

# The effects of topical aloe vera on an experimentally designed penile fracture model in rats

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## ABSTRACT

**BACKGROUND:** This study assessed the histopathological and oxidative effects of topical Aloe Vera (AV) on penile fractures (PF) formed experimentally in a rat model.

**METHODS:** Forty Wistar albino rats (220-250 g) were used. The PF model was created experimentally with a number 15 lancet. Then, the rats were randomly and equally divided into five groups. In the first group (C), no incision was formed. In the second group (P), an incision was formed. In the third group (PR), the incision line was closed primarily. In the fourth group (PA), AV was locally applied onto the incision without suturing for three days. In the last group (PRA), AV was applied to the primary repair region for three days. All groups were compared to each other according to histopathological and biochemical data.

**RESULTS:** Hyperemia-bleeding was observed to be suppressed in the PRA group compared to the other groups ( $p<0.001$ ). Inflammation was observed only in Groups PR and PRA ( $p<0.001$ ). Significant fibrosis was observed in the PA and PRA groups compared to the other groups ( $p<0.001$ ). Superoxide Dismutase (SOD) and Glutathione (GSH) values increased in favor of Group PRA ( $p=0.009$  and  $p=0.035$ , respectively). Total Oxidative Status (TOS) and Malondialdehyde (MDA) values decreased in favor of Group PA ( $p=0.036$  and  $p=0.026$ , respectively). Tumor Necrosis Factor-alpha (TNF- $\alpha$ ) and Interleukin-1 beta (IL-1 $\beta$ ) levels decreased mostly in the PRA group, but these changes did not reach statistical significance ( $p>0.05$ ).

**CONCLUSION:** Topical AV application reduces tissue inflammation and oxidative stress but appears to increase the development of fibrosis after PF.

**Keywords:** Aloe vera; penile fracture; rat model.

## INTRODUCTION

Penile fracture (PF) is a rare urological emergency defined as a traumatic rupture of the corpus cavernosum following high-pressure trauma to the erect penis. Masturbation and sexual intercourse are the most commonly reported causes in Western countries.<sup>[1]</sup> Since its first description, PF has been managed conservatively, without surgery.<sup>[2]</sup> However,

it has been observed that nearly one-third of patients with PF treated conservatively experienced penile deformities and erectile dysfunction. Surgical repair, as the primary treatment modality for PF, was first introduced in 1971. Today, immediate surgical repair is recommended due to its excellent long-term results and significantly better prognosis for long-term sexual health.<sup>[3-5]</sup>

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Increased inflammation has been reported in PF in rats.<sup>[6,7]</sup> The inflammatory response involves perivascular infiltration of lymphocytes, monocytes, and neutrophils at the site of injury.<sup>[8]</sup> This is followed by the excessive generation of free radicals known as reactive oxygen species (ROS). ROS are scavenged by cellular antioxidant defense systems, which consist of enzymatic mechanisms such as superoxide dismutase (SOD), catalase, glutathione peroxidase, and glutathione reductase, as well as non-enzymatic mechanisms such as reduced glutathione (GSH), and vitamins A, C, and E.<sup>[9]</sup> Cytokines and growth factors mediate the healing process of injured tissue, which supports and enhances the primary cellular activities essential for healing.<sup>[10]</sup> DeClue and Shornick reported that diabetic wound healing was associated with the over-release of proinflammatory cytokines such as Interleukin-1 beta (IL-1 $\beta$ ), Interleukin-6 (IL-6), and Tumor Necrosis Factor-alpha (TNF- $\alpha$ ).<sup>[11]</sup>

Medicinal plants are used to accelerate wound healing, and Aloe Vera (AV) is among the most beneficial.<sup>[12]</sup> Traditionally, it has been used for a wide variety of biological activities, including wound healing, antifungal activity, anti-inflammatory activity, immunomodulatory effects, anti-cancer properties,<sup>[13]</sup> and antibacterial effects.<sup>[14,15]</sup> It is believed that the activities of AV are due to the synergistic action of many bioactive compounds present in the gel.<sup>[16]</sup> The gel is the inner layer of AV leaves, which contains amino acids, anthraquinones, carbohydrates, chromones, dietary fibers, enzymes, hormones, minerals, sterols, proteins, and organic compounds.<sup>[17]</sup> A vast majority of studies have illustrated the therapeutic activity of AV in the regeneration of tissues.<sup>[15]</sup> Acceleration of wound healing has been observed through both oral and local administration of AV gel.<sup>[18]</sup>

For this purpose, in this study, levels of some oxidative stress (OS) biomarkers such as malondialdehyde (MDA), total oxidant status (TOS), GSH, and SOD, as well as proinflammatory responses and histopathological evaluation, were performed in penile fracture and topical AV-treated groups.

