9.0-13.0)	21.0 (19.0-22.0)
Group 9 (5% <i>Aloe vera</i>)	3.0 (2.0-4.0)	11.0 (11.0-12.0)	21.0 (19.0-22.0)
Group 10 (10% <i>Aloe vera</i>)	3.0 (2.0-3.0)	10.0 (9.0-11.0)	20.5 (19.0-22.0)
P value	0.002	<0.001	0.014

*All values indicate the days and are median (min-max).

Planimetry: The total wound healing percentage was determined with the formula stated in Swaim *et al.*'s (1993) study (as cited in Gul *et al.* {2008}). The median (min-max) values, and pairwise comparison of unhealed wound area and total wound healing percentage of all groups on days 7, 14, and 21 are given in Table 3.

Table 3: Comparison of the unhealed wound area values and the percentage of total wound healing on days 7, 14, and 21

Group	Unhealed wound area* (mm ²)			Pairwise comparisons				Total wound healing percentage* (%)			Pairwise comparisons			
	Day 7	Day 14	Day 21	P-value	Days 7 and 14	Days 7 and 21	Days 14 and 21	Day 7	Day 14	Day 21	P-value	Days 7 and 14	Days 7 and 21	Days 14 and 21
1 Control (0.9% NaCl)	251.0 (196.0-398.0)	26.5 (5.0-53.0)	3.0 (0.0-9.0)	<0.001	0.012	0.012	0.012	37.3 (0.5-51.0)	93.4 (86.8-98.8)	99.3 (97.8-100.0)	<0.001	0.012	0.012	0.012
2 Madecassol®	258.0 (208.0-376.0)	17.5 (5.0-35.0)	1.0 (0.0-5.0)	<0.001	0.012	0.012	0.018	35.5 (6.0-48.0)	95.6 (91.3-98.8)	99.8 (98.8-100.0)	<0.001	0.012	0.012	0.018
3 Carravet®	215.0 (142.0-325.0)	15.5 (8.0-34.0)	1.0 (0.0-8.0)	<0.001	0.012	0.012	0.012	46.3 (31.3-64.5)	96.1 (91.5-98.0)	99.8 (98.0-100.0)	<0.001	0.012	0.012	0.012
4 0.1% <i>Aloe vera</i>	191.5 (121.0-283.0)	9.0 (4.0-28.0)	0.0 (0.0-1.0)	<0.001	0.012	0.012	0.012	52.1 (29.3-69.8)	97.8 (93.0-99.0)	100.0 (99.8-100.0)	<0.001	0.012	0.012	0.012
5 0.25% <i>Aloe vera</i>	158.0 (129.0-232.0)	14.0 (8.0-21.0)	0.0 (0.0-3.0)	<0.001	0.012	0.012	0.012	60.5 (42.0-67.8)	96.5 (94.8-98.0)	100.0 (99.3-100.0)	<0.001	0.012	0.012	0.012
6 0.5% <i>Aloe vera</i>	189.0 (47.0-227.0)	9.0 (0.0-31.0)	0.0 (0.0-13.0)	<0.001	0.012	0.012	0.017	52.8 (43.3-88.3)	97.8 (92.3-100.0)	100.0 (96.8-100.0)	<0.001	0.012	0.012	0.017
7 1% <i>Aloe vera</i>	154.5 (76.0-349.0)	6.0 (0.0-39.0)	0.0 (0.0-0.0)	<0.001	0.012	0.012	0.012	59.9 (12.8-81.0)	98.5 (90.3-100.0)	100.0 (100.0-100.0)	<0.001	0.012	0.012	0.012
8 2.5% <i>Aloe vera</i>	290.5 (250.0-322.0)	22.5 (7.0-44.0)	0.0 (0.0-7.0)	<0.001	0.012	0.012	0.012	27.4 (19.5-37.5)	94.4 (89.0-98.3)	100.0 (98.3-100.0)	<0.001	0.012	0.012	0.012
9 5% <i>Aloe vera</i>	385.0 (207.0-397.0)	34.0 (4.0-47.0)	0.0 (0.0-5.0)	<0.001	0.012	0.012	0.012	8.3 (0.8-48.3)	91.5 (88.3-99.0)	100.0 (98.8-100.0)	<0.001	0.012	0.012	0.012
10 10% <i>Aloe vera</i>	249.0 (217.0-384.0)	20.5 (9.0-56.0)	0.0 (0.0-6.0)	<0.001	0.012	0.012	0.012	37.8 (4.0-45.8)	94.9 (86.0-97.8)	100.0 (98.5-100.0)	<0.001	0.012	0.012	0.012
P-value	<0.001	0.024	0.069					<0.001	0.024	0.069				

*All values are median (min-max).

Planimetry results of the unhealed wound area indicated a highly significant difference between days 7, 14, and 21 within the groups ($P < 0.001$). The unhealed wound area decreased from the 7th to the 21st day, and the total wound healing percentage increased at the same rate. The day with the most significant statistical difference was day 7 ($P < 0.001$) (Table 3).

