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Research Article / Araştırma Makalesi

The Effects of S-Allyl Cysteıne on Inflammatory Cascade in Lipopolysaccharide Induced Rat Sepsis Model

Lipopolisakkarit ile İndüklenen Sıçan Sepsis Modelinde S-Allil Sisteinin İnflamatuar Kaskat Üzerine Etkileri

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Abstract

Lipopolysaccharide (LPS) is a main constituent of Gram-negative bacterial cell walls and is considered a leading cause of sepsis. S-allyl cysteine (SAC) is a water-soluble organosulfur component present in garlic which has a potent antioxidant and free radical scavenger activity. The purpose of this study is to examine the antioxidant and anti-inflammatory potential of SAC on endotoxin LPS-induced sepsis. Female Wistar albino rats were divided into 6 groups. LPS (5 mg/kg) was applied to rats in sepsis and treatment groups intraperitoneally. After 24 hours from LPS injection 50 mg/kg and 100 mg/kg SAC was orally administrated to treatment groups. Lung and liver 18F-fluoro-deoxy-D-glucose (18F-FDG) uptake was measured by 18FDG-PET scan. Serum levels of nuclear factor-kappa B (NF-κβ), tumor necrosis factor-alpha (TNF-α), matrix metalloproteinase-9 (MMP-9), plasma levels of interleukin-1β (IL-1β), IL-6 and tissue levels of oxidative stress markers catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA) and nitric oxide (NO) were determined. As a result of the study, MDA and NO levels of sepsis group were significantly higher than treatment groups in lung tissue. SOD activities of sepsis group was determined to significantly lower in the liver and lung tissues than the groups which were treated with SAC. Likewise, it was concluded that serum MMP-9, TNF-α and NF-κβ levels of sepsis group was significantly higher compared to levels of treatment groups. It was determined that SAC administration reduced 18F-FDG uptake in septic rats. In conclusion, SAC was observed to diminish effects of the acute toxicity and oxidative stress formed with LPS.

Keywords: Sepsis; lipopolysaccharide; S-allyl cysteine; rat; antioxidant; anti-inflammatory

Özet

Lipopolisakkarit (LPS), Gram-negatif bakteriyel hücre duvarlarının ana bileşenidir ve sepsisin önde gelen bir nedeni olarak kabul edilir. S-alil sistein (SAC), sarımsakta bulunan güçlü bir antioksidan ve serbest radikal temizleyici aktiviteye sahip suda çözünür bir organosülfür bileşenidir. Bu çalışmanın amacı, SAC'nin endotoksin LPS kaynaklı sepsis üzerindeki antioksidan ve antiinflamatuar potansiyelini incelemektir. Dişi Wistar albino sıçanları 6 gruba ayrıldı. LPS (5 mg/kg), sepsis ve tedavi gruplarındaki sıçanlara intraperitoenal olarak uygulandı. LPS enjeksiyonundan 24 saat sonra 50 mg/kg ve 100 mg/kg SAC oral olarak tedavi gruplarına uygulandı. Akciğer ve karaciğer 18F-floro-deoksi-D-glukoz (18F-FDG) alımı, 18FDG-PET taraması ile ölçüldü. Serum nükleer faktör-kappa B (NF-κβ), tümör nekroz faktörü-alfa (TNF-α), matriks metalloproteinaz-9 (MMP-9), interlökin-1β (IL-1β) seviyeleri, plazmada IL-6 seviyesi ve dokuda oksidatif stres markerleri katalaz (CAT), süperoksit dismutaz (SOD), malondialdehid (MDA) ve nitrik oksit (NO) seviyeleri belirlendi. Çalışma sonucunda, akciğer dokusunda MDA ve NO düzeyleri sepsis grubunda tedavi gruplarından anlamlı olarak yüksekti. Karaciğer ve akciğer dokularında SOD aktivitelerinin sepsis grubunda SAC ile tedavi edilen gruplara göre anlamlı derecede düşük olduğu belirlendi. Benzer şekilde sepsis grubu serum MMP-9, TNF-α ve NF-κβ düzeylerinin tedavi gruplarının düzeylerine göre anlamlı derecede yüksek olduğu sonucuna varıldı. Septik sıçanlarda SAC uygulamasının 18F-FDG alımını azalttığı belirlenmiştir. Sonuç olarak, SAC'nin LPS ile oluşan akut toksisite ve oksidatif stresin etkilerini azalttığı gözlenmiştir.

