

Evaluation of the efficacy of silymarin and dexmedetomidine on kidney and lung tissue in the treatment of sepsis in rats with cecal perforation

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Abstract. Sepsis is a systemic inflammatory response syndrome that develops in the host against microorganisms. This response develops away from the primary infection site and results in end-organ damage. The present study aimed to investigate the protective and therapeutic effects on lung and kidney tissue of silymarin (S) and dexmedetomidine (DEX) applied 1 h before and after sepsis induced by the cecal ligation and puncture (CLP) method in rats. A total of 62 rats was randomly divided into eight groups: i) Control (n=6); ii) cecal perforation (CLP; n=8); iii) S + CLP (n=8; S + CLP; S administered 1 h before CLP); iv) CLP + S (n=8; S administered 1 h after CLP); v) DEX + CLP (n=8; D + CLP; DEX administered 1 h before CLP); vi) CLP + D (n=8; DEX administered 1 h after CLP); vii) SD + CLP (n=8; S and DEX administered 1 h before CLP) and viii) CLP + SD (n=8; S and DEX administered 1 h after CLP). After the cecum filled with stool, it was tied with 3/0 silk under the ileocecal valve and the anterior surface of the cecum was punctured twice with an 18-gauge needle. A total of 100 mg/kg silymarin and 100 µg/kg DEX were administered intraperitoneally to the treatment groups. Lung and kidney tissue samples were collected to evaluate biochemical and histopathological parameters. In the histopathological examination, all parameters indicating kidney injury; interstitial edema, peritubular

capillary dilatation, vacuolization, ablation of tubular epithelium from the basement membrane, loss of brush border in the proximal tubule epithelium, cell swelling and nuclear defragmentation; were increased in the CLP compared with the control group. Silymarin administration increased kidney damage, including ablation of tubular epithelium from the basement membrane, compared with that in the CLP group. DEX significantly reduced kidney damage compared with the CLP and silymarin groups. The co-administration of DEX + silymarin decreased kidney damage, although it was not as effective as DEX-alone. To conclude, intraperitoneal DEX ameliorated injury in CLP rats. DEX + silymarin partially ameliorated injury but silymarin administration increased damage. As a result, silymarin has a negative effects with this dosage and DEX has a protective effect. In the present study, it was determined that using the two drugs together had a greater therapeutic effect than silymarin and no differences in the effects were not observed any when the application times of the agents were changed.

Introduction

Sepsis is a systemic inflammatory response syndrome (SIRS) that develops in the host against microorganisms. This response develops away from the primary infection area and results in end-organ damage (1). The response that occurs during infection in healthy individuals continues with pathogen recognition, control and rapid tissue repair (2,3). Upon activation of the cell-mediated immune response, anti-/pro-inflammatory mediators are released (4,5). Overactivation by powerful pathogens leads to endothelial damage, tissue hypoperfusion, disseminated intravascular coagulation, treatment-resistant shock, multiple organ damage and death (6). Although a number of treatment methods have been developed such as antibiotics, corticosteroids, fluid and adjunctive therapies; SIRS and sepsis have high mortality and morbidity in intensive care units (7). In 2017, 48.9 million cases of sepsis were reported worldwide,

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of which 11 million resulted in death (8). Similar proportions of mortality and incidence have been reported in European countries (9-14). The current clinical approach to treatment starts with early diagnosis, identification of the source of infection and early antibiotic treatment, with corticosteroids also playing an important role (1). However, although there are studies showing that steroid treatment reduces mortality in sepsis, its effects on long-term mortality are controversial (15,16). Therefore, the effectiveness of novel drugs is being investigated in experimental and clinical studies (17-21).

Milk thistle (*Silybum marianum*) is a historical medicinal plant and its well-known flavonoid silymarin is an agent that has promising therapeutic efficacy in different clinical studies (22-24). *S. marianum* is a herbal product used in Ancient Greek medicine to treat gallbladder disorder and protect the liver from toxic agents (25). Furthermore, silymarin preparations have been used to treat liver and other gastrointestinal diseases due to hepatoprotectivity, neuroprotectivity, anti-fungal and anti-cancer activity (23,26,27). The anti-inflammatory activity of silymarin may underlie the positive effects of the agent (26,28). Several studies have demonstrated the anti-inflammatory activities of silymarin, which inhibits interferon- γ , IL-4 and IL-10 in a dose-dependent manner (29-31). Silymarin suppresses NF- κ B binding transporter gene transcription in a rat model of sepsis (32). In addition to its cell-protective effects via antioxidative and radical scavenging activity, silymarin also acts via specific receptor interactions such as P-glycoproteins and estrogen and nuclear receptors (29). Derivatives of silymarin could provide new avenues for therapeutic applications. However, although certain researchers have reported silymarin to be well-tolerated and safe clinically, there are also conflicting results (24,33-35). While gastrointestinal and neurological side effects were reported in the study by Schrieber *et al* (33); there are also studies in the literature, in which no adverse events were observed despite using similar or higher doses (34,35). Therefore, it is crucial that this agent be studied experimentally in organs and tissues before use in clinical practice. Furthermore, the origin of the milk thistle plant, from which silymarin is obtained, is along the Mediterranean coast of Europe and therefore, the fact that this herbal flavonoid is quite common in Anatolia (36) was also effective in its selection in the present study as it is possible to obtain pure raw materials from this plant in Turkey, where the present study was performed.

The activation of adrenergic α 2 receptors causes hypotension, bradycardia, sedation, arterial and venous vasoconstriction, decreased presynaptic transmitter release, thrombus stabilization, hypothermia, decreased gastric acid secretion and motility and inhibition of lipolysis and pancreatic insulin release (37,38). A number of studies has shown that sepsis is associated with sympathetic overactivation, which may contribute to end-organ damage (39,40). In septic shock, increased endogenous sympathetic outflow plays a major role in maintaining vascular tone and tissue perfusion (41). Despite elevated concentrations of endogenous vasoconstrictors, such as noradrenaline, downregulation of adrenergic receptors and post-receptor signaling pathways leads to significant decline in vascular response (40,41). To prevent the negative consequences of excessive sympathetic flow, researchers have investigated the

use of sympathetic blockade in the treatment of sepsis (42,43). According to Pichot *et al*, inhibiting sympathetic activity with an α 2 agonist corrects vascular reactivity by upregulating α 1 receptors in septic shock, thereby decreasing the need for vasopressors (44). Similarly, response to norepinephrine decreases following application of lipopolysaccharide and the administration of α 2 agonists increases this response in rats (45). Dexmedetomidine (DEX), is one of the most commonly used sedation agents in intensive care (46-48). As a highly selective α 2-adrenoreceptor agonist, DEX serves as an adjunctive therapy through pro-inflammatory downregulation and control of the anti-inflammatory response in patients with sepsis (49). DEX suppresses the release of TNF- α , IL-6, IL-8 and high mobility group box-1 (HMGB-1) in human whole blood cultured with lipopolysaccharide (50). The suppressive effect of DEX on proinflammatory mediator production occurs via α 2 adrenergic receptors (49). There are numerous experimental and retrospective observational studies on the benefits of this agent in sepsis, which is the most common cause of mortality in intensive care units (8,51). To the best of our knowledge, however, there are still insufficient data on the specific protective benefits of this agent on tissue and organs. Various studies have shown that DEX, similar to silymarin, has potential benefits by inducing antioxidant pathways in different clinical situations such as ischemia-reperfusion, cancer and sepsis (52-55). Therefore, it was hypothesized these two agents together may show strong antioxidant activity and decrease tissue and organ damage.

