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# Alliinin Çekal Ligasyon ve Delme (CLP) Kaynaklı Akciğer Hasarına Antioksidan ve Antiinflamatuar Etkileri

# The Antioxidant and Antiinflammatory Effects of Alliin on Cecal Ligation and Puncture (CLP)-Induced Lung Injury

Ayhan TANYELİ[1,*] and Derya GÜZEL ERDOĞAN[2]

[1] *Department of Physiology, Faculty of Medicine, Atatürk University, Erzurum, Turkey*
[2] *Department of Physiology, Faculty of Medicine, Sakarya University, Sakarya, Turkey*
**Sorumlu yazar / Corresponding Author*: ayhan.tanyeli@atauni.edu.tr

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**Öz: Amaç**: Bu araştırmanın amacı, Alliin'in çekal ligasyonu ve delme (CLP) kaynaklı akciğer hasarı üzerindeki koruyucu etkilerini incelemektir.
**Gereç ve Yöntem**: Deneyimizde, sıçanlar sham kontrol, CLP, CLP + Alliin 100 mg/kg ve CLP + Alliin 200 mg/kg olmak üzere 4 gruba ayrıldı. Bazı oksidan, antioksidan ve enflamatuar parametreler deney sonunda elde edilen akciğer dokularında değerlendirildi.
**Bulgular**: Mevcut çalışmada, CLP grubunda oksidan ve inflamatuar parametrelerin arttığını ve antioksidan parametrelerin azaldığını, ancak antioksidan parametrelerin arttığını ve tedavi gruplarında antioksidan parametrelerin azaldığını ve Alliin uygulamasının CLP'nin neden olduğu akciğer oksidatif hasarına karşı koruyucu olduğunu gözlemledik.

**Anahtar Kelimeler** — Çekal ligasyon ve delme, Alliin, akciğer, oksidatif stres, inflamasyon, sıçan.

**Abstract: Purpose**: The purpose of this research is to examine protective effects of Alliin on cecal ligation and puncture (CLP)-induced lung injury.
**Material and Method**: In our experiment, the rats were separated as 4 groups including sham control, CLP, CLP + Alliin 100 mg/kg and CLP + Alliin 200 mg/kg. Some oxidant, antioxidant and inflammatory parameters were evaluated in lung tissues obtained at the end of the experiment.
**Findings**: In current study, we observed that the oxidant and inflammatory parameters increased and antioxidant parameters decreased in the CLP group but the antioxidant parameters increased and oxidant parameters decreased in treatment groups suggesting that administration of Alliin is protective against CLP-induced lung oxidative damage.

**Keywords** — Cecal ligation and puncture, Alliin, lung, oxidative stress, inflammation, rat.

## INTRODUCTION

Sepsis is a serious inflammatory condition that is currently stemming from pathogenic microorganisms and disrupts organ function [1]. Despite advances in critical care treatment and increased understanding of the pathophysiology of sepsis, the mortality rate of affected patients remains high (40 to 60%) even in developed countries [2]. Sepsis is a collection of disorders associated with infection arising from bacteria, viruses, or fungi. It often leads to an overwhelming response of innate inflammation [3] which results in a densely produced

inflammatory factors such as cytokines [4]. CLP-induced sepsis is one of the powerful experimental methods used in sepsis model. The studies indicates increased tumor necrosis faktör-alpha (TNF-α) and interleukin-6 (IL-6) levels in CLP-induced sepsis [5]. TNF-α is well known as one of the key cytokines mediating inflammatory responses [6]. Inflammatory cascades, aggreved by cytokines, results in increased oxidant biomarker levels [7, 8]. Myeloperoxidase (MPO), one of the inflammatory biomarkers, is released by neutrophils and plays role in these cascades [9]. Lungs are believed to be the first and mostly affected organ due to intra-abdominal sepsis [10]. Fortunately, there are several animal models demonstrating the status of reactive oxygen species (ROS) during cecal ligation and puncture (CLP)-induced sepsis in lung injury [11, 12]. An increase in the ROS level triggers apoptosis and inflammation even at the cellular level [13]. In this process, due to the cell membrane lipid oxidation, oxidant markers (MDA, etc) increase [14, 15]. While total antioxidant status (TAS) is used as an indicator of total antioxidant activity, total oxidant status (TOS) is a strong marker to determine the total oxidant activity. Oxidative stress index (OSI) is a value indicating the balance in the current oxidative status [16, 17].

Recent studies have shown that under the control of inflammation in many inflammatory diseases, including sepsis, survival and natural antioxidant agents administration is effected which are efficient against sepsis [18, 19]. Alliin (S-allyl cysteine sulfoxide, C6H11NO3S) is, one of the antioxidant agents, an important organosulfur compound derived from garlic [20]. Recent studies have shown that alliin has improving properties such as antioxidant, antiinflammatory, antidiabetic, and anti-aggregation effects [20, 21, 22, 23, 24].