When the 5% *Aloe vera* group (Group 9) was compared with 0.1, 0.25, 0.5, and 1% *Aloe vera* groups (Groups 4-7, respectively), the statistical differences were significant on day 7 ($P < 0.05$). The difference between the 0.25% *Aloe vera* group (Group 5) and the 2.5% *Aloe vera* group (Group 8) was significant ($P < 0.05$) (Table 3).

In the statistical comparisons between the study groups, the percentage of total wound healing and the unhealed wound area on day 14 were significantly reduced in all groups ($P = 0.024$) (Table 3). Although a difference was observed among the groups, no significant difference was substantiated in the treatment effect in the paired comparisons. When the day 21 values were examined, no difference was found among the groups ($P = 0.069$) (Table 3).

Histopathological findings: Inflammatory cell score and capillary proliferation rate were used to evaluate wound healing degree. Inflammatory cells in four areas under X400 magnification were counted in slides stained with hematoxylin-eosin. The character of inflammatory cells changed over time in all groups. While lymphocytes, plasma cells, and neutrophils were observed on day 7, mononuclear inflammatory cell infiltrates consisting almost entirely of lymphocytes, plasma cells, and macrophages were seen in biopsies taken on days 14 and 21, except for the areas close to the wound surface.

Inflammatory cell scores and comparisons among the groups are given in Table 4. The inflammatory cell score was determined to be lower in the animals treated with *Aloe vera* (Groups 4-10) when compared to the groups treated with Madecassol® (Group 2) and Carravet® (Group 3). The concentration of *Aloe vera* had no significant effect on the inflammatory cell score (Groups 5-10) (Table 4).

Immunohistochemical evaluation: In all groups, on day 7, almost all of the capillaries had very small lumens, and there were often no erythrocytes in their lumen, while on days 14 and 21, the capillaries were determined to be larger and have a wider lumen (Fig. 2).

CD31+ cell numbers were counted in four areas, and the comparison of the groups is shown in Table 4. The number of CD31+ cells decreased in all groups weekly. This decrease was significant in all groups and within weeks, except for days 14 and 21 in Group 4 (0.25% *Aloe vera*) and Group 10 (10% *Aloe vera*).

In the control (Group 1), Madecassol® (Group 2), and Carravet® (Group 3) groups, CD31+ cell counts were lower than in the *Aloe vera* groups (Groups 4-10), and no difference was observed among the groups in the comparison of the first three groups. When the *Aloe vera* groups were compared, significant differences were found among the groups for the solution concentration except for Groups 4 and 10 (Table 4).

Serological evaluation: The serum amounts of TGF-β1 and VEGF proteins in biopsy samples taken from the wound areas in all subjects were measured using ELISA and the results are summarized in Table 5.

The correlations of TGF-β1 and VEGF with unhealed wound area, percentage of total healing, the total number of vessels, and inflammatory cell score were also calculated and are shown in Table 6. On day 14, a

significant inverse relationship was found between the unhealed wound area ($r=-0.403$; $P<0.001$). A significant correlation was found between the percentage of total wound healing and TGF-β1 ($r=0.403$; $P<0.001$) and between the inflammatory cell score and TGF-β1 in the same direction on day 7 ($r=0.284$; $P=0.11$). A significant inverse relationship was found between the total number of vessels and TGF-β1 on days 7 and 14 ($r=-0.498$; $P<0.001$; $r=-0.279$; $P=0.012$, respectively). An inverse correlation was found between the unhealed wound area and VEGF on day 14 ($r=-0.249$; $P=0.026$). A significant correlation was found in the same direction between the total healing percentage and VEGF ($r=0.249$; $P=0.026$) and between the total number of vessels and VEGF ($r=0.726$; $P<0.001$) on day 7. While there was a significant inverse relationship between the inflammatory cell score and VEGF on day 7 ($r=-0.524$; $P<0.001$), a significant relationship was found in the same direction on day 21 ($r=0.375$; $P=0.001$).

