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Effect of Strontium Ranelate on Multiple Organ Damage in a Rat Sepsis Model

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Abstract

Background: Sepsis and multiple organ dysfunction syndrome (MODS) are life-threatening conditions common in intensive care units. In this regard, studies have shown that Strontium ranelate has anti-inflammatory activity by blocking tumor necrosis factor-alpha (TNFα).
Objectives: This study aimed to investigate the effect of Strontium ranelate on MODS in an experimental sepsis model.
Methods: The study protocol was approved by Cumhuriyet University Institutional Ethics Committee for Animal Experiments (Sivas-Turkiye, date 07/12/2017). Twenty female Wistar-Albino rats were randomly divided into four groups of sham operation, cecal ligation and perforation (CLP), CLP + Strontium (S) (oral 40 mg/kg for 7 days), and S (40 mg/kg oral preoperative 5 days) + CLP + S (oral 40 mg/kg for 7 days). Blood samples were taken, and alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), and creatinine were studied. Tissues were removed, and inflammation scores were determined.
Results: The mean inflammation scores of lung, liver, and renal tissues were found to be the lowest in the sham group (0.8 ± 0.45), and they increased in the S + CLP + S (1.6 ± 0.55) and CLP + S (3.4 ± 0.55) groups, respectively, with the highest score in the CLP (3.8 ± 0.45) group. It was found that there was no statistical difference between the sham and S + CLP + S groups ($P > 0.05$); however, there was a significant difference between the other groups ($P < 0.05$). The mean ALT, AST, BUN and creatinine values were found to be the lowest in the sham group, and they increased in the S + CLP + S and CLP + S groups, respectively, with the highest score in the CLP group. Regarding the mean ALT results, it was noted that there was no significant difference between the sham and S + CLP + S groups ($P > 0.05$); however, there was a significant difference between the other groups in terms of mean ALT, and there was a significant difference between all the groups in terms of mean AST, BUN, and creatinine ($P < 0.05$).
Conclusions: It was concluded that Strontium ranelate reduced the development of life-threatening MODS in patients with sepsis, especially when it was administered before the development of sepsis, by suppressing inflammatory mediators.

Keywords: Atrophy, Inflammation Mediators, Multiple Organ Failure, Sepsis, Strontium

1. Background

Sepsis presenting with dysfunction in the regulation of inflammation, impaired blood clotting, and multiple organ dysfunction syndrome (MODS) is a life-threatening condition that is common in intensive care units (ICUs) (1, 2). The distinguishing characteristics of sepsis are usually due to the irregularization of the systemic inflammatory response and are characterized by the over-accumulation of proinflammatory mediators such as tumor necrosis factor (TNF), interleukin (IL) -1, interferon-gamma (IFN-γ), and nitric oxide (NO) (1). The mortality rate of sepsis and its complications is 30 - 50% (2-4). MODS is observed in more than 45% of patients with sepsis and septic shock, and it causes significant mortality and morbidity in patients admitted to ICUs (5-7). MODS is a clinical syndrome characterized by a progressive and potentially reversible loss of function in two or more organ systems (8). MODS develops as a common final pathway of different diseases including severe infection, shock, trauma, burns, major surgical procedure, and pancreatitis (4, 6, 9).

Strontium chloride or ranelate is a compound that has been shown to have a positive effect on inflammation. In recent studies, it has been shown to have anti-inflammatory activity in allergic rhinitis, interstitial cystitis, and ulcerative colitis (10-12). Strontium ranelate is also used in the treatment of pain due to bone metastasis (13, 14). Strontium ranelate plays a very similar role to the physiological behavior of calcium ions. It mimics calcium by physiologically replacing calcium. Recent studies showed that Strontium ranelate inhibits inflammatory mediators

by blocking TNFα (15-17).

2. Objectives

In this study, we aimed to investigate the effect of Strontium ranelate on multiple organ dysfunction (i.e., liver, kidney, and lung) in an experimental sepsis model. This is the first attempt to study the effect of Strontium ranelate on sepsis and MODS.