There are three current approaches frequently used to construct sepsis models: Lipopolysaccharide administration, intravascular or intraperitoneal administration of live bacteria and the cecal ligation and puncture (CLP) method (56). The CLP method provides the closest results to sepsis in humans (57). Although the efficacy of experimental sepsis models in animals and their adaptability to human studies have been discussed for some time (58), the cecal ligation and puncture method still remains valid (59).

The aim of the present study was to investigate the protective and therapeutic effects of silymarin and DEX in CLP-induced sepsis in rat lung and kidney tissues.

Materials and methods

Animal studies. The present study was conducted at the Gazi University Animal Experiments Laboratory (Ankara, Turkey) in July 2021 in accordance with the ARRIVE guidelines (60). The present study was approved by The Local Ethics Committee of Gazi University Animal Experiments (approval no. G.Ü.E.T-20.022; Ankara, Turkey). Animal studies were performed in accordance with The Guide for the Care and Use of Laboratory Animals by the National Institutes of Health (61). A total 62 male Wistar Albino rats (Gazi University Animal Experiments Laboratory, Ankara, Turkey) weighing 225-300 g were used. Rats were kept in a temperature-controlled ($21 \pm 1^\circ\text{C}$) and humidity-controlled (45-55%) room and were maintained under a 12-h light/dark cycle. The animals were fed a standard pellet diet and allowed to drink water *ad libitum*. Rats were randomly divided into eight groups as follows: i) Control (n=6); ii) cecal perforation (CLP; n=8); iii) silymarin + CLP (n=8; S + CLP; silymarin

administered 1 h before CPL); iv) CLP + S (n=8; silymarin administered 1 h after CLP); v) DEX + CLP (n=8; D + CLP; DEX administered 1 h before CLP); vi) CLP + D (n=8; DEX administered 1 h after CLP); vii) SD + CLP (n=8; silymarin and DEX administered 1 h before CLP) and viii) CLP + SD (n=8; silymarin and DEX administered 1 h after CLP).

Rats were anesthetized by 50 mg/kg intramuscular ketamine hydrochloride (Ketalar® vial; Parke-Davis; Pfizer, Inc.) and 10 mg/kg xylazine hydrochloride (Alfazyne; 2%; EGE VET) and placed on a heating pad to maintain their body temperature. Midline laparotomy was performed in rats whose skin was aseptically prepared. The intestines were removed using wet gauze. In the control group, the cecum was manipulated. However, drilling and ligation were not performed.

After the cecum filled with stool, it was tied with 3/0 silk under the ileocecal valve and the anterior surface of the cecum was punctured twice using an 18-gauge needle. No treatment (e.g., dexmedetomidine or silymarin) was applied to the sham or CLP group. Saline was applied to the peritoneal space to minimize heat and fluid loss. A total of 100 mg/kg silymarin (Sigma-Aldrich; Merck KGaA; cat. no. SO292-50G) and 100 µg/kg DEX (Sedodamid; 100 µg/2 ml; Koçak Farma®) was administered intraperitoneally to the treatment groups. All the rats were sacrificed 24 h after the operation; rats were anesthetized with ketamine (50 mg/kg) and xylazine (10 µg/kg) and sacrificed by collecting blood (5-10 ml) from the abdominal aorta. After heartbeat and respiration ceased, rats were monitored for a further 2 min to confirm death. Tissue samples were stored at -70°C for biochemical analysis and immersed in 10% neutral buffered formalin for histopathological assessment.

In the present study, two rats were lost in the CLP and S + CLP groups and one rat in the CLP + S group. No losses were observed in any of the other groups. In the first 24 h, mortality rates in the CLP and S groups were similar to those reported by Kang *et al* (32), Al-Kadi *et al* (62) and Canikli Adigüzel *et al* (63).

Histopathological evaluation. Lung and kidney tissue specimens were fixed in 10% neutral-buffered formalin for 48 h at room temperature and embedded in paraffin after routine tissue processing. Tissue specimens were dehydrated through an increasing-grade series of ethanol. Dehydrated specimens were cleared in xylene, infiltrated in liquid paraffin at 60°C, and embedded in paraffin. Thereafter, 5 µm-thick tissue sections were cut from paraffin blocks using a microtome (Leica SM 2000; Leica Microsystems GmbH) and stained with hematoxylin and eosin (H&E) to analyze histopathological changes. Lung and kidney sections were incubated with hematoxylin and eosin stain solutions for 12 min each, at room temperature. The stained sections were assessed under a light microscope (Leica DM 4000 B; Leica Microsystems GmbH) equipped with a computer, and micrographs were captured using Leica LAS V4.9 software (Leica Microsystems GmbH).

H&E-stained kidney sections were examined under x400 magnification and renal injury was evaluated semi-quantitatively. Histopathological parameters, including interstitial edema, peritubular capillary dilatation, vacuolization, ablation of tubular epithelium from the basement membrane, loss of brush border in the proximal tubule epithelium, cell swelling

and nuclear defragmentation, were scored 0-3 (0, none; 1, mild; 2, moderate; 3, severe) and the mean score was determined for each parameter in each group (64).

H&E-stained lung samples were examined under 200x and 400x magnification and lung injury was assessed semi-quantitatively. Alveolar wall thickening, capillary congestion, intra-alveolar hemorrhage and interstitial and intra-alveolar neutrophil infiltration were scored 0-3 (0, none; 1, mild; 2, moderate; 3, severe), and the mean score was determined for each parameter (65).