## MATERIALS AND METHODS

The experimental study was carried out after obtaining approval from the Local Ethics Committee (Ethical approval number: 2020-32-03/12), and 40 Wistar albino rats (220-250 g) were enrolled. All surgical interventions were performed

under sterile conditions by the same team, at the same time, and in the same environment. The rats were randomly divided into five equal groups; Group C (healthy control), Group P (pathology control), Group PR (primary repair group), Group PA (P+AV), and Group PRA (PR+AV). The design of the groups is summarized in Table 1.

A single dose of ceftriaxone 20 mg/kg (Unisef 1 g, Mustafa Nevzat İlaç Sanayi A.Ş./Türkiye) was administered intramuscularly (i.m.) one hour before the surgical procedures. The rats were placed in a supine position after general anesthesia i.m. with ketamine 75 mg/kg (Ketalor 500 mg/10 ml, Pfizer, U.S.A.) and xylazine 7 mg/kg (XylazinBio 2%, Czech Republic). The genital area was shaved, and the penile skin was cleaned with 10% povidone-iodine. A 3F urethral catheter was inserted approximately 2 cm up to the mid-penile level from the external meatus. Experimental penile fracture was created with a number 15 lancet as previously described.<sup>[7]</sup> All procedures were performed with surgical loupes (2.5x). The rats were housed in individual cages after they had recovered from general anesthesia.

During the 3-week holding period, the animals were fed normally. After that time, the rats were placed in a supine position following general anesthesia administered i.m. with ketamine 75 mg/kg and xylazine 7 mg/kg. The whole penile tissue of all groups was harvested and cut into two pieces. The proximal part of the penile tissue was washed with ice-cold normal saline and placed in 25 mL of 10% formaldehyde for later histopathological examination. The distal part of the penile tissue was stored at -80°C for further biochemical analysis.

After the penile tissue was removed under bleeding control, a blood sample was drawn from the abdominal aorta. Approximately 5 mL of blood was collected, placed in a tube, allowed to clot, then centrifuged at 3,000 rpm for 20 minutes, and the separated serum was directly subjected to laboratory cytokine levels testing. At the end of the experiment, rats were euthanized by exsanguination from the abdominal aorta.

### AV Solution

In our study, commercially-prepared freeze-dried pure Aloe Vera *Barbadensis* Leaf Juice Powder (Aloe Vera Powder Organic 200:1 Concentration [bi-origins/MYSTIC MOMENTS UK/Madar Corporation Ltd.]) was used. By adding 0.5 g of AV powder to 99.5 g of pure water, we obtained a ready-to-use 1:1 AV gel solution in our study.

**Table 1.** Groups definitions

Group C	No incision was made. This group served as a healthy reference tissue.
Group P	The incision was left unrepaired to heal secondarily.
Group PR	The incision was repaired primarily using 6-0 polydioxanone.
Group PA	Aloe Vera (AV) was applied locally only to the incision area.
Group PRA	AV was applied to the incision area after primary repair.

## Biochemical Analysis

### MDA and GSH Levels

The malondialdehyde (MDA), a by-product of lipid peroxidation, was evaluated by a spectrophotometric method.<sup>[19]</sup> Briefly, using a mechanical homogenizer, penile tissue samples were homogenized in ice-cold 10% trichloroacetic acid (TCA) solution and then centrifuged. After centrifugation, the supernatants were added to an equal volume of thiobarbituric acid (0.67% TBA) solution. The mixture was boiled to 100°C for 15 minutes. The absorbance of the samples was measured spectrophotometrically at 535 nm. The reduced glutathione (GSH) levels of penile tissue were determined in the supernatant obtained using the same homogenization procedure as described above. Dithiobisnitrobenzoate and Na<sub>2</sub>HPO<sub>4</sub> solution were added to the supernatant samples. The mixture was vortexed, and the absorbance of the mixture was measured spectrophotometrically at 412 nm.<sup>[20]</sup>