Anahtar Kelimeler: Sepsis; lipopolisakkarit; S-allil sistein; sıçan; antioksidan; antiinflamatuar

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1. Introduction

Sepsis is the invasion of microorganisms or toxins into sterile tissues and blood, so that organism responds by activating the inflammatory pathways (1). Inflammatory response is given in order to initiate a healing process but chronical or high doses of exposure to an endotoxin causes imbalances on inflammatory cytokines, reactive oxygen species and even on homeostasis which lead to organ dysfunction and cell death (2).

Lipopolysaccharide (LPS) is an endotoxin present in cell wall of Gram-negative bacteria which is used to induce sepsis model on rats (3). Exposure to LPS causes activation of inflammatory cascade by inducing receptors on monocytes and machrophages cell membrane, which triggers activation of nuclear factor-kappa B (NF-κB) pathway that in turn activates release of inflammatory mediators such as cytokines and reactive oxygen species from plasma, leukocytes and vascular cells (4). Sepsis is an overproduction of both proinflammatory and anti-inflammatory cytokines that work together on physiological conditions and control the effects of each other. Interleukin-1 (IL-1), IL-6 and tumor necrosis factor-alpha (TNF- α) are major cytokines that are important regulators both in inflammatory response and also in sepsis. Both IL-1 and IL-6 induces release of interferon-γ (IFN- γ), IL-2, IL-4 from T cells by activating them. IL-1 has also role in nitric oxide (NO) synthesis by activating cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) (5-7). TNF-α triggers activation of adhesion molecules on neutrophils so that they can accumulate on endothelial cells but this accumulation causes damage on endothelial cells due to both peptidase activities of degranulated neutrophils and free oxygen radical release (2). Matrix metalloproteinase-9 (MMP-9) is a kind of that protease, whose activities are triggered by cytokines, plays a significant role in inflammatory response by regulating extracellular matrix composition which lets lymphocytes to penetrate required area (8).

Except for cytokines, the other component of body to deal with an inflammation is to produce free radicals in order to kill the invading organisms. The drawback of producing free radicals are generation of aldehyde formation like malondialdehyde (MDA) and also DNA and protein damage which in turn lets development of many diseases such as cancer, atherosclerosis, diabetes, arthritis, and osteoporosis (4,9). These free radicals are scavenged by antioxidant defense system such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) (10).

S-allyl cysteine (SAC), which is a natural product present in garlic (Allium sativum L.), has shown to have anticancer activity by inducing apoptosis and anti-inflammatory activities by suppressing cytokine release in various studies, SAC also prevents lipid and protein oxidation and blocks NF-κβ activation (11,12). SAC contains the thiol group and this nucleophile responsible for the antioxidant properties is rapidly attacking the electrophilic sites and neutralizing them to become less reactive. In vitro and in vivo studies have shown that SAC inhibits the oxidation and nitration of proteins and lipids and has an antioxidant effect (13).

In this study, our aim was to determine anti-inflammatory and antioxidant effects of SAC on experimental sepsis rat model induced by LPS. Treatment of sepsis, which is leading cause of deaths due to infectious diseases in the world, with natural agents is crucial for development of new drugs and complementary therapies.

2. Material and Methods

Animals and study design

Four to five months old, 250-300 g weighing, 42 Wistar albino rats were divided into 6 groups. The rats were acclimated in a room, which had a 12-hour dark 12-hour light, temperature of 22 ± 2 °C and humidity of 45-50% and fed on standard pellet laboratory diet. All experimental procedures were approved by the Eskisehir Osmangazi University Medical Faculty Animal Use and Care Committee (Date: 09.04.2015, No: 82/452).

In the study, sepsis model induced by administration of single dose of LPS (E. coli, serotype 055-B5, 5 mg/kg) intraperitoneally to sepsis, Sepsis+SAC 50 and Sepsis+SAC 100 groups. SAC was given to treatment groups orally (50 mg/kg and 100 mg/kg) every 12 h for 7 days after 24-h of intraperitoneal LPS injection. At the 12th hour following the last SAC

application, animals were screened for 18F-fluoro-deoxy-D-glucose-positron emission tomography (18FDG-PET). Table 1 describes the substances and amounts applied to the groups.

Table 1. Groups and administered substances

Groups (n=7)	Treatments
Control	Saline, intraperitoneal (i.p.)
Sepsis	5 mg/kg LPS [(*E. coli*, serotype 055-B5), intraperitoneal (i.p.)]
SAC control 50	50 mg/kg S-allyl cysteine intragastric (i.g.)
SAC control 100	100 mg/kg S-allyl cysteine (i.g.)
Sepsis+SAC 50	5 mg/kg LPS (i.p.) + 50 mg/kg S-allyl cysteine intragastric (i.g.)
Sepsis+SAC 100	5 mg/kg LPS (i.p.) + 100 mg/kg S-allyl cysteine intragastric (i.g.)