Biochemical determination. Total antioxidant status (TAS) and total oxidative status (TOS) were analyzed in blood samples. TAS and TOS were measured using test kits according to the manufacturer's instructions (Rel Assay Diagnostics®). TAS levels were calculated as follows: $TAS = [(\Delta \text{Absorbance (Abs)} \text{ H}_2\text{O} - \Delta \text{Abs sample}) / (\Delta \text{Abs H}_2\text{O} - \Delta \text{Abs standard})]$, and the results were expressed in mmol Trolox Eq/l. TOS levels were calculated as follows: $TOS = (\Delta \text{Abs sample} / \Delta \text{Abs standard}) \times \text{standard concentration (10 } \mu\text{mol/l)}$, and the results were expressed in µmol H₂O₂ Eq/l.

Statistical analysis. All data are expressed as the mean ± standard deviation (SD) or standard error of mean (SEM). The experiments was performed once. All statistical analyses were performed using SPSS (version 26.0; IBM Corp.). The distribution of data was analyzed using the Shapiro-Wilk test. Comparisons of >2 groups were performed using Kruskal-Wallis test followed by Dunn's post hoc test or one-way ANOVA followed by Tukey's post hoc test. P<0.05 was considered to indicate a statistically significant difference. The intention to treat analysis method was used (66-68).

Results

Kidney tissue histopathological results. The mean scores for histopathological changes in kidney specimens are summarized in Table I. The severity of interstitial edema in kidney was significantly different between the groups (P=0.003); it was more severe in the CLP, S + CLP, SD + CLP and CLP + SD groups than in the control group (P=0.008, P=0.001, P=0.016 and P=0.004, respectively). Interstitial edema was decreased in the D + CLP group compared with that in the CLP group (P=0.013). The interstitial edema score was significantly lower in the D + CLP and CLP + D groups than in the S + CLP group (P=0.001 and P=0.013, respectively). Peritubular capillary dilatation mean scores were also different (P=0.034), with a significantly higher score in the CLP, S + CLP and CLP + S groups than in the control group (P=0.047, P=0.012 and P=0.012, respectively), whereas it was lower in the CLP + D and CLP + SD groups than in both the S + CLP (P=0.020 and P=0.020, respectively) and CLP + S groups (P=0.020 and P=0.020, respectively; Table I; Fig. 1). Focal cystic formations along the more prominent tubular dilatation were observed in the cortex and medulla of the kidney from S + CLP and CLP + S groups (Fig. 1).

Lung tissue histopathological results. The histopathological changes in the lung samples are summarized in Table II. Alveolar wall thickening in lung scores were significantly

Table I. Histopathological findings in kidney tissue (mean \pm SEM).

Histopathological finding	Control, n=6	CLP, n=8	S + CLP, n=8	CLP + S, n=8	D + CLP, n=8	CLP + D, n=8	SD + CLP, n=8	CLP + SD, n=8	Kruskal Wallis test P-value
Interstitial edema	0.33 \pm 0.21	1.63 \pm 0.26 ^a	2.00 \pm 0.50 ^a	1.25 \pm 0.17	0.50 \pm 0.19 ^{a,b,c}	0.88 \pm 0.30 ^{ab}	1.50 \pm 0.19 ^a	1.75 \pm 0.25 ^a	0.003
Dilatation of peritubular capillaries	0.83 \pm 0.31	1.75 \pm 0.45 ^a	2.00 \pm 0.33 ^a	2.00 \pm 0.27 ^a	1.63 \pm 0.33	1.00 \pm 0.19 ^{c,d}	1.25 \pm 0.16	1.00 \pm 0.27 ^{c,d}	0.034
Vacuolization	0.17 \pm 0.17	1.75 \pm 0.45 ^a	2.50 \pm 0.27 ^a	2.25 \pm 0.49 ^a	0.38 \pm 0.18 ^{b,c,d}	0.63 \pm 0.26 ^{a,c,d}	0.38 \pm 0.18 ^{b,c,d}	1.00 \pm 0.00 ^{a,b,d}	<0.001
Ablation of tubular epithelium from the basement membrane	0.50 \pm 0.22	1.00 \pm 0.50	1.88 \pm 0.40 ^a	2.75 \pm 0.17 ^{a,b}	1.13 \pm 0.44 ^d	0.50 \pm 0.19 ^{c,d}	0.38 \pm 0.19 ^{c,d}	0.50 \pm 0.19 ^{c,d}	<0.001
Loss of brush border in proximal tubule epithelium	1.16 \pm 0.31	2.88 \pm 0.13 ^a	2.38 \pm 0.32 ^a	2.75 \pm 0.16 ^a	1.75 \pm 0.25 ^{b,d}	1.75 \pm 0.16 ^{b,d}	2.12 \pm 0.30 ^{a,b}	1.88 \pm 0.23 ^{b,d}	<0.001
Cell swelling and nuclear defragmentation	1.33 \pm 0.21	2.63 \pm 0.25 ^a	2.50 \pm 0.27 ^a	1.75 \pm 0.31 ^b	1.50 \pm 0.19 ^{b,e}	1.38 \pm 0.19 ^{b,e}	1.88 \pm 0.30 ^b	1.75 \pm 0.31 ^b	0.004

P<0.05 vs. ^acontrol, ^bCLP, ^cS + CLP and ^dCLP + S. CLP, cecal ligation and puncture; S, silymarin; D, dexmedetomidine; SD, S + D.Table II. Histopathological findings of lung tissue (mean \pm SEM).

Histopathological finding	Control, n=6	CLP, n=8	S + CLP, n=8	CLP + S, n=8	D + CLP, n=8	CLP + D, n=8	SD + CLP, n=8	CLP + SD, n=8	Kruskal Wallis test P-value
Thickening in the alveolar wall	0.50 \pm 0.34	2.88 \pm 0.13 ^a	1.88 \pm 0.30 ^{a,b}	1.50 \pm 0.27 ^{a,b}	1.38 \pm 0.27 ^{a,b,c}	1.50 \pm 0.19 ^{a,b,c}	1.00 \pm 0.00 ^{b,c}	1.38 \pm 0.18 ^{a,b}	<0.001
Capillary congestion	1.00 \pm 0.52	2.63 \pm 0.18 ^a	2.00 \pm 0.19 ^a	1.75 \pm 0.32 ^b	1.25 \pm 0.16 ^{b,c}	1.50 \pm 0.16 ^b	1.38 \pm 0.26 ^b	1.38 \pm 0.18 ^b	0.001
Intra-alveolar hemorrhage	0.00 \pm 0.00	1.38 \pm 0.32 ^a	1.00 \pm 0.36 ^a	0.88 \pm 0.35 ^a	0.38 \pm 0.26 ^b	0.13 \pm 0.13 ^{b,c}	0.38 \pm 0.26 ^b	0.00 \pm 0.00 ^{b,c,d}	0.006
Interstitial neutrophil infiltration	1.17 \pm 0.17	2.25 \pm 0.16 ^a	1.62 \pm 0.18 ^b	1.50 \pm 0.19 ^b	1.63 \pm 0.18 ^b	1.50 \pm 0.19 ^b	1.25 \pm 0.25 ^b	1.50 \pm 0.19 ^b	0.015
Intra-alveolar neutrophil infiltration	0.17 \pm 0.17	0.50 \pm 0.19	0.25 \pm 0.16	0.50 \pm 0.19	0.38 \pm 0.26	0.00 \pm 0.00	0.13 \pm 0.13	0.00 \pm 0.00	0.158

P<0.05 vs. ^acontrol, ^bCLP, ^cS + CLP and ^dCLP + S. CLP, cecal ligation and puncture; S, silymarin; D, dexmedetomidine; SD, S + D.