### The Enzyme-Linked Immunosorbent Assay (ELISA) Analysis

#### SOD and TOS Levels

Enzyme-Linked Immunosorbent Assay (ELISA) was used to measure the levels of SOD and TOS in penile tissues (Sunred Biological Technology Co., Ltd, China). ELISA was performed in accordance with the manufacturer's recommendations. Briefly, penile tissues were homogenized in a Phosphate-Buffered Saline (PBS) buffer (pH 7.2–7.4) in a tissue homogenizer. After homogenization, the tissues were centrifuged at a speed of 2000–3000 rpm for 20 minutes. Then, the supernatants were carefully removed. The standards and supernatants were placed into wells, followed by adding antibody and streptavidin-Horseradish Peroxidase (HRP) to each well. The plate was incubated for 60 minutes at 37°C. Then, the plate was washed. Chromogen solutions were added to each well, and the plate was incubated again for 10 minutes at 37°C in the dark. After incubation, a stop solution was added. Finally, the absorbance was measured at a wavelength of 450 nm by a microplate reader (4300 Chromate Microplate Reader, Awareness Technology, Inc., FL, USA). The sample density was calculated using the Optical Density (OD) value and the standard curve.

#### Serum Cytokine Levels

Blood samples were collected from the aorta into a tube on the day of animal sacrifice. The blood samples were centrifuged, and the serum was obtained and stored at -80°C until the time of analysis. The plasma levels of TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 were measured using an ELISA kit according to the manufacturer's recommendations (BT-LAB ELISA Kit, Bioassay Technology Laboratory, Changsheng, China). Briefly, serum samples and standards were placed into the wells. Other steps performed are the same as the procedure mentioned above. Finally, absorbance was read at a wavelength of 450 nm by a microplate reader (4300 Chromate Microplate Reader, Awareness Technology, Inc., FL, USA). The concentration of

the serum samples was calculated using the standard curves.

### Histopathological Evaluation of Penile Tissue

Slices of penile tissues, including the fractured and healed areas, were made at a thickness of 1 mm and transported to the pathology lab in a 10% formaldehyde solution. Each slice was coded and embedded separately in paraffin wax after an overnight process. Four-micrometer-thick slices were cut from paraffin blocks and automatically stained with Masson's Trichrome and Hematoxylin and Eosin for histopathological evaluation and fibrosis evaluation under light microscopy using a Ventana Roche BenchMark Special Stains histochemistry instrument. An expert pathologist, who was unaware of the specimen's group, assessed the final pathology. Five criteria were used to evaluate the histochemical changes: fibrosis, inflammation, hyperemia, erythrocyte accumulation, and siderophage presence.

### Statistical Analysis

Statistical analysis was performed with SPSS 19.0. Continuous variables were expressed as median (min-max), categorical variables as frequency and percent. Kruskal-Wallis test was used to determine for differences between five groups. The Dunn test was used for post-hoc test after Kruskal-Wallis test. Categorical variables were compared using Pearson Chi-square test or Fisher Exact Chi-Square test. p value of less than 0.05 was considered statistically significant for all tests.

## RESULTS

In this study, one rat each from Group PR and Group PA died during the procedures. Complications such as allergic reactions, urinary retention, and infection were not observed.

Various amounts of inflammation were observed only in Groups PR and PRA. In the PRA group, while inflammation was observed in five rats, no inflammatory findings were observed in three rats. In the PR group, inflammation was observed in all rats. The inflammatory findings are presented in Figure 1a-d.

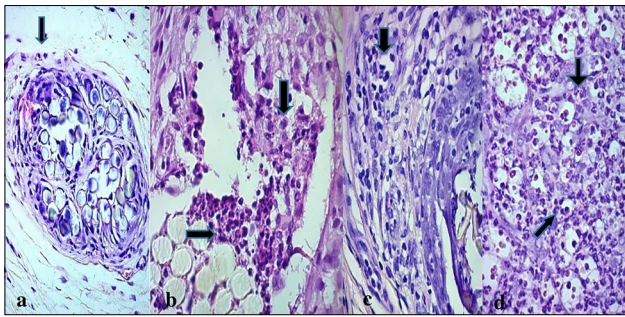
Fibrosis was observed only in the PA and PRA groups where Aloe Vera was applied. In Group PA, mild fibrosis was detected in two rats, while in one rat, severe fibrosis was seen. In the PRA group, all rats exhibited only moderate fibrosis. The fibrotic findings are presented in Figure 2a-e.

