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# Comparison of hyperbaric oxygen, ozone, and dexpanthenol therapies in rats with acute lung injury

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**Abstract:**

**BACKGROUND AND AIM:** Acute respiratory distress syndrome (ARDS) is a fatal disease presenting with respiratory failure. Patients with ARDS account for a considerable portion of patients staying in the intensive care unit (ICU). Therefore, advances in the treatment of these patients are of great importance. Direct or indirect injury to the lung initiates an inflammatory process. This results in impaired integrity of the alveolar-capillary membrane, pulmonary edema, and severe hypoxia. The present study compared hyperbaric oxygen (HBO), ozone, and dexpanthenol therapies administered to rats with experimentally induced ARDS, as well as the efficacy of these therapies.

**METHODS:** Thirty-two male Wistar Albino rats were used in the study. The rats were divided into four groups. All groups were administered antibiotherapy for 5 days after administering live *Escherichia coli*. Group 1 (control group) rats received intraperitoneal saline. Group 2 rats were treated with HBO. Group 3 rats received an oxygen + ozone mixture. Group 4 rats received dexpanthenol. After 5 days, anesthesia was administered to all rats, blood gases were collected from the abdominal aorta, and then the rats were sacrificed. Some of the collected blood was used for cytokine assays. The right lung tissues were used for histopathological examination. The left lung tissues were used to measure enzyme levels.

**RESULTS:** Histopathologically, there were intra-alveolar hemorrhage, edema, intensive inflammatory cell infiltration, fibrosis, collapse, type 2 alveolar cells, and macrophage accumulation in all groups. In terms of fibrosis/alveolar septal thickening, the dexpanthenol group had a significantly lower mean score than the control and HBO groups. In terms of alveolar collapse, the dexpanthenol group had a significantly lower mean score than all other groups. In terms of increased macrophage and type II alveolar cell counts, the ozone group had a significantly lower mean score than all other groups. There was no significant difference in immunohistochemical staining between the groups. In terms of superoxide dismutase levels, the dexpanthenol group had a significantly lower score than the control group. Regarding IL-10 levels, the ozone group had a significantly higher score than the control and HBO groups. The dexpanthenol group had a

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significantly higher score only than the HBO group. Regarding PaO<sub>2</sub> levels, the ozone group had a significantly higher score than all groups. The ozone group had a significantly lower score on PaCO<sub>2</sub> levels than all groups.

**CONCLUSIONS:** Among the treatments, the HBO therapy increased cell injury. The ozone therapy produced anti-inflammatory effect and histopathologically positive outcomes. The ozone therapy provided significant improvement in arterial oxygenation. The dexpanthenol therapy produced antioxidant effect and histopathologically positive outcomes. The antifibrotic effect was prominent in the dexpanthenol therapy. Further studies are needed to generalize the use of these treatments in ARDS.

**Keywords:**

Anti-inflammatory, antioxidant, dexpanthenol, HBO, lung injury, ozone

## Introduction

Acute respiratory distress syndrome (ARDS) is a severe, life-threatening form of acute respiratory failure. There is pulmonary edema based on an alveolar-capillary membrane injury, with an acute onset of hypoxemia resistant to oxygen therapy. ARDS is accompanied by multiorgan system failure and therefore is associated with high mortality and morbidity in the intensive care unit (ICU). It increases the cost of treatment of critical care patients by prolonging their stay in the ICU and hospital.<sup>[1,2]</sup> The clinical features of ARDS include acute-onset respiratory failure, bilateral diffuse infiltrates on the chest X-ray, severe hypoxemia resistant to treatment, and reduced pulmonary compliance. This condition should not be accompanied by clinical manifestations of cardiogenic pulmonary edema.

Sepsis is mentioned as one of the common risk factors. The rate of acute lung injury is 46% in sepsis of pulmonary origin, compared with 33% in sepsis of nonpulmonary origin.<sup>[2]</sup> In a multicenter international study of approximately 30 000 ICU patients, 10% of the ICU admissions were due to ARDS. Most (80%) patients with ARDS needed mechanical ventilation.<sup>[3]</sup> Although the incidence is not high, ARDS remains relevant in ICUs because it may occur at young ages, patients have a prolonged stay in the ICU, and unfortunately, the mortality rate is high. The prognosis is poorer and mortality is higher in older adult patients with chronic diseases and patients with sepsis and multiple organ failure.

Considering the pathophysiology of ARDS, the main feature is the impairment of the alveolar-capillary barrier due to direct or indirect lung injury.<sup>[4]</sup>

With the onset of inflammation, neutrophils are increasingly produced and begin to accumulate in the inflammation sites and in the lung. The release of various cytokines increases neutrophil accumulation and activation. Acti-

vated neutrophils cause cell injury by secreting free radicals, inflammatory mediators, proteases (elastase, collagenase, and reactive oxygen species [ROS]), and cytokines such as tumor necrosis factor (TNF)-alpha. In neutrophils, hydrogen peroxide formed by the superoxide dismutation reacts via myeloperoxidase (MPO) to form hypochlorous acid (HClO) (reactive oxygen particle), which is a bactericidal agent. In case of excessive secretion, reactive oxygen particles become harmful rather than beneficial.<sup>[5]</sup> Increased free radicals create oxidative stress. To maintain the balance in the body, antioxidant defense develops against oxidative stress. The first defense includes the antioxidant defense systems of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which reduce the formation of free radicals.<sup>[6]</sup>

There is no definitively proven treatment for ARDS yet. This is because the condition presents with diffuse inflammation and develops due to many etiological factors.

