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*Research article*

# EFFECTS OF TOPICAL *ALOE VERA* ON EPITHELIAL HEALING AND CORNEAL HAZE AFTER ALKALI BURN IN RABBITS: A COMPARISON WITH AUTOLOGOUS SERUM

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This randomized controlled study evaluated whether topical *Aloe vera* (AV) eye drops improve corneal epithelial healing and corneal haze after alkali burn in New Zealand White rabbits, and whether their effects are comparable to 50% autologous serum (AS). Sixteen rabbits (32 eyes; 8 males and 8 females; 36.2 ± 2.8 weeks old) received bilateral central corneal alkali burns induced with 1 N NaOH and were assigned to balanced salt solution (BSS; 12 eyes), AV (10 eyes), or 50% AS (10 eyes), administered four times daily for 4 weeks. Residual epithelial defect area was measured from fluorescein-stained slit-lamp images, and corneal haze was graded using the Fantes scale. AV and AS reduced residual epithelial defect area more rapidly than BSS during days 1 and 2, with estimated mean differences of −15.6 and −9.8 percentage points, respectively. All corneas re-epithelialized by day 7, and no significant difference was observed between AV and AS. Corneal haze did not show sustained treatment-related differences. These findings suggest that topical AV may support early epithelial recovery after acute alkali injury, with effects comparable to 50% AS, although its effect on corneal haze appears limited.

**Keywords:** alkali burn, *Aloe vera*, autologous serum, corneal wound healing, rabbit model

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## INTRODUCTION

*Aloe vera* (AV) is a perennial succulent plant belonging to the family Asphodelaceae (formerly Aloaceae) and has long been used for medicinal and cosmetic purposes [1]. Its therapeutic applications are well-documented across multiple fields [2,3]. AV exhibits diverse pharmacological effects, including wound-healing, anti-inflammatory, antimicrobial, and antioxidant properties, and has demonstrated efficacy in burn-related tissue injury [4-6]. However, the reported effects of AV on corneal wound healing have been inconsistent across studies. Recent studies have indicated that AV may promote corneal re-epithelialization and attenuate inflammation after alkali burns in rats [7], whereas other studies in rabbits have demonstrated no significant improvement in corneal wound healing with AV treatment [8]. Consequently, the efficacy of AV in ocular surface injury remains uncertain.

Autologous serum (AS) has been shown to be beneficial in the treatment of various ocular surface disorders, including keratitis, ulcerative corneal disease, persistent corneal epithelial defects (PEDs), superficial chronic corneal epithelial defects (SCCEDs), and certain forms of keratoconjunctivitis [9-11]. AS is widely used in clinical practice, and serum eye drops have been shown to remain stable under appropriate storage conditions [12]. In veterinary medicine, serum eye drops are routinely prepared from autologous or heterologous sources depending on clinical circumstance, and heterologous options can improve accessibility and support broader clinical use [13,14]. These preparations are usually obtained using standard blood collection and processing procedures, so they are generally practical for routine clinical use.

Robust injury models are essential when evaluating corneal repair therapies such as AV and AS, because mild models may heal too quickly and can hide small treatment effects. A more severe model can also provide a clearer pathological setting in which inflammation and stromal responses are present, allowing potential therapeutic benefits to be assessed more reliably. Corneal alkali burn in rabbits is a well-established model for the study of severe corneal wound healing [15-21]. Alkali injury induces acute corneal damage characterized by recurrent epithelial sloughing, stromal cell death, intense inflammatory cell infiltration, and endothelial dysfunction [22-24]. Such injury also triggers the release of proteolytic enzymes from the damaged cornea, leading to ulceration and occasionally keratomalacia [25-27]. These features make this model suitable for evaluating therapies aimed at promoting corneal regeneration while limiting fibrosis and scarring.

Despite the potential therapeutic benefits of AV, few studies have directly compared its efficacy with established therapies such as autologous serum (AS) in corneal wound healing. A recent study in dogs exhibiting spontaneous chronic corneal epithelial defects compared adjunctive treatment with AS versus an AV-based ophthalmic solution (Vizoovet®, containing propolis, AV, and chamomile) after corneal debridement. Both treatments improved healing relative to controls; however, no statistically significant difference in healing time was reported between the AS and Vizoovet groups [10].