Severe hyperemia-bleeding was observed in 8 (100%) rats in Group P, 7 (100%) rats in Group PR, and 7 (100%) rats in Group PA. In group PRA, mild hyperemia-bleeding was observed in 5 (62.5%) rats, moderate in 2 (25%) rats, and severe in 1 (12.5%) rat. Hyperemia and bleeding findings are shown in Figure 3-d.

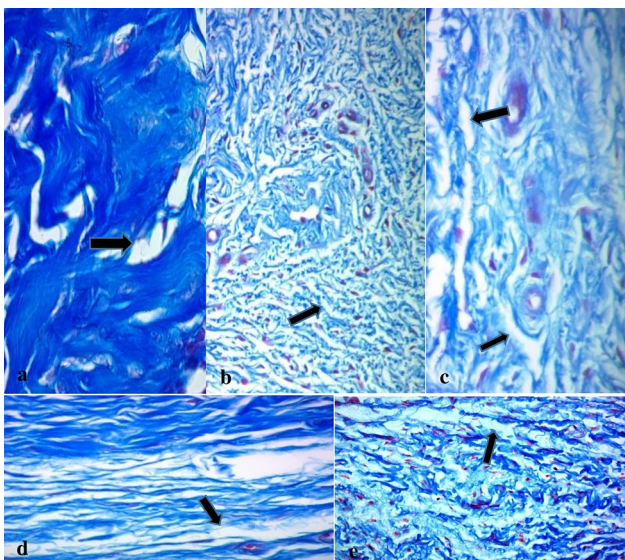
Severe hyperemia-bleeding was observed in the P, PR, and PA groups, although it was found to be suppressed in the PRA group (p<0.001).

When compared with the other groups, varying degrees of





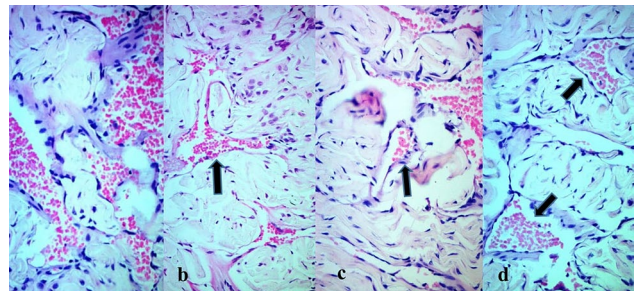
**Figure 1.** Evaluation of inflammation in cavernosal tissue: **(a)** Note the sparse and scattered inflammatory cells, with a count < 5 per high power field (HPF), marked with an arrow and graded as "mild". This image belongs to Group 3 (H&E, x200). **(b, c)** Observe that the inflammatory cells are not as severe as in Figure 1d, yet not as mild as in Figure 1a, representing moderate inflammation (H&E, x200). **(d)** This image shows the area around the suture material with severe inflammation composed of mixed-type inflammatory cells. Note that the inflammatory cells number more than 100 in one HPF (H&E, x400). These cells are indicated with an arrow. This image belongs to Group 5.



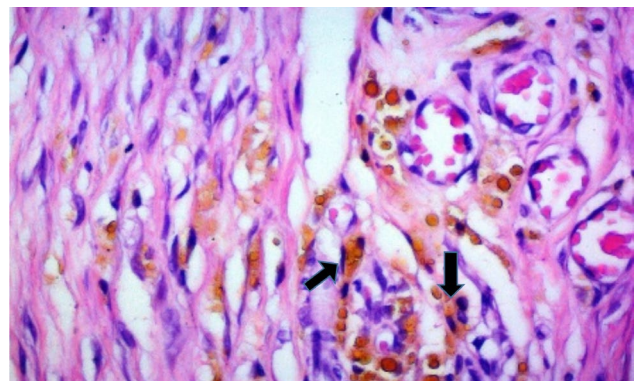
**Figure 2.** Grading of fibrosis with Masson's Trichrome Stain. The concentrated blue fibers represent normal collagen fibers of the tissue, whereas fibrosis is characterized by weak, thin, and pale fibers. **(a)** Observe the normal collagen tissue of the control group (x400). **(b, c)** No collagen fiber is present in the fibrosis of pathology and pathology plus suture in the cavernosal tissue (x200, x400). **(d, e)** In Groups 4 and 5, note fibrosis together with haphazard collagen fibers in the tunica albuginea (x400).

inflammation were observed only in Groups PR and PRA ( $p < 0.001$ ). The PRA group does not differ significantly from the PR group (Table 1). Significant fibrosis was observed in the PA and PRA groups compared to the other groups ( $p < 0.001$ ). However, there was no statistically significant difference between the PA and PRA groups ( $p = 0.077$ ).