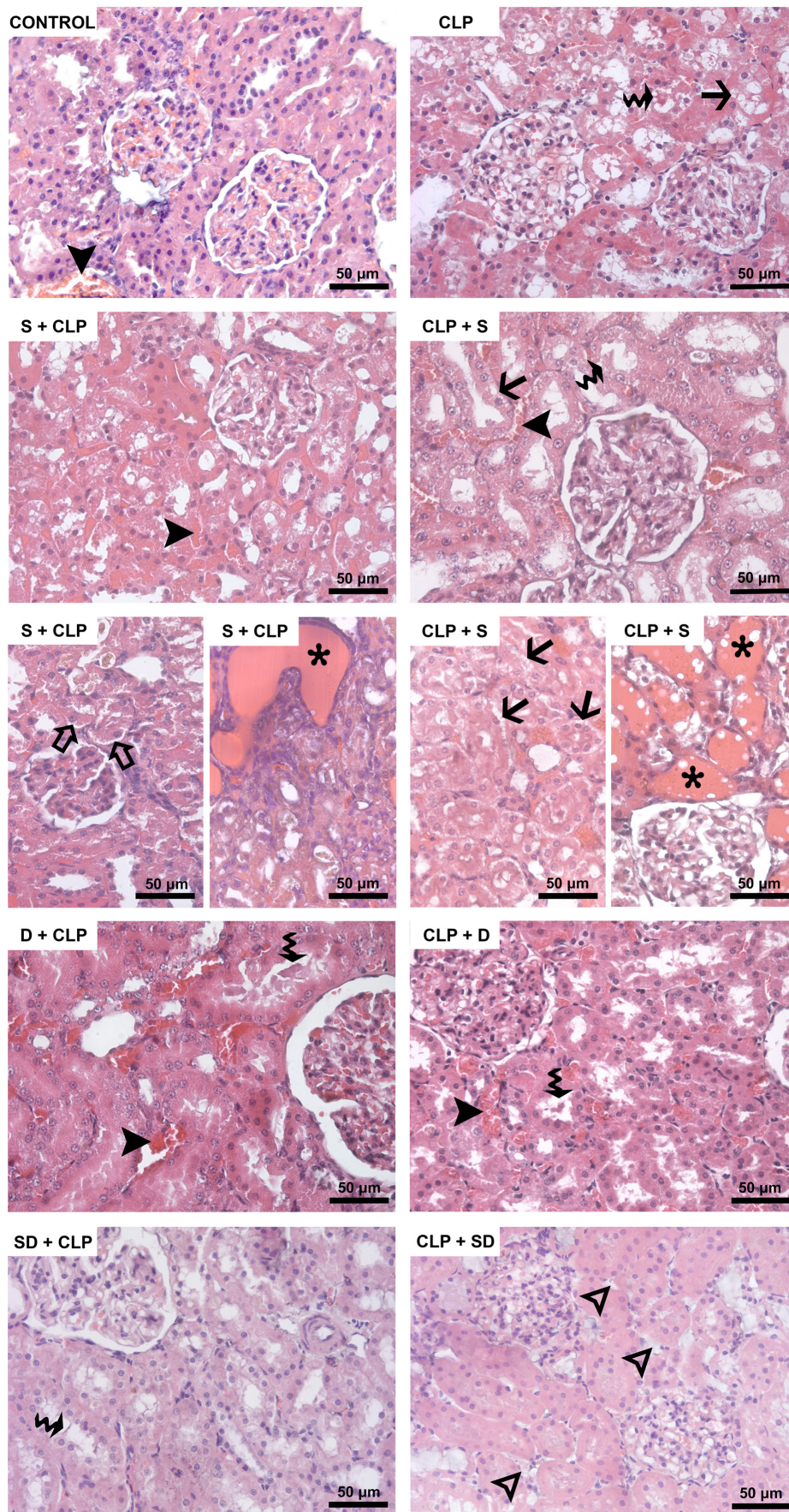


Figure 1. Hematoxylin and eosin-stained kidney sections. Black arrowhead, dilatation of peritubular capillaries. Waved arrow, loss of brush border in proximal tubule epithelium. Black arrow, vacuolization in tubular epithelial cells. Hollow arrowhead, interstitial edema. Hollow arrow, ablation of tubular epithelium from the basement membrane. Asterisk, focal cysts in both the cortex and medulla. CLP, cecal ligation and puncture; S, silymarin; D, dexmedetomidine; SD, S + D.

different between the groups ($P<0.0001$). Alveolar wall thickening in the CLP, S + CLP, CLP + S, D + CLP, CLP + D and CLP + SD groups was greater than that in the control group ($P<0.0001$, $P<0.0001$, $P=0.004$, $P=0.012$, $P=0.004$ and $P=0.012$, respectively). However, it was significantly reduced in S + CLP, CLP + S, D + CLP, CLP + D, SD + CLP and CLP + SD groups compared with the CLP group ($P=0.002$, $P<0.0001$, $P<0.0001$, $P<0.0001$ and $P<0.0001$, respectively). Furthermore, this decrease was more prominent in the SD + CLP group than that of the S + CLP group ($P=0.007$). The difference in severity of interstitial neutrophil infiltration between the groups was also significant ($P=0.015$). It was significantly more severe in the CLP than in the control group ($P<0.0001$), whereas it was improved in the S + CLP, CLP + S, D + CLP, CLP + D, SD + CLP and CLP + SD groups compared with that in the CLP group ($P=0.024$, $P=0.007$, $P=0.024$, $P=0.007$, $P<0.0001$ and $P=0.007$, respectively). By contrast, intra-alveolar neutrophil infiltration scores of all the groups were similar ($P=0.158$; Table II; Fig. 2).

