

## The efficacy of spironolactone and surfactant treatment on HMGB1, CRP, IL-1 $\beta$ and TNF- $\alpha$ levels in acute lung injury

*Akut akciğer hasarında spironolakton ve sürfaktan tedavisinin HMGB1, CRP, IL-1 $\beta$  ve TNF- $\alpha$  düzeyleri üzerine etkisi*

Muhammet Emin Naldan,<sup>1</sup> Canan Atalay,<sup>2</sup> Ayşenur Coşkun,<sup>3</sup> Muhammet Karaca,<sup>2</sup> Yener Aydın,<sup>4</sup>  
Emrullah Dorman,<sup>5</sup> Ferah Daloğlu,<sup>6</sup> Hüsnü Kürşad,<sup>2</sup> Ömer Araz<sup>7</sup>

*Institution where the research was done:*  
Medical Faculty of Atatürk University, Erzurum, Turkey

*Author Affiliations:*

<sup>1</sup>Department of Anesthesia and Reanimation, Erzurum Training and Research Hospital, Erzurum, Turkey  
Departments of <sup>2</sup>Anesthesia and Reanimation, <sup>4</sup>Thoracic Surgery, <sup>5</sup>Biochemistry, <sup>6</sup>Pathology and <sup>7</sup>Chest Diseases,  
Medical Faculty of Atatürk University, Erzurum, Turkey

<sup>3</sup>Department of Anesthesia and Reanimation, Denizli Sarayköy State Hospital, Denizli, Turkey

### ABSTRACT

**Background:** This study aims to compare high-mobility group box-1 (HMGB1), interleukin-1 beta (IL-1 $\beta$ ), tumor necrosis factor alpha (TNF- $\alpha$ ), and C reactive protein (CRP) levels with spironolactone and surfactant treatment, investigate if spironolactone and surfactant treatment is effective, and evaluate the effect of this treatment on histopathologic changes in the lungs in acute lung injury (ALI) and acute respiratory distress syndrome (ARDS).

**Methods:** Forty rabbits were randomized into five groups to include eight rabbits in each group: group N: normal group, group T: tracheotomized group, group A: ARDS group, group S1: spironolactone group, group S2: surfactant group. Lungs of the rabbits were dissected and examined histopathologically.

**Results:** HMGB1, CRP, IL-1 $\beta$  and TNF- $\alpha$  levels were statistically significantly increased in group A. There was a statistically significant decrease in HMGB1, CRP, IL-1 $\beta$  and TNF- $\alpha$  levels in S1 and S2 groups ( $p < 0.05$ ). While PO<sub>2</sub>, SpO<sub>2</sub>, pH and PO<sub>2</sub>/FIO<sub>2</sub> levels were statistically significantly decreased in group A, they were increased in the treatment groups ( $p < 0.05$ ).

**Conclusion:** As in many studies, our study suggests that surfactant is effective in the treatment of ALI and ARDS, and spironolactone also has a similar effect as surfactant. Spironolactone may be used as a cost effective and efficient agent in ALI and ARDS. Further comprehensive studies are required regarding this subject.

**Keywords:** Acute lung injury; acute respiratory distress syndrome; spironolactone; surfactant.

### ÖZ

**Amaç:** Bu çalışmada akut akciğer hasarı (AAH) ve akut solunum sıkıntısı sendromunda (ASSS) high-mobility group box-1 (HMGB1), interlökin-1 beta (IL-1 $\beta$ ), tümör nekroz faktör alfa (TNF- $\alpha$ ) ve C reaktif protein (CRP) düzeyleri ile spironolakton ve sürfaktan tedavisi karşılaştırıldı, spironolakton ve sürfaktan tedavisinin etkili olup olmadığı araştırıldı ve bu tedavinin akciğerdeki histopatolojik değişiklikler üzerine etkisi değerlendirildi.

**Çalışma planı:** Kırk tavşan her bir grupta sekiz tavşan olacak şekilde beş gruba randomize edildi: grup N: normal grup, grup T: trakeostomili grup, grup A: ASSS grubu, grup S1: spironolakton grubu, grup S2: sürfaktan grubu. Tavşanların akciğerleri diseke edildi ve histopatolojik olarak incelendi.