In present study, we evaluated the effects of alliin, which has various biological properties such as antioxidant, antiinflammatory in lung in order to alleviate the oxidative damage in the CLP model in rats.

## 2.MATERIAL AND METHODS

### Experimental Animals and Ethical Approval

Atatürk University Experimental Animal Ethics Committee approved (28.03.2019/64) the experiments of our study where was performed at Atatürk University Experimental Animals Research and Application Center. Male rats of Wistar albino species, obtained from Atatürk University Experimental Animals Research and Application Center were kept in polypropylene cages in standardized conditions such as 12 light/12 darkness, temperature of

22±2 °C, humidity of 55±5 %, and feed and water. 12 hours before experiment food consumption was not allowed to animals but it was free to drink water.

**Experimental Animals and Experimental Design**

For our experiments we used 32 healthy male rats (240-270 gr) which were randomly assigned to 4 groups in which each includes 8 subjects. The rats in group 1 (Sham control group, n=8) had 2 cm incision at the abdominal area to reach the peritoneum. Then incision was closed with a 3.0 silk suture without any procedure. The rats in group 2 (CLP group, n=8) had their cecum isolated after reaching their peritoneum through 2 cm incision. Following that, ileocecal valve was ligated up to 2 cm distally, and pierced by 18-gauge needle (4 holes). Then the cecum was put back to the abdomen and abdomen was closed with 3.0 silk suture. The rats of group 3 (100 mg/kg alliin+CLP group, n=8) had alliin administiration intraperitoneally in low dose (100 mg/kg) 30 minutes before the same CLP model in group 2. The rats of group 4 (200 mg/kg alliin+CLP group, n=8) was administered alliin intraperitoneally in high dose (200 mg/kg) 30 minutes before the same CLP model in group 2. In the CLP groups (group 2, 3, 4), the abdominal regions were washed with povidone-iodine after being shaved. Analgesic lidocaine solution was applied to the suture areas in order to prevent pain stress of the rats to remove the error margin. As postoperatively the rats had no food, but was free to reach water for 18 hours until they were sacrificed.

**Biochemical Assessments**

TAS and TOS are evaluated by a commercial kit (Rel Assay Diagnostics). OSI which demonstrates the TOS to TAS ratio was calculated as follows: OSI = [(TOS, μmol $H_2O_2$ equivalent L)/(TAS, mmol Trolox equivalent/L) × 10]. We preferred OSI as other oxidative stress indicator. The evaluation of superoxide dismutase (SOD) was predicated on superoxide radicals production. Those radicals are generated by xanthine oxidase system which performs reaction with nitroblue tetrazolium for formazan dye formation [25]. Malondialdehyde (MDA) is used to measure the amount of lipid peroxidation in lung tissue through thiobarbituric acid test [26]. The activities of MPO in the lung tissues were estimated due to the methods described by Bradley et al. [27].

**Statistical Analysis**

All the results have been presented as mean ± SD (standard deviation). Results heve been analyzed by One-way ANOVA and then Tukey test for pairwise comparisons of groups. The differences heve been approved significant when $p<0.05$.

## 3.RESULTS

It has been no morbidity or mortality in rats during experimental applications. When the CLP group compared to the sham control group, TAS (from 0.844±0.094 to 0.271±0.037, p=0.000) level decreased, whereas the TOS (from 7.099±0.718 to 11.497±0.760, p=0.000), OSI (from 0.852±0.142 to 4.316±0.747, p=0.000) levels increased. When the CLP + 100 mg/kg alliin group, compared to the CLP group, TAS (from 0.271±0.037 to 0.733±0.046, p=0.000) level increased, while TOS (from 11.497±0.760 to 8.415±0.575, p=0.000), OSI (from 4.316±0.747 to 1.151±0.101, p=0.000) levels decreased (Table 1).

When CLP + 200 mg/kg alliin group, compared to the CLP group, TAS (from 0.271±0.037 to 0.817±0.069, p=0.000) increased, while TOS (from 11.497±0.760 to 7.536±0.834, p=0.000) and OSI (from 4.316±0.747 to 0.930±0.149, p=0.000) levels decreased statistically significantly. In the CLP + 200 mg/kg alliin group, TAS levels (p=0.013) were higher but TOS (p=0.028) and OSI (p=0.004) levels were lower than the CLP + 100 mg/kg alliin group (Table 1).