^{18}FDG-PET scanning

Similar to glucose, ^{18}F-fluoro-deoxy-D-glucose (^{18}F-FDG) is also converted into glucose-6-phosphate by hexokinase, and take up into cells by cell membrane proteins (especially GLUT-1). Contrary to normal glucose metabolism, this glucose-6-phosphate can not get through to the other steps of glycolysis and can not breakdown because present in a small amount in tumor cells. Rapid metabolism of glucose in cancer cells with high metabolic rate causes more ^{18}F-FDG uptake. Thus, as the location of the tumor tissue is determined, high ^{18}F-FDG uptake is also observed in infected and inflamed areas (14,15). For the determination of the ^{18}F-FDG uptake, rats were anesthetized with ketasol (1 ml/kg) and ^{18}F-FDG (0.8 ml/kg) applied intracardially. Half hour after ^{18}F-FDG injection, ^{18}F-FDG uptake in lung and liver was measured using ^{18}FDG-PET scanner (Biograph 6 Hires PET / CT Siemens, Knoxville, Tennessee, USA).

In PET evaluations, activity concentration of ^{18}F-FDG in tissue (mCi/g) was calculated [injected dose (mCi) and body weight (g)]. Visual (qualitative) evaluation was performed by determining areas that were initially increased uptake according to surrounding tissues and background. Semiquantitative evaluation was then carried out and "Standard Uptake Value" (SUV) was determined (17).

SUV= mean selected region activity (mCi/ml) / injected dose (mCi) / body wt (g)

Preparation of Samples

Following scanning, animals were sacrificed under anesthesia to receive blood and tissue samples. Blood samples were centrifuged at 3500 g for 15 min. to separate serum. For MDA level detection, liver and lung tissues were divided into equal parts and homogenized with ultrasonic homogenizer in 0.01 M, pH = 7.4 phosphate buffer solution. Tissue samples were homogenized in an ultrasonic homogenizer in potassium chloride (1 %) buffer to determine SOD activities. Homogenates for CAT activities were prepared as described in LZ Biotech ELISA Kit (Cat. No: YHB0207Ra). Tissues were homogenized in 1 mM phosphate buffer (pH: 7.4), centrifuged at 12000 g for 15 min. and supernatant removed to evaluate NO levels. Protein quantities in tissues were determined according to Sigma to Total Protein Kit (Product code: TP0100).

Oxidative stress markers determination

SOD activity was evaluated with Sigma SOD Determination Kit (Cat. No. 19160) established on the WST (water-soluble tetrazolium salt) reaction that provides reduction with superoxide anion. The reaction inhibited by SOD so calculated SOD activities were expressed as % inhibition. CAT activity was determined by "LZ Biotech ELISA Assay Kit (YHB0207Ra)" and MDA levels were measured by the "Elabscience Assay Kit (E-EL-0060)". All steps were carried out in accordance with explanations in the catalogs. All measurements were analyzed with BioLab Elisa reader at a wavelength of 532 nm for MDA and 450 nm for SOD and CAT.

Nitric oxide levels determination

In many studies endogenously produced NO concentration in the body has calculated as total nitrite and nitrate. Because the produced NO quickly turn into nitrate and nitrite. Proteins were removed from the media by deproteinization and then nitrite and nitrate evaluations were made. Measurements were performed as previously described (16). First, the samples were deproteinated in the Somogyi reagent. Nitrate was then converted to nitrite in glycine buffer (pH 9.7) with Cu coated cadmium granules (2.5-3 g granule for 4 ml reaction mix.). Sulfanilamide solution (1 ml) and N-naphthylethylenediamine solution (1 ml) were added and after an hour incubation, absorbance was established at 545 nm. spectrophotometrically. Nitrite concentration was calculated for each sample using sodium nitrite as standard and was determined as µmol /mg relative to the protein amounts of the samples.

Serum NF-κβ, TNF-α, MMP-9 and plasma IL-1β, and IL-6 levels determination

NF-κβ, TNF-α, MMP-9, IL-1β, and IL-6 levels were determined according to the protocol "LZ Biotech ELISA Kit Cat. No. YHB0794Ra, Cat. No. YHB1098Ra, Cat. No. YHB0724Ra YHB0616Ra, and Cat. No: YHB1747Hu", respectively. All measurements were analyzed with BioLab Elisa reader at a wavelength of 450 nm.