**Bulgular:** Grup A'da HMGB1, CRP, IL-1 $\beta$  ve TNF- $\alpha$  düzeyleri istatistiksel olarak anlamlı şekilde artmış idi. S1 ve S2 gruplarında HMGB1, CRP, IL-1 $\beta$  ve TNF- $\alpha$  düzeylerinde istatistiksel olarak anlamlı azalma vardı ( $p < 0.05$ ). PO<sub>2</sub>, SpO<sub>2</sub>, pH, PO<sub>2</sub>/FIO<sub>2</sub> düzeyleri grup A'da istatistiksel olarak anlamlı şekilde azalmışken tedavi gruplarında yükselmiş idi ( $p < 0.05$ ).

**Sonuç:** Birçok çalışmada olduğu gibi, çalışmamız AAH ve ASSS tedavisinde sürfaktanın etkili olduğuna ve spironolaktonun da sürfaktan ile benzer etkiye sahip olduğuna işaret etmektedir. Spironolakton AAH ve ASSS'de uygun maliyetli ve etkin bir ajan olarak kullanılabilir. Bu konu hakkında ileri, kapsamlı çalışmalara ihtiyaç vardır.

**Anahtar sözcükler:** Akut akciğer hasarı; akut solunum sıkıntısı sendromu; spironolakton; sürfaktan.



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Correspondence: Yener Aydın, M.D. Atatürk Üniversitesi Tıp Fakültesi Göğüs Cerrahisi Anabilim Dalı, 25240 Erzurum, Turkey.

Tel: +90 535 - 784 89 70 e-mail: dryeneraydin@hotmail.com

Acute respiratory distress syndrome (ARDS) is a general term that indicates acute respiratory failure and is characterized by pulmonary edema due to increased capillary permeability in the lungs.<sup>[1-4]</sup> In addition, an increase in C-reactive protein (CRP) levels, tumor necrosis factor alpha (TNF- $\alpha$ ) and other markers occurs as a result of serious inflammation. Different medical therapies are available to treat ARDS, but no general consensus has been formed with regard to which is best.<sup>[3-10]</sup> With that in mind, the goal of this study was to investigate the efficacy of the use of a surfactant, which is routinely utilized in children with ARDS, when diuresis is present and spironolactone for other indications associated with acute lung injury (ALI) and ARDS.

## MATERIALS AND METHODS

This study was conducted on 40 New Zealand rabbits weighing between 1.5 and 3 kg that were randomly divided into five groups. In order to determine the initial values of the normal group (group N), after 30 minutes of stabilization, arterial blood gases were drawn from the femoral artery of the rabbits to study the high-mobility group box 1 (HMGB1) protein, CRP, interleukin 1 beta (IL-1 $\beta$ ), and TNF- $\alpha$  levels.

In the tracheostomized group (group T), the rabbits' cervical regions were shaved, and a tracheostomy was performed approximately 1 cm above the carina using surgical animal equipment. A handmade 20-gauge (20-G) intravenous (i.v.) tracheostomy cannula was first inserted into the tracheotomy region. Then prior to the administration of anesthesia, 60 mg/kg/hour i.v. thiopental and pancuronium bromide 0.5 mg/kg/hour were given, and intramuscular volume-controlled mechanical ventilation was applied to the rabbits via a Servo 900 D ventilator (Siemens-Elcoma AB, Solna, Sweden).

In the ARDS group (group A), in order to create lung damage, the rabbits were given 0.4 mL/kg, 0.1 mol/L hydrogen chloride (HCl) with a pH of approximately 1.25 via an endotracheal cannula after the tracheostomy, and volume-controlled mechanical ventilation was performed.

In the spironolactone group (group S1), the rabbits were given 100 mg/kg spironolactone via a nasogastric catheter 30 minutes after ARDS following the tracheotomy.

Finally, in the surfactant group (group S2), 100 mg/kg spironolactone was given intratracheally 30 minutes after ARDS after performing the tracheotomy.

The mechanical ventilator was adjusted to 8 mL/kg of the tidal volume (vt) and 40/min. of the respiratory frequency (f) for all of the groups. The arterial blood gases were drawn from the femoral arteries of the rabbits at the sixth and 24<sup>th</sup> hours, and after the procedure, the lungs of the rabbits were dissected and examined by a pathologist.

