

# Effects of S-allyl cysteine on lung and liver tissue in a rat model of lipopolysaccharide-induced sepsis

Orhan Bayraktar · Neslihan Tekin · Ozlem Aydin · Fahrettin Akyuz · Ahmet Musmul · Dilek Burukoglu

Received: 3 July 2014 / Accepted: 25 November 2014 / Published online: 6 December 2014  
© Springer-Verlag Berlin Heidelberg 2014

**Abstract** Sepsis is characterized by a severe production of reactive oxygen species (ROS) and other radical species with consequent oxidative stress. S-allyl cysteine (SAC) is a water-soluble organosulfur component present in garlic which is a potent antioxidant and free radical scavenger. In the present study, the purpose was to explore the anti-inflammatory, antioxidant, and anti-apoptotic actions of SAC on lipopolysaccharide (LPS)-induced sepsis in rats. Thirty-two male Wistar rats were separated into 4 groups. These were control, SAC control, sepsis, and sepsis + SAC-induced groups. Sepsis was induced by administration of LPS (5 mg/kg) into 2 groups. SAC (50 mg/kg) was given orally to SAC control and SAC treatment groups per 12 h during 2 days after intraperitoneal LPS injection. Serum AST, ALT, ALP, and hsCRP levels and liver and lung MPO, NO, and DNA fragmentation levels were evaluated. In sepsis group, elevated levels of ALT, AST, ALP, and hsCRP were observed. The abnormal increases were decreased in sepsis + SAC group compared to sepsis group. In lung tissue, MPO and NO levels were increased in sepsis group compared to the control group. MPO activity and NO levels were decreased by SAC application in sepsis + SAC

group compared with sepsis group. In liver tissue, DNA fragmentation was significantly higher in sepsis group than that in the control group. In contrast, a decreased level of DNA fragmentation was noted in sepsis + SAC group when compared with the sepsis group. In conclusion, SAC ameliorates LPS-induced indicators of liver damage and suppresses the discharge of NO and MPO in lung tissue via its antioxidant properties.

**Keywords** Lipopolysaccharide · S-allyl cysteine · Nitric oxide · DNA fragmentation · Antioxidant

## Abbreviations

LPS	Lipopolysaccharide
ROS	Reactive oxygen species
SAC	S-allyl cysteine
NOP	Nitric oxide
iNOS	Inducible NO-synthase
MPO	Myeloperoxidase
PMNs	Polymorphonuclear neutrophils
H&E	Hematoxylin and eosin
SPSS	Statistical Package for the Social Sciences
ANOVA	One-way analysis of variance
ILs	Interleukins
CRP	C-reactive protein
LDL	Low-density lipoprotein
HOCl	Hypochlorous acid

O. Bayraktar · O. Aydin · F. Akyuz  
Department of Medical Biochemistry, Faculty of Medicine, Eskisehir Osmangazi University, Eskisehir, Turkey

N. Tekin (✉)  
Department of Chemistry, Biochemistry Division, Faculty of Science and Letters, Aksaray University, Aksaray, Turkey  
e-mail: neslihan\_tekin@hotmail.com

A. Musmul  
Department of Biostatistics, Faculty of Medicine, Eskisehir Osmangazi University, Eskisehir, Turkey

D. Burukoglu  
Department of Histology, Faculty of Medicine, Eskisehir Osmangazi University, Eskisehir, Turkey

## Introduction

Sepsis is a major cause of death in the world that is characterized by a dysregulated immune response, oxidative stress, and mitochondrial dysfunction. During sepsis, disequilibrium among ROS and antioxidant protective mechanisms occurs,

and increased ROS lead to cellular damage and organ dysfunction (Lowes et al. 2013).

The majority of cases of sepsis are due to contamination of the blood with bacteria (Liu et al. 2009). Lipopolysaccharide (LPS) is a constituent of the cell wall of Gram-negative bacteria and is a main agent causing sepsis (Ren et al. 2008). LPS triggers the manufacture of cytokines and ROS such as superoxide, hydroxyl radicals, and peroxynitrite (Sepehr et al. 2013). These effects of LPS can induce lipid peroxidation and oxidative damage of proteins and DNA (Ozdemir et al. 2007).