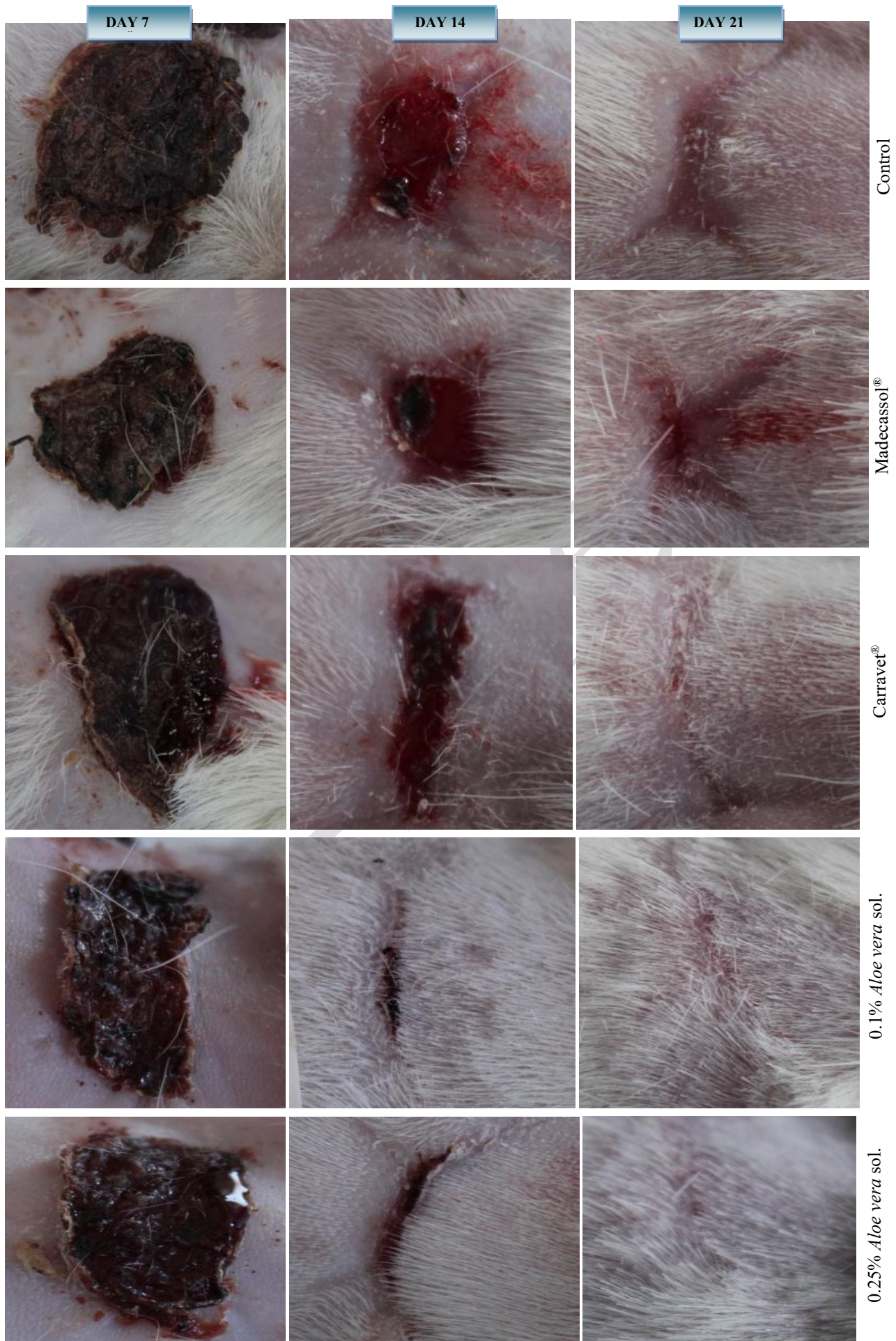
Biomechanical findings: The median (min-max) maximum force (N) and elongation values (%) of the tissues in all groups are given in Table 7. There was a significant difference among the groups regarding maximum force (N) and elongation values (%) ($P<0.001$ and 0.042, respectively). When the statistical pairwise comparisons of the maximum force values were

Table 4: Comparison of inflammatory cell score values and number of vessels as determined with anti-CD31 staining on days 7, 14, and 21 based on groups