Lung tissue biochemical results. There was a significant difference in lung TOS and TAS levels ($P=0.001$ and $P=0.001$, respectively). The TOS levels were significantly higher in the CLP, S + CLP, CLP + S, SD + CLP and CLP + SD groups than in the control group ($P<0.0001$, $P<0.0001$, $P<0.0001$, $P=0.044$ and $P=0.005$, respectively). TOS levels were significantly lower in the D + CLP, CLP + D and SD + CLP groups than in the CLP group ($P=0.032$, $P=0.002$ and $P=0.043$, respectively). TOS levels were significantly lower in the D + CLP and CLP + D groups than in the S + CLP group ($P=0.041$ and $P=0.006$, respectively; Fig. 3). Similarly, TOS levels were significantly lower in the D + CLP and CLP + D groups than in the CLP + S group ($P=0.036$ and $P=0.003$, respectively; Fig. 3).

TAS levels were significantly lower in the CLP, S + CLP and CLP + S groups than in the control group ($P=0.002$, $P=0.039$ and $P=0.047$, respectively). TAS levels were significantly higher in the D + CLP, CLP + D, SD + CLP and CLP + SD groups than in the CLP group ($P<0.0001$, $P<0.0001$, $P=0.001$ and $P<0.0001$, respectively). Similarly, TAS levels were significantly higher in the D + CLP, CLP + D, SD + CLP and CLP + SD groups than in the S + CLP group ($P=0.014$, $P=0.008$, $P=0.035$ and $P=0.021$, respectively; Fig. 4).

Kidney tissue biochemical results. There was a significant difference in kidney TOS and TAS levels ($P<0.0001$ and $P<0.0001$, respectively). TOS levels were significantly higher in the CLP, S + CLP and CLP + S groups than in the control group ($P=0.004$, $P=0.010$ and $P=0.027$, respectively). TOS levels were significantly lower in the D + CLP, CLP + D and SD + CLP groups than in the CLP group ($P<0.0001$, $P<0.0001$ and $P=0.006$, respectively). TOS levels were significantly lower in the D + CLP, CLP + D and SD + CLP groups than in the S + CLP group ($P<0.0001$, $P=0.001$ and $P=0.015$, respectively). Similarly, TOS levels were significantly lower in the D + CLP, CLP + D, SD + CLP and CLP + SD groups than in the CLP + S group ($P=0.001$, $P=0.003$, $P=0.035$ and $P=0.042$, respectively; Fig. 5).

TAS levels were significantly lower in the CLP, S + CLP and CLP + S groups than in the control group ($P<0.0001$,

$P=0.044$ and $P=0.035$, respectively). TAS levels were significantly higher in the D + CLP, CLP + D, SD + CLP and CLP + SD groups than in the CLP group (all $P<0.0001$). The TAS levels were significantly higher in the D + CLP, CLP + D and CLP + SD groups than in the CLP group ($P=0.005$, $P=0.003$ and $P=0.012$, respectively). Similarly, TAS levels were significantly higher in the D + CLP, CLP + D, and CLP + SD groups than in the S + CLP group ($P=0.044$, $P=0.002$, and $P=0.009$, respectively; Fig. 6).

Discussion

In the clinical use of agents, prophylactic efficacy is as important as therapeutic efficacy. Therefore, the present study aimed to observe both the therapeutic and preventive effects of dexmedetomidine and silymarin. The present study observed differences following application of agents both before and after sepsis modeling.

Silymarin and DEX have been used in different doses in different studies and a definite effective dose has not been determined yet (24,69). In the present study, dose selection was based on similar studies (70-75). Treatment time was also determined based on previous studies, but since both preventive and therapeutic effects were investigated in the clinical sepsis model, separate groups were created for application times (62,76).

Since the polymicrobial peritonitis table created by the CLP model is termed sepsis in studies in the literature (77-84), it was assumed that the clinical picture created by the CLP method in the present study constitutes a sepsis model. CLP, which is an experimental technique, may not mimic sepsis in exactly the same way. Of course, due to the dynamic and developing nature of science, it may be possible to perform more accurate sepsis modeling in the coming years if different techniques are discovered.

In the present study, histopathological damage in lung and kidney tissue following sepsis modeling was observed. However, this damage was accompanied by decreased TAS and increased TOS. Tissue oxidant-antioxidant balance may result in organ damage, which is in line with the literature (85,86). Sepsis is a common clinical problem and silymarin and DEX have shown promising results in recent experimental studies (87-90).

Sepsis is the most common cause of mortality in intensive care units (91). Lung and kidney involvement is relatively common in sepsis, and dysfunction of these organs is associated with poor survival outcome (92,93). Therefore, it has become increasingly important to identify agents that have therapeutic or protective effects on the lungs and kidney during sepsis.

Silymarin is a herbal flavonoid obtained from the seeds or fruits of *S. marianum* (thistle) (25). Flavonoids, a class of secondary metabolites of plants and fungi, have both prooxidant and antioxidant activity due to their polyphenolic structure (94,95). These effects vary depending on the dose and cell or tissue types (72). For example, Malekinejad *et al* (96) determined that silymarin applied at the same dose and time had a protective effect on the liver, while increasing damage in the brain. Numerous studies have examined the curative and protective effects of