LPS generates large quantities of nitric oxide (NO) subsequent to the stimulation of the inducible NO-synthase (iNOS) in various cell types and tissues. Increased production of NO exhibits a significant role in the pathogenesis of endotoxin-induced tissue injury (Yamashita et al. 2000). Myeloperoxidase (MPO), abundantly expressed in polymorphonuclear neutrophils (PMNs), plays a central role in innate immunity and regulation of the generation of NO-derived oxidants (Tiruppathi et al. 2004). NO overproduction interferes with mitochondrial respiration and induces apoptosis and necrosis (Escames et al. 2006). It has been suggested that reactive nitrogen species cause DNA or tissue damage and contribute to the process of carcinogenesis (Ippoushi et al. 2002).

Garlic has long been used as a natural remedy to treat thrombosis, inhibit inflammation, and prevent cellular oxidative stress (Kim et al. 2001). SAC is the most plentiful organosulfur constituent of garlic and has been described to possess antioxidant, anticancer, antihepatotoxic, and neurotrophic properties (Javed et al. 2011). Several mechanisms of action of SAC have been suggested. It was proposed that SAC displays its strong antioxidant property by scavenging superoxide radical, hydroxyl radicals, and hydrogen peroxide. Another mechanism proposes that SAC prevents oxidized low-density lipoprotein (LDL)-induced endothelial cell damage and LDL oxidation. In addition, SAC prevents H<sub>2</sub>O<sub>2</sub>-induced activation of nuclear factor kappa B and regulates nitric oxide (NO) production with associated anti-inflammatory responses (Atif et al. 2009; García et al. 2010; Pérez-Severiano et al. 2004). However, there are no scientific studies directly addressing the effects of SAC on rat model of sepsis.

The aim of the current investigation was to determine the antioxidant, anti-inflammatory, and anti-apoptotic effects of SAC on liver and lung tissues of LPS-induced septic rats.

## Materials and methods

### Animals

Male Wistar rats at 3 months of age, weighing 200 to 250 g were acquired from Eskisehir Osmangazi University Experimental Research Center. The rats were kept in polycarbonate

cages at controlled room temperature and humidity (21±2 °C, 45 to 55 %). They were fed with a normal rat chow and allowed free access to drink water ad libitum. This study was permitted by the Animal Use and Care Committee, Faculty of Medicine, Eskisehir Osmangazi University.

### Experimental design

A total of 32 rats were randomly separated into 4 main groups, each comprising of 8 rats.

**Control group** Control rats were given 100 µL of physiological saline orally using an intragastric tube (gavage) twice a day for a period of 2 days.

**SAC control** Group 2 rats were administered with SAC (Sigma Chemical Company, St. Louis, MO, USA) dissolved in 100 µL of vehicle saline, and rats were subjected to it orally using an intragastric tube twice a day during 2 consecutive days. We evaluated the acute toxicity level of SAC on rats according to some studies, and SAC dose was selected at 50 mg/kg for use due to no toxic effect at this concentration (Colín-González et al. 2012; Kodera et al. 2002; Yan and Zeng 2005).

**Sepsis** Sepsis was induced in rats by a single intraperitoneal administration of LPS (*Escherichia coli*, serotype 055-B5, 5 mg/kg) from *E. coli* (Li et al. 2013).

**Sepsis + SAC** SAC (50 mg/kg) was given orally using an intragastric tube twice a day during 2 consecutive days after the injection of LPS (5 mg/kg).

### Sample preparations

At the end of the experimental period, intracardiac blood samples were obtained under ketamine/xylazine anesthesia, and the rats were killed by cervical dislocation. Blood samples were centrifuged at 3500×g for 10 min, and serum was isolated to determine AST, ALT, ALP, and hsCRP. Liver and lung tissue samples were cleaned using an ice-cold solution of isotonic NaCl for the removal of blood spots, and they were then dried with blotting paper. Liver and lung tissues were divided into two parts, one part for biochemical analysis and the other for histology.

### Determination of biochemical parameters

Serum enzymes (AST, ALT, and LDH) and high sensitivity C-reactive protein (hsCRP) levels were analysed using Roche Hitachi kit via Roche Diagnostic Modular Analyzer and expressed as units per liter, units per liter, units per liter, and milligrams per liter, respectively.