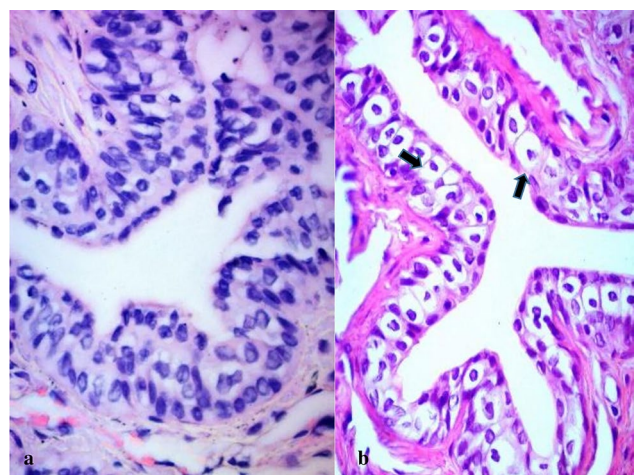
Siderophages were observed in two rats each in the P and



**Figure 3.** Evaluation of hyperemia and bleeding in cavernosal tissue: **(a)** As expected, the pathology group exhibits the most prominent congestion and erythrocyte accumulation around the collagen fibers. In contrast, all other therapeutic groups show minimal congestive alterations. **(b, c, d)** Bleeding and erythrocyte clusters are indicated with arrows (H&E, x200).

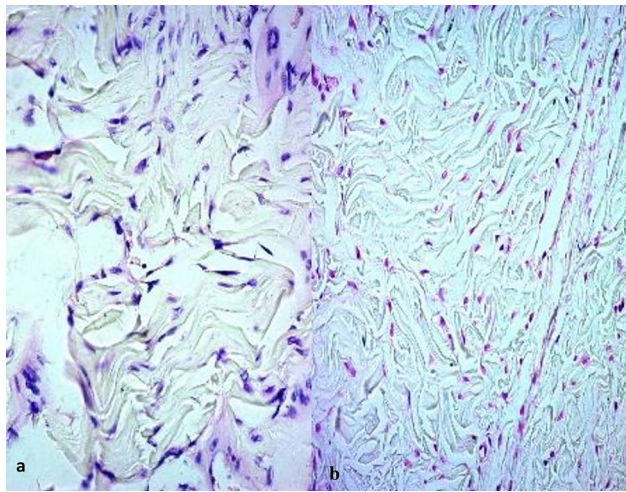


**Figure 4.** Siderophages indicating hemosiderin in the tissue as a sign of ancient hemorrhage, demonstrated with an arrow (H&E, x200).



**Figure 5.** Evaluation of vacuoles in urothelial epithelial tissue as a sign of healing damage, similar to many other organs, with vacuolar changes observed solely in Aloe Vera groups: **(a)** This image shows the normal urothelial epithelium. **(b)** The vacuolar epithelium, confirming vacuoles around the epithelial cell's nucleus (H&E, x400).

PA groups (Fig. 4). Edema was observed in all rats in the PA group (Fig. 5a-b), while vacuolar changes were observed in



**Figure 6.** Edema observed only in the Aloe Vera group without suture: **(a)** Control group example without edema. **(b)** Loose tissue view with edema (H&E, x400).

urothelial cells in all rats in the PA and PRA groups only (Fig. 6a-b).

All histopathological findings in the groups are summarized in Table 2.

**Influence on Serum Biochemical Parameters**

When Group C and Group P were compared, it was observed that the TOS and MDA values increased in favor of pathology but gained statistical significance only for the TOS value ( $p=0.018$ ), while SOD and GSH values tended to decrease in Group P, but not to a statistically significant extent ( $p>0.05$ ). When Group C and Group PR, Group PA, and Group PRA were compared, it was seen that there was no statistical difference in terms of SOD, GSH, TOS, and Malate Dehydrogenase (MDH) values ( $p>0.05$ ).