3. Methods

An experimental study was carried out in Cumhuriyet University Experimental Animals Laboratory. The study was initiated with the approval of Cumhuriyet University Institutional Ethics Committee for Animal Experiments (Sivas-Turkiye, date 07/12/2017; code: 65202830-050.04.04/109). Twenty female Wistar-Albino rats weighing 150 - 230 gr were used in the study. The rats were maintained under standard conditions (at a temperature of 22°C, 65% humidity, and 12 hours dark: 12 hours light cycle). All the rats were starved 18 hours before the beginning of the experiment, but they were allowed to drink water until the last 20 minutes. The rats were randomly divided into four groups in a way that each group would include five of them, and they were taken to separate cages.

3.1. Formation of Sepsis with CLP

The rats were anesthetized with ketamine (30 mg/kg) and xylazine (6 mg/kg), and laparotomy was performed with a 2-cm midline incision. Then, the cecum was taken out of the abdomen, ligated with a 3/0 silk, and punctured with 22 G needle twice. Then, the stool was removed from these holes by compressing the cecum, and the intestines were placed in the peritoneal cavity again.

3.2. Experimental Procedure

No procedure was performed in Group 1. Only cecal ligation and perforation (CLP) were performed in Group 2. In Group 3, after performing CLP, 40 mg/kg Strontium ranelate was administered for seven days by the oral gavage method. In Group 4, 40 mg/kg Strontium ranelate (granule for Protelos® 2 GR Oral suspension Servier Ilac ve Arastirma A.S. Maslak, Istanbul) was initiated by the oral gavage method five days before CLP, and the treatment was continued for seven days after performing CLP.

The rats were euthanized by administering high doses of Sodium pentothal on the seventh day. Lung, liver, and renal tissues were removed for pathological examination, and blood samples were taken for biochemical examination.

3.3. Histopathological Evaluation

The rats were euthanized by administering high doses of Sodium pentothal on the seventh day. By performing laparotomy again using the former incision, lung, liver, and renal tissues were obtained and fixed in 10% buffered neutral formalin for 24 - 48 hours. Then, the routine histological follow-up procedures were performed, and the tissues were embedded in paraffin blocks. Sections with a thickness of about 5 μm were taken with a microtome (Leica RM2235, Germany) from the obtained paraffin blocks. The sections obtained were evaluated by staining with hematoxylin-eosin (H&E). All the sections (at least four sections for each tissue) were examined, and the sections with apparent artifacts due to staining were excluded from evaluation. All preparations that were prepared after staining were evaluated in terms of presence of damage under the light microscope (Olympus BX-51 Tokyo, Japan), and they were photographed.

Liver tissue samples were evaluated by examining changes occurring under the capsule in the parenchyma and hepatocytes. Renal tissue samples were evaluated under the light microscope by examining structural changes in proximal tubules, tubular atrophy, tubular brush border loss, tubular dilatation, mononuclear cell infiltration, erythrocyte extravasation, changes in renal corpuscle morphology, and changes in the interstitial site.

The general morphological changes of the alveolus and parenchyma in the lung tissue sections (i.e., alveolar structures, inflammation, alveolar septum, alveolar macrophages and neutrophils, parenchymal hemorrhage, edema, and congestion) were stained with Hematoxylin-Eosin and evaluated by the light microscope.

The images obtained from the sections were scored according to the damage rate semiquantitatively as 0, +, ++, ++ +, ++++.

3.4. Biochemical Analysis

Blood samples were subjected to centrifugation at 3000 g for 10 minutes Then, aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), and creatinine values were determined by the enzymatic colorimetric method in resolved serum (Beckman Coulter AU5800, USA).