## Histopathological evaluation

The rabbits were sacrificed while under thiopental anesthesia, and their lungs were excised after the termination of the procedure in each group. The lungs were fixated using 10% formaldehyde. Random samples were then taken from the fixated segments and made into blocks by an automatic follow-up device. Next, the 5-7  $\mu$ m slices, which had been prepared by a microtome, were dyed with hematoxylin-eosin (H-E) and examined under a light microscope with x200 magnification where acute pulmonary damage was present. The following five criteria were investigated: (i) alveolar congestion, (ii) hemorrhage, (iii) neutrophil infiltration and/or aggregation in the air space and/or blood vessel wall, (iv) alveolar wall thickness and hyaline membrane formation, and (v) vascular congestion. In addition, each criteria was evaluated as follows: minimally damage (0 point), mild damage (1 point), moderate damage (2 points), severe damage (3 points), and maximum damage (4 points).

The total acute lung injury (ALI) of this group was calculated by ranking each criterion in all groups, and the total scores belonging to this criterion were calculated accordingly.

## Statistical analysis

All statistical analyses were performed using the PASW Statistics for Windows version 18.0 (SPSS Inc., Chicago, IL, USA). The data was obtained by using two repetitive measurements at the postprocedural sixth and 24<sup>th</sup> hours, and the difference between the measurements was analyzed using a matching test for each variable. For a non-parametric test, Kruskal-Wallis was used to analyze the differences between the groups because the data in each group was limited (n=8) and was not normally distributed. The Mann-Whitney U test was used to compare the data between two groups. A *p* value of <0.05 was considered to be statistically significant.

## RESULTS

No statistically significant differences were noted with regard to the blood gases, and the 24<sup>th</sup> hour results are shown in Table 1. The mean blood gas levels were 7.41,

**Table 1. The 24-hour blood gas analysis**

	Group N (n=8)	Group T (n=8)	Group A (n=8)	Group S1 (n=8)	Group S2 (n=8)	<i>p</i>
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
pH	7.4±0.5	7.3±0.1	7.2±0.1	7.3±0.1	7.4±0.1	<0.05
SpO <sub>2</sub>	92.5±1.8	96.3±6.4	81.4±13.9	96.6±2.2	92.9±6.8	<0.05
PaCO <sub>2</sub>	31.7±4.6	39.1±15.4	83.8±23.1	52.4±7.3	35.8±7.8	<0.05
PaO <sub>2</sub>	66.0±5.1	156.1±64.9	75.9±24.4	118.3±26.9	113.2±51.1	<0.05
PaO <sub>2</sub> /FIO <sub>2</sub>	313.3±24.1	295.4±31.1	195.7±23.3	221.8±113.7	220.5±89.1	<0.05

Group N: Normal group; Group T: Tracheotomized group; Group A: ARDS group; Group S1: Spironolactone group; Group S2: Surfactant group; SD: Standard deviation; SpO<sub>2</sub>: Peripheral capillary oxygen saturation; pCO<sub>2</sub>: Partial pressure of carbon dioxide; pO<sub>2</sub>: Partial pressure of oxygen; FIO<sub>2</sub>: Fraction of inspired oxygen.

7.33 and 7.18 in the N, T, and A groups, respectively while the mean pH levels were 7.27 and 7.39 in the S1 and S2 groups. Additionally, a serious improvement in acidosis was observed. However, the surfactant was more beneficial than the spironolactone, and the difference was statistically significant ( $p<0.05$ ).

The mean peripheral capillary oxygen saturation (SpO<sub>2</sub>) levels were 92.5%, 96% and 81% in the N, T, and A groups, respectively and 96.6 and 92.9 mmHg in the S1 and S2 groups, and these levels were statistically significant ( $p<0.05$ ). Furthermore, the spironolactone was found to be slightly more beneficial than the surfactant but not at statistically significant levels.

The mean partial pressure of carbon dioxide (PaCO<sub>2</sub>) levels were 31.7, 39.1, and 83.8 mmHg in the N, T, and A groups, respectively ( $p<0.05$ ), and after treatment, these levels were 52.3 and 35.8 mmHg in the S1 and S2 groups ( $p<0.05$ ). However, the surfactant was more effective than the spironolactone in terms of reducing the pCO<sub>2</sub> levels, and the difference was statistically significant ( $p<0.05$ ).