The present study investigated hyperbaric oxygen (HBO), ozone, and dexpanthenol therapies, which are not commonly used for ARDS, and compared the anti-inflammatory and antioxidant effects of these therapies. An animal model was preferred for this study. Animal models play an important role between patients and laboratory data of diseases. Although available animal models do not exactly reflect human pathology, these models are necessary to understand the pathophysiological changes in ARDS and to develop new treatment strategies.

Sepsis is one of the main risk factors for ARDS. Several animal models of sepsis have been developed to explore acute lung injury. In a study investigating lung injury in septic rats, the rats were intraperitoneally (IP) administered live *Escherichia coli* cells at a concentration of 2.1×10<sup>9</sup> cfu, resulting in edema, hemorrhage, leukocyte infiltration, and alveolar septal thickening in the lung tissue to varying degrees.<sup>[7]</sup>

**Table 1: Treatment groups**

Group 1	Control group (n=8)	<i>E. coli</i> bacteria (10 <sup>9</sup> cfu/100 g body weight)	Cefepime HCL (50 mg/kg)P for 5 days	Saline (10 mL/kg) IP for 5 days
Group 2	The HBO group (n=8)	<i>E. coli</i> bacteria (10 <sup>9</sup> cfu/100 g body weight)	Cefepime HCL (50 mg/kg) IP for 5 days	At 2.5 ATA for 90 minutes every 8 h on the first day and every 12 h on other days
Group 3	The ozone group, (n=8)	<i>E. coli</i> bacteria (10 <sup>9</sup> cfu/100 g body weight)	Cefepime HCL (50 mg/kg) IP for 5 days	IP 0.7 mg/kg oxygen+ozone mixture once a day for 5 days
Group 4	The dexpanthenol group, (n=8)	<i>E. coli</i> bacteria (10 <sup>9</sup> cfu/100 g body weight)	Cefepime HCL (50 mg/kg) IP for 5 days	Dexpanthenol (500 mg/kg) IP once a day for 5 days

HCL: Hypochlorous acid, HBO: Hyperbaric oxygen

Another study created a model of sepsis-induced lung injury was created, administered 10<sup>7</sup> and 10<sup>9</sup> cfu *E. coli* to rats IP at 1 mL/100 g and compared the blood gas changes in these rats and the samples from bronchoalveolar lavage fluid.<sup>[8]</sup>

## Materials and Methods

This study was initiated after ethical approval (Date: June 30, 2020 and Decision No: 2020/06-02) was obtained from the Animal Experiments Local Ethics Committee of Çanakkale Onsekiz Mart University (ÇOMÜ). All animal experiments were conducted at the Experimental Application and Research Center of ÇOMÜ between March and April 2021. The study employed 32 male Wistar Albino rats with an average weight of 200–250 g. During the study, rats were housed in wire cages at ÇOMÜ Experimental Research Center, under a circadian rhythm of 12 h darkness and 12 h light, at an ambient temperature of 24–26°C and a humidity of 50%–60%. The rats were fed a standard commercial pellet diet and the city's drinking water. All rats were treated in accordance with the Regulation on the Welfare and Protection of Animals Used for Experimental and Other Scientific Purposes (13.12.2011-28141) prepared by the Ministry of Food, Agriculture, and Livestock.

Blood gas analyses were conducted in the Biochemistry laboratory of ÇOMÜ. The assays of cytokines and tissue antioxidant enzymes were conducted at an external center upon transfer under suitable storage conditions at –80°C. Histopathological examinations were performed in the laboratory of the Department of Histology and Embryology of ÇOMÜ. Live *E. coli* were grown in the Microbiology laboratory of ÇOMÜ. The treatment groups are given in Table 1.

Considering the biological rhythm, all injections were administered at the same time. All treatments were initiated 10 h after the administration of live *E. coli*. All groups received IP cefepime HCL at a dose of 50 mg/kg for 5 days. The antibiotic dose was adjusted based on the study by Yamanel et al.<sup>[7]</sup> All subjects were clinically followed up by monitoring daily activity, appetite, and respiratory rate. Blood gases were collected from the abdominal aorta on day 5, just before sacrifice. At the end of day 5, the subjects were administered anesthesia by IP 50 mg/kg ketamine and 5 mg/kg xylazine. Following anesthesia, the abdominal region of the subjects was opened under sterile conditions. The abdominal aorta was clamped, and blood samples were collected using heparinized syringes. Then, the subjects were quickly sacrificed. Some of the samples were sent for blood gas analysis and the blood samples collected for other parameters were centrifuged at 3000 rpm for 15 min. Heparinized plasma specimens obtained after centrifugation were transferred to Eppendorf tubes. The Eppendorf tubes were stored at –80°C in the cabinet in our clinic for cytokine assays. After death, the chest wall of the rats was opened. Lung tissues were separated from the surrounding tissues and frozen at –80°C to measure MPO and SOD in left lung tissues. Right lung tissues were fixed in 10% formaldehyde [Figs. 1–4].