3.5. Statistical Analysis

To analyze the data, SPSS Statistics Software for Windows, version 23.0 (IBM Corp., Armonk, N.Y., USA) was used. Firstly descriptive statistics, median, and interquartile range (IQR) values in the groups were calculated. Nonparametric test groups were used due to the small number of rats in the groups ($n_i < 30$). The differences between the groups were examined by using the Kruskal-Wallis H test and Mann-Whitney U test.

4. Results

4.1. Histopathological Evaluation

The descriptive statistics and the significance values of the inflammation scores obtained from the pathological examination of the lung, liver, and renal tissues are presented in Table 1. The mean lung tissue inflammation scores were 0.80 ± 0.45 and (median; IQR) 1 and 0 in the sham group, 3.80 ± 0.45 and (median; IQR) 4 and 0 in the CLP group, 3.40 ± 0.55 and (median; IQR) 3 and 1 in the CLP + STR group, and 1.60 ± 0.55 and (median; IQR) 2 and 1 in the STR + CLP + STR group. The mean liver tissue inflammation scores were 0.60 ± 0.55 and (median; IQR) 1 and 1 in the sham group, 3.40 ± 0.85 and (median; IQR) 4 and 1 in the CLP group, 2.80 ± 0.54 and (median; IQR) 3 and 1 in the CLP + STR group, and 1.20 ± 0.84 and (median; IQR) 1 and 1 in the STR + CLP + STR group. The mean renal tissue inflammation scores were 0.40 ± 0.55 and (median; IQR) 0 and 1 in the sham group, 3.80 ± 0.45 and (median; IQR) 4 and 0 in the CLP group, 3.40 ± 0.55 and (median; IQR) 3 and 1 in the CLP + STR group, and 1.40 ± 0.85 and (median; IQR) 2 and 1 in the STR + CLP + STR group (Table 1 and Figure 1). Accordingly, significant differences were calculated in all the variables ($P < 0.05$). Mann-Whitney U test did not reflect any significant difference between the control and STR + CLP + STR groups ($P > 0.05$); however, there were significant differences in the pair-wise comparison of all the other groups ($P < 0.05$).

4.2. Biochemical Analysis

The descriptive statistics and significance values of ALT, AST, BUN, and creatinine are presented in Table 2. The mean ALT was 37.00 ± 13.47 (IU/Lit) and (median; IQR) 33 and 13 in the sham group, 133.60 ± 5.22 and (median; IQR) 132 and 5 in the CLP group, 119.00 ± 11.34 and (median; IQR) 114 and 14 in the CLP + STR group, and 84.60 ± 5.68 and (median; IQR) 85 and 5 in the STR + CLP + STR group (Table 2 and Figure 2). Accordingly, a significant difference was calculated in ALT results ($P < 0.05$). Mann-Whitney U test did not show any significant differences between the control and STR + CLP + STR groups in this regard ($P > 0.05$); however, there was a significant difference between the other groups in pair-wise comparisons ($P < 0.05$). The mean AST level was 111.80 ± 16.36 and (median; IQR) 112 and 29 in the sham group, 338.20 ± 25.92 (IU/Lit) and (median; IQR) 354 and 34 in the CLP group, 266.20 ± 11.34 and (median; IQR) 263 and 3 in the CLP + STR group, and 209.00 ± 11.42 and (median; IQR) 213 and 15 in the STR + CLP + STR group. AST was found to be significantly different between all the groups in pair-wise comparisons ($P < 0.05$).

The mean creatinine results were 0.37 ± 0.04 (mg/dL) and (median; IQR) 0.38 and 0.04 (mg/dL) in the sham group, 0.89 ± 0.04 and (median; IQR) 0.89 and 0.03 in the

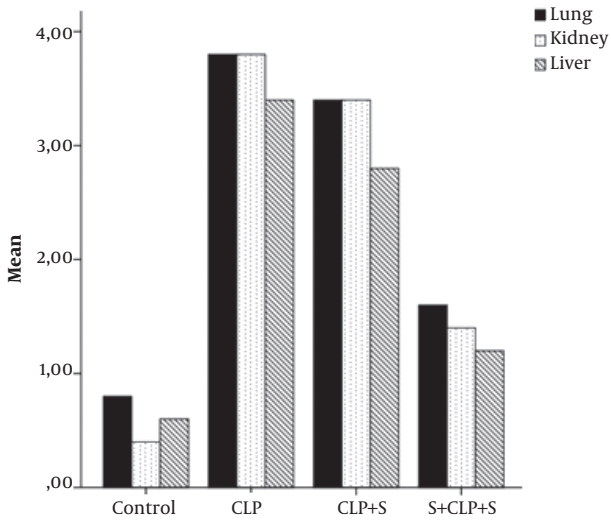

Figure 1. Inflammation Scores in All the Groups

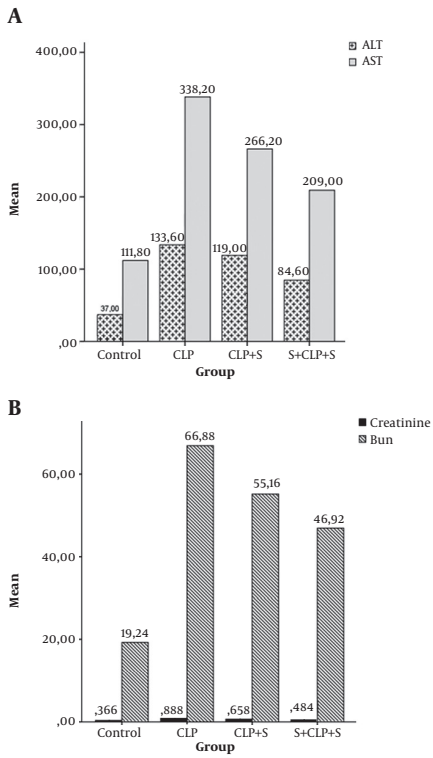

Figure 2. Mean values of alanine aminotransferase (IU/Lit), aspartate aminotransferase (IU/Lit), blood urea nitrogen (mg/dL), creatinine (mg/dL) in all the groups, A: Mean value of alanine aminotransferase, aspartate aminotransferase; B: Mean value of blood urea nitrogen, creatinine. CLP= Cecal Ligation and Perforation, CLPS=CLP+ Strontium S= Strontium

Table 1. Descriptive Statistics of Pathological Examination Results and Kruskal-Wallis H Test Results

Variables / Category	Minimum	Maximum	Median	IQR	Mean	Standard Deviation	P Value
Lung							< 0.001[a]
Sham	0.00	1.00	0	1	0.80	0.45	
CLP	3.00	4.00	4	0	3.80	0.45	
CLP + S	3.00	4.00	3	1	3.40	0.55	
S + CLP + S	1.00	2.00	2	1	1.60	0.55	
Kidney							< 0.001[b]
Sham	0.00	1.00	0	1	0.40	0.55	
CLP	3.00	4.00	4	0	3.80	0.45	
CLP + S	3.00	4.00	3	1	3.40	0.55	
S + CLP + S	0.00	2.00	2	1	1.40	0.89	
Liver							< 0.001[c]
Sham	0.00	1.00	1	1	0.60	0.55	
CLP	2.00	4.00	4	1	3.40	0.89	
CLP + S	2.00	4.00	3	1	2.80	0.84	
S + CLP + S	0.00	2.00	1	1	1.20	0.84	

Abbreviations: CLP, Cecal ligation and perforation; S, Strontium ranelate.
[a] There was a significant difference between the Control and Sham ($P < 0.001$) and Sepsis + STR ($P < 0.001$) groups. There is a significant difference between the STR + Sepsis + STR and Sham ($P \leq 0.001$) and Sepsis + STR ($P < 0.001$) groups.
[b] There is a significant difference between the Control and Sham ($P < 0.001$) and Sepsis + STR ($P < 0.001$) groups. There is a significant difference between the STR + Sepsis + STR and Sham ($P \leq 0.001$) and Sepsis + STR ($P < 0.001$) groups.
[c] There is a significant difference between the Control and Sham ($P < 0.001$) and Sepsis + STR ($P = 0.002$) groups. There is a significant difference between the STR + Sepsis + STR and Sham ($P = 0.002$) and Sepsis + STR ($P = 0.026$) groups.

CLP group, 0.66 $\pm$ 0.09 and (median; IQR) 0.65 and 0.07 in the CLP + STR group, and 0.48 $\pm$ 0.03 and (median; IQR) 0.49 and 0.003 in the STR + CLP + STR group. The mean BUN results were 19.24 $\pm$ 1.12 and (median; IQR) 18.94 and 0.50 in the sham group, 66.88 $\pm$ 3.56 and (median; IQR) 65.30 and 3.30 in the CLP group, 55.16 $\pm$ 2.14 and (median; IQR) 54.76 and 1.22 in the CLP + STR group, and 46.92 $\pm$ 1.2 and (median; IQR) 47.19 and 1.05 in the STR + CLP + STR group (Table 2 and Figure 2). The differences between the groups in terms of BUN and creatinine values were found to be statistically significant ($P < 0.05$). Mann-Whitney U test demonstrated significant differences between all paired variables ($P < 0.05$).

5. Discussion

We found that Strontium ranelate protects the liver, kidney, and lung tissues from damages due to sepsis. It had the highest protective effect against multiple organ damage, especially when it was administered before the development of sepsis.

The natural immune system is the first line of defense against infection by the release of inflammation mediators and phagocytosis. In general, the excessive release of inflammatory mediators that trigger pathophysiological abnormalities of sepsis occurs during serious infection. Increased TNF-α and IL-6 levels are a well-characterized symptom of proinflammatory response and play a key role in the development of systemic dysfunctions in sepsis (18).

In response to environmental signals, macrophages are divided either into M1 or M2 phenotype. M1 macrophages produce proinflammatory cytokines (e.g., TNF-α and IL-6), and M2 macrophages secrete anti-inflammatory cytokines (e.g., IL-10 and IL-1ra) that improve angiogenesis and tissue repair (19, 20). It is well known that TNF-α is involved in immune modulation and inflammation reactions. Moreover, it may cause cytotoxic effects leading to cell death (21).

Calcium-mediated signal transduction is indispensable for cellular functions (22-24). Cell membrane depolarization opens L-type calcium channels. The increase in intracellular calcium concentration enables a large area in signal pathways. Here, phosphatase calcineurin plays a key role in cell response. With this stimulus, gene expression occurs in the cell nucleus, and active T cells nuclear factor NFAT is released. Calcium/calcineurin/NFAT pathway is activated by the increase of intracellular calcium (25-27). With these effects, the inflammatory process begins, leading to the release of mediators such as TNFα, IL-1, and IL-6 and the onset of the inflammatory process. Calcineurin af-

Table 2. Examination Results of Alanine Aminotransferase, Aspartate Aminotransferase, BUN and creatinine

Variables / Category	Minimum	Maximum	Median	IQR	Mean	Standard Deviation	P Value
ALT							< 0.001[a]
Sham	22.00	57.00	33	13	37.00	13.47	
CLP	129.00	142.00	132	5	133.60	5.22	
CLP + S	109.00	136.00	114	14	119.00	11.34	
S + CLP + S	76.00	91.00	85	5	84.60	5.68	
AST							< 0.001[b]
Sham	93.00	129.00	112	29	111.80	16.36	
CLP	301.00	360.00	354	34	338.20	25.92	
CLP + S	245.00	294.00	263	3	266.20	17.63	
S + CLP + S	192.00	219.00	213	15	209.00	11.42	
Creatinine							< 0.001
Sham	0.32	0.41	0.38	0.04	0.37	0.04	
CLP	0.82	0.94	0.89	0.03	0.89	0.04	
CLP + S	0.55	0.78	0.65	0.07	0.66	0.09	
S + CLP + S	0.44	0.52	0.49	0.03	0.48	0.03	
BUN							< 0.001
Sham	18.21	21.13	18.94	0.50	19.24	1.12	
CLP	63.40	72.40	65.30	3.30	66.88	3.56	
CLP + S	53.02	58.68	54.76	1.22	55.16	2.14	
S + CLP + S	45.54	48.42	47.19	1.05	46.92	1.10	

Abbreviations: ALT: alanine aminotransferase; AST: aspartate aminotransferase; BUN: blood urea nitrogen; CLP: cecal ligation and perforation; S: Strontium ranelate.
[a] There is a significant difference between the Control and Sham ($P < 0.001$) and Sepsis + STR ($P < 0.001$) groups. There is a significant difference between the STR + Sepsis + STR and Sham ($P \leq 0.001$) and Sepsis + STR ($P < 0.001$) groups.
[b] All groups are different from each other.

fects many transcription factors including NF-κB (27, 28). Nuclear factor kappa B (NF-κB) is a critical transcription factor in the regulation of proinflammatory cytokine production (29).

Calcium and Strontium Ranelate are active as competitive inhibitors, and Strontium ranelate inhibits the calcium flow (15). In former studies, it was concluded that Strontium disrupts TNFα blockage and NF- κB signal transduction and prevents the release of inflammatory mediators from monocytes (16, 17). Sibel et al. reported that Strontium chloride hexahydrate topically suppressed inflammation in the skin and TNF-α in a rat wound healing model (30). Porta et al. demonstrated that the p50 subunit of NF-$\kappa\beta$ is the main controller of this process (3, 19).

In a CLP-induced sepsis study carried out by Khader et al. with SRT1720, which is one of the sirtuin-activating compounds that relieve the activity of NF- κB, they showed that SRT1720 decreased liver and renal tissue damage (29). Li et al. found that ketamine prevented acute lung damage in sepsis in a rat model of sepsis-induced lung damage. This result was associated with the suppression of the anti-inflammatory response after ketamine, NF-κB, and mitogen-activated protein kinases (MAPK) treatment (31).

Zhu et al. showed that Strontium effectively prevented particle-induced bone loss. They also concluded that the protective effects of Strontium ranelate were mainly through the down-regulation of the NF-κB pathway via the inhibition of osteoclast formation and inflammatory response (32).

In this study, Strontium ranelate effectively prevented liver, kidney, and lung tissue damages due to sepsis, especially when it was used before sepsis. It also decreased serum ALT, AST, BUN, and creatinine levels. It is assumed that it provided this healing through TNFα blockage and NF- κBd down-regulation. It was concluded that Strontium decreased the development of life-threatening multiple organ dysfunction syndrome in patients with sepsis through the pathophysiologic pathway described in the inflammatory process and decreased multiple organ damage more effectively when it was administered before the development of sepsis through suppressing inflammatory mediators. This finding could be a new treatment and prophylac-

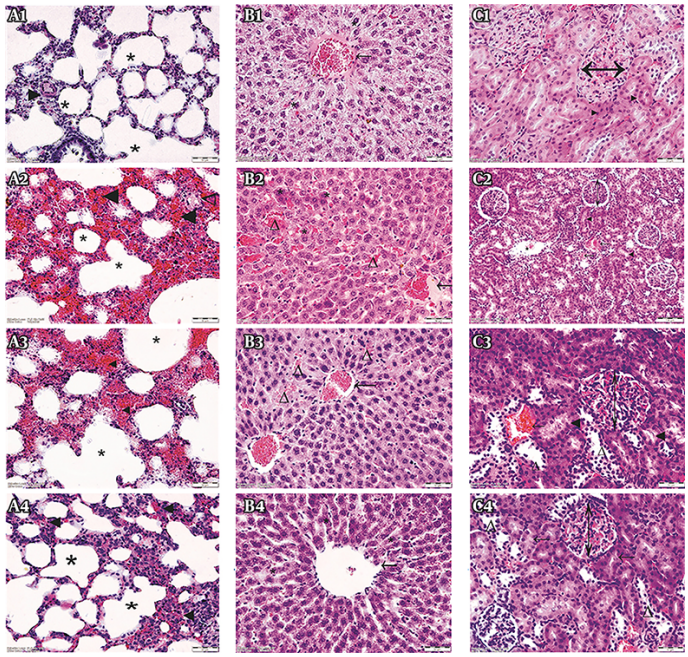

Figure 3. Histopathological images of biopsy samples obtained from lung (A), liver (B) and renal (C) tissues (40×, hematoxylin-eosin), A1, (Lung tissue in the control group 40× H&E) Bronchioli (←), alveoli (*) and blood vessels in the interalveolar septum were observed in normal lung tissue; A2, (Lung tissue in CLP group 40×) Dilatation and deterioration of the integrity of alveoli (*), mononuclear cell infiltration, diffuse alveolar damage, increase in permeability of capillaries and erythrocyte extravasation in the interalveolar septum (Δ) and increase in the number of alveolar macrophages were observed; A3, (Lung tissue in CLP + S group 40×) Compared to the CLP group, the CLP + s group has been shown to better protect the integrity of the alveoli. Less dilation in alveoli, alveolar damage, decreased mononuclear cell (Δ) in the interalveolar septum, decrease capillary permeability and extravasation of erythrocytes and a decrease in alveolar macrophage count were observed; A4, (Lung tissue in S + CLP + S group 40×) It has been determined that the integrity of the alveoli (*) is better preserved similar to the Control group. Reduced mononuclear cell infiltration and decreased diffuse alveolar damage in the interalveolar septum (Δ), decrease capillary permeability and extravasation of erythrocytes and decrease in the number of alveolar macrophages were observed; B1, (Liver tissue in Control group 40× H&E) The control group has normal vena centralis (←) and hepatocytes (*) in the liver; B2, (Liver tissue in CLP group 40× H&E) Fullness in vena centralis (←) and erythrocyte extravasation (Δ) is observed between hepatocytes (*) in liver tissues; B3, (Liver tissue in CLP + S group 40× H&E) According to the CLP group, vena centralis circumference (←), hepatocytes were seen normal in appearance. Hyperemia and erythrocyte extravasation (Δ) in the parenchyma were not observed between hepatocytes; B4, (Liver tissue in S + CLP + S group 40× H&E) Vena centralis (←) and hepatocytes (*) were normal appearance. However, hyperemia, vena centralis enlargement, and erythrocyte extravasation were observed in the parenchyma; C1, (Renal tissue in Control group 40× H&E) Normal proximal tubules (Δ) and renal corpuscle (↔) in kidney tissue; C2, (Renal tissue in CLP group 40× H&E): Mononuclear cell infiltration in the peritubular area (*), extravasation of erythrocytes (←), brushed edge cell loss and tubular atrophy in proximal tubules (Δ), The expansion of the Bowman capsule between parietal and visceral leaf (↔) is observed; C3, (Renal tissue in CLP + S group 40× H&E) Mononuclear cell infiltration in the peritubular area is very low compared to the CLP group very less rate of erythrocyte extravasation (←) is observed. Brush-edged cell loss is less in proximal tubules (Δ). Distal tubules enlargement and tubular atrophy (Δ), Between the parietal and visceral leaf of the Bowman capsule (↔) a close range is observed in the control group ; C4, (Renal tissue in S + CLP + S group 40× H&E): Similar to the control group, normal-appearing Bowman capillary (↔), proximal (Δ) and distal tubules (Δ) are observed.

tic option, especially for patients at risk for sepsis development receiving treatment in ICUs. However, further studies are required in this regard.

The limitations of this study include not measuring the levels of inflammatory mediators such as TNF alpha IL1 and not evaluating the activity of Strontium ranelate at different doses.

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