The mean partial pressure of oxygen (PaO<sub>2</sub>) levels were 66.0, 156.1 and 75.0 mmHg in the N, T, and A groups, respectively ( $p<0.05$ ), whereas these levels were 118.3 and 113.2 mmHg for the S1 and S2 groups. No significant differences were noted between the latter two groups.

The groups were also evaluated according to acute phase reactants, and the HMGB1, IL-1 $\beta$ , TNF- $\alpha$ , and CRP levels were greater in group T but even higher in group A ( $p<0.05$ ). Furthermore, there was a statistically significant decrease between groups S1 and S2 and group A ( $p<0.05$ ). The acute phase reactant levels at the end of the 24<sup>th</sup> hour are shown in Table 2.

The reduced HMGB1, IL-1 $\beta$  and TNF- $\alpha$  levels in group S2 were higher than in group S1, but the differences between the groups were not statistically significant ( $p>0.05$ ). The decline in CRP levels was similar in these two groups.

A histopathological evaluation of the ALI scores yielded results of 1.62±0.744, 2.50±0.534, 2.87±0.991, 2.25±0.88, and 1.62±1.06 for the N, T, A, S1, and S2 groups, respectively ( $p<0.05$ ). These scores in group S2 were lower than for S1 group, but the difference did not reach statistical significance ( $p>0.05$ ). The histopathological samples are presented in Figure 1.

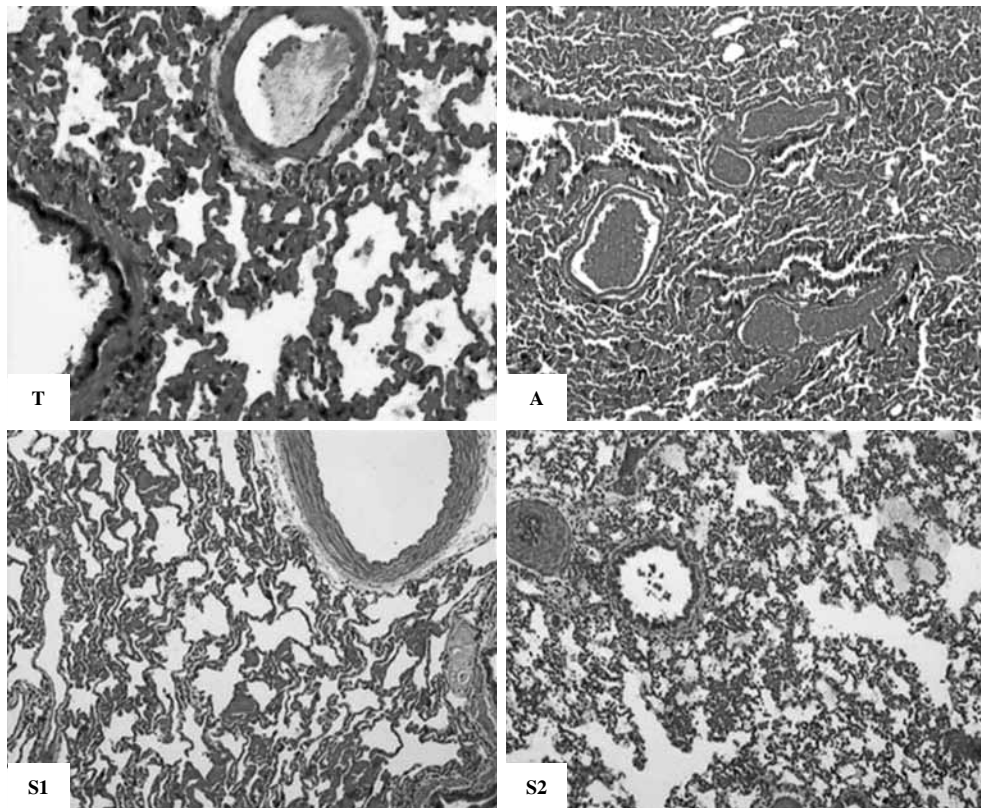
## DISCUSSION

Acute respiratory distress syndrome is a disease with high mortality, and several studies have been conducted to find a more effective treatment option. In this study, we wanted to determine whether surfactants and spironolactone were effective, and, if so, how did their use affect the HMGB1, CRP,