The blood was placed into yellow-cap, polymer gel-containing tubes (BD RST™) and centrifuged at 3000 rpm for 15 min to obtain heparinized plasma specimens. These plasma specimens were measured using the BIOTEK ELX800 according to the associated protocols.

The blood gas tests were performed using a Radiometer ABL 800 FLEX (Radiometer, Copenhagen, Denmark) blood gas analyzer. Partial carbon dioxide pressure (PaCO<sub>2</sub>) and partial oxygen pressure (PaO<sub>2</sub>) were measured using the principle of amperometric measurement.

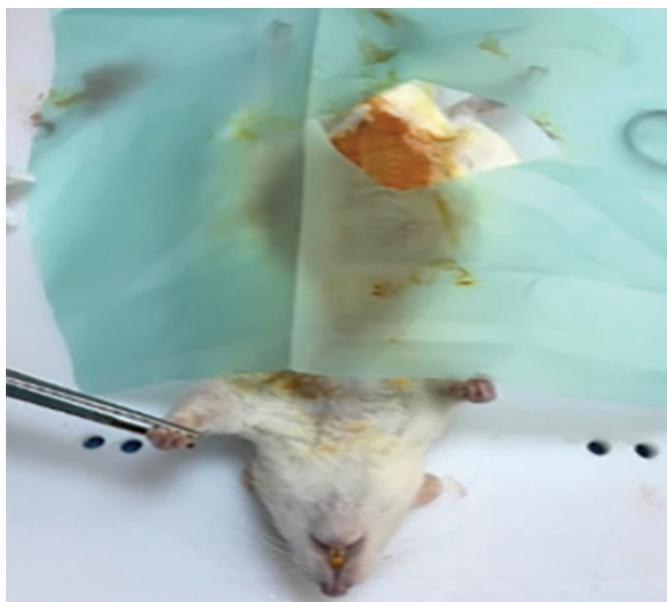


Figure 1: Depth of anesthesia

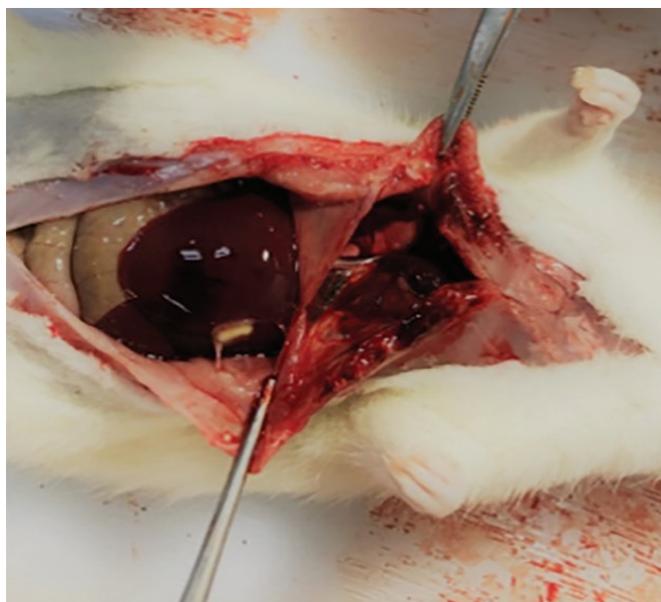


Figure 3: Intrathoracic organs



Figure 2: Blood collection from the abdominal aorta



Figure 4: The lung tissue from the HBO Group

After the experiment, the chest wall of the animals was opened. Left lung tissues were collected by cleaning from other tissues. The tissue activities of SOD and MPO were measured by Mindray BS300 fully automated biochemistry analyzer.

The right lungs of the rats were removed just before sacrifice and fixed in 10% neutral buffer formaldehyde solution for 48 h. The right middle lobes were selected and 0.5-cm thick sections were placed into tissue processing

cassettes. After fixation, tissue samples were washed under running tap water for 4 h. To dehydrate the tissues, the tissues were passed through a series of alcohols of increasing grades (70%, 80%, 90%, 96%, and 100%). Then, tissues were passed through xylol for transparency and embedded in 60°C molten paraffin to form blocks. Serial sections were cut from the prepared paraffin blocks at 200- $\mu$ m intervals to examine the entire lung tissue. The sections from all groups were exposed to hematoxylin & eosin (H&E) staining and immunohistochemical staining.