When Group P and Group PR were compared, it was observed that SOD and GSH values increased in favor of Group

**Table 2.** Comparison of groups according to histopathological features

Histopathological Features	Group C (n=8)	Group P (n=8)	Group PR (n=7)	Group PA (n=7)	Group PRA (n=8)	p
<b>Hyperemia-Bleedinga</b>						
Mild	8 (100%)	0	0	0	5 (62.5%)	<0.001
Moderate	0	0	0	0	2 (25%)	
Severe	0	8 (100%)	7 (100%)	7 (100%)	1 (12.5%)	
<b>Inflammationb</b>						
No	8 (100%)	8 (100%)	0	7 (100%)	3 (37.5%)	<0.001
Mild	0	0	3 (42.9%)	0	2 (25%)	
Moderate	0	0	3 (42.9%)	0	1 (12.5%)	
Severe	0	0	1 (14.3%)	0	2 (25%)	
<b>Fibrosisc</b>						
No	8 (100%)	8 (100%)	7 (100%)	0	0	<0.001
< 30	0	0	0	2 (28.6%)	0	
31-50	0	0	0	4 (57.1%)	8 (100%)	
51-70	0	0	0	1 (14.3%)	0	
<b>Siderophage</b>						
Absent	8 (100%)	6 (75%)	7 (100%)	5 (71.4%)	8 (100%)	0.155
Present	0	2 (25%)	0	2 (28.6%)	0	
<b>Edema</b>						
Absent	8 (100%)	8 (100%)	7 (100%)	0	8 (100%)	<0.001
Present	0	0	0	7 (100%)	0	
<b>Vacuolar Changes</b>						
Absent	8 (100%)	8 (100%)	7 (100%)	0	0	<0.001
Present	0	0	0	7 (100%)	8 (100%)	

P: Group C-P: <sup>a</sup><0.001, Group C-PR: <sup>a</sup><0.001, <sup>b</sup><0.001, Group C-PA: <sup>a</sup><0.001, <sup>c</sup><0.001, Group C-PRA: <sup>a</sup>0.200, <sup>b</sup>0.026, <sup>c</sup><0.001, Group P-PR: <sup>b</sup><0.001, Group P-PA: <sup>c</sup><0.001, Group P-PRA: <sup>a</sup>0.001, <sup>b</sup>0.026, <sup>c</sup><0.001, Group PR-PA: <sup>b</sup>0.001, <sup>c</sup>0.001, Group PR-PRA: <sup>a</sup>0.001, <sup>b</sup>0.343, <sup>c</sup><0.001, Group PA-PRA: <sup>a</sup>0.001, <sup>b</sup>0.044, <sup>c</sup>0.077.



**Table 3.** Comparison of groups according to oxidative and pro-inflammatory status

	Group C (n=8)	Group P (n=8)	Group PR (n=7)	Group PA (n=7)	Group PRA (n=8)	p
SOD <sup>a</sup>	40	34	37	44	41.6	0.009
GSH <sup>b</sup>	11.7	7	8.7	10	12.3	0.035
TOS <sup>c</sup>	3.7	4.2	3.7	3.7	4.1	0.036
MDA <sup>d</sup>	160.6	269.1	235.9	167.3	202.3	0.026
TNF- $\alpha$	106.5	125.1	117.7	118.7	111.7	0.340
IL-6 3.4	2.7	2.7	2.6	2.8	0.650	
IL-1 $\beta$	30.8	35.1	35.4	35.3	33.4	0.499

P: Group C-P: <sup>a</sup>0.340, <sup>b</sup>0.212, <sup>c</sup>0.018, <sup>d</sup>0.079, Group C-PR: <sup>a</sup>1.000, <sup>b</sup>0.833, <sup>c</sup>0.558, <sup>d</sup>1.000, Group C-PA: <sup>a</sup>1.000, <sup>b</sup>0.091, <sup>c</sup>0.585, <sup>d</sup>1.000, Group C-PRA: <sup>a</sup>1.000, <sup>b</sup>0.083, <sup>c</sup>0.394, <sup>d</sup>1.000, Group P-PR: <sup>a</sup>1.000, <sup>b</sup>0.147, <sup>c</sup>0.004, <sup>d</sup>1.000, Group P-PA: <sup>a</sup>0.010, <sup>b</sup>0.006, <sup>c</sup>0.006, <sup>d</sup>0.043, Group P-PRA: <sup>a</sup>0.034, <sup>b</sup>0.005, <sup>c</sup>0.112, <sup>d</sup>1.000, Group PR-PA: <sup>a</sup>0.833, <sup>b</sup>0.134, <sup>c</sup>0.987, <sup>d</sup>0.848, Group PR-PRA: <sup>a</sup>1.000, <sup>b</sup>0.125, <sup>c</sup>0.156, <sup>d</sup>1.000, Group PA-PRA: <sup>a</sup>1.000, <sup>b</sup>0.981, <sup>c</sup>0.177, <sup>d</sup>1.000.