Groups Group	Inflammatory Cell Score (Median [min-max])			P-value	Pairwise Comparisons			CD31+ Cell Count			P-value	Pairwise Comparisons			
	Day 7	Day 14	Day 21			P-value			Day 7	Day 14		Day 21	P-value		
						Days 7-14	Days 7-21	Days 14-21					Days 7-14	Days 7-21	Days 14-21
Group 1 -Control	13.0	11.0	9.5					88.5	54.5	38.0	0.001	0.012	0.012	0.161	
Median (min-max)	(11.0-14.0)	(6.0-12.0)	(5.0-15.0)	0.005	0.011	0.028	0.156	(60.0-139.0)	(33.0-81.0)	(25.0-72.0)					
Group 2 -Madecassol®	13.0	11.0	8.0					68.5	56.5	37.0		0.012	0.012	0.012	
Median (min-max)	(11.0-15.0)	(10.0-13.0)	(6.0-10.0)	<0.001	0.016	0.011	0.011	(46.0-85.0)	(43.0-64.0)	(23.0-56.0)	<0.001				
Group 3 -CarraVet®	13.0	12.5	8.0					79.5	44.5	33.0		0.043	0.012	0.050	
Median (min-max)	(10.0-16.0)	(8.0-16.0)	(6.0-12.0)	0.002	0.414	0.017	0.011	(57.0-88.0)	(30.0-94.0)	(20.0-44.0)	0.003				
Group 4 -0.1% Aloe vera	6.5	5.0	4.0					91.0	67.0	52.0	-	-	-	-	
Median (min-max)	(5.0-7.0)	(4.0-6.0)	(4.0-5.0)	0.002	0.047	0.011	0.038	(49.0-146.0)	(53.0-89.0)	(32.0-145.0)	0.325				
Group 5 -0.25% Aloe vera	8.5	7.0	4.5					117.5	83.5	61.5	0.001	0.012	0.012	0.017	
Median (min-max)	(6.0-11.0)	(6.0-9.0)	(4.0-6.0)	0.002	0.041	0.012	0.027	(75.0-202.0)	(67.0-96.0)	(42.0-82.0)					
Group 6 -0.5% Aloe vera	9.0	6.0	5.0					135.0	93.0	66.0	<0.001	0.012	0.012	0.012	
Median (min-max)	(6.0-11.0)	(5.0-9.0)	(4.0-6.0)	<0.001	0.011	0.011	0.014	(91.0-194.0)	(65.0-104.0)	(36.0-81.0)					
Group 7 -1% Aloe vera	8.0	7.0	5.0					183.0	125.0	78.5		0.012	0.012	0.036	
Median (min-max)	(7.0-8.0)	(5.0-9.0)	(4.0-7.0)	0.006	0.163	0.011	0.028	(145.0-272.0)	(83.0-159.0)	(51.0-159.0)	0.001				
Group 8 -2.5% Aloe vera	11.0	9.0	7.0					232.5	138.5	109.5		0.012	0.012	0.012	
Median (min-max)	(8.0-12.0)	(7.0-12.0)	(6.0-9.0)	0.001	0.068	0.011	0.011	(193.0-273.0)	(107.0-253.0)	(74.0-130.0)	<0.001				
Group 9 -5% Aloe vera	8.0	8.0	5.5					189.5	127.5	99.0		0.012	0.012	0.050	
Median (min-max)	(5.0-9.0)	(5.0-10.0)	(4.0-7.0)	0.003	0.480	0.016	0.018	(150.0-214.0)	(98.0-159.0)	(73.0-130.0)	0.001				
Group 10 -10% Aloe vera	8.5	7.5	5.0					217.0	152.5	126.0	0.005	0.025	0.012	0.069	
Median (min-max)	(6.0-12.0)	(5.0-11.0)	(5.0-7.0)					(132.0-264.0)	(119.0-252.0)	(80.0-196.0)					
P-value	<0.001	<0.001	<0.001			<0.001	<0.001	<0.001							

Table 5: Serum TGF-β1 and VEGF levels were measured in pg/mL using ELISA kits from the biopsy samples in all groups

Group	TGF-β1 Serum Levels (pg/mL)			VEGF Serum Levels (pg/mL)		
	Day 7	Day 14	Day 21	Day 7	Day 14	Day 21
Group 1 (Control)	138.74	91.664	41.816	138.74	91.664	41.816
Group 2 (Madecassol®)	202.43	158.12	188.59	202.43	158.12	188.59
Group 3 (CarraVet®)	484.90	545.83	579.06	484.90	545.83	579.06
Group 4 (0.1% <i>Aloe vera</i>)	213.51	205.20	196.89	213.51	205.20	196.89
Group 5 (0.25% <i>Aloe vera</i>)	166.43	194.12	263.36	166.43	194.12	263.36
Group 6 (0.5% <i>Aloe vera</i>)	47.35	224.59	180.28	47.35	224.59	180.28
Group 7 (1% <i>Aloe vera</i>)	135.97	293.82	210.74	135.97	293.82	210.74
Group 8 (2.5% <i>Aloe vera</i>)	202.43	133.20	207.97	202.43	133.20	207.97
Group 9 (5% <i>Aloe vera</i>)	61.20	44.58	171.97	61.20	44.58	171.97
Group 10 (10% <i>Aloe vera</i>)	61.20	97.20	166.43	61.20	97.20	166.43