To our knowledge, no study has directly compared pure AV and AS in a controlled experimental corneal injury model. In the present study, AS was selected as a comparator because of its long-standing clinical use in severe ocular surface disorders, including persistent epithelial defects, chemical burns, and other severe ocular surface diseases [28,11]. Previous studies have demonstrated that AS promotes corneal epithelial healing; however, its effects on stromal fibrosis and corneal haze remain less clearly defined [29,30]. Although Aloe vera is widely available, preparation of a sterile ophthalmic formulation requires purification and filtration steps, and standardized commercial ophthalmic products are not yet widely available. Establishing evidence for its efficacy in a controlled model may therefore support future development of standardized formulations. The aim of this study was to determine whether AV improves corneal epithelial healing and achieves outcomes comparable to AS, as well as to evaluate its effects on corneal haze following alkali burn.

## MATERIAL AND METHODS

### Corneal alkali burn model

This study was approved by the Institutional Animal Care and Use Committee of Chonbuk National University (CBU 2013-0037).

A total of 16 New Zealand White rabbits (8 males, 8 females; 2.6 ± 0.3 kg; 36.2 ± 2.8 weeks of age) were used. On the day of the procedure, general anesthesia was induced with tiletamine–zolazepam (Zoletil 50; Virbac, France) and xylazine (Rompun; Bayer, Germany). Topical anesthesia was administered with a 0.5% proparacaine hydrochloride solution (Alcaine; Alcon, USA) immediately before the corneal procedure.

Corneal alkali burns were induced by applying a 6-mm-diameter filter paper soaked in 1 N NaOH (pH 13.6) to the central cornea of each eye for 30 s; the average corneal diameter of New Zealand White rabbits is approximately 13–15 mm [31]. The eyes were then immediately irrigated with 10 mL of sterile 0.9% saline solution. Animals were randomly assigned to three groups: balanced salt solution (BSS; Alcon Research, LLC, USA; 6 rabbits, 12 eyes), AV (5 rabbits, 10 eyes), and AS (5 rabbits, 10 eyes). Alkali burns were induced bilaterally in all animals. The BSS group included a larger number of eyes because it served as a shared control for both comparisons. The sex distribution was comparable among groups (BSS: 3 males/3 females; AV: 2 males/3 females; AS: 3 males/2 females).

All eyes received the assigned treatment or control on the same schedule and at the same volume (four times daily; approximately 35–40 μL per instillation) for 4 weeks, beginning immediately after irrigation on day 0. All instillations were performed by personnel not involved in outcome assessment. Slit-lamp imaging was performed and corneal haze was graded by masked evaluators using the Fantes corneal haze grading scale (0–4) [32]. Eyes showing clinical evidence of infection or severe complications (e.g., purulent discharge or hypopyon) were excluded from analysis.

After the procedure, systemic analgesia was provided for 3 consecutive days with meloxicam (0.2 mg/kg, subcutaneously; Hana Pharm, South Korea) administered once daily and buprenorphine (0.005 mg/kg, intramuscularly; Myungmoon Pharm, South Korea) administered twice daily. Meloxicam was continued once daily for an additional 11 days during the recovery period. Sucralfate (50 mg/kg, orally; Daewoong Pharm, South Korea) was co-administered during meloxicam treatment to minimize gastrointestinal adverse effects. For descriptive histopathologic evaluation of representative healing patterns, one rabbit from each treatment group was euthanized at predefined time points (3 days, 2 weeks, and 4 weeks) following injury. At the end of the experimental period, animals were euthanized under deep general anesthesia induced by an additional intravenous dose of Zoletil 50 and Rompun, followed by intravenous administration of potassium chloride (35 mg/kg). All procedures complied with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and euthanasia was performed in accordance with the AVMA Guidelines for the Euthanasia of Animals.

## Preparation of AV ophthalmic solution

Fresh AV leaves were harvested and processed using a standardized hand-filleting method based on a previously patented ophthalmic formulation protocol (US Patent No. 6013259A) [33]. After surface disinfection with a 200 ppm chlorinated solution and rinsing in sterile distilled water, the leaves were air-dried. The outer green rind was carefully removed, and the inner mucilaginous gel was collected.

To stabilize bioactive components and inhibit microbial growth, the gel was treated with 0.1% citric acid and 0.2% potassium sorbate and then homogenized using a sterile high-speed blender (MX2000; Braun, Germany). The homogenate was immediately frozen at −18 °C. Upon thawing, it was centrifuged at 10,000 × g for 15 minutes, and the supernatant was passed through a 0.2-μm membrane filter (Millex; Millipore, USA) under aseptic conditions to remove particulate material and reduce microbial contamination. This process yielded an aloin-free, pulp-free AV extract concentrated at a 40:1 ratio relative to the starting fresh Aloe vera gel.