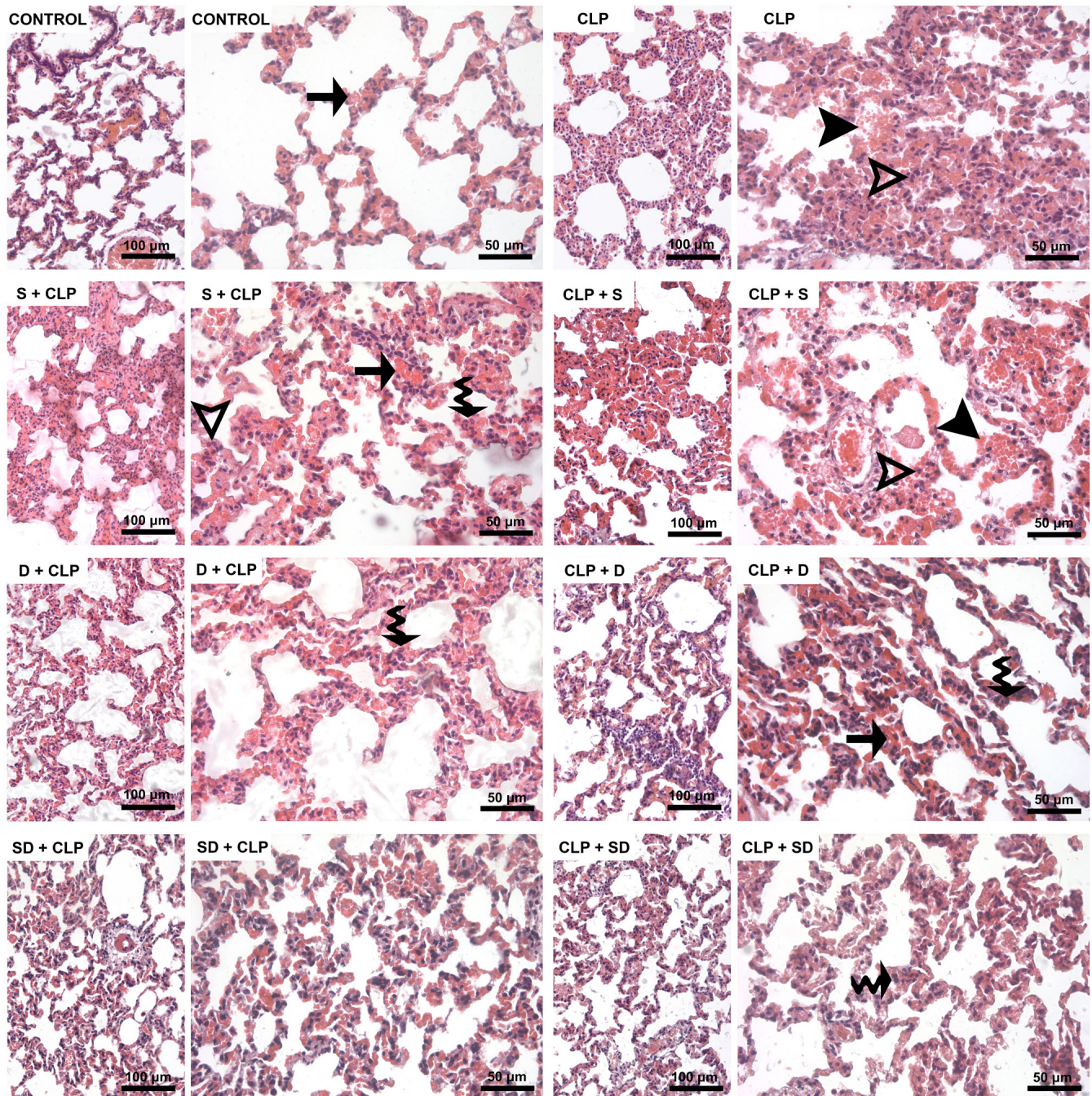


Figure 2. Representative micrographs of hematoxylin and eosin-stained lung sections. Arrowhead, intra-alveolar hemorrhage. Arrow, capillary congestion. Waved arrow, interstitial neutrophil infiltration. Hollow arrowhead, neutrophils within the alveolar space. CLP, cecal ligation and puncture; S, silymarin; D, dexmedetomidine; SD, S + D.

silymarin on kidney and lung tissue through various mechanisms (94,95,97).

Al-Kadi *et al* showed that 1 h after CLP induction, 100 mg/kg silymarin has a protective effect on kidney tissue (62). Toklu *et al* (94) studied serum and plasma oxidation markers in lung tissue and concluded that 50 mg/kg per oral silymarin has potential therapeutic efficacy in a similar sepsis model and they found that silymarin may reduce sepsis-induced oxidative organ injury and that this can be attributed to its ability to balance oxidant-antioxidant status. By contrast with previous studies (32,88,94) in the present study, silymarin was administered 1 h before and after sepsis induction. In our

study, a decrease in organ damage was observed in the kidney and lung tissues examined in histopathological samples, but no statistically significant difference was detected between the groups. In addition, TAS and TOS measurements did not improve in the silymarin-treated groups (S + CLP, CLP + S). This may indicate that the biochemical improvement reported in the literature (98,99) does not significantly contribute to tissue damage observed in sepsis.

Silymarin improves kidney tissue damage (62,71,95), however, this was not observed in the present study. This may be due to differences in the mechanisms that cause damage (ischemia reperfusion, sepsis, toxicity, malignancy) or changes in the selection of

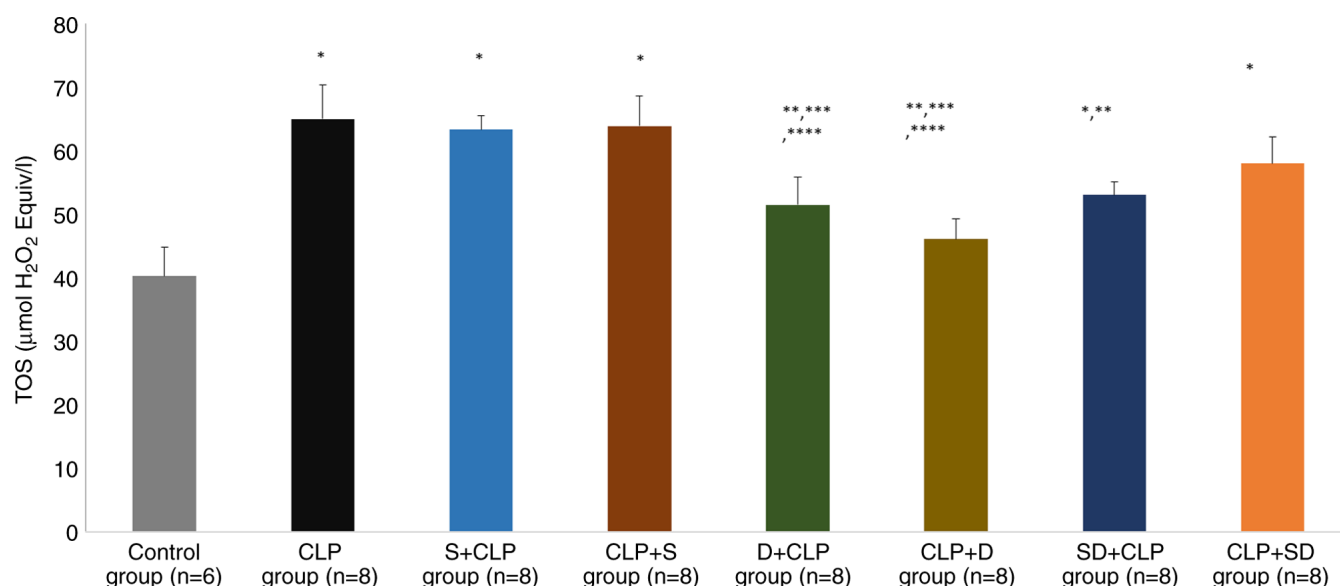


Figure 3. Lung tissue TOS levels (mean \pm SD). $P < 0.05$ vs. *control, **CLP, ***S + CLP and ****CLP + S. CLP, cecal perforation; S, silymarin; D, dexmedetomidine; SD, S + D; TOS, total oxidative status.