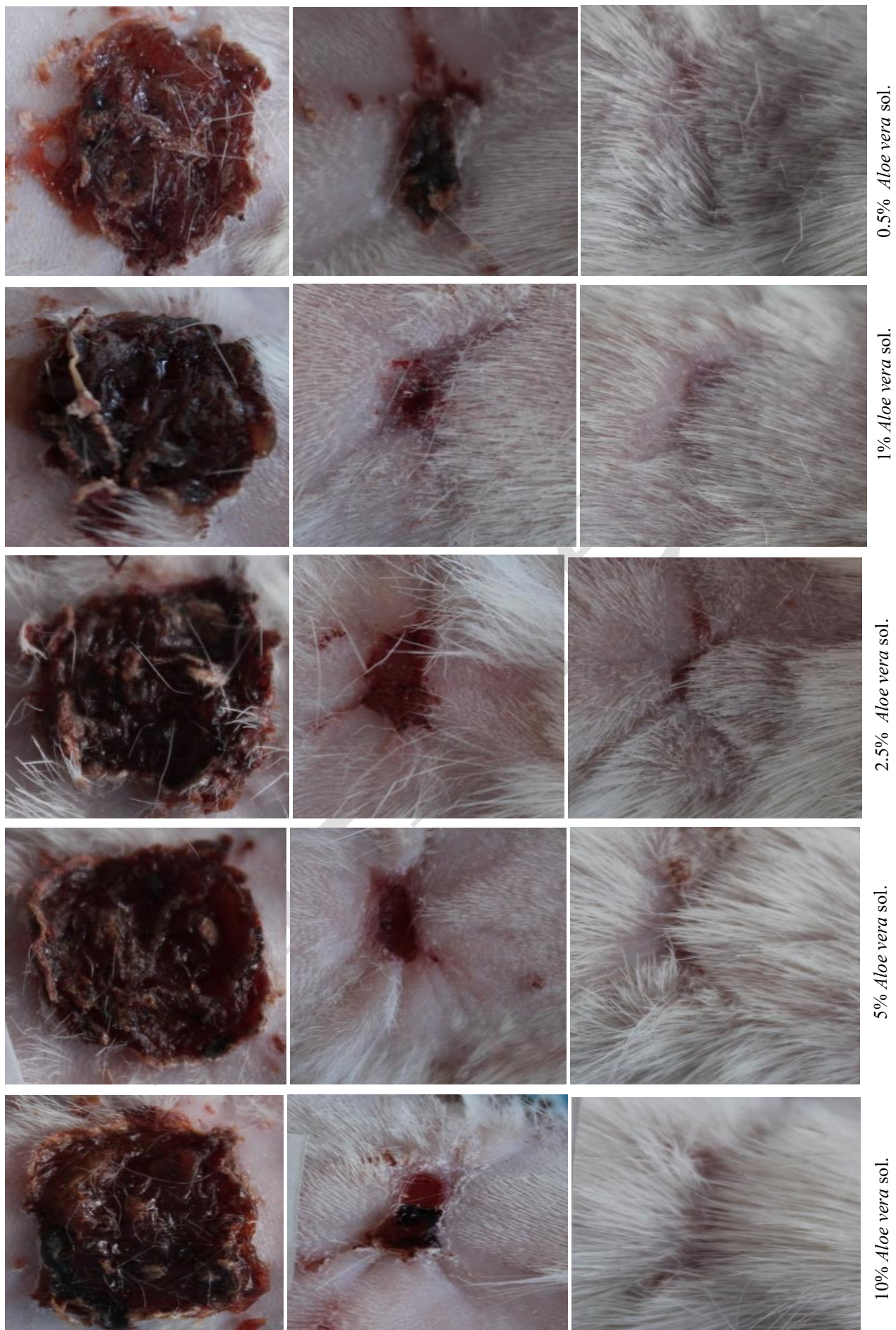


Fig. 1: Appearances of the wounds created on the right side in all groups on days 7, 14, and 21 (from left to right).

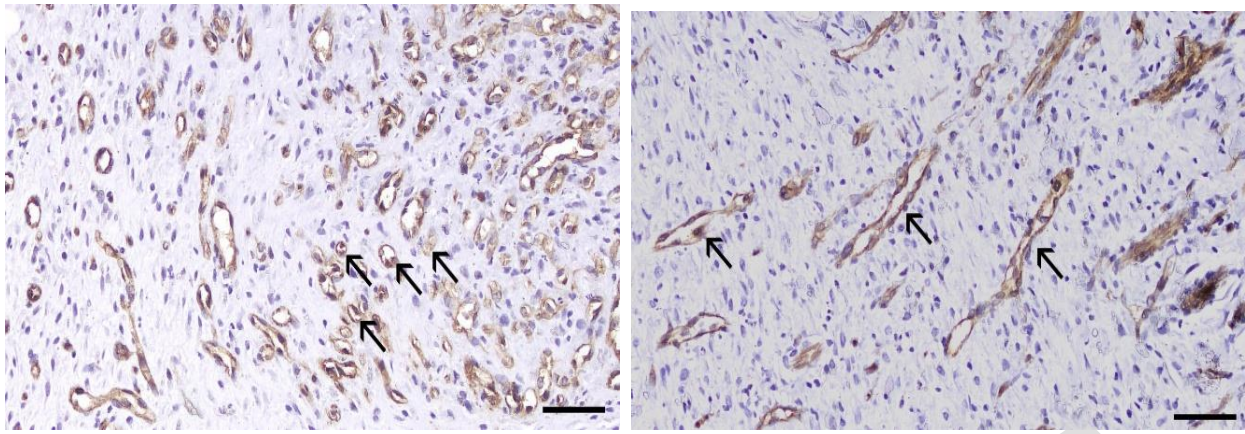


Fig. 2: Almost all capillaries (arrows) observed have very narrow lumens and generally do not contain erythrocytes (Day 7, Group 9 [5% *Aloe vera*]). The capillary lumens (arrows) are wider on day 21 of the same animal: Streptavidin-peroxidase, DAB chromogen, bar: 80 μ m.