**Table 1:** TOS, TAS and OSI levels comparisions among the experimental groups.

| Experimental Groups (n=8) | TAS | TOS | OSI |
|---|---|---|---|
| *Sham control (1)* | 0.844±0.094 | 7.099±0.718 | 0.852±0.142 |
| *CLP(2)* | 0.271±0.037 | 11.497±0.760 | 4.316±0.747 |
| *CLP+Alliin 100 mg/kg (3)* | 0.733±0.046 | 8.415±0.575 | 1.151±0.101 |
| *CLP+Alliin 200 mg/kg (4)* | 0.817±0.069 | 7.536±0.834 | 0.930±0.149 |
| *p value (Meaningful intergroup comparisons)* | 0.000 (1-2)<br>0.010 (1-3)<br>0.000 (2-3)<br>0.000(2-4)<br>0.013 (3-4) | 0.000 (1-2)<br>0.001 (1-3)<br>0.000 (2-3)<br>0.000 (2-4)<br>0.028(3-4) | 0.000(1-2)<br>0.000 (1-3)<br>0.000 (2-3)<br>0.000 (2-4)<br>0.004 (3-4) |

TAS = Total Antioxidant Status; TOS = Total Oxidant Status; OSI = Oxidative Stress Index. Data are presented as mean ± S.D. $p<0.05$.

When the CLP group compared to the sham control group, SOD (from 284.716±17.744 to 193.710±20.898, p=0.000) level decreased, while MPO (from 155276.845±23418.744 to 382656.553±38649.132, p=0.000), MDA (from 57.191±6.647 to 92.179±11.357, p=0.000), TNF-α (from 20277.135±1624.811 to 81107.189±5346.898, p=0.002), and IL-1β (from 23799.306±1744.156 to 98889.863±6808.398, p=0.002 ) levels increased. When the CLP + 100 mg/kg alliin group, compared to the CLP group, while the level of SOD (from 193.710±20.898 to 245.202±35.384, p=0.003) increased, MPO (from 382656.553±38649.132 to 213298.825±24232.652, p=0.000), MDA (from 92.179±11.357 to 63.014±7.941, p=0.000), TNF-α (from 81107.189±5346.898 to 23892.692±2847.935, p=0.000), and IL-1β (from 98889.863±6808.398 to 24533.186±2329.884, p=0.000) levels decreased (Table 2).

When the CLP + 200 mg/kg alliin group, compared to the CLP group, while the level of SOD (from 193.710±20.598 to 271.120±21.588, p=0.000) increased, MPO (from 382656.553±38649.132 to 173187.873±20069.664, p=0.000), MDA (from 92.179±11.357 to 56.979±4.296 ; p=0.000), TNF-α (from 81107.189±5317.898 to 22302.043±1417.417, p=0.000), and IL-1β (from 98889.863±6808.398 to 22578.180±1111.485; p=0.000) levels decreased (Table 2).

**Table 2:** Comparisons of other oxidative markers and cytokines among the experimental groups.

| Experimental Groups (n=8) | SOD | MPO | MDA | TNF-α | IL-1β |
|---|---|---|---|---|---|
| *Sham control (1)* | 284.716±17.744 | 155276.845±23418.744 | 57.191±6.647 | 20277.135± 1624.811 | 23799.306±1744.156 |
| *CLP(2)* | 193.710±20.898 | 382656.553±38649.132 | 92.179±11.357 | 81107.189± 5346.898 | 98889.863±6808.398 |
| *CLP+Alliin 100 mg/kg (3)* | 245.202±35.384 | 213298.825±24232.652 | 63.014±7.941 | 23892.692±2847.935 | 24533.186±2329.884 |
| *CLP+Alliin 200 mg/kg (4)* | 271.120±21.588 | 173187.873±20069.664 | 56.979±4.296 | 22302.043±1417.417 | 22578.180±1111.485 |
| *p value (Meaningful intergroup comparisons)* | 0.000 (1-2)<br>0.014 (1-3)<br>0.003 (2-3)<br>0.000(2-4) | 0.000 (1-2)<br>0.000 (1-3)<br>0.000 (2-3)<br>0.000 (2-4)<br>0.003(3-4) | 0.000(1-2)<br>0.000 (2-3)<br>0.000 (2-4)<br>0.008 (3-4) | 0.002(1-2)<br>0.008 (1-3)<br>0.019 (1-4)<br>0.000 (2-3)<br>0.000 (2-4) | 0.002(1-2)<br>0.000 (2-3)<br>0.000 (2-4)<br>0.050 (3-4) |

SOD=Superoxide Dismutase; MPO=Myeloperoxidase; MDA=Malondialdehyde; TNF-α=Tumor necrosis factor-α; IL-1β=Interleukin-1β. Data are presented as mean ± S.D. $p<0.05$.

When the results are evaluated, a great similarity is observed between the control group and the treatment groups.