**Table 2: Oxidant-antioxidant, inflammatory- anti-inflammatory, and blood gas scores, and mean and standard deviation values for acute lung injury in the treatment groups**

	Control	Hyperbaric	Ozone	Dexpanthenol	p
SOD*	215±3.2	212±2.3	211±5.3	208±4.6	0.01
MPO*	82±15.1	144±82.5	102±12.7	116±31	0.07
TNF-alpha*	140±13	123±29	127±31	152±38	0.21
IL-1β*	108±2.1	109±2.5	105±7.2	103±10	0.32
IL-10*	19±7.1	14±5.7	37±18	33±13.8	0.002
TGF-β*	1.4±0.7	2.3±3	3±2.8	1.5±0.6	0.39
PaO <sub>2</sub> *	69±13	77±15	95±13	70±2	0.001
PaCO <sub>2</sub> *	49±4.4	48±7	42±2.4	52±2.3	0.001

One-way ANOVA test was used, \*Mean±SD, SOD: Superoxide dismutase, MPO: Myeloperoxidase, TNF-alpha: Tumor necrosis factor-alpha, IL-1β: Interleukin-1β, IL-10: Interleukin-10, TGF-β: Transforming growth factor-β, PaO<sub>2</sub>: Arterial oxygen pressure, PaCO<sub>2</sub>: Arterial carbon dioxide pressure

The sections collected from the right lung at 200-μm intervals and stained with H&E were examined histomorphologically. Using a method consistent with previous studies, a total of 20 fields for each rat were evaluated at 10× magnification under a Zeiss Primostar light microscope.<sup>[9]</sup> Edema, hemorrhage, inflammation, fibrosis (alveolar septal thickening), and alveolar collapse in lung tissue were scored as follows: 0, normal lung tissue; 1, injury of ≤25%; 2, injury of 26%–75%; and 3, injury of ≥76%. The increase in type II alveolar cells and macrophages was scored “0” for absent and 1 for present. Immunohistochemical staining with surfactant protein D (SFPD) was evaluated using a method consistent with previous studies.<sup>[10]</sup> The intensity of immunostaining with SFPD was scored as: 0, none; 1, mild; 2, moderate; and 3, intense. Tissues were evaluated by two histologists (A.G.B. and M.I.G.) in a double-blind fashion, and the mean scores were calculated. Photographs were acquired using the ZEN blue edition software and the Zeiss Scope A1 Light microscope with AxioCam 503 color camera attachment.

### Statistical analysis

Statistical analysis was carried out using IBM SPSS Statistics ver. 24.0 (IBM Co., Armonk, NY, USA). Descriptive statistical methods (mean, standard deviation, frequency, percent, minimum, and maximum) were used to evaluate the study data. Pearson’s Chi-squared test was used to compare qualitative data, and Fisher’s exact test was applied if the number of subgroups was low. The normality of data distribution was assessed by the Shapiro-Wilk test. One-way ANOVA was used for more than two groups with the normal distribution. Subgroups compared with the post hoc (Tukey’s) tests. On the results of the analysis,  $p < 0.05$  was considered to be statistically significant.

### Results

The level of SOD, an antioxidant enzyme, was statistically significantly different between the groups. The post hoc (Tukey’s) tests revealed that the statistically significant difference was between the control group and the dexpanthenol group ( $p=0.006$ ), with no significant difference between the other groups. There was no statistically significant difference between the groups in the levels of MPO activity, an index of polymorphonuclear leukocyte infiltration and accumulation, which has a bactericidal effect by producing hypochlorous acid (HClO) from hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and chloride anion, Cl<sup>-</sup> (or halide).

Table 2 presents the mean and standard deviation values for interleukin (IL)-1β, TNF-α, IL-10, and transforming growth factor (TGF)-β levels of the treatment groups. A statistically significant difference was established only in IL-10 levels between the treatment groups. No statistically significant difference was found in other parameters.

There was a significant difference in the levels of IL-10, an anti-inflammatory parameter, between the groups. The post hoc (Tukey’s) tests revealed a statistically significant difference between the ozone group and the control group ( $p=0.034$ ), and the HBO group ( $p=0.004$ ). A statistically significant difference was established between the dexpanthenol group and only the HBO group ( $p=0.027$ ). There was no significant difference between the dexpanthenol group and the ozone group, the dexpanthenol group and the control group, and the control group and the HBO group.

Table 2 presents the mean and standard deviation values for PaO<sub>2</sub> and PaCO<sub>2</sub> levels of the treatment groups. A statistically significant difference was established in PaO<sub>2</sub>

**Table 3: Histological scoring of acute lung injury in treatment groups**

	Control	Hyperbaric	Ozone	Dexpanthenol	p
HP edema					0.3
No	3	0	3	1	
<25%	5	7	5	7	
25%–75%	0	1	0	0	
HP hemorrhage					0.2
<25%	0	0	3	2	
25%–75%	2	1	0	2	
>75%	6	7	5	4	
HP inflammation					0.1
<25%	1	1	5	2	
25%–75%	4	2	0	3	
>75%	3	5	3	3	
HP fibrosis					0.02
<25%	1	1	3	4	
25%–75%	1	0	2	4	
>75%	6	7	3	0	
HP collapse					0.03
<25%	1	1	3	4	
25%–75%	2	0	1	4	
>75%	5	7	4	0	
HP sfpd					0.25
25%–75%	0	3	1	2	
>75%	8	5	7	6	
HP type 2 ACs-macrophages					0.02
Absent	0	0	3	0	
Present	8	8	5	8	

The Chi-squared test and Fisher's exact test were used. HP: Histopathological

and PaCO<sub>2</sub> levels between the treatment groups. The post hoc (Tukey's) tests revealed a statistically significant difference in PaO<sub>2</sub> levels between the ozone group and the control group (p=0.001), the HBO group (p=0.027), and the dexpanthenol group (p=0.002). There was no significant difference between other groups. The post hoc (Tukey's) tests revealed a statistically significant difference in PaCO<sub>2</sub> levels between the ozone group and the control group (p=0.014), the HBO group (p=0.041), and the dexpanthenol group (p=0.001). There was no significant difference between other groups (Table 2).