Statistical analysis

Data was analyzed by package program IBM SPSS 21. Shapiro Wilk test was used to test normality of quantitative variables and variables were given as mean±standard deviation. Overall and pairwise group differences were compared by using one-way Anova and Tukey test, respectively. Statistically significant differences were confirmed by p values lower than 0.05.

3. Results

^{18}F-FDG uptake values

Results presented in Table 2 and Table 3 showed that there were significant differences between ^{18}F-FDG uptake of sepsis groups compared with control groups both in liver and lung tissues ($p<0.001$). ^{18}F-FDG uptake of sepsis group was significantly higher than of treatment groups (Sepsis+SAC 50 and Sepsis+SAC 100) in liver ($p<0.001$). Similarly, a significant difference was found in lung ($p<0.01$).

Table 2. Liver ^{18}F-FDG uptake

			Multiple comparison					
	Groups	**SUV max.**	**1**	**2**	**3**	**4**	**5**	**6**
1	**Control**	0.37±0.05		***	ns	ns	***	***
2	**Sepsis**	1.19±0.04	***		***	***	***	***
3	**SAC control 50**	0.38±0.03	ns	***		ns	***	***
4	**SAC control 100**	0.38±0.04	ns	***	ns		***	***
5	**Sepsis+SAC 50**	0.97±0.06	***	**	***	***		ns
6	**Sepsis+SAC 100**	0.81±0.09	***	**	***	***	ns	

Data are presented as mean ± SD (n = 7).
*Significant differences were found: **P < 0.01, ***P < 0.001*
ns, non significant; SUV, standart uptake value; ^{18}F-FDG: ^{18}F-fluoro-deoxy-D-Glucose

Table 3. Lung ^{18}F-FDG uptake

			Multiple comparison					
	Groups	**SUV max.**	**1**	**2**	**3**	**4**	**5**	**6**
1	**Control**	0.30±0.04		***	ns	ns	***	***
2	**Sepsis**	1.50±0.17	***		***	***	**	**
3	**SAC control 50**	0.28±0.04	ns	***		ns	***	***

4	**SAC control 100**	0.27±0.06	ns	***	ns		***	***
5	**Sepsis+SAC 50**	0.90±0.09	***	**	***	***		ns
6	**Sepsis+SAC 100**	0.84±0.08	***	**	***	***	ns	

Data are presented as mean ± SD (n = 7).
*Significant differences were found: **P < 0.01, ***P < 0.001*
ns, non significant; SUV, standart uptake value; ^{18}F-FDG: ^{18}F-fluoro-deoxy-D-Glucose.

Effects of SAC on MDA levels

MDA levels in liver and lung were shown at Table 4. MDA levels of control and sepsis group did not differ significantly (p>0.05) whereas SAC control 50 (p<0.01), SAC control 100 (p<0.001), Sepsis+SAC 50 (p<0.01), and Sepsis+SAC 100 (p<0.01) groups were found to be significantly lower compared to sepsis group in liver tissue. In lung tissue, MDA levels in control group were found to be significantly lower than sepsis group (p<0.01), and MDA levels of SAC control 50 (p<0.01), and SAC control 100 (p<0.01) groups were found to be significantly lower compared to sepsis group.

Table 4. Liver and lung MDA levels

Groups	Liver MDA levels (nmol / mg protein)	Lung MDA levels (nmol / mg protein)
Control	131.25±4.48	114.20±9.41
Sepsis	141.38±6.33	141.91±9.91**
SAC control 50	122.85±8.02^{++}	108.77±13.30^{++}
SAC control 100	121.56±7.31^{+++}	112.53±11.03^{++}
Sepsis+SAC 50	129.06±4.19^{++}	138.94±12.95**
Sepsis+SAC 100	128.46±2.92^{++}	133.61±7.07

Data are presented as mean ± SD (n = 7).
*Significant differences were found: **P< 0.01 compared with control group; $^{++}$P< 0.01, $^{+++}$P< 0.001 compared with sepsis group. MDA: malondialdehyde.*