For ophthalmic application, the concentrate was adjusted to pH 7.1. Accordingly, the final applied solution was a 30% dilution of the 40:1 extract, corresponding to approximately 12 g of raw Aloe vera gel per milliliter. The physiological tear pH range in rabbits is approximately 7.3–7.8, and ophthalmic formulations are typically adjusted near this range to maintain ocular compatibility [34]. The selected dilution falls within the range reported for ophthalmic formulations in previous patents (6–30%) [33]. The final preparation was stored at 4 °C and used within 7 days to minimize degradation of bioactive components and potential microbial growth. The pH of autologous serum was not directly measured in this study. Reported pH values of rabbit serum are approximately 7.4 [35], and BSS has a physiological pH of approximately 7.2–7.4 according to the manufacturer.

### Preparation of AS

AS samples were prepared according to a standardized protocol. Whole blood (5 mL) was collected once from each rabbit at baseline without anticoagulants and allowed to clot for 2 hours at room temperature. Serum was obtained by centrifugation at 4,000 × g for 10 minutes, diluted to 50% with BSS, and divided into sterile amber vials, each containing approximately one week's supply, to prevent degradation of light-sensitive components. The prepared serum was stored frozen and used throughout the study. For administration, individual vials were thawed as needed and stored at 4 °C for up to 7 days [36,12]. Each vial was discarded after 7 days, and a new vial was used for subsequent treatment periods.

### Evaluation of epithelial defect healing

Fluorescein staining was used to assess corneal epithelial defect areas. A sterile fluorescein sodium ophthalmic strip (Fluorescein Sodium Ophthalmic Strips U.S.P.; Madhu Instruments Pvt. Ltd., India) moistened with sterile balanced salt solution was applied to the ocular surface, and photographs were obtained using a slit-lamp biomicroscope (Topcon SL-8Z, Topcon Corporation, Japan) with a cobalt blue filter. All images were captured under standardized conditions using a fixed magnification (10×), identical illumination settings at maximum intensity, consistent camera parameters, and a constant working distance of 10 cm from the corneal surface to ensure comparable imaging conditions [37]. Epithelial defects were evaluated at baseline (day 0), day 1, day 2, day 3, and week 1 post-injury. Images were calibrated in ImageJ software (National Institutes of Health, USA) using a predefined pixel-to-millimeter conversion factor derived from a reference image acquired under identical settings to ensure consistent area quantification.

### Corneal haze grading

Corneal haze was graded from standardized slit-lamp photographs obtained on days 1 and 3, and weekly thereafter for up to 4 weeks, under identical illumination and 10 × magnification. Haze was assessed using the Fantes 0–4 ordinal scale [32] (0, clear cornea; 0.5, trace haze visible only with broad tangential illumination; 1, mild; 2, moderate with partial loss of iris details; 3, substantial with complete loss of iris details; 4, severe with a completely opaque cornea and no view of anterior chamber structures). No standardized toxicological scoring systems (e.g., Draize or SPOTS tests) were applied in this study, and ocular comfort or irritation was not formally assessed.

### Histopathologic analysis

Corneal tissues were harvested at 3 days (n = 3 rabbits; 1 per treatment group), 2 weeks (n = 3 rabbits; 1 per treatment group), and 4 weeks (n = 3 rabbits; 1 per treatment

group) post-injury. Samples were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). Sections were digitally imaged and independently evaluated by a veterinary pathologist. For descriptive presentation, H&E-stained sections encompassing the central alkali-injured cornea were reviewed, and sections with adequate tissue orientation and minimal processing artifacts were selected by the authors in consultation with the veterinary pathologist. Selection was based on histologic quality and anatomical representation rather than the severity of the observed lesions. Histopathologic evaluation was primarily descriptive and focused on representative morphologic features within the central alkali-injured cornea, including epithelial continuity and stratification, stromal inflammatory cell infiltration, preservation of resident stromal keratocyte nuclei, stromal lamellar organization, and vascular profiles within the corneal stroma. For semi-quantitative histopathologic assessment, five non-overlapping high-power fields (200×) were evaluated in the central cornea of each animal. In this study, the term "keratocyte" refers to resident stromal cells in the quiescent cornea, whereas "fibroblast" denotes activated stromal cells involved in stromal repair and remodelling. Inflammatory cell infiltration and fibroblast proliferation were recorded semiquantitatively as absent (−), mild (+), moderate (++), or marked (+++). For descriptive presentation, inflammatory cell counts per 200× field were categorized as follows: −, 0–10 cells; +, 11–50 cells; ++, 51–100 cells; +++, >100 cells. Fibroblast counts per 200× field were categorized as follows: −, 0–20 cells; +, 21–50 cells; ++, 51–80 cells; +++, >80 cells. Because histologic evaluation was based on a limited number of samples, these data are presented descriptively without statistical comparison.