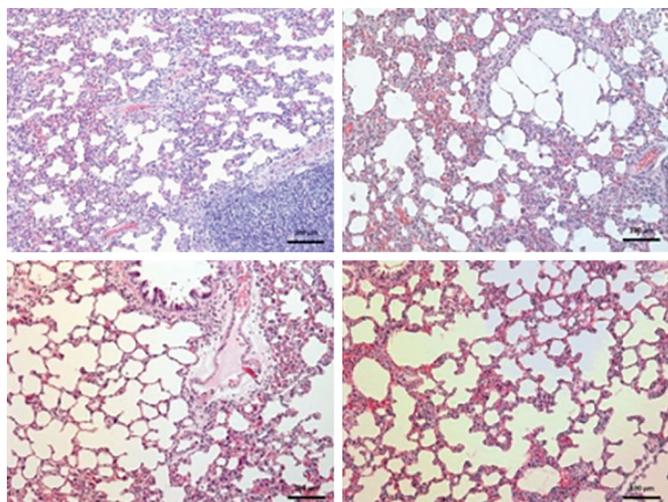
The values obtained by histopathological examination of the serial sections cut from the right middle lobes at 200-µm intervals were interpreted statistically. No significant difference was established between the groups in edema, hemorrhage, and inflammation in lung tissues. There was a significant difference between the groups in fibrosis, alveolar collapse, and increased macrophage and type II alveolar cell counts in lung tissues (p=0.02, p=0.03, and p=0.02, respectively).

When the groups were compared:

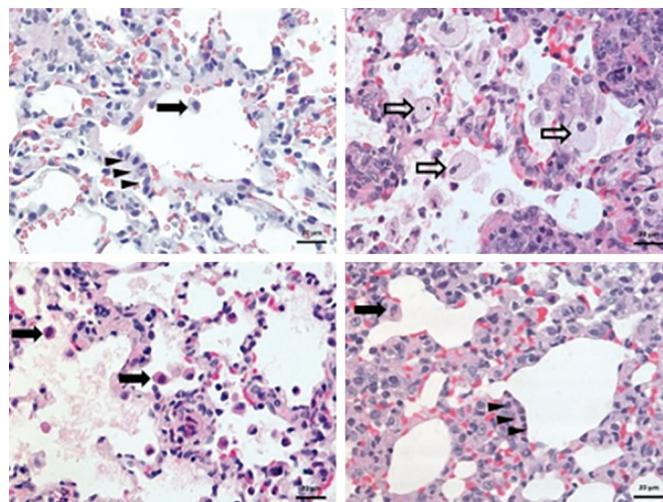
- The dexpanthenol group had a significantly lower mean score on fibrosis/alveolar septal thickening than the control and HBO groups (p=0.008 and p=0.002, respectively).
- The dexpanthenol group had a significantly lower mean score on alveolar collapse than all other groups (p=0.024 for the control group, p=0.002 for the HBO group, and p=0.05 for the ozone group).
- The ozone group had a significantly lower mean score on increased macrophage and type II alveolar cell counts than all other groups (p=0.05 for all).
- Type II alveolar cells, macrophages, and Clara cells were immunohistochemically stained with SFPD. There was no statistically significant difference in this type of staining between the groups (Table 3) [Figs. 5–8].

## Discussion

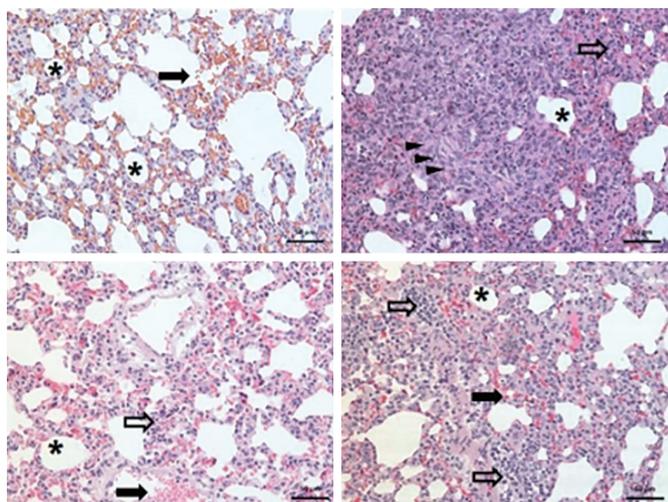
Human studies on ARDS reveal important information about physiological and inflammatory changes in the