## 4.DISCUSSION

Septicemia is an uncontrolled hyper-inflammatory response and and a devastating cause of mortality [2, 28]. Despite the advancements in anti-microbial drugs; sepsis and septic shock still keep being a tough issue for clinicians. The annual morbidity due to sepsis reaches 50–95 cases per 100,000 citizens in USA [29]. The diagnosis of sepsis is avalaible if there are clinical evidences of systemic inflammation. The most common body field of infection is the lungs, composing 40% of all body involvement [30, 31]. The pulmonary inflammation due to excessive production of ROS, acute lung injury (ALI) is a world-wide sepsis complication [32].

Among the several murine models of sepsis, CLP is a commonly used animal model leading to systemic inflammation in which controlled bowel perforation following cecal ligation occurs [33, 34, 35]. Infection may lead to sepsis due to bacterial translocation, mostly originated from spleen, liver or mesenteric lymph nodes. Sepsis activates inflammatory response initially via inflammatory mediators released by cytokines [36, 37]. Live body needs

inflammation, because it is the preservative response of immune system which mostly acts against microorganisms [38]. Uncontrolled or intense inflammation leads to diseases [39]. Proinflammatory cytokines such as TNF-α, IL-1β are considered as pivotal mediators for sepsis-induced lung injury [40, 41]. MDA occurs after lipid peroxidation. SOD is the only antioxidant enzyme that scavenges superoxide [42, 43]. ROS occurs as a result of aerobic metabolism and can be removed thanks to antioxidant enzymes such as SOD [44]. ROS decreases natural antioxidants, glutathione (GSH) and SOD [45].

Studies of the CLP-sepsis model in vivo showed increased ROS levels in lung tissue [46]. ROS triggers the endogenous antioxidant defense system. As a result of this cascade, as seen in our results, the concentration of SOD decreased in tissue [47, 48]. The intense release of ROS causes lipid peroxidation and the increases MDA concentration, which is the final product of unsaturated lipid oxidation. MDA also indicates oxidative damage indirectly [49]. Our results were correlate with this data [50]. MPO also increased in our study. MPO is determined intensively in neutrophils, and high concantration of MPO indicates neutrophil activation [51]. This result plays major role in the etiopathogenesis of ALI / acute respiratory distress syndrome (ARDS) [52]. Clinical and experimental studies support that antioxidant administration looks like helpful in septic states in which the lungs are the most affected remote organs [12, 53].

Many alliin-related studies are available in the literature supporting the results of our study. Alliin has shown an antiinflammatory effect by reducing TNF-α, IL-1β and MPO levels in a study [54]. Alliin reduced inflammation by reducing TNF-α and MDA levels during DENV infection [55]. Alliin decreases MDA, MPO levels and heals dextran sulfate sodium-induced ulcerative colitis and stops the inflammatory responses in lipopolysaccharide-stimulated RAW264.7 cells [56]. AHE (contains organosulfur composites like alliin, S-allylcysteine, etc.) considerably inhibited NO, cytokines and ROS production in lipopolysaccharide-induced RAW264.7 cells [23]. Alliin hindered the increment of proinflammatory gene (IL-6, TNF-α) expression in lipopolysaccharide- stimulated 3T3-L1 adipocytes [57]. Alliin attenuated nuclear factor-κB ligand (RANKL)-induced osteoclastogenesis receptor activator by scavenging ROS [24]. The study showed the protective effect of alliin on isoproterenol-induced cardiotoxicity in Wistar albino male rats by increasing antioxidants [58, 59]. Alliin exhibited antioxidant activities as protective compounds against free radical damage [60]. In parallel with these studies, in our study, antioxidant and antiinflammatory properties of alliin have been shown in CLP-induced sepsis model in rats. In the CLP group, TAS and SOD

decreased while MDA, MPO, TNF-α, IL-1β, TOS, OSI levels were increased and alliin treatment reversed these levels.

Due to our results, reduction of TNF-α, IL-1β concentrations in septic rats by alliin, suggesting that alliin alleviated CLP-induced ALI. We assessed oxidative stress in the lung tissue to investigate the improving effects of the alliin against CLP-induced lung injury and observed that oxidative stress decreased with alliin. The fact that there is no study related with the protective effects of alliin in the literature review of CLP-induced sepsis makes this study original.

Understanding of cellular damage mechanisms of sepsis is important for planning new and effective treatment methods. Sepsis studies demonstrated that inflammation and oxidative stress suppression can provide significant contributions to the sepsis treatment. In our study, inflammation, oxidative stress pathways are suppressed by alliin and this encourages hope in the treatment of sepsis.

## 5.CONCLUSIONS

Alliin provides a protection against lung injury arising from CLP-induced sepsis via its antioxidant and antiinflammatory properties. We have indicate that treatment with alliin at different doses reduces lung damage in experimental animals exposed to CLP-induced sepsis model. Moreover, further researches are necessarry for explain the other protective mechanism on lung tissue damage induced by sepsis.

**Conflict of interest:** The authors declare that there are no conflicts of interest.

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