**Table 2. Between-group comparison of acute phase reactants**

	Group N (n=8)	Group T (n=8)	Group A (n=8)	Group S1 (n=8)	Group S2 (n=8)	<i>p</i>
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
HMGB1 (ng/mL)	23.8±11.5	39.8±21.9	84.2±7.9	17.2±5.4	12.0±5.0	<0.05
CRP (ng/mL)	8.9±5.7	59.9±22.4	128.5±35.1	8.3±4.2	8.9±3.5	<0.05
IL-1 $\beta$ (pg/mL)	21.7±12.9	40.0±41.4	127.0±11.6	33.4±13.0	13.3±5.2	<0.05
TNF- $\alpha$ (pg/mL)	24.6±12.8	59.9±44.9	131.9±31.0	40.4±9.2	31.8±11.9	<0.05

Group N: Normal group; Group T: Tracheotomized group; Group A: ARDS group; Group S1: Spironolactone group; Group S2: Surfactant group; SD: Standard deviation; HMGB1: High-mobility group box 1; CRP: C-reactive protein; IL-1 $\beta$ : Interleukin-1 beta; TNF- $\alpha$ : Tumor necrosis factor-alpha.



**Figure 1.** Histopathological samples belonging to the tracheostomized (T), acute respiratory distress (A), and spironolactone (S1) groups along with a photomicroscopic image of a sample from the surfactant (S2) group (H-E x 200).

IL-1 $\beta$  and TNF- $\alpha$  levels. In addition, we investigated the use of these parameters for follow up therapy.

In ARDS cases, the neutrophils accumulate and cause pulmonary damage. The life span of neutrophils is prolonged by delayed apoptosis, which can lead to ALI/ARDS because of the constant damage. C-reactive proteins play an important role in neutrophil chemotaxis. Buchta et al.<sup>[8]</sup> reported that low doses of CRP stimulate this process, whereas high doses inhibit it, indicating that high doses of CRP have a protective role.

Acute phase reactants play a major part in the inflammation that accompanies ARDS, with the most common being CRP. Bajwa et al.<sup>[5]</sup> reported that in ARDS cases, the CRP levels were significantly lower in died patients comparing to live ones, and they observed that increased CRP levels were associated with lower mortality rates. After the beginning of ARDS, increased plasma CRP levels improve the chance of survival in the first 48 hours, and these levels are related to lower organ dysfunction and less mechanical ventilation. In our study, the CRP levels were at the highest level in group A, and a

significant decrease was seen in the S1 and S2 groups after treatment ( $8.3 \pm 4.2$  ng/mL and  $8.9 \pm 3.5$  ng/mL, respectively).

Mittal and Sanyal<sup>[11]</sup> showed that the pro-inflammatory cytokines TNF- $\alpha$  and IL-1 $\beta$  are secreted as an early response to the inflammation process created by lipopolysaccharides (LPS). Furthermore, they determined that LPS-dependent TNF- $\alpha$  secretion may be related to other inflammatory mediators secreted by the same cell types. These authors induced septicemia in their study and noted the presence of TNF- $\alpha$  secretion. In addition, this inflammatory mediator synthesized with another important chemokine. We observed increased TNF- $\alpha$  and IL-1 $\alpha$  levels which were lowered via the surfactant or spironolactone treatment in the rabbits with ARDS induced by HCl.

Meduri et al.<sup>[9]</sup> reported that when the TNF- $\alpha$  and IL-1 $\beta$  levels in their study were high for a long period of time, mortality occurred, but when these levels dropped rapidly, the patients survived. Furthermore, Parsons et al.<sup>[12]</sup> determined that there was a link between elevated plasma TNF levels and mortality.

In our study, we found that after surfactant and spironolactone administration, a significant reduction occurred in the TNF- $\alpha$  levels, with the largest decrease being in the rabbits with ARDS (17.21 ng/mL and 31.84 $\pm$ 11.87 ng/mL; respectively).

Luo et al.<sup>[13]</sup> investigated the role of IL-1 $\beta$  in the pathogenesis of ARDS by monitoring the changes in IL-1 $\beta$  levels in the bronchoalveolar lavage fluid and serum in cases with this disease and found that the IL-1 $\beta$  levels were statistically significantly higher in both the bronchoalveolar lavage fluid and serum compared with the control group. They also determined that IL-1 $\beta$  plays a role in the pathogenesis of ARDS and that monitoring the IL-1 $\beta$  levels in the bronchoalveolar lavage fluid in the early period of this disease might prove to be significant in the evaluation of the ARDS process. We did not investigate the IL-1 $\beta$  levels in the bronchoalveolar fluid in our study. However, the serum levels showed the highest values in the rabbits with ARDS, and we observed that following the surfactant and spironolactone treatment, the IL-1 $\beta$  levels decreased significantly.