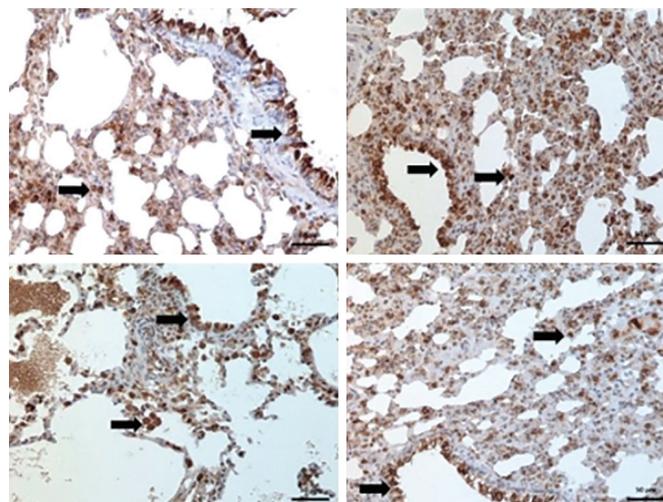
**Figure 5:** Comparison of histological views of all groups. (H&E staining, scale bar 100 μm). Intensive inflammation, alveolar collapse, and fibrosis are prominent in the control group. Hemorrhage and edema are notable in the HBO group.



**Figure 7:** An increase in type II alveolar cells (triple arrowhead) and macrophages (thick arrow) and foamy macrophages (hollow arrow) are visible in all groups (H & E staining, scale bar 20 μm).



**Figure 6:** Hemorrhage (thick arrow), inflammation (hollow arrow), fibrosis (triple arrowhead), and alveolar collapse (asterisk) are visible in all groups. (H & E staining, scale bar 50 μm).



**Figure 8:** Immunohistochemical staining with SFPD reveals stained (thick arrows) type II alveolar cells, macrophages, and Clara cells in all groups (SFPD immunostaining, scale bar 50 μm).

lung. This information allows developing hypotheses on the mechanisms of injury, but the uncontrollable clinical variables present in patients have made it difficult to study such hypotheses in humans. Therefore, experimental animal models have been designed for the development of ARDS/acute lung injury.

Our study induced acute lung injury by intraperitoneal administration of live *E. coli*, which is consistent with the literature. In the study by Yamanel et al.,<sup>[7]</sup> rats were administered IP injection of *E. coli*, and the histopathological assessment of the lungs of the bacteria-treated groups

revealed significant edema, hemorrhage, leukocyte infiltration, alveolar septal thickening, and severe sepsis. The authors did not histopathologically detect any pathology in the group that did not receive bacteria. Another study<sup>[8]</sup> examined the effect of pentoxifylline on lung inflammation in a sepsis-induced acute lung injury model. For this purpose, the rats were administered different dosages of live *E. coli* to induce sepsis, bronchoalveolar lavage samples were collected at certain intervals, and neutrophil and macrophage counts, TNF-alpha levels, malonyldialdehyde levels, protein concentration in lavage, and wet/dry lung ratios were evaluated. These

studies show that live *E. coli* can be administered IP to induce lung injury through sepsis and support our study. Developing a sepsis model with well-defined respiratory dysfunction is important both as a tool to understand the pathogenesis of the dysfunction and as a model to test potential interventions.

One of the treatments compared in our study was the administration of HBO in addition to antibiotherapy in rats with induced lung injury. When we examined the HBO group rats, we found evidence of increased injury in histopathological, blood, and tissue parameters in the lung with ARDS. Although the level of SOD, an antioxidant enzyme, and the level of MPO, an index of polymorphonuclear leukocyte infiltration and accumulation, were not statistically significantly different in this group compared with the other groups, the MPO level was the highest in the HBO group. Also, the level of IL-10, an anti-inflammatory parameter, was the lowest in this group. Results were similar histopathologically. Considering the overall histopathological picture, injury of >75% was observed mostly in the HBO group. In particular, hemorrhage, fibrosis, and collapse were profound in the HBO group.

Although our study found that the HBO therapy increased inflammation and oxidative damage, there are reports in the literature indicating antioxidant and anti-inflammatory effects of the HBO therapy. The study by Perng *et al.*<sup>[11]</sup> induced lung injury by intratracheal administration of lipopolysaccharide to rats, and observed reduced findings of lung injury, such as increased leukocytes and improved survival rates after HBO therapy. In the study by Yamanel *et al.*,<sup>[7]</sup> rats were administered IP injection of *E. coli*, and the histopathological assessment of the lungs of the bacteria-treated groups revealed severe sepsis. The bacteria-treated groups were compared for HBO and ozone therapies. The authors found an increased level of antioxidant enzymes, a decreased level of MPO, and reduced inflammatory parameters in the HBO group. Histopathologically, the degrees of hemorrhage, leukocyte infiltration, and alveolar septal thickening were found to be much lower than in the control group. Although this study is one of the closest studies to our study, we obtained different results on HBO. In our study, the HBO group had the highest level of MPO activity and the highest degree of histopathological injury. The review of the literature suggests that a reduction in the increased MPO activity is one of the criteria for

treatment success.<sup>[12,13]</sup> In the light of this information, the high MPO levels in our study support our view that HBO therapy increases oxidative damage and inflammation.