SAC treatment elevated SOD and CAT activities

Results for SOD activities at liver and lung were shown at Table 5 respectively. SOD activities were found to be lower in sepsis group compared to control (p<0.001) and also SAC control 50 (p<0.001), SAC control 100 (p<0.01), Sepsis+SAC 50 (p<0.001) and Sepsis+SAC 100 (p<0.001) groups have shown higher SOD activities compared to sepsis group in liver tissue. Similarly, decreased SOD activity was detected in sepsis group compared with control group in lung (p<0.001). In addition, SAC treatment (Sepsis+SAC 50, and Sepsis+SAC 100) significantly increased SOD activities in lung homogenates (p<0.001). The decrease in SOD activities seen in the sepsis group was increased by SA

Table 5. Liver and lung SOD activities

Groups	Liver SOD (% inhibition)	Lung SOD (% inhibition)
Control	84.86±2.96	77.77±1.58
Sepsis	72.43±2.53***	68.62±1.69***
SAC control 50	80.65±2.21**,$^{+++}$	79.15±2.02^{+++}
SAC control 100	77.85±2.27***,$^{++}$	79.36±1.89^{+++}
Sepsis+SAC 50	82.37±1.31^{+++}	74.38±2.12**,$^{+++}$
Sepsis+SAC 100	83.56±2.06^{+++}	75.31±2.21^{+++}

Data are presented as mean ± SD (n = 7).
*Significant differences were found: **P< 0.01, ***P< 0.001 compared with control group; $^{++}$P< 0.01, $^{+++}$P< 0.001compared with sepsis group. SOD: superoxide dismutase.*
C treatment.

CAT activities between control and sepsis group were found to be not different for both liver and lung ($p>0.05$). Liver tissue CAT level in SAC control 100 group were found to be higher compared to sepsis group ($p<0.01$), whereas increased CAT levels in SAC control 50 ($p<0.05$), SAC control 100 ($p<0.05$), and Sepsis+SAC 50 ($p<0.01$) groups was evaluated compared with sepsis group for lung tissue (Table 6).

Table 6. Liver and lung CAT activities

Groups	Liver CAT (pg / mg protein)	Lung CAT (pg / mg protein)
Control	3.67±0.71	11.27±1.87
Sepsis	2.36±0.88	10.16±2.16
SAC control 50	3.83±0.39	15.08±2.55^{+}
SAC control 100	4.74±1.17^{++}	15.01±2.82^{+}
Sepsis+SAC 50	3.77±1.02	15.86±4.16$^{*,++}$
Sepsis+SAC 100	3.64±1.25	14.31±2.34

Data are presented as mean ± SD (n = 7).
*Significant differences were found: *P< 0.05 compared with control group; $^{+}$P< 0.05, $^{++}$P< 0.01 compared with sepsis group. CAT: catalase.*

SAC treatment reduced NO levels in lung

NO levels in liver and lung was given in Table 7. No significant difference was found for liver tissue ($p>0.05$). For lung tissue. there was no difference between sepsis and control group ($p>0.05$), while NO levels in SAC control 50 ($p<0.01$), SAC control 100 ($p<0.01$), Sepsis+SAC 50 ($p<0.01$) and Sepsis+SAC 100 ($p<0.01$) groups were significantly lower than sepsis group.

Table 7. Liver and lung NO levels

Groups	Liver NO (µmol / mg protein)	Lung NO (µmol / mg protein)
Control	0.77±0.09	0.32±0.06
Sepsis	0.78±0.14	0.39±0.11
SAC control 50	0.73±0.12	0.27±0.06^{++}
SAC control 100	0.65±0.19	0.25±0.09^{++}
Sepsis+SAC 50	0.76±0.15	0.27±0.05^{++}
Sepsis+SAC 100	0.75±0.09	0.27±0.03^{++}

Data are presented as mean ± SD (n = 7).
Significant differences were found: $^{++}$P< 0.01, compared with sepsis group. NO: nitric oxide.

Serum NF-κβ and TNF-α levels were decreased with SAC treatment

NF-κβ levels in sepsis group were significantly higher than all groups ($p<0.001$) and Sepsis+SAC 50. and Sepsis+SAC 100 were found to be significantly lower compared to sepsis group ($p<0.001$) (Figure 1).

TNF-α levels in sepsis group elevated significantly compared to all groups ($p<0.001$) and decreased TNF-α levels of treatment groups (Sepsis+SAC 50 and Sepsis+SAC 100) were determined in comparison with sepsis group ($p<0.001$) (Figure 1).

SAC reduced serum MMP-9 levels

MMP-9 levels in sepsis group were found to be not different compared to control ($p>0.05$) and Sepsis+ SAC 50 and Sepsis+ SAC 100 were found to be significantly lower compared to sepsis group ($p<0.05$, $p<0.001$ respectively) (Figure 1).