### Statistical analysis

To account for within-animal correlation between eyes, values from both eyes were combined at the animal level to generate a single representative value per rabbit at each time point. The unit of analysis was the individual animal. Residual epithelial defect area was analyzed as a continuous variable using linear mixed-effects models with treatment group and time as fixed effects and animal as a random intercept. Time-specific contrasts were used to estimate between-group differences at each time point, and effect estimates with 95% confidence intervals were reported. Corneal haze scores were analyzed as ordinal data and summarized as median (interquartile range). As the primary analysis, a cumulative link mixed model (CLMM) was used to evaluate treatment group effects on ordinal haze scores across time points, with treatment group and time as fixed effects and animal as a random intercept. This approach accounts for the ordinal nature of the outcome, within-animal correlation, and the reduction in sample size at later time points due to scheduled euthanasia. As supplementary analyses, group comparisons at each time point were performed using the Kruskal–Wallis test, followed by pairwise Mann–Whitney U tests with Holm correction when appropriate. Values of $P < .05$ were considered statistically significant.

## RESULTS

A total of 16 rabbits (32 eyes) were included: BSS (6 rabbits, 12 eyes), AV (5 rabbits, 10 eyes), and AS (5 rabbits, 10 eyes). One rabbit per group was euthanized at predefined time points (3 days, 2 weeks, and 4 weeks) for histopathologic evaluation, resulting in a reduced number of animals available for analysis at later time points. In addition, one female rabbit in the BSS group was euthanized at week 3 due to worsening corneal haze accompanied by severe blepharospasm and epiphora, suggestive of secondary symblepharon formation; data from this animal were included through week 2 and excluded thereafter. Consequently, analyses at week 4 were based on 3 rabbits (6 eyes) per treatment group prior to euthanasia for histopathologic evaluation.

### Effects on epithelial defect recovery

Epithelial defect area decreased more rapidly in the AV and AS groups than in the BSS control group during the early phase of healing (Figure 1). In the mixed-effects model, treatment with AV was associated with a greater reduction in residual epithelial defect area during days 1 and 2 compared with BSS (estimated mean difference, −15.6 percentage points; 95% CI, −23.6 to −7.5; P = .002). Similarly, AS treatment was associated with a greater reduction during days 1 and 2 relative to BSS (−9.8 percentage points; 95% CI, −17.9 to −1.8; P = .032). No statistically significant difference was observed between the AV and AS groups at any time point (−5.7 percentage points; 95% CI, −14.1 to 2.6; P = .2). Representative fluorescein-stained slit-lamp images illustrating the temporal progression of epithelial defect healing in each group are shown (Figure 2). Corresponding absolute values and temporal changes are summarized (Table 1). These findings were consistent with per-animal analyses accounting for bilateral treatment. However, because epithelial defects were not assessed between days 3 and 7, the exact time to complete epithelial closure could not be determined. Complete re-epithelialization was observed in all groups by day 7.

### Effects on corneal haze

Corneal haze scores decreased in all groups through week 1 (Figures 3 and 4). In the cumulative link mixed model, a statistically significant difference between the AV and BSS groups was observed (coefficient = −3.42; SE = 1.05; z = −3.26; P = .001); however, this did not correspond to consistent or sustained differences in time-specific analyses, likely reflecting limited statistical power at individual time points rather than a true sustained treatment effect. No significant difference was observed between the AS and BSS groups (coefficient = 0.12; SE = 0.93; z = 0.13; P = .90). At week 1, median haze scores were numerically lower in the AV group than in the BSS group, while the AS group showed values comparable to BSS. In supplementary time-specific analyses, a significant overall difference among groups was observed at week 2 (Kruskal–Wallis, P = .023); however, pairwise comparisons with Holm correction

did not reach statistical significance (AV vs BSS, P = .091). From week 2 onward, haze scores increased across all groups, and no statistically significant differences were detected between groups (all P > .05). These results should be interpreted with caution given the small sample sizes at later time points.