It is known that the HBO therapy results in increased production of ROS<sup>[14]</sup> and that ROS are able to act as important signaling molecules to strengthen a host defense system.<sup>[15]</sup> In addition, it was suggested that some beneficial effects of HBO might occur with the effect of stimulated reactive molecules, especially superoxide ( $O_2^-$ ) and  $H_2O_2$ .<sup>[16]</sup> The lack of a statistically significant result regarding the activity of SOD, an antioxidant parameter, in our study is attributed to the complex and bidirectional action of the HBO therapy that induces the oxidative stress itself but reduces the existing oxidative stress.

Our study administered the HBO therapy for 90 min at 2.5 ATA (1 ATA: atmosphere absolute=760 mmHg) at three doses per day on the first day and two doses per day on the other days. Histopathologically, the severity of the injury was expected to reduce in the injured lung tissue after HBO therapy but opposite findings were obtained. This suggests that the doses and pressures used in the HBO therapy were not suitable for our ARDS model. It is believed that the therapy increased inflammation through hyperoxia and that the HBO administration might cause barotrauma in the injured lung.

Considering the general mechanism of action of the HBO therapy, the amount of dissolved oxygen in the plasma is increased by breathing 100% oxygen at high pressures. As it is known, the transport of oxygen to the tissues at normal atmospheric pressure occurs by binding to hemoglobin at the rate of 97% and the level of oxygen dissolved in the plasma is quite low. With the administration of pure oxygen, oxygen can bind to the hemoglobin at the rate of 100%, and after the full saturation of hemoglobin, the increased free oxygen levels in the plasma are distributed to the tissues. In HBO therapy, the plasma concentration of oxygen increases up to 3 ATA and the oxygenation in the tissues up to 10–15 times.<sup>[17]</sup> In this case, an increased blood level of  $PaO_2$  after HBO therapy is an expected finding. In our study,  $PaO_2$  levels were slightly higher than expected in the HBO group than in the control group, but the difference was not statistically significant. The only moderate increase in  $PaO_2$  levels in our HBO group was attributed to the increased alveolar injury in this group, as supported by histopathological

findings. On the other hand, another study, which examined the effect of HBO on hypoxemia, showed that hypoxemia was improved shortly after HBO administration, but a temporary hypoxic phase was observed, and it approached normal values at hour 24.<sup>[11]</sup>

Another treatment group of our study received ozone therapy in addition to antibiotherapy. Although the levels of SOD and MPO did not statistically significantly differ in this group, the IL-10 level was statistically significantly higher in the ozone group than in the control and HBO groups. There was also no statistical difference in the levels of TGF-beta, another anti-inflammatory parameter, but the highest level was observed in this group. This suggests that ozone therapy may be an option as an anti-inflammatory treatment. We observed another significant difference in blood gas parameters. The highest PaO<sub>2</sub> and lowest PaCO<sub>2</sub> levels were in the ozone group, which supports our view that ozone therapy reduces ventilation/perfusion imbalance due to alveolar injury. Considering the overall histopathological picture, the rate of injury of >75% was lower than in the HBO and control groups. Although statistically insignificant, inflammatory cell accumulation was relatively less in the ozone group, and, in particular, the macrophage density was lower than in other groups.

There are studies in the literature showing that ozone therapy has antioxidant and anti-inflammatory effects, which is in line with our study.<sup>[7,12]</sup>

The lack of a statistically significant difference in SOD levels of the ozone group in our study suggests that our ozone therapy did not cause any significant antioxidant activity. However, H<sub>2</sub>O<sub>2</sub>, an essential ROS molecule, can act as a precursor of ozone. In addition, ozone has been shown to support cellular antioxidant systems including SOD, CAT, and enzymatic reactions.<sup>[18]</sup> Here, the dosage of ozone becomes important. Because a concept of physiological threshold has been specified for ozone therapy. In most cases, an ozone dose of <10 µg/mL in blood means that it is biologically ineffective as it is completely neutralized by plasma antioxidants. In other words, the concept of threshold helps to understand that a very low dose of ozone may be ineffective (the placebo effect), while a dose higher than the therapeutic dose may be toxic.<sup>[19]</sup> The ozone dose we preferred had a high anti-inflammatory effect, but the antioxidant dose might be insufficient.

Our study obtained statistically significant results on PaO<sub>2</sub> and PaCO<sub>2</sub> levels in the ozone group. While the PaO<sub>2</sub> level was significantly higher in the ozone group, the PaCO<sub>2</sub> level was statistically significantly lower. Statistically significant results were not obtained for other groups. These data suggest that ozone therapy is more effective in blood gas parameters compared with other treatments and that alveolar injury was less in the ozone therapy group. In addition, ozone increases the production of 2,3-diphosphoglycerate in erythrocytes, shifting the oxyhemoglobin dissociation curve to the left and increasing oxygen delivery to the tissues. Ozone therapy increases oxygen saturation in blood, serum, and erythrocytes. There are reports on increased PaO<sub>2</sub> and decreased PaCO<sub>2</sub> in blood gas in patients receiving ozone therapy in the form of major autohemotherapy.<sup>[20]</sup> Deng et al.<sup>[21]</sup> conducted a study showing that ozone therapy improved PaO<sub>2</sub> levels in patients with aortic dissection. This effect of ozone therapy was also mentioned in another study,<sup>[22]</sup> and these studies support our view.