PR, but this increase was not statistically significant. TOS and MDA values decreased in favor of Group PR, but only the decrease in TOS values became statistically significant (p=0.004).

When Group P and Group PA were compared, it was observed that SOD and GSH values increased in favor of Group PA, and TOS and MDA values decreased in favor of Group PA, with the changes being statistically significant (p<0.05).

When Group P and Group PRA were compared, it was seen that SOD and GSH values increased in favor of Group PRA, and this change gained statistical significance (p<0.05). A decrease was observed in TOS and MDA values, but this decrease did not gain statistical significance (p>0.05).

When Groups PR, PA, and PRA were compared, it was observed that the SOD level increased mostly in the PA group, the GSH level increased mostly in the PRA group, and the MDA level decreased mostly in the PA group, but these changes did not gain statistical significance (p>0.05).

When proinflammatory blood levels were compared between the groups, it was observed that TNF- $\alpha$  levels decreased in all treatment groups compared to the P group, but decreased most in the PRA group. When the IL-1 $\beta$  level was compared with the P group, a decrease was seen in the PRA group, but all these changes did not gain statistical significance (p>0.05).

The levels of proinflammatory cytokines and the oxidant and antioxidant levels of the tissues are summarized in Table 3.

## DISCUSSION

This study aimed to investigate the efficacy of the application of topical AV solution after PF on rat penile cavernous structure. Proper recovery in the penile cavernous structure can prevent the development of fibrosis and complications such as erectile dysfunction.<sup>[21]</sup> The current treatment choice to prevent these complications is early surgery of the damaged

tunica albuginea.<sup>[22]</sup> Various studies have evaluated wound healing after PF in the English literature.<sup>[6,7,21,23-25]</sup> Scar tissue development has been reported in 25% of patients in the area after PF repair.<sup>[26]</sup> Due to the positive effects on wound healing, we examined the topical effect of a solution obtained from the freeze-dried powder form of AV.<sup>[27]</sup>

Bleeding and hyperemia in tissues due to trauma and surgical interventions are expected outcomes. In our study, hyperemia and bleeding were observed in all groups, similar to the study by Akgül et al.<sup>[23]</sup> In another study conducted by Akgül et al., when cyanoacrylic glue (CG) was applied to PF animals, a decrease in hyperemia was observed, attributed to the hemostatic property of CG.<sup>[6]</sup> Therefore, we observed hyperemia and bleeding in all rat tissues. However, in our study, hyperemia was significantly less common in the PRA group.

Inflammation is a result of the wound healing process. In our study, inflammation was observed only in the PR and PRA groups. We believe this may be related to wound suturing. Although there was no significant difference between the two groups, the density was lower in the PRA group. This can be explained by the anti-inflammatory activity of the AV solution. In our study, it was observed that the application of a local AV solution after PF significantly decreased the levels of oxidants (TOS, MDA) and increased the levels of antioxidants (SOD, GSH) in the penile tissue. Furthermore, it was observed that TNF- $\alpha$  and IL-1 $\beta$  levels in the PRA group and TNF and IL-6 levels in the PA group were lower compared to Group P, although not statistically significant, in the rats treated with the local AV solution.

The development of fibrosis as a result of wound healing is considered a pathological healing process.<sup>[28]</sup> Fibrosis is a condition that should be prevented, as it may lead to complications after PF. Akgül et al. reported that fibrosis following PF repair may be related to the suture material.<sup>[23]</sup> However, in other studies by Akgül et al. and Taşdemir et al., it was observed that fibrosis decreased in the tissues of rats that

underwent primary repair after PF.<sup>[6,7]</sup> Similarly, in our study, it was observed that fibrosis did not increase in rats that underwent primary repair. Intense fibrosis was observed in the PA and PRA groups at the end of the study, although this was not statistically significant between the two groups ( $p=0.077$ ). It was concluded that local AV application was associated with the development of fibrosis. Contrarily, Akgül et al. observed that AV application reduces fibrosis.<sup>[23]</sup> After wound formation, inflammation mediates the balance between wound healing and fibrosis.<sup>[29]</sup> The use of anti-inflammatory agents aids in wound healing but also negatively affects cells that prevent fibrosis development. Fibroblasts and myofibroblasts play a crucial role in the development of fibrosis.<sup>[29]</sup> In one study, local application of AV was reported to result in prolonged retention of proinflammatory agents such as vascular endothelial growth factor (VEGF) in the tissue and a higher fibroblast density.<sup>[27]</sup> This study supports the results of local AV application for fibrosis in our study.