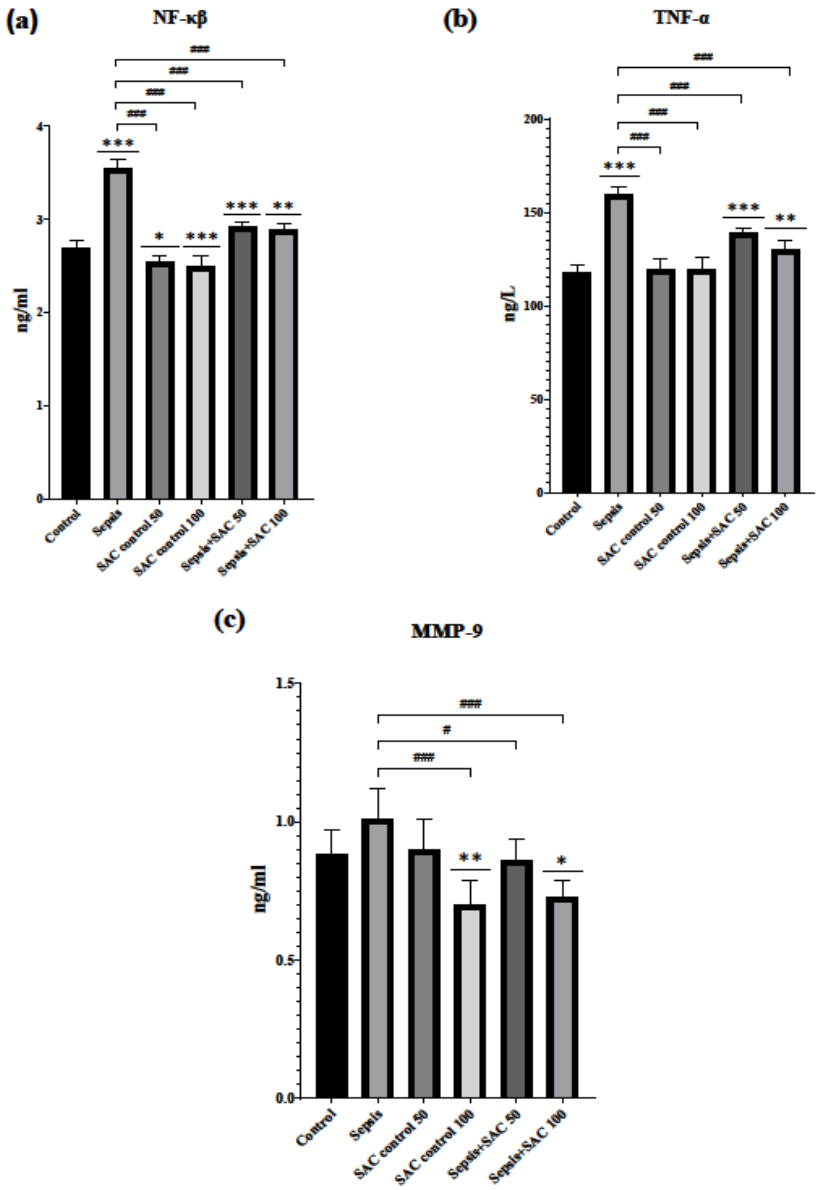

Figure 1. Serum levels of **a)** NF-κβ, **b)** TNF-α and **c)** MMP-9. Data are presented as mean ± SD (n = 7). Significant differences were found: : **P*< 0.05, ***P*< 0.01, ****P*< 0.001 compared with control group; ###*P*< 0.001 compared with sepsis group. NF-κβ: nuclear factor-kappa B, TNF-α: tumor necrosis factor-alpha, MMP-9: matrix metalloproteinase-9.

Effects of SAC on plasma IL-1β and IL-6 levels

Although there was a decrease in IL-1β and IL-6 levels of the treatment groups compared to sepsis group there were no significant differences, elevated IL-1β and IL-6 levels were found in sepsis group compared with controls significantly (Figure 2).