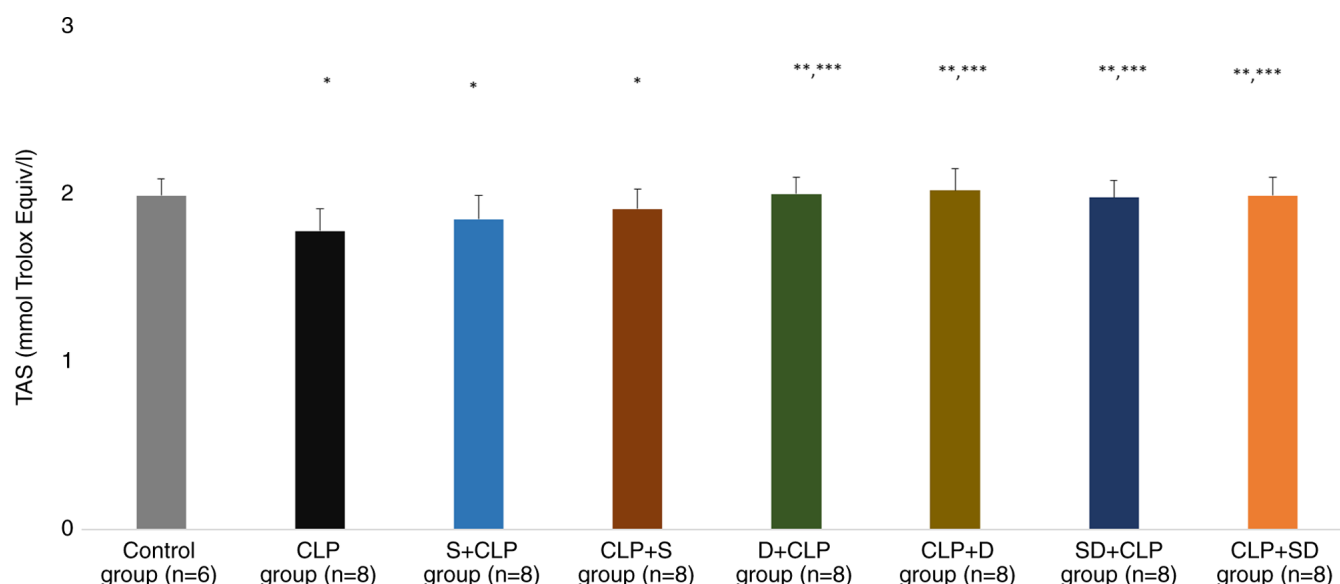


Figure 4. Lung tissue TAS levels (mean \pm SD). $P < 0.05$ vs. *control, **CLP, ***S + CLP. CLP, cecal perforation; S, silymarin; D, dexmedetomidine; SD, S + D; TAS, total antioxidant status.

drug doses. Flavonoids have also been shown to have pro-oxidant activity and these pro-oxidant mechanisms are thought to provide anticarcinogenic activity by triggering cell death in malignant cells (100). Therefore, silymarin has different effects on different tissues at different doses (72,96,100). Although the antioxidant activity of silymarin is well-known (22,100,101), further studies are required to understand its protective effects against sepsis and associated organ damage. In the review of Soleimani *et al* (24), the side effects and doses used in the studies conducted with silymarin were examined and it was seen that it can be used safely at a number of different doses. However, present study suggested that it may have a prooxidant effect on the lung and kidney at the dose used in the experimental sepsis model (100 mg/kg, intraperitoneal). The continued use of silymarin, one of the oldest known

plant-derived medicinal agents, in experimental studies may be due to novel effects, as demonstrated in the present study.

Although silymarin has demonstrated promising results in numerous clinical situations (31,35,72), it needs larger studies with different doses and drug combinations before it can be used clinically for its therapeutic or prophylactic effects. The present study evaluated both preventive and therapeutic efficacy, performed with the one of the highest intraperitoneal doses found in the literature (102) and also including interaction with a different agent. It was hypothesized that the dose of silymarin used had a pro-oxidant effect, as in other studies (72,96,100), and that this is why the animal losses occurred. Using the two drugs together had a greater therapeutic effect than silymarin.

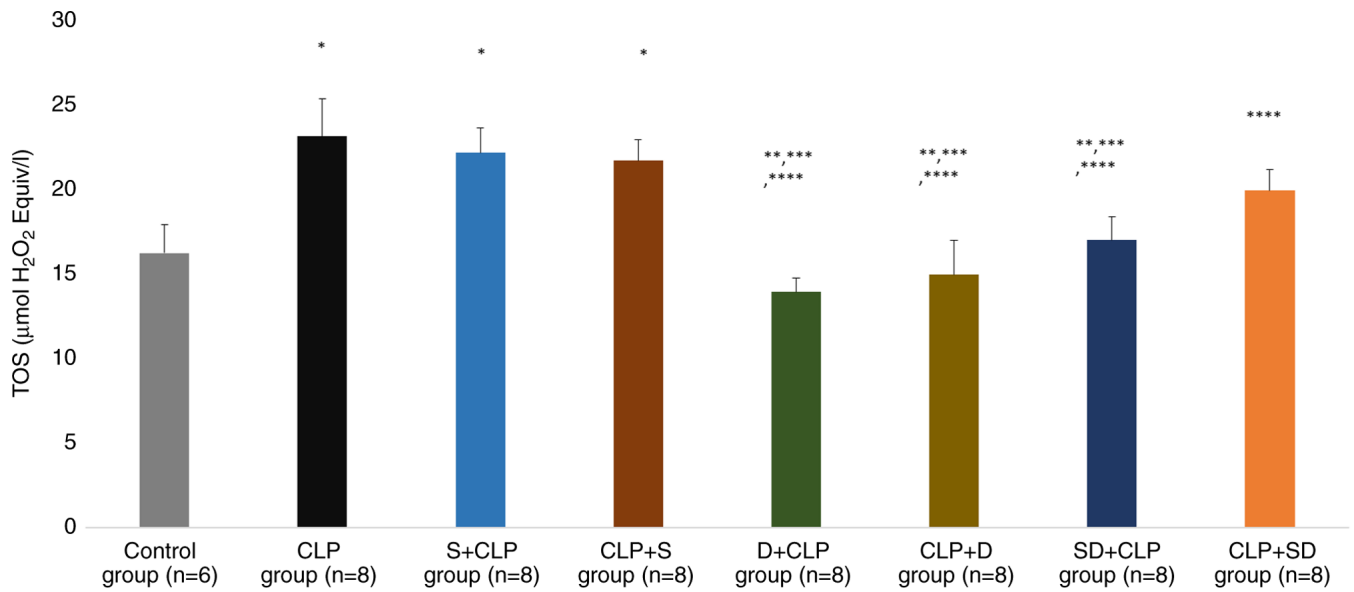


Figure 5. Kidney tissue TOS levels (mean \pm SD). $P < 0.05$ vs. *control, **CLP, ***S + CLP and ****CLP + S. CLP, cecal perforation; S, silymarin; D, dexmedetomidine; SD, S + D; TOS, total oxidative status.