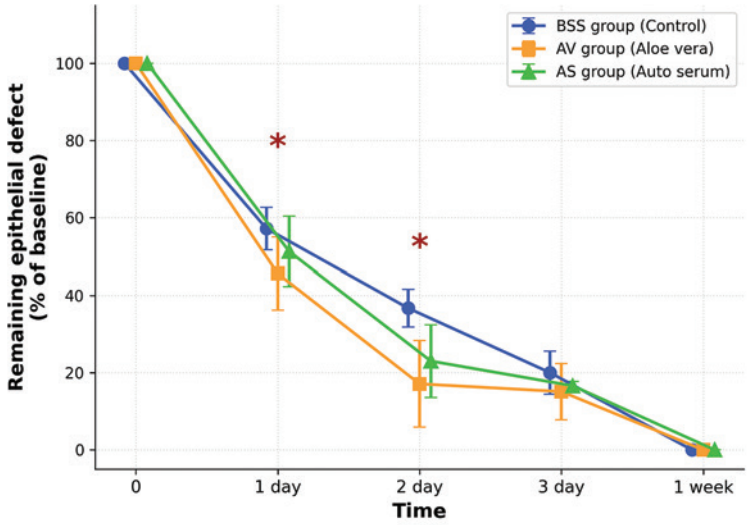



**Figure 1.** Time course of corneal epithelial wound healing after alkali burn injury. Residual epithelial defect area decreased more rapidly in the AV and AS groups than in the BSS control group during the early phase of healing (days 1 and 2). Complete re-epithelialization was observed in all groups by day 7. Data are presented as mean ± SD. Asterisks indicate statistically significant differences compared with the BSS group.

**Table 1.** Residual corneal epithelial defect area (% of baseline) after alkali burn

| Time | BSS group | AV group | AS group |
|---|---|---|---|
| **Baseline (Day 0)** | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 |
| **Day 1** | 57.2 ± 5.5$^{a}$ | 45.6 ± 9.4$^{b}$ | 51.3 ± 9.1$^{b}$ |
| **Day 2** | 36.7 ± 4.9$^{a}$ | 17.1 ± 11.2$^{b}$ | 23.0 ± 9.4$^{b}$ |
| **Day 3** | 20.0 ± 5.6 | 15.1 ± 7.3 | 16.6 ± 1.1 |
| **Week 1** | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |

Values are presented as mean ± SD (% of baseline area; day 0 = 100%). AS = autologous serum; AV = Aloe vera; BSS = balanced salt solution. Different superscript letters indicate significant differences between groups.

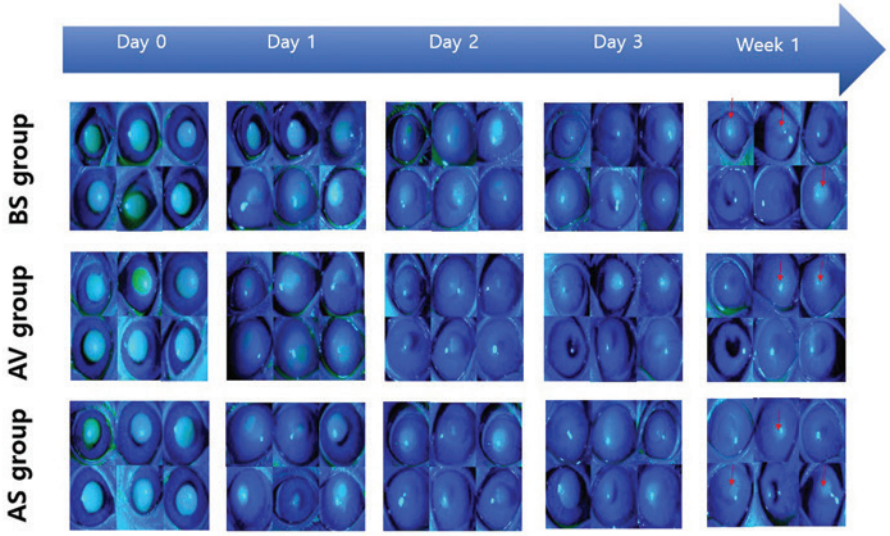



Figure 2. Representative fluorescein-stained slit-lamp photographs of corneal epithelial defects after alkali burn injury. Images are shown for the BSS, AV, and AS groups. Within each treatment group, the same eye is presented in the same position across the time series to illustrate the progression of epithelial healing. Fluorescein staining highlights epithelial defects during the early healing phase. Complete re-epithelialization was confirmed in all eyes by day 7. Residual axial stromal opacity observed in some eyes at week 1 (red arrows) represents stromal haze associated with early fibrotic remodelling rather than persistent epithelial ulceration.