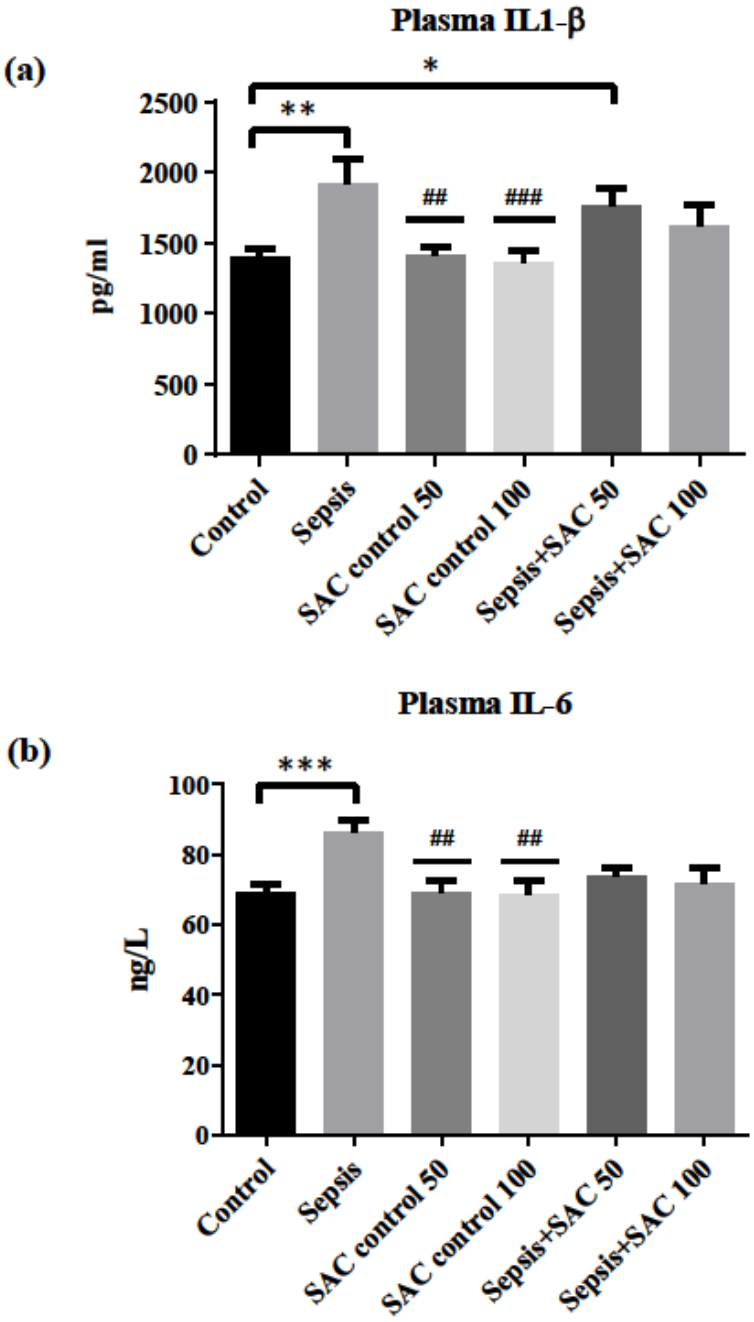

Figure 2. *Plasma levels of* ***a)*** *IL-1β and* ***b)*** *IL-6. Data are presented as mean ± SD (n = 7). Significant differences were found: : *P< 0.05, **P< 0.01, ***P< 0.001 compared with control group; ##P< 0.01, ###P< 0.001 compared with sepsis group. IL-1β: interleukin-1β, IL-6: interleukin-6.*

4. Discussion and Conclusion

Sepsis which is characterized by unbalanced immune response. oxidative stress and mitochondrial dysfunction is a major death causing disease (13). The leading factor for sepsis is being unbalanced inflammatory responses immune system modulators are

major key candidates for treatment of sepsis. Since immune response in sepsis process is extremely complicated only anti-microbial therapy seems to be insufficient to increase survival. It is thought that anti-oxidant agents may play a protective role in sepsis and inflammation with increasing understanding of role of oxidant agents day by day especially while studies about mechanisms of action of mediators and cytokines that are taking place in the physiopathology of sepsis (18).

Garlic is a natural product and has been used widely as traditional drug for treatment of inflammation for years. SAC which is the active compound of garlic is an organosulfur chemical and has anti-oxidant, anticancer and antihepatotoxic activities (19). SAC's antioxidant activities have shown both in vivo and in vitro. In vitro studies shown that SAC could scavenge hydrogen peroxide, superoxide anion, and hydroxyl radicals and also decrease endothelial cell damage and lipid peroxidation (20,21).

TNF-a is a pleiotropic pro-inflammatory cytokine with a wide range of biological functions secreted by monocytes, macrophages, natural killer cells, neutrophils and T-cells following bacterial lipopolysaccharide stimulation (22). Plasma and serum levels of TNF-α which were elevated within 60-90 minutes after endotoxin injection in healthy patients were determined and it was detected that there was an inverse relationship between TNF-α level and survival rate (23). In our study, it was indicated that levels of TNF-α were significantly reduced in 50 and 100 mg SAC treatment groups compared to sepsis group. TNF-α was reduced from the level of sepsis to healthy and sham group levels.