Table 6: Comparison of TGF- β 1 and VEGF with unhealed wound area, total healing percentage, total number of vessels, and inflammatory cell scores

	Unhealed wound area		The percentage of total healing		Total number of vessels		Inflammatory cell score	
TGF- β 1	r	P	r	P	r	P	r	P
Day 7	-0.035	0.755	0.054	0.0637	-0.498	<0.001	0.284	0.11
Day 14	-0.403	<0.001	0.403	<0.001	-0.279	0.012	-0.133	0.241
Day 21	-0.203	0.071	0.203	0.071	-0.188	0.095	-0.090	0.429
VEGF	r	P	r	P	r	P	r	P
Day 7	-0.039	0.733	0.020	0.864	0.726	<0.001	-0.524	<0.001
Day 14	-0.249	0.026	0.249	0.026	0.045	0.695	-0.009	0.937
Day 21	0.099	0.383	-0.099	0.383	0.154	0.171	0.375	0.001

examined, the 0.5, 2.5, and 5% *Aloe vera* groups (Groups 6, 8, and 9, respectively) had lower tensile strength than the control group (Group 1), Carravet® (Group 3) and 1% *Aloe vera* (Group 7) ($P < 0.05$) groups. Although there was a difference among the groups in the statistical pairwise comparisons of tissue elongation percentage measurements among the groups ($P < 0.05$), the treatments were not effective enough to create a significant difference (Table 7).

Table 7: Median (min-max) values of maximum forces and percentages of elongation in all groups

Group	Maximum Force (N) Values (median [min-max])	The Percentage of Elongation (%) (median [min-max])
Group 1 (Control)	19.0 (11.5-26.0)	73.8 (40.1-235.0)
Group 2 (Madecassol®)	11.6 (7.7-15.1)	66.1 (36.5-135.1)
Group 3 (Carravet®)	16.8 (8.9-27.7)	152.1 (59.7-211.3)
Group 4 (0.1% <i>Aloe vera</i>)	9.2 (6.6-14.5)	136.3 (53.8-234.8)
Group 5 (0.25% <i>Aloe vera</i>)	8.5 (5.9-19.6)	89.2 (62.5-230.3)
Group 6 (0.5% <i>Aloe vera</i>)	8.0 (5.0-18.6)	58.1 (37.8-151.6)
Group 7 (1% <i>Aloe vera</i>)	13.7 (7.3-14.5)	78.2 (55.8-216.3)
Group 8 (2.5% <i>Aloe vera</i>)	5.8 (5.5-12.6)	52.3 (47.2-143.9)
Group 9 (5% <i>Aloe vera</i>)	8.2 (5.1-14.3)	81.5 (41.1-210.8)
Group 10 (10% <i>Aloe vera</i>)	9.7 (6.6-15.7)	181.1 (49.7-228.5)
P value	<0.001	0.042

DISCUSSION

Aloe vera, in the Liliaceae family, is a complex plant containing approximately 200 different ingredients, such as growth factors, enzymes, glycoproteins, vitamins, and minerals (Thant *et al.*, 2023). AV is a plant often cultivated in people's homes around the world as a natural compound intended for widespread use by both human and animals and recognized in clinical practice as a tool for wound healing (Altinkaynak *et al.*, 2023; Hattingh *et al.*, 2023). In this study, we aimed to examine *Aloe vera*'s effect on wound healing and investigate the dose's role.

Acemannan is the main bioactive polysaccharide in *Aloe vera* and is found in the gel layer in the center of the leaves (Liu *et al.*, 2019; Thant *et al.*, 2023). This compound plays an essential role in healing by promoting tissue reepithelialization, angiogenesis, and wound contraction mechanisms (Bai *et al.*, 2023). Medicines in gel form increase the healing rate by protecting the moist environment. Sierra-Garcia *et al.* (2014) mentioned the benefits of the wound treatment of Carravet® gel (a commercial acemannan product) by reducing pain and providing a moist environment in the wound area in cats, dogs, and horses. Therefore, the Carravet® group was included as a positive control in our study in addition to the *Aloe vera* solution groups.

Wound healing occurs with the contraction feature of panniculus carnosus in species such as rats and rabbits (Kwak *et al.*, 2014). In the present study, full-thickness skin excision wounds were created in rats by including panniculus carnosus to observe contraction. This procedure of creating an excisional wound model in rats has also been applied in similar studies (Oryan *et al.*, 2016; Shen *et al.*, 2016).

Wound healing study by Gul *et al.* (2017) in rats with *Tarantula cubensis* extract was conducted and wounds were observed daily until completely healed. Considering the time when granulation tissue first appeared and when the wound area was filled with granulation tissue, a significant difference was found among the groups ($P < 0.05$) in our study. When both Carravet® and *Aloe vera* solution groups were compared with the control group, the healing occurred faster in the groups applied with *Aloe vera*.

The most important points to consider in the observations of open wounds during daily wound care are the cleanliness of the area and the presence of any discharge (Gul *et al.*, 2017). Sari *et al.* (2018) detected the

existence of exudate in all groups during daily wound care observations in a wound study in which they used *Aloe vera* gel for treatment purposes in rats. Oryan *et al.* (2016) suggested that the early disappearance of discharge in the groups containing *Aloe vera* gel compared to the control group provided faster healing of the wounds in these groups. In our study, serous discharge was detected in all *Aloe vera* solution groups until the 11th day, and serosanguinous discharge was detected in the Carravet® group. In the control group, the last serosanguinous discharge was observed on the 13th day. The earliest time when discharge disappeared was seen in the 0.1 and 0.5% *Aloe vera* solution groups on day 5. This situation suggested that *Aloe vera* was more effective in stopping bleeding in the region and suppressing exudate formation more rapidly during the wound-healing process.