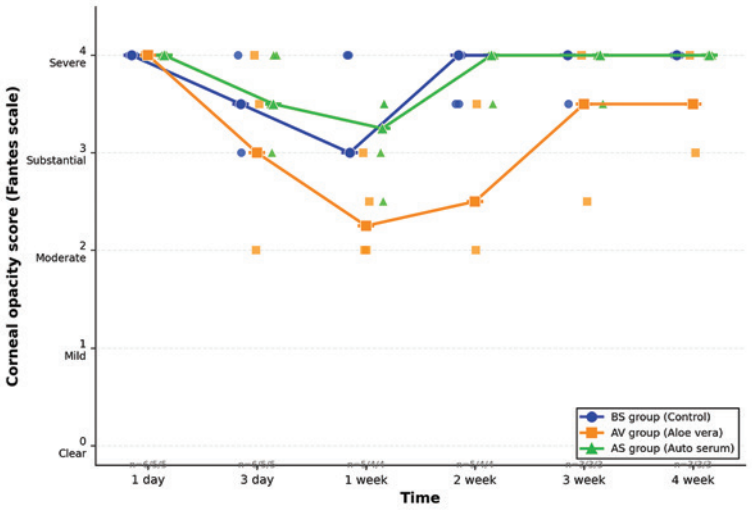



**Figure 3.** Individual corneal haze scores after alkali burn injury. Corneal haze was graded using the Fantes 0–4 ordinal scale from standardized slit-lamp photographs obtained at 1 day, 3 days, and weekly from 1 to 4 weeks after injury. Each data point represents the mean score of both eyes for an individual rabbit. Horizontal bars indicate median values for each group. The number of animals evaluated at each time point is shown below the x-axis (BSS/AV/AS)

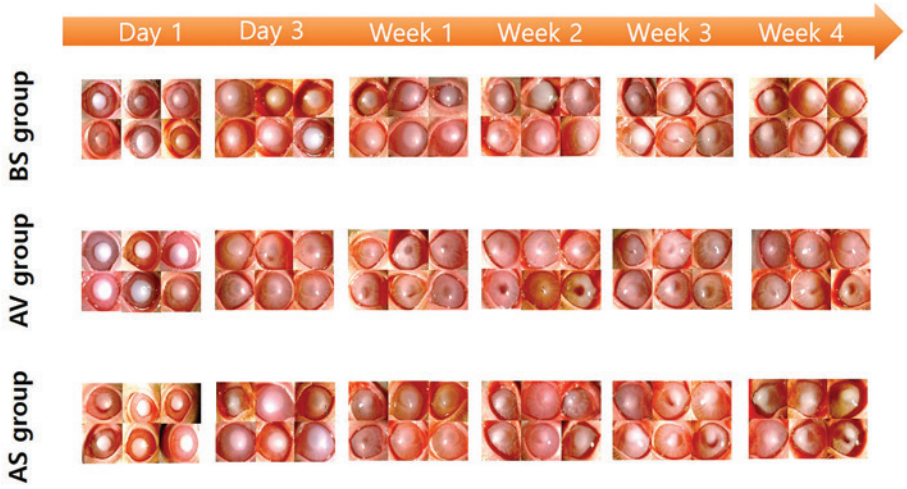
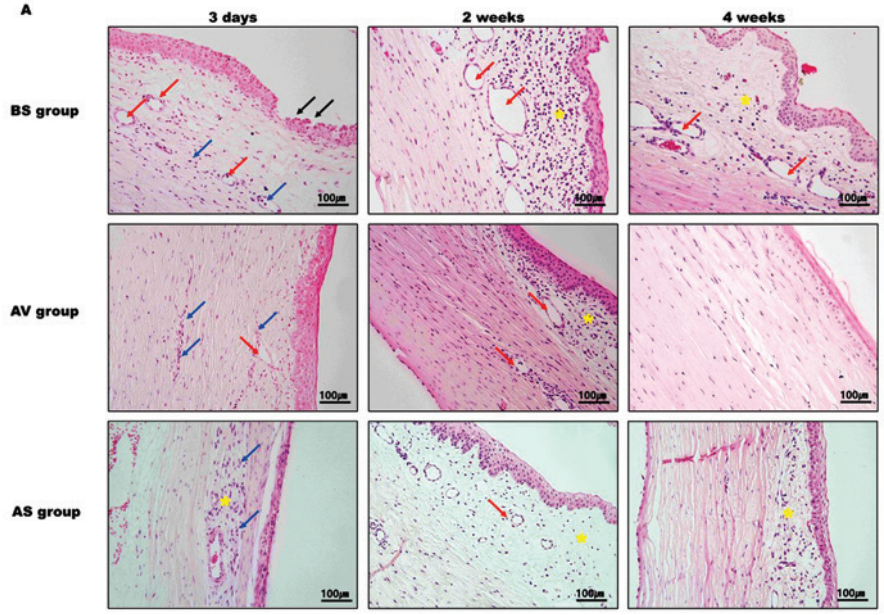



**Figure 4.** Representative slit-lamp photographs illustrating corneal haze over 4 weeks following alkali burn injury. Representative images illustrate for the BSS, AV, and AS groups at each time point. Corneal haze decreased through week 1 and increased thereafter in all groups. At later time points, substantial haze was observed across all groups, consistent with the absence of sustained between-group differences in the statistical analyses

### Stromal inflammatory response and fibroblastic remodelling

Histopathologic evaluation of representative H&E-stained corneal sections collected at sequential time points after alkali injury identified alterations in epithelial integrity, stromal cellular infiltration, stromal lamellar arrangement, and vascularisation. Because histologic assessment was performed on one rabbit per group at each time point, these observations are descriptive and should be interpreted qualitatively.