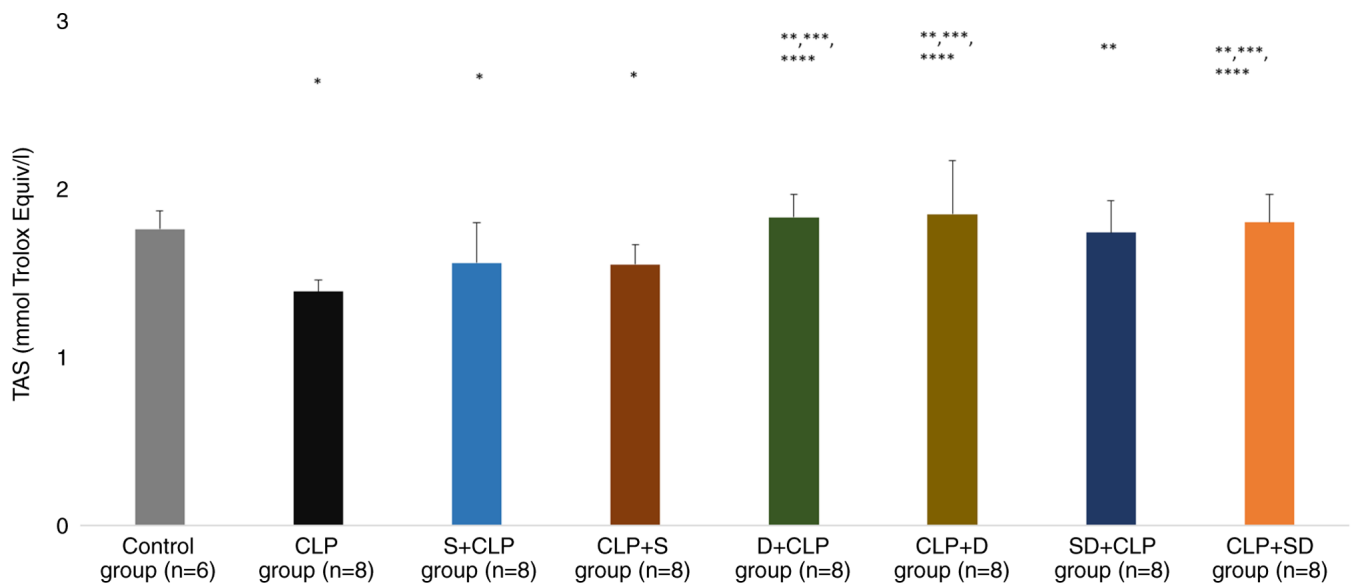


Figure 6. Kidney tissue TAS levels (mean \pm SD). $P < 0.05$ vs. *control, **CLP, ***S + CLP and ****CLP + S. CLP, cecal perforation; S, silymarin; D, dexmedetomidine; SD, S + D; TAS, total antioxidant status.

DEX is a α_2 -adrenergic receptor agonist that exerts sympatholytic effects such as anxiolysis, sedation and analgesia in certain regions of the brain (103). Owing to the absence of side effects such as respiratory depression, it is a frequently preferred agent for sedation in intensive care units (104). The positive effects of DEX on *in vitro* experimental sepsis models have been reported in literature (105,106). For example, Koca *et al* (107) applied 50 $\mu\text{g/kg}$ DEX to rats and observed improvements in both histomorphological and immunohistochemical findings in a sepsis model using CLP technique. Different hypotheses have been proposed for the similar organ-protective effects of DEX and positive results have been obtained. Li *et al* (108) suggested that the lung protective effect of dexmedetomidine in septic rats was achieved through

increasing vagal tone; Wu *et al* (109), in the same experimental model, argued that the protective effect occurs through the TLR4/NF- κ B pathway. Qiu *et al* (76) observed that DEX decreased acute renal failure and increased survival in a sepsis model. They also suggested that this effect occurred via the NF- κ B pathway induced by lipoxin A4.

In the present study, it was hypothesized that DEX, which is known to regulate the oxidant-antioxidant balance in ischemia-reperfusion models (74,75), may show organ-protective effects in sepsis through oxidant-antioxidant balance pathway. Statistically significant positive effects were observed at both the histopathological and biochemical levels in the DEX treatment groups (D + CLP, CLP + D, SD + CLP, CLP + SD). DEX improved lung and kidney tissue damage in the treatment

groups. The effects of DEX administration before and after sepsis were not significantly different.

In the present study, DEX application statistically significantly increased the total antioxidant score in tissues and decreased the oxidant score. In a study conducted by Şengel *et al* (110), TAS and TOS scores in kidney tissue after DEX application showed similar changes as in the present study and a statistically significant improvement in histopathological damage was observed. These data support the hypothesis that DEX may exert positive effects on the lung and kidneys during sepsis. The positive effects of DEX on both TAS and TOS levels and pathological examinations may be a guide for further studies on its mechanism of action.

In previous studies, silymarin and dexmedetomidine have been studied together with different combinations (98,111-116), but no study has been performed in which these two agents were used together. Thus, the present study aimed to investigate the effects of these two agents used together. However, a limitation of the present study was that the mechanism of action of these agents was not examined, and only tissue and organ results were studied. Further studies should study the mechanisms of the effects of these agents.

Although the present study aimed to observe the prophylactic effects of the agents before and after sepsis induction, no differences in the effects were observed when the application times of the agents were changed; however, it may be possible to obtain different results using larger sample sizes. The present study compared both the interactions and preventive and therapeutic effects of promising agents in an experimental sepsis model.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

MAr and AK designed the study and analyzed and interpreted the data. ADD, AY, MAI and ZY performed the experiments. MAI, AİE and ZY confirm the authenticity of all the raw data. AİE, AK, MAr and MAI critically revised the article for important intellectual content. AİE and collected samples. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Ethical approval for the study was obtained from The Gazi University Experimental Animals Ethics Committee (Ankara, Turkey; approval no: G.Ü.E.T-20.022).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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