The HMGB1 protein is secreted by monocytes and macrophages via LPS stimulation and supports the inflammatory response. Cohen et al.<sup>[6]</sup> investigated the role of HMGB1 secretion in 168 patients with a serious injury and tissue hypoperfusion in the early stages of ARDS and found elevated plasma HMGB1 levels within 30 minutes in the patients with serious trauma. In the cases that resulted in mortality, the plasma HMGB1 levels were significantly higher compared with the patients that survived. Their findings led them to conclude that in patients suffering from severe trauma, HMGB1 released in the early stages is indeed associated with severe injury and tissue hypoperfusion.

Ueno et al.<sup>[7]</sup> showed that ALI produced by LPS can be relieved by anti-HMGB1 antibodies and that HMGB1 might play a key role in the pathogenesis of both clinical and experimental ALI. In our study, we observed that the HMGB1 levels were the highest in group A, but a statistically significant decrease took place following the spironolactone and surfactant treatment.

Hagiwara et al.<sup>[14]</sup> treated rats with ARDS with i.v. immunoglobulin G (IgG) before they underwent cecal ligation and investigated the HMGB1 and TNF- $\alpha$  levels of the rats along with the association between HMGB1 and ARDS. They determined that these levels increased significantly and that following the

administration of the IgG, the patients' inflammation decreased. In addition, the HMGB1 and TNF- $\alpha$  levels were significantly reduced in the rats who underwent cecal ligation.

In our study, the HMGB1 and TNF- $\alpha$  levels in the rats reached a peak after ARDS was triggered by the HCI, but they subsequently decreased after receiving the surfactant and spironolactone treatment.

Calfee and Matthey<sup>[15]</sup> examined ARDS therapies by dividing them into ventilatory fluid, medical, and new potential categories and reported that there has been no better agent than medical therapy for reducing mortality in the last decade. The latest medical therapies include the use of surfactants, spironolactone, inhaled nitric oxide (NO), antifungal drugs, corticosteroids, and phosphodiesterase inhibitors.

Deal et al.<sup>[16]</sup> investigated 10 randomized and controlled studies conducted between 1968-2008, and with the help of data obtained from clinical observations, they concluded that high doses of corticosteroids were not satisfactory for protecting patients from ARDS. Instead, they were associated with septicemia and/or septic shock more than the other forms of therapy.

Häfner et al.<sup>[17]</sup> compared four surfactants that contain protein in the treatment of late-term ARDS and determined that the surfactants containing protein caused an increase in the reduced PaO<sub>2</sub> levels. Furthermore, another study focused on HCI-induced ARDS as we did and found that surfactants improved oxygenation significantly and provided meaningful increases in the PO<sub>2</sub>/fraction of inspired oxygen (FiO<sub>2</sub>) ratio.<sup>[18]</sup>

In patients with ALI and ARDS, inflammation causes endothelial dysfunction, fluid extravasation from the capillaries, and impaired fluid drainage. Pulmonary edema increases thickness of the alveolar-capillary interface and this increases oxygen diffusion to blood. In these instances, gas exchange is impaired, and hypoxia occurs, causing fibrosis in the alveolar air space. Under certain physiological conditions, cells that contain aldosterone receptors in the lungs actively move to the alveolar capillary barrier during sodium transport. Hence, 12 hours after the fluid aspiration, the prognosis for patients with ARDS improves in practice most likely because the aldosterone stimulates interstitial fibrosis by local dehydration. Interestingly, various studies have shown that spironolactone regulates pulmonary fibrosis, congestion, edema, and the capacity for gas diffusion.<sup>[19-21]</sup>

## Conclusion

In our study, we determined that the rabbits with ARDS induced by HCI showed improvement in acute phase reactant and blood gas levels after being given spironolactone or a surfactant. In addition, our findings showed that spironolactone and surfactants have similar effects. Therefore, we believe that spironolactone may be used as a cost-effective and efficacious agent for treating patients with ALI/ARDS. However, more comprehensive studies are needed to verify our results.

## Declaration of conflicting interests

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