At 3 days post-injury, the corneal section from one rabbit in the BSS group showed marked attenuation of the corneal epithelium with segmental partial-thickness epithelial loss. The underlying stroma exhibited edema and dense heterophil-predominant inflammatory cell infiltration extending into the anterior stroma. Separation and disruption of stromal lamellae were also observed. The corneal section from one rabbit in the AV group showed mild epithelial attenuation with focal surface irregularity. Inflammatory cells were present within the anterior stroma, accompanied by mild stromal edema and focal alteration of lamellar organization. The corneal section from one rabbit in the AS group showed epithelial attenuation and surface alteration, with stromal inflammatory cell infiltration, edema, and focal stromal architectural disruption (Figure 5).




B

Semiquantitative inflammatory cell infiltration scores

| Time point | BS | AV | AS |
|---|---|---|---|
| 3 days | ++ | + | + |
| 2 weeks | +++ | + | ++ |
| 4 weeks | ++ | - | + |

C

Semiquantitative fibroblast scores

| Time point | BS | AV | AS |
|---|---|---|---|
| 3 days | ++ | + | + |
| 2 weeks | +++ | ++ | ++ |
| 4 weeks | + | + | + |

**Figure 5.** Histopathologic assessment of corneal sections after alkali injury. (A) Representative H&E-stained corneal sections from the BSS, AV, and AS groups at 3 days, 2 weeks, and 4 weeks. Blue arrows indicate heterophil infiltration, red arrows indicate neovascularisation, black arrows indicate epithelial loss, and asterisks indicate substantial granulation tissue formation; scale bar = 100 μm. (B) Inflammatory cell counts per 200× field in corneal sections from the BSS, AV, and AS groups. (C) Fibroblast counts per 200× field in corneal sections from the BSS, AV, and AS groups

## DISCUSSION

In this rabbit alkali-burn model, both topical AV and 50% AS promoted earlier epithelial defect reduction than BSS during the first two days, with no significant difference between the two active treatments, and complete re-epithelialization was achieved in all groups by day 7. These findings suggest that AV may support early epithelial recovery through mechanisms broadly comparable to those of AS, potentially involving modulation of inflammatory and stromal remodelling processes rather than solely accelerating epithelial closure. Histopathologic observations from representative sections were consistent with this interpretation, although their descriptive nature precludes definitive conclusions.

Previous studies have reported beneficial effects of AV on corneal healing. Atiba et al. demonstrated that topical AV accelerated epithelial regeneration and reduced

inflammatory infiltration in alkali-burned corneas [7]. Similarly, Rezaei Moghadam et al. reported faster wound closure and preservation of stromal keratocytes in rabbits treated with an AV-based ophthalmic formulation [38]. In contrast, some studies have reported limited or inconsistent effects of AV on corneal wound healing [8,39]. These discrepancies likely reflect variations in experimental conditions, including the type and severity of corneal injury, formulation and concentration of AV, dosing schedule, and methodological differences between in vitro and in vivo models. For example, superficial abrasion models primarily involve epithelial injury and often undergo rapid spontaneous healing within approximately 48 hours, whereas alkali-burn models produce more extensive stromal and inflammatory damage. Such differences in the biological response to injury may influence the extent to which the therapeutic effects of AV can be detected and may partly explain the variability among studies.

Species-related differences in corneal wound healing should also be considered when interpreting these results. Rabbit corneas show robust epithelial repair and stromal remodelling following injury, with substantial capacity for spontaneous epithelial and stromal self-recovery after alkali burns [40]. Differences across animal models have been described, including variations in inflammation, neovascularisation, epithelial thickness, and goblet cell formation between rabbit and rodent models [41]. In clinical veterinary patients, corneal wound healing responses may be more variable and influenced by factors such as injury type, underlying disease, and breed-related characteristics. Accordingly, the magnitude and temporal pattern of treatment effects observed in this model may differ from those in clinical veterinary patients.

The concentration of AS used in this study (50%) was selected for practical reasons related to sample handling and study design, including the need to obtain sufficient volume from a single collection and to maintain suitable viscosity for topical administration. Although both diluted and undiluted serum preparations have been reported in previous studies [10,42,43], the use of diluted AS is not consistently adopted in clinical practice, and its relative efficacy compared with higher concentrations remains incompletely defined. Therefore, the use of 50% AS in this study represents a methodological choice rather than an evidence-based optimal concentration, and this should be considered when interpreting comparisons with other treatments.