Another treatment group of our study received dexpanthenol in addition to antibiotherapy. Remarkable data were obtained in this group as well. Antioxidant SOD levels were statistically significantly lower than in the control group. This suggested that this group had the least oxidative damage in our study. The IL-10 level was statistically higher in the dexpanthenol group than in the HBO group and was higher than in the control group although the difference was statistically insignificant. These data can be evaluated that dexpanthenol reduced oxidative damage and promoted anti-inflammatory parameters, and the histopathological results were also positive. Considering the overall histopathological picture, the rate of injury of >75% was the lowest in this group. Another remarkable finding was the statistically significantly lower scores especially on fibrosis and collapse in this group than in other groups.

There are studies in the literature showing the antioxidant and anti-inflammatory effects of dexpanthenol.<sup>[4,23,24]</sup> These studies support our view that dexpanthenol reduces oxidative damage and inflammation. Unlike these studies, we associated the anti-inflammatory activity of dexpanthenol mostly with an increase in IL-10 levels.

Dexpanthenol is enzymatically oxidized to the common pantothenic acid (PA) and distributed into tissues. PA

protects tissues against cell injury caused by ROS. PA supports cellular antioxidant systems, including GSH (reduced glutathione), GPx, SOD, CAT, and other enzymatic reactions, and prepares the host for the enzymatic reactions required to cope with ROS-mediated pathophysiological conditions. GSH and GPx are the main defense systems against lipid peroxidation and oxidative stress. SOD and CAT are antioxidant enzyme components of the defense mechanism against ROS activities.<sup>[9]</sup> Our study established a statistically significantly lower level of SOD in the dexpanthenol group than in the control group. This suggested that the level of oxidative damage was lower in the dexpanthenol group. There are studies in the literature that support our view. Leff et al.<sup>[25]</sup> reported increased SOD and CAT levels and reduced GPx levels in sepsis patients with ARDS compared with those without ARDS. Other studies also established a correlation between low SOD levels and reduced cell injury, supporting our view.<sup>[18,26]</sup>

One of the remarkable findings of our study was that fibrosis was less severe in the dexpanthenol group than in other treatment groups. There are studies that mention the positive effects of dexpanthenol on fibrosis.<sup>[27,28]</sup> The study by Ermis et al.<sup>[29]</sup> showed that dexpanthenol had an effect on preventing bleomycin-induced pulmonary fibrosis. This was attributed to the bleomycin triggering inflammation and collagen deposition with a significant increase in MPO activity, like an increased inflammatory activity, which was prevented by dexpanthenol. Although the effect of dexpanthenol on MPO activity was similar to that of other treatment groups in our study, the significantly decreased level of fibrosis suggests that further studies are needed to understand the antifibrotic effect of dexpanthenol.

In addition, the absence of significant change in blood gas parameters in the dexpanthenol group, despite the reduced tissue injury, was attributed to the lack of an effect of dexpanthenol on partial oxygen solubility in the blood like HBO and ozone therapies.

## Conclusion

The HBO therapy increased cell injury considering antioxidant and anti-inflammatory effects and histopathologically. The ozone therapy produced positive outcomes in terms of anti-inflammatory effect and histopathologically.

The ozone therapy provided significant improvement in arterial oxygenation. The dexpanthenol therapy, in turn, produced an antioxidant effect and histopathologically positive outcomes. The antifibrotic effect was prominent with the dexpanthenol therapy. Further studies are needed to generalize the use of these treatments in ARDS.

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## Conflicts of interest

There are no conflicts of interest.

## Ethics Committee Approval

The study was approved by the Çanakkale Onsekiz Mart University (ÇOMÜ) the Animal Experiments Local Ethics Committee (No: 2020/06-02, Date: 30/06/2020).

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## Peer-review

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## Authorship Contributions

Concept – M.Y., P.M., N.A.M., H.O.K., A.G.B., M.İ.G., Ş.K., H.Y.Ç.; Design – M.Y., P.M., N.A.M., H.O.K., A.G.B., M.İ.G., Ş.K., H.Y.Ç.; Supervision – M.Y., P.M., N.A.M., H.O.K., A.G.B., M.İ.G., Ş.K., H.Y.Ç.; Funding – M.Y., P.M., N.A.M.; Materials – M.Y., P.M., A.G.B., M.İ.G., H.Y.Ç.; Data collection &/or processing – M.Y., P.M., H.O.K., Ş.K.; Analysis and/or interpretation – M.Y., P.M., N.A.M., H.O.K., A.G.B., M.İ.G., Ş.K., H.Y.Ç.; Literature search – M.Y., P.M., N.A.M.; Writing – M.Y., P.M., N.A.M., H.O.K., A.G.B., M.İ.G., Ş.K., H.Y.Ç.; Critical review – M.Y., P.M., N.A.M.

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