NF-κβ is redox-sensitive transcription factor required in expression of inflammatory mediators and regulation of immune response (24). NF-κβ activity stimulators are reactive oxygen species (ROS), TNF-α (25), IL-1β, bacterial lipopolysaccharides (26), hypoxia (27) and ionizing radiation (28). Geng et al. (29) showed that SAC inhibited both TNF-α and H2O2-induced NF-κB activation in human T lymphocytes before. A previous study reported that SAC reduces TNF-α, IL-6 and NF-κB levels in diabetic nephropathy. It was described that SAC increased inflammatory effects by lowering ROS levels and decreasing NF-κB levels (30). Furthermore, NF-κB increases expression of various extracellular matrix degradation proteins with inflammatory cytokines (31). MMP-9 is found predominantly in inflamed cells and epithelial cells and is capable of degrading many extracellular matrix components, in particular type IV collagen and plays an important role in formation and development of tissue fibrosis (32). Another important function of MMP-9 is to modulate cell-cell interactions by modifying cell adhesion molecules, cell surface receptors, cytokines, growth factors and other proteases (33). According to the findings obtained in our study, NF-κB levels in sepsis group were found to be significantly higher than all groups and SAC therapy caused a substantial decrease in NF-κB levels. Despite elevation of serum MMP-9 levels in sepsis group compared to control group there were no statistically significant difference. We think that increase in NF-κB and TNF-α levels in septic rats induced an elevation of MMP-9. However, there was a decrease in MMP-9 levels in SAC-administered groups. This reduction suggested that SAC reduced MMP-9 synthesis in macrophages and neutrophils thereby prevented tissue damage while minimizing the effects of LPS.

IL-6 and TNF-α give rise to an acute phase response and then to sepsis-like symptoms. Various studies showed that IL-6 and IL-1β levels increase in sepsis (34,35). Despite higher levels of IL-6 and IL-1β were detected in sepsis group induced by LPS than in all groups, statistically significant differences were found between sepsis with control group and also sepsis with SAC administered groups alone.

Several methods are used as indicators of cellular oxidative stress. Among these, measuring the amount of MDA, one of the end products of lipid peroxidation, is most used method in current clinical and experimental studies (2,36). Crimi et al. reported that MDA levels were increased in

sepsis patients and that their antioxidant system was weakened in reverse (37). Studies had shown evidence that lipid peroxidation was prevented by causing a reduction in MDA levels of SAC therapy (38,39). Our results suggested that lipid peroxides which cause endothelium and organ damage in sepsis could be significantly reduced by SAC and that the effects of SAC should be studied at different doses.

Antioxidant enzymes such as SOD and CAT are thought to be the primary defense system that protects macromolecules from oxidative damage. LPS-induced toxicity can cause a significant reduction in efficacy of enzymatic antioxidants. An irreversible inhibition of their activity may occur due to their increased use in LPS-induced free radical scavenging processes. Baluchnejadmojarad et al. reported that SAC enhanced the SOD and CAT antioxidant defense system in their studies on diabetic rats (39). In our study, there was no statistically significant difference between CAT levels of control group and septic rats but a significant decrease in SOD levels was determined. Our results showed that SAC administration caused an increase in the activity of antioxidant enzymes. It was thought that SAC ameliorated oxidative stress by increasing ROS scavenging due to its effective antioxidant activity which in turn increased the activities of antioxidant enzymes.

The first step in the development of oxidative stress in sepsis is mitochondrial deterioration 749910

and increase in nitric oxide (40). NO synthesized by macrophages is one of the most important part of host defense and causes non-specific cytotoxicity against protozoa and tumor cells (41). Yoshimura et al. reported that liver NO levels were increased in sepsis group compared to control group in LPS-induced sepsis mice model (42). In present study, there were no significant differences between NO levels of sepsis and treatment groups in liver, elevated NO levels were found in sepsis group compared to control and treatment groups in lung. Suppression of NO production stimulated by SAC may be associated with the ability to lower NF-κB signal. thus leading to cell protection (13).

In conclusion, our study suggest that SAC prevents the sepsis induced by LPS probably by regulating cytokines in inflammatory cascade such as by blocking NF-κβ and also by increasing antioxidant enzyme activities.

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