The present study has several limitations. Histopathologic analysis was descriptive and not based on quantitative morphometric evaluation. Corneal thickness was not measured histologically because the available sections were prepared for descriptive pathology rather than standardized morphometric analysis. Stromal tissue loss was not a predefined endpoint, and section orientation was not standardized to evaluate epithelial–stromal angles; therefore, subtle stromal loss cannot be excluded. Untreated normal-control corneas and immediate post-injury histologic samples at time 0 were not included, and stromal cellularity at later time points was therefore interpreted only relative to post-injury sections examined within this study rather than to a true histologic baseline. In addition, the intrinsic healing capacity of the rabbit cornea may have reduced the apparent magnitude of treatment-related differences. Finally, the AV

formulation used in this study was prepared from a 40:1 concentrated extract, and the reported 30% concentration refers to dilution of this concentrate rather than raw plant material; because direct dose–response comparisons were not performed, the optimal therapeutic concentration of AV for corneal wound healing remains to be determined.

## CONCLUSION

In this rabbit alkali-burn model, topical AV and 50% AS resulted in broadly similar epithelial healing outcomes, and both treatments showed an earlier reduction in epithelial defect area than BSS. However, this early advantage did not appear to persist over time, since corneal haze did not differ significantly between groups and no clear sustained treatment effect was observed during the follow-up period. Overall, these findings suggest that AV may be a practical adjunctive or alternative option for acute alkali corneal injury, particularly when AS is not available in a clinical setting or cannot be prepared promptly. Further studies incorporating quantitative histologic analysis, more standardized outcome measures, and clinical evaluation in companion animals are needed to clarify the therapeutic mechanisms, optimise treatment protocols, and confirm the clinical applicability of AV.

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**Authors′ contributions**

JB, MSK and SJ contributed to conceptualization. JB, JK and DBJ contributed to methodology. JB, JK and MSK performed the investigation. JB and MSK curated the data. JB, DBJ and SMB contributed to formal analysis. JB and SMB wrote the original draft. SJ and MSK reviewed and edited the manuscript. JB prepared the visual materials. SJ supervised the study. All authors read and approved the final manuscript.

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**Declaration of competing interests**

No author has any direct or indirect financial interest, intellectual property rights, or commercial involvement related to the Aloe vera formulation or the referenced patent (US Patent No. 6013259A). The authors have nothing to disclose. No generative AI tools were used in the scientific writing, data analysis, or interpretation of this manuscript. An AI-assisted tool was used in a limited manner during the final stage of reference formatting to standardize citation style. All references were subsequently manually verified by the authors.

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## EFEKTI LOKALNE PRIMENE *ALOE VERA* NA ZACELJIVANJE EPITELA I ZAMUĆENJE ROŽNJAČE NAKON OPEKOTINA ALKALIJAMA KOD ZEČEVA: POREĐENJE SA AUTOLOGNIM SERUMOM

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Ova randomizovana kontrolisana studija je imala za cilj procenu da li lokalne kapi za oči sa *Aloe vera* (AV) poboljšavaju zarastanje epitela rožnjače i zamućenje rožnjače nakon opekotina alkalijama kod novozelandskih belih zečeva i da li su njihovi efekti

uporedivi sa 50% autolognim serumom (AS). Šesnaest zečeva (32 oka; 8 mužjaka i 8 ženki; starosti 36,2 ± 2,8 nedelja) podvrgnuto je bilateralnim centralnim opekotinama rožnjače alkalijama izazvane sa 1 N NaOH. Raspoređeni su u grupu sa uravnoteženim rastvorom soli (BSS; 12 očiju), AV (10 očiju) ili 50% AS (10 očiju), primenjene četiri puta dnevno tokom 4 nedelje. Površina rezidualnog epitelnog defekta merena je na osnovu slika dobijenih slit lampom, obojeni fluoresceinom, a zamućenje rožnjače je ocenjeno pomoću Fantesove skale. AV i AS su smanjili površinu rezidualnog epitelnog defekta brže od BSS tokom 1. i 2. dana, sa procenjenim srednjim razlikama od -15,6 odnosno -9,8 procentnih poena. Sve rožnjače su se reepitelizovale do 7. dana, i nije primećena značajna razlika između AV i AS. Zamućenje rožnjače nije pokazalo trajne razlike povezane sa tretmanom. Ovi nalazi ukazuju na to da lokalno AV može podržati rani oporavak epitela nakon akutne alkalne povrede, sa efektima uporedivim sa 50% AS, iako je njen efekat na zamućenje rožnjače ograničen.