### Determination of NO levels

Liver and lung tissue nitrite plus nitrate contents, as an index of tissue NO levels, were measured spectrophotometrically by the Griess method. These tissues were homogenized in Tris-HCl buffer (5 mM containing 2 mM EDTA, pH 7.4) and centrifuged at 2000 g for 5 min. The nitrate was converted to nitrite by Cu-coated cadmium granules in glycine buffer at pH 9.7 following the deproteinization of the samples with Somogyi reagent. Samples were reacted with Griess reagent (N-naphthylethylenediamine and sulfanilamide) for 45 min at room temperature and measured with a spectrophotometer at a wavelength of 545 nm. Nitrite levels of the tissues were identified by using standard curve, which was created with different concentrations of sodium nitrite as the standard (Cortas and Wakid 1990). Tissue protein contents were measured using the Biuret method (Kingsley 1942). Results were specified as micromoles per milligram protein.

### Determination of MPO activity

MPO activity was estimated spectrophotometrically by the Dessler method in liver and lung homogenates. Tissue samples were homogenized in 0.02 M EDTA and centrifuged at 20,000 g for 15 min. Pellets were discarded and then resuspended in 0.1 M sodium phosphate buffer containing 0.5 % hexadecyltrimethylammonium bromide. These suspensions were centrifuged at 20,000 g for 15 min, and the consisting of supernatant solution was analyzed (Ekingen et al. 2006). The samples (25  $\mu$ l) of the supernatant were combined with 1.0 ml of 0.1 M sodium phosphate buffer (pH 7.0) containing 1.6  $\mu$ l of guaiacol and 0.0005 % hydrogen peroxide as substrates. MPO activity was determined with a spectrophotometer at a wavelength of 470 nm (Werner et al. 2002). Tissue protein contents were measured using the Biuret method (Kingsley 1942). Results were specified as units per milligram protein.

### Determination of DNA fragmentation levels

DNA fragmentation was measured spectrophotometrically in liver and lung tissues according to previous reports. This assay is mainly based on the hydrolysis of DNA. Briefly, tissues were homogenized in lysis buffer containing 5 mM Tris-HCl, 20 mM EDTA, 0.5 % Triton X 100, pH 8. Homogenized tissues were centrifuged at 26,000 g for 25 min. Pellet and supernatant were assayed to determine DNA content using diphenylamine reaction. DNA fragmentation was expressed as the percentage of fragmented DNA appearing in studied tissues (Burton 1968; Atroschi et al. 1999).

### Histopathological assays

Liver and lung tissues were dissected out, washed in saline, and fixed in formalin. After embedded in paraffin, they were sectioned at 4  $\mu$ m and stained with hematoxylin and eosin (H&E). All sections were scanned after examination with Olympus BH-2 photomicroscope.

### Statistical analysis

The data were analyzed by Statistical Package for the Social Sciences (SPSS) 20.0 packed program. Variable distribution was evaluated using the Shapiro-Wilk test. Samples with normal distribution (parametric) were evaluated by one-way analysis of variance (ANOVA), followed by the Tukey post hoc test. Nonparametric data were evaluated using the Kruskal-Wallis test. Data are presented as mean  $\pm$  SD or median (interquartile range). A value of  $p < 0.05$  was considered to be significant.

### Results

No difference was found between the control group and SAC control group for serum levels of ALT, AST, ALP, and hsCRP ( $p > 0.05$ ). The ALT, AST, ALP, and hsCRP levels were significantly elevated in the sepsis group in comparison with the control group ( $p < 0.05$ ,  $p < 0.05$ ,  $p < 0.05$ ,  $p < 0.001$ , respectively). On the other hand, the values of ALT, AST, ALP, and hsCRP decreased significantly in the sepsis + SAC group as compared with the sepsis group ( $p < 0.05$ ,  $p < 0.05$ ,  $p < 0.05$ ,  $p < 0.001$ , respectively) (Table 1).

In lung tissue, we found increased NO levels and MPO activities in the sepsis group compared to the control group ( $p < 0.001$ ,  $p < 0.001$ ). In addition, NO levels and MPO activities were significantly decreased in the sepsis + SAC group when compared to the sepsis group ( $p < 0.001$ ,  $p < 0.001$ ). However, NO and MPO levels were found to be higher in the sepsis + SAC group compared to the control group ( $p < 0.05$ ,  $p < 0.001$ ) (Table 2). In liver tissue, there were no significant differences in the levels of NO and MPO between the groups ( $p < 0.05$ ,  $p < 0.05$ ) (Table 3).