Oryan *et al.* (2016) showed that granulation tissue developed earlier in both low-dose (25mg/mL) and high-dose (50mg/mL) *Aloe vera* gel groups when compared to the control groups in rats. According to the data obtained from Oryan's study, *Aloe vera*'s aqueous extract accelerated wound healing. We observed the shortest complete closure times in the 1% *Aloe vera* solution group, indicating the completion of the wound healing process. Drudi *et al.* (2018) found that *Aloe vera* liquid extract is more effective than other traditional treatments and the *Aloe vera* gel in wound healing. It is a known fact that the *Aloe vera* gel form, which is frequently preferred in studies (Sari *et al.*, 2018), accelerates wound healing. However, the observations of our study during daily wound care revealed that the *Aloe vera* solution form was more efficient in open wounds than the gel form containing acemannan.

Oryan *et al.* (2016) also determined that the wounds were completely closed on the 15th day in the high-dose *Aloe vera* group and on the 20th day in the low-dose group. In our study, the first granulation occurred later with the 0.1% solution than with the 0.25% solution. However, granulation tissue developed much earlier in the groups treated with high doses (1, 2.5, 5, and 10%) of *Aloe vera* solution than in the control, Madecassol® and Carravet® applied groups (Table 2).

In the present study, in all groups where *Aloe vera* solution was applied, granulation tissue was found in the wound area for the first time on day 4, the area was completely filled with granulation tissue on day 12, and the wounds were completely closed by day 21. When the complete closure times were compared, the wounds in the 1% *Aloe vera* group closed much earlier than the other solution groups. This finding shows the importance of dosing as a solution prepared from *Aloe vera* powder than using the powder, as the dissolved medicine is more easily accessible by the cells.

When the average unhealed wound area was examined daily, the most significant statistical difference was observed on day 7 ($P < 0.05$). The group with the largest unhealed wound area was the 5% *Aloe vera* solution group (Group 9) on days 7 and 14. However, by day 21, the unhealed wound area of the control group was the largest. When the entire process was examined until the 21st day, the fastest shrinking unhealed wound area was in the 1% concentration of the *Aloe vera* solution group (Group 7). In similar wound healing studies

(Cangul *et al.*, 2006; Gul *et al.*, 2008), while the statistical difference among the groups was significant on day 7 regarding the unhealed wound area, it was determined that the unhealed wound areas of the control groups remained larger on days 14 and 21.

The percentage of total wound healing was examined as one of the parameters in wound healing (Gul *et al.*, 2008), and we also used this formula. On day 21, the healing rate was 100% in all *Aloe vera* solution groups, whereas the healing percentages of the control, Madecassol®, and Carravet® groups were lower (median 99.3, 99.8, and 99.8%, respectively). Despite the difference in the ratio at planimetry, there was a statistical difference among the groups on days 7 and 14, but no difference was found on day 21 ($P > 0.05$). We observed that the low dose (<2.5%) *Aloe vera* solution was more efficient in completing the wound healing process than the high dose groups.

Many different parameters, such as reepithelialization, cellularity, collagen deposition, and new vessel formation are used in the histopathological evaluation of wound healing (Rodgers *et al.*, 2003). In our study, the change in inflammatory cell infiltration and vascularization were used for the histopathological evaluation of wound healing. While the inflammatory cell score was highest in all groups on day 7, a decrease was observed on days 14 and 21. The number of inflammatory cells in open wounds is expected to be high initially and then decrease over time, as shown in previous studies (Cangul *et al.*, 2006; Gul *et al.*, 2008). The number of inflammatory cells in the *Aloe vera* applied groups was lower than in the control group and the animals treated with Madecassol® and Carravet®. This situation can be interpreted as *Aloe vera*'s anti-inflammatory effect. In a similar study, 1 and 2% *Aloe vera* cream applied to experimental wounds in rats was more effective than Madecassol® against neutrophil infiltration (Prakoso *et al.*, 2018). The dose of *Aloe vera* did not significantly affect the inflammatory cell count.

We immunohistochemically demonstrated CD31 antigen to reveal neovascularization. In our study, *Aloe vera* application increased the neovascularization rate, indicating this plant's angiogenic potential and this increase was found to be significant on some days and groups. This result is similar to several studies (Morgan and Nigam, 2013; Oryan *et al.*, 2010; Prakoso *et al.*, 2018) revealing the angiogenic potential of *Aloe vera* in wound healing.

Cytokines and growth factors play an essential role in the normal progression of the healing process in humans and animals, acting alone or in combination. Wound healing consists of intertwined phases (Hashemi *et al.*, 2015), and these phases are controlled by many growth factors and cytokines, especially TGF- β 1 and VEGF. TGF- β 1 controls severe inflammation by suppressing neutrophils and fibroblasts in the inflammatory phase and provides the transition to the remodeling phase. Afterwards, it supports angiogenesis together with VEGF. TGF- β 1 and VEGF were specifically selected as markers in our study for examining the healing process.