In a recently published review, the moisturizing properties of Aloe Vera hydrogel have been highlighted.<sup>[30]</sup> Dal'Belo et al. mentioned the moisture-retaining properties of formulations containing AV.<sup>[31]</sup> In the same study, even a single application of the freeze-dried powder form of AV showed long-term moisture-retaining effectiveness.<sup>[31]</sup> Although we observed that only the PA tissue was edematous in our study, vacuolar changes were observed in urothelial epithelial cells in both the PA and PRA groups. Since these findings were not encountered in other groups, we believe this may be due to the moisturizing effect of local AV application.

There are some limitations in our study. The AV used in our study was a pre-prepared form, which may not replicate the effects of the fresh form. However, if it is to be used as a treatment in the future, we believe working with commercial forms would provide more accurate results. In our study, we applied topical AV for three days, a period determined by considering the literature. Nonetheless, we believe studies investigating both duration and dose regulation would be beneficial. We believe that the lack of significant response in proinflammatory responses is related to the small size of the rat tissues and the limited area of our trauma. More appropriately designed animal studies may yield a more objective response. Nevertheless, we think our data will contribute to the literature.

## CONCLUSION

Our study shows that topical AV application reduces tissue inflammation and oxidative stress after PF. However, it appears to increase the development of fibrosis. Additionally, the clinical implications of vacuolar changes in urothelial cells remain unknown. Based on these findings, we cannot conclude that the application of topical AV solution positively affects wound healing after PF. Further research is needed to fully understand the clinical effects of topical AV solution on this tissue.

**Ethics Committee Approval:** This study was approved by the Zonguldak Bulent Ecevit University Ethics Committee (Date: 03.12.2020, Decision No: 2020-32-03/12).

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## DENEYSSEL ÇALIŞMA - ÖZ

### Sıçanlarda deneysel olarak tasarlanmış penil fraktur modelinde topikal aloe veranın etkileri

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**AMAÇ:** Bu çalışmada topikal aloe veranın (AV) sıçan modelinde deneysel olarak oluşturulan penil fraktur (PF) üzerindeki histopatolojik ve oksidatif etkileri değerlendirildi.

**GEREÇ VE YÖNTEM:** Kırk adet Wistar albino sıçan (220-250 g) kullanıldı. PF modeli deneysel olarak 15 numaralı lanset ile oluşturuldu. Sıçanlar rastgele ve eşit şekilde 5 gruba ayrıldı. Birinci grupta (C) herhangi bir kesi oluşturulmadı. İkinci grupta (P) insizyon oluşturuldu. Üçüncü grupta (PR) kesi hattı primer olarak kapatıldı. Dördüncü grupta (PA) 3 gün süreyle insizyon hattına onarım yapmadan lokal olarak AV uygulandı. Son grupta (PAV) primer onarım bölgesine 3 gün süreyle AV uygulandı. Tüm gruplar histopatolojik ve biyokimyasal verilere göre birbirleriyle karşılaştırıldı.

**BULGULAR:** PRA grubunda hiperemi-kanamanın diğer gruplara göre baskılandığı görüldü ( $p < 0.001$ ). Enflamasyon sadece PR ve PRA grubunda görüldü ( $p < 0.001$ ). PA ve PRA gruplarında diğer gruplarla karşılaştırıldığında anlamlı fibrozis gözlemlendi ( $p < 0.001$ ). SOD ve GSH değerleri PRA grubu lehine arttı (sırasıyla  $p = 0.009$  ve  $p = 0.035$ ). TOS ve MDA değerleri PA grubu lehine azaldı (sırasıyla  $p = 0.036$  ve  $p = 0.026$ ). TNF- $\alpha$  en çok PRA grubunda, IL-1 $\beta$  düzeyi ise en çok PRA grubunda azaldı ancak tüm bu değişiklikler istatistiksel anlamlılık kazanmadı ( $p > 0.05$ ).

**SONUÇ:** Topikal AV uygulamasının doku inflamasyonunu ve oksidatif stresi azalttığı ancak PF sonrası fibrozis gelişimini artırdığı görülmektedir.

**Anahtar sözcükler:** Aloe vera; penil fraktur; sıçan modeli.

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