In lung tissue, no statistically meaningful differences were found in DNA fragmentation between the groups ( $p < 0.05$ ) (Table 2). The liver DNA fragmentation levels were increased in the sepsis group when compared to the control group ( $p < 0.001$ ). However, liver tissue levels of DNA fragmentation were decreased in the sepsis + SAC group in comparison with the sepsis group ( $p < 0.001$ ) (Table 3).

Light-microscopic observation on sections from the control group showed normal lung histology. The lung sections of

**Table 1** Serum biochemical parameters and hsCRP

	Control	SAC control	Sepsis	Sepsis + SAC
ALP (U/L)	581.88±47.35	541.78±70.99	698.13±107.47*	566.63±99.54 <sup>#</sup>
ALT (U/L)	44 (40.5–49)	44 (43–45.75)	65.5 (62–68.5)*	47.5 (31.5–52.5) <sup>#</sup>
AST (U/L)	123.5 (113.5–144)	118 (112.75–146.25)	162.5 (154.5–188)*	120.5 (115–145) <sup>#</sup>
hsCRP (mg/L)	0.95±0.23	0.96±0.29	2.49±0.49**	1.16±0.46 <sup>###</sup>

Values are shown as ± SD and median (interquartile range)

\* $p < 0.05$  versus control group; \*\* $p < 0.001$  versus control group; <sup>#</sup> $p < 0.05$  versus sepsis group; <sup>###</sup> $p < 0.001$  versus sepsis group

SAC-treated control group showed normal tissue histology (Fig. 1). Intravascular congestion, pulmonary edema, inflammatory cellular infiltration, and intra-alveolar hemorrhage were seen in lung sections of the sepsis group. We also observed alveoli filled with red blood cells and leukocyte in septic rats (Fig. 2). Although damage was still observed in some regions of lung tissue, it was significantly reduced with SAC treatment in the sepsis + SAC group. In addition, SAC-treated septic rats showed normal alveolar architecture (Fig. 1).

The histopathology of the liver sections of the control group exhibited a normal architecture of hepatocytes. The liver sections of SAC-treated control group showed normal tissue histology. All sepsis group samples exhibited liver degeneration, focal necrosis, infiltration of mononuclear leukocytes, and blood vessel congestions (Fig. 3). The sepsis group hepatocytes contained cytoplasmic microvesicles and karyolysis (Fig. 4). SAC treatment significantly decreased these pathological changes in the sepsis + SAC group but did not completely restore the injury (Fig. 3).

## Discussion

LPS, also known as endotoxin, is a strong stimulator of acute sepsis or chronic inflammation (Bashir et al. 2011). LPS activates macrophages, initiates lymphocyte differentiation and blood coagulation, and induces oxidative stress and pro-inflammatory molecules release from macrophages, leading to tissue damage, organ failure, and death (González-Renovato et al. 2013). Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukins

(ILs), mainly IL-1 $\beta$ , and IL-6 are thought to be involved in the pathogenesis of sepsis with LPS. In accordance with the complement activation, the cytokine stimulation of circulating and resident immune cells and endothelial cells results in elevated accumulation of ROS and reactive nitrogen species, such as superoxide anion and NO (von Dessauer et al. 2011).

Antioxidant defense mechanisms of the organism have developed to restrict the levels of reactive oxidants and the harm they induce. In addition to the protective effects of endogenous antioxidant defense mechanisms, supplementation of exogenous nutritional antioxidants seems to be of great significance (Sandoval et al. 1997). SAC is recognized as a significant garlic-derived organosulfur compound and has been shown to possess antioxidant activity (Ho et al. 2001). SAC includes a thiol group accountable for its antioxidant action because this nucleophile can readily deliver its proton to electrophilic species, thus neutralizing them or making them less reactive. SAC inhibits oxidation and nitration of lipid and protein, supporting its antioxidant properties (Colín-González et al. 2012). SAC is also a scavenger of superoxide anion, hydrogen peroxide, hydroxyl radical, and peroxy nitrite anion. Furthermore, SAC have been reported to scavenge the hypochlorous acid