Bryan *et al.* (2005) stated that increased TGF- β 1 levels can accelerate healing. In this study, we examined the wound healing process from day zero to day 21, and

the TGF- β 1 levels were higher in the 0.25 and 10% *Aloe vera* groups than in the other groups. The TGF- β 1 level was elevated with decreased unhealed wound area only on day 14. When the relationship between the inflammatory cell score and TGF- β 1 was examined, the TGF- β 1 level was high in direct proportion to the inflammatory cell count due to the presence of inflammation only on day 7, and the decrease in the total number of vessels due to the reduction in angiogenesis from day 7 to 14. These data showed that TGF- β 1 plays an active role in wound healing and can be used as a specific marker. Both low and high-ratio solutions of *Aloe vera* powder positively affected TGF- β 1 levels in the wound area compared to the untreated wound area.

Some researchers (Gao *et al.*, 2015; Negahdari *et al.*, 2017) have mentioned the effect of VEGF on wound healing process and scar tissue formation. In the study by Gharaboghaz *et al.* (2020), in which the authors applied *Aloe vera* gel to wounds in mice, *Aloe vera* significantly increased VEGF levels. In our study, an increase in VEGF levels was observed in the control, Madecassol®, Carravet®, 0.25%, and 5% *Aloe vera* solution groups from day 7 to 21. However, the VEGF levels were high only in the 2.5% and 10% concentration groups on day 21. In line with these data, the fact that VEGF levels generally increased in both treated and untreated groups during the 21-day period in which the wound-healing process was examined suggested that *Aloe vera* did not have a primary effect on VEGF. However, on the 21st day, the ratio in only the 2.5 and 10% *Aloe vera* solution groups showed us that VEGF, which is normally present in the environment, was present in the wound area for a longer period of time due to high concentrations of *Aloe vera*.

In similar studies (Demling, 2000; Oryan *et al.*, 2010) to ours, biomechanical tensile strength test was used as a parameter for wound healing. Oryan *et al.* (2010) applied tensiometry on the Instron device by storing the skin samples they took from rats at -20°C at the end of their wound study and then bringing them to room temperature on the measurement day. Researchers such as Bellare *et al.* (2018) have stated that more efficient results can be obtained with fresh skin samples than with frozen skin samples. In our study, biomechanical testing was applied to the skin samples taken immediately after euthanasia to maximize measurement sensitivity.

In this study, the tensile strength of the healed wounds on day 21 was found by measuring the maximum force before the tissue strips were torn, as in Demling's (2000) wound study. The strength was more significant in the 1% *Aloe vera* solution group than in the other solution groups. Our study found a significant difference among the groups regarding elongation percentage measurements ($P < 0.05$). The elongation percentage of the 10% (median: 181.1) density group from the *Aloe vera* solution groups was higher than the others, followed by the Carravet® and 0.1% *Aloe vera* groups. Although there was a significant difference among the groups, no data were found that would create a substantial difference in pairwise comparisons. In that study, where the maximum force that the healing skin segment could withstand was measured, a significant difference was found in the wound strength of the wounds treated with *Aloe vera* cream on the 9th and 14th days. In our study, no significant difference was

found on day 21. A more detailed evaluation of the strength of the healing process would have been possible by measuring these parameters on days 7 and 14.

Conclusions: In the present study, the effects of different concentrations of *Aloe vera* solution on experimentally induced excisional wounds in rats were evaluated clinically, histopathologically, immunohistochemically, serologically, and biomechanically. As a conclusion, we suggest that the solution form of *Aloe vera* is more effective than the gel form in wound healing, and that the 1% *Aloe vera* solution applied to open wounds is sufficient to stimulate the wound healing process and the high solution density does not provide an additional advantage. In terms of time for the total wound closure, indicating the completion of wound healing in open wounds, the *Aloe vera* solution form had a significantly shorter time; the 1% *Aloe vera* dose provided the shortest time. We observed that *Aloe vera* shortens the wound healing process and has a high anti-inflammatory effect. It also accelerates angiogenesis, which is an essential step in wound healing and plays an active role in developing and stimulating vascular endothelial growth factors. Regardless of the solution density, *Aloe vera* increased the level of TGF- β 1 in the environment. The high-density (10%) *Aloe vera* was more effective in tissue strength following wound closure. As the broad phytotherapeutic effects of *Aloe vera* extract are detailed, experimental studies will provide us with more precise results on dosage management and the level of response of the organism over time. In addition, future studies will further develop existing *Aloe vera* commercial products and provide different formulations that would allow the development of effective, practical, and cost-effective agents that can be used in human and animal health.

Authors contribution: ITC was involved in all stages of this study, from conceptualization to writing and editing. NYGS conceived and designed the project/study, investigation, methodology, validation, and visualization. HC was responsible for conducting the experiment. ITC, AA, and OY performed histopathological analysis and interpreted results. SG performed the immunohistochemical analysis. AT, STS, EU, and EK were involved in the investigation and visualization. All authors have approved the final manuscript.

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