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Çekal Ligasyon ve Delme ile oluşturulan Polimikrobiyal Sepsis Modelinde Akut Organ Hasarına Karşı Fraxin'in Antioksidan Etkileri

The Antioxidant Effect of Fraxin against Acute Organ Damage in Polymicrobial Sepsis Model induced by Cecal Ligation and Puncture

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Öz: Amaç: Bu makalede, ratlarda çekal ligasyon ve delme (CLP) modeli ile indüklenen akut organ hasarına karşı fraksin' in antioksidan etkisinin değerlendirilmesi amaçlandı. **Gereç ve Yöntemler**: Bu amaçla çalışmamızda, tüm deney hayvanları randomize şekilde gruplandırıldı. Bu gruplar sham kontrol, CLP, CLP+Fraxin 50 mg/kg, CLP+Fraxin 100 mg/kg gruplar olarak belirtildi. Tüm sıçanların böbrek ve akciğer dokularında TAS, TOS, OSI, MDA düzeyleri, MPO ve SOD aktiviteleri değerlendirildi. **Bulgular**: Böbrek ve akciğer dokularının TOS, OSI, MDA düzeyleri ve MPO aktivitesi, CLP grubunda sham kontrol grubundan daha yüksekti, ancak TAS düzeyi ve SOD aktivitesi daha düşüktü. Bununla birlikte, bu sonuçlar fraxin tedavi gruplarında önemli ölçüde değişmiştir. **Sonuç**: Bu sonuçların ışığında, fraksin' in, CLP' nin neden olduğu sepsis modelinde böbrek ve akciğer dokusu üzerindeki TOS, MPO ve MDA seviyelerini düşürerek SOD ve TAS seviyesini yükselterek olumlu bir etki gösterdiği ifade edilebilir.

Anahtar Kelimeler — Çekal ligasyon ve delme, fraksin, sepsis, rat.

Abstract: Aim: In this paper, it was purposed to evaluate the antioxidant effect of fraxin against acute organ damage induced by cecal ligation and puncture model (CLP) in rats**. Material and Methods**: For this purpose in our study, all experimental animals were randomly grouped. These groups were stated as sham-control, CLP, CLP+Fraxin 50 mg/kg, CLP+Fraxin 100 mg/kg groups. TAS, TOS, OSI, MDA levels, MPO and SOD activities were evaluated in kidney and lung tissues of all rats. **Results**: TOS, OSI, MDA levels and MPO activity of kidney and lung tissues were higher in the CLP group than in the sham-control group, but TAS level and SOD activity were lower. However, these results were significantly changed in the fraxin treatment groups. **Conclusion**: In light of these results, it can be stated that fraxin shows a positive effect by raising the level of SOD and TAS by decreasing TOS, MPO and MDA levels on kidney and lung tissue in the model of sepsis caused by CLP.

Keywords — Cecal ligation and puncture, fraxin, sepsis, rat.

1.Introduction

Sepsis is a life-threatening clinical condition leading to organ dysfunction in which a host response to infection occurs [1, 2]. The cecal ligation and puncture (CLP) that we have applied in our study is very similar to sepsis seen in the clinic, and CLP in experimental animals is commonly used to simulate sepsis animal modelling and imitation clinical appendicitis or diverticulitis perforation [3, 4]. For this reason, to date, the efficacy of a large number of agents to minimize or completely eliminate organ damage caused by sepsis with high morbidity and mortality rates has been investigated [5, 6]. Fraxin has anti-inflammatory,

hepatoprotective, antioxidant, immunomodulatory activity, analgesic effects and is known to be the main active compound obtained from Cortex Fraxini, which is preferred in clinic therapies such as hyperuricemia, diarrhea and liver therapies [7-9]. However, when a literature review was performed, it was not come across the studies that evaluated the therapeutic or protective efficacy of fraxin in a polymicrobial sepsis model induced by CLP So, the aim of this study was to evaluate an antioxidant effect of the fraxin on the kidney and lung tissues in the sepsis model induced by CLP in rats.

2. Material and Methods

The experimental animals used in this study were obtained from the Atatürk University Experimental Animal Research and Application Center (ATADEM) and the experimental stages of our study were performed in the same place. This study has been approved by Atatürk University Local Animal Ethics Committee (Date/Number of ethical approval:28.06.2018/138). All experimental animals were kept in the same standard laboratory conditions and were fed with standard rat food and water.

2.1. Design of Experimental Study

Firstly, 32 Wistar albino rats used in this study were divided into 4 groups. These groups were designed as follows;

1. Sham-Control group (n=8): In this group, only laparatomy and bowel manipulation were applied. But cecal ligation puncture was not done.

2. CLP (cecal ligation puncture) group (n=8): The animals in this group were anesthetized with a mixture of ketamine / xylazine (60/10 mg / kg; i.p.). Polymicrobial sepsis was formed by the CLP method which was used in previous studies [10, 11]. After the abdominal area was cleared, an incision was made on the midline. The cecum was taken out and two holes were drilled using an 18-ga needle. Then the punctured cecum was placed in the peritoneum and the abdominal wall was closed again.

3. CLP+Fraxin 50 mg/kg group (n=8): After CLP was performed, the subjects were treated with fraxin as 50 mg/kg intraperitoneally.

4. CLP+Fraxin 100 mg/kg group (n=8): After CLP was performed, the subjects were treated with fraxin as 100 mg/kg intraperitoneally.

Approximately 18 hours after the formation of sepsis induced by CLP, all rats were sacrificed. Later the lungs and kidney tissues were rapidly removed.

2.2. Biochemical Analysis

MDA/ Malondialdehyde (μmol/g protein) level, SOD/ Superoxide dismutase (U/mg protein) and MPO/ Myeloperoxidase (U/mg protein) activities were measured taking reference from previous studies [12-14]. Also, TAS/ Total antioxidant status (mmol/L) and TOS/ Total oxidant status (μmol/L) levels were analysed using commercial kits. OSI/ oxidative stress index value was determined as formula: OSI = [(TOS, μmol H_2O_2 equivalent/L)/(TAS, mmol Trolox equivalent/L) × 10].

2.3. Statistical Analysis

All of our biochemical data were analyzed statistically with One-Way ANOVA and Tukey HSD tests. Statistical significance was considered significant at the 0.05 level. All our results are given as Mean±Standard Error Mean (SEM).

3. Results

MDA/ Malondialdehyde (µmol/g protein) levels, SOD/ Superoxide dismutase (U/mg protein), MPO/ Myeloperoxidase (U/mg protein) activities, TAS (mmol/L) and TOS (µmol/L) levels and OSI value of lung and kidney tissues.

MDA level as an indicator of lipid peroxidation and MPO activities were significantly higher in CLP groups compared to sham-control groups in lung and kidney tissues. On the contrary, in the analysis of both lung and kidney tissues, MDA levels and MPO activities were found to be significantly decreased in the groups given fraxin at 50 and 100 mg/kg doses (See Fig 2a and b; $p<0.05$).
When SOD activity of lung and kidney tissues were evaluated, it was determined that SOD activities decreased especially in the CLP group when compared with the sham-control group and also increased with fraxin treatments (See Fig 2c; $p<0.05$).

Table 1 and 2 show that TAS levels of lung and kidney tissues were significantly decreased in CLP group and increased due to Fraxin treatments. However, in the CLP group, TOS and OSI values decreased significantly compared to the rise in TAS. Besides, fraxin treatment was able to decrease the TOS level and OSI value.

Table 1a: TAS, TOS levels and OSI values belonging to kidney tissue of all groups are presented as Mean±SEM. There is a statistically significant relationship between the groups with the same letters.

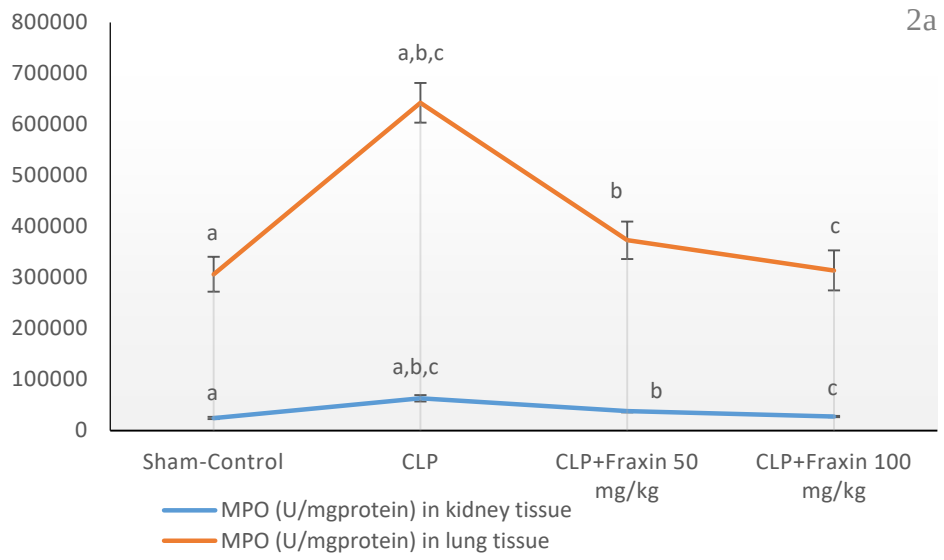
Kidney Tissue			
	TAS (mmol/L)	**TOS (µmol/L)**	**OSI**
Sham-Control	2,94±0,06[a]	5,33±0,19[a]	0,18±0,008[a]
CLP	0,86±0,02[a,b,c]	9,01±0,23[a,b,c]	1,04±0,04[a,b,c]
CLP+Fraxin 50 mg/kg	2,78±0,12[b]	6,66±0,27[b]	0,24±0,01[b]
CLP+Fraxin 100 mg/kg	3,00±0,11[c]	5,58±0,26[c]	0,18±0,01[c]

[a]: Comparison with Sham-Control group ($p<0.05$), [b]: Comparison with CLP group ($p<0.05$), [c]: Comparison with CLP group ($p<0.05$).

Table 1b: TAS, TOS levels and OSI values belonging to lung tissue of all groups are presented as Mean±SEM. There is a statistically significant relationship between the groups with the same letters.

Lung Tissue			
	TAS (mmol/L)	**TOS (µmol/L)**	**OSI**
Sham-Control	0,36±0,01[a]	11,48±0,45[a]	3,22±0,27[a]
CLP	0,17±0,01[a,b,c]	19,61±0,60[a,b,c]	11,37±0,69[a,b,c]
CLP+Fraxin 50 mg/kg	0,30±0,02[b]	13,71±0,38[b]	4,58±0,33[b]
CLP+Fraxin 100 mg/kg	0,36±0,03[c]	11,80±0,56[c]	3,51±0,37[c]

[a]: Comparison with Sham-Control group ($p<0.05$), [b]: Comparison with CLP group ($p<0.05$), [c]: Comparison with CLP group ($p<0.05$).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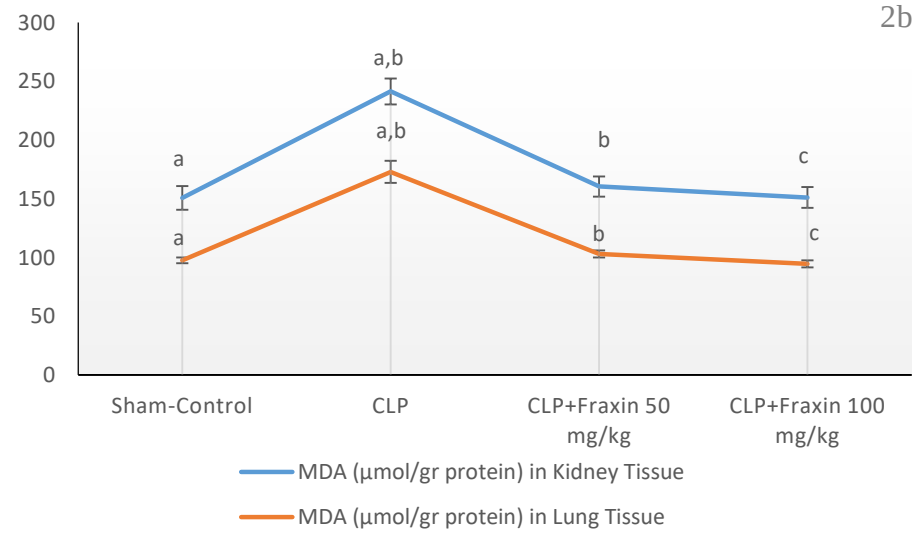
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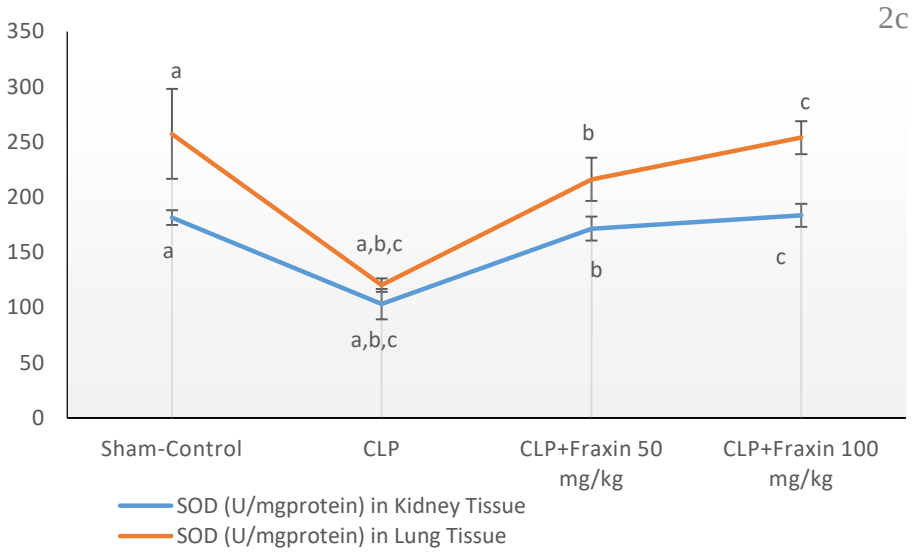
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CLP
CLP+Fraxin 50 mg/kg
CLP+Fraxin 100 mg/kg
SOD (U/mgprotein) in Kidney Tissue
SOD (U/mgprotein) in Lung Tissue

[a]: Comparison with Sham-Control group ($p<0.05$), [b]: comparison with CLP group ($p<0.05$), [c]: comparison with CLP group ($p<0.05$).

Figure 2a: MPO activities of all groups were given in lung and kidney tissues. **2b:** MDA level of all groups were given in lung and kidney tissues. **2c:** SOD activities of all groups were given in lung and kidney tissues. There is a statistically significant relationship between the groups with the same letters.

4. Discussion and Conclusion

Sepsis has been one of the problems that have been difficult to treat and mortality for many years. Sepsis and septic shock, which need to be treated urgently, are still considered to be a pathology with high mortality [15]. This is a clinical and pathophysiological process in which the majority of patients have a continuous increase in the severity of the clinical conditions leading to sepsis, severe sepsis, septic shock and multiorgan failure involving acute lung injury, acute respiratory distress syndrome, acute/chronic kidney disease and end-stage renal disease and even death [15-17]. Therefore, this situation, which has a high mortality rate, is defined as acute in the early period and the chance of survival of the patients can be increased with the application of an emergency and effective treatment [15]. Today, studies on sepsis have focused on experimental models. Sepsis was formed with different models on experimental animals. Although it is currently aimed at revealing the pathophysiology of sepsis with a wide variety of experimental studies and developing new treatment strategies, the current treatment protocol is often limited to routine life support treatment [6, 18]. However, cecal ligation and puncture (CLP) method was frequently used on some studies [19, 20]. Acute lung and kidney damage is the most common type of endotoxemia induced by CLP. Therefore, research focuses on the prevention or improvement of lung and kidney damage caused by sepsis [5, 21]. It is reported that acute lung and kidney damage were occured by the overproduction of reactive oxygen species (ROS), neutrophil accumulation and increased production of proinflammatory cytokines in kidney and lung [5, 22, 23]. ROS is thought to be an important defence mechanism for the protection of the organism against bacterial infections and the generation of free oxygen radicals is increased against sepsis. In some studies, malondialdehyde (MDA) levels, which are a marker of lipid peroxidation due to oxidative stress, were found to be high in the presence of sepsis [24, 25]. Previous studies have reported that lipid peroxidation is increased, especially in patients with sepsis, whereas antioxidant enzyme activities have decreased [26]. Oxidative stress, which results from the unbalance of ROS production and antioxidant enzymes. Increased oxidant levels in sepsis directly result in cellular damage by attacking biological molecules such as cellular proteins, lipids and nucleic acids. In this respect, treatment procedures with antioxidant agents are studied experimentally and clinically in sepsis. In several studies, it was demonstrated that MDA levels increased in CLP-induced sepsis and glutathione (GSH) and superoxide dismutase (SOD) activities decreased [5, 6, 27]. Myeloperoxidase (MPO) enzyme is a hemoprotein found in defensive cells such as neutrophils and monocytes. It shows indispensable properties in the formation of an inflammatory response by catalyzing the formation of ROS which is effective in microbial killing [28]. MPO activity and oxidant products are considered responsible for shaping many pathologies [29]. As accordance with the results of the previous scientific studies in our study, it was found that MDA level and MPO activities increased and SOD activity decreased in both lung and kidney tissues in CLP-induced sepsis model. The total antioxidant status (TAS) is known as a marker of total antioxidant capacity and is a total measure of the cumulative effect of all antioxidants, either by incorporation of all antioxidants, including in unexplored form and total oxidant status (TOS) reflects the total effect of all oxidants [30-32]. OSI value is obtained based on the ratio

of TOS level to the TAS level. In this study, it was determined that TAS level decreased due to sepsis and a significant increase in the TOS and OSI levels were observed in lung and kidney tissues. It was observed that these oxidant levels, which we evaluated in sepsis, decreased significantly with the fraxin treatment and antioxidant activities increased.
In conclusion, we think that fraxin as an antioxidant support therapy can be overcome in order to alleviate the damage of kidney and lung tissue in polymicrobial sepsis induced by CLP and may have a positive effect on sepsis treatment. We believe that this experimental study data will shed light on the wider experimental series and clinical prospective randomized human studies.

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Conflict of interest

There is no conflict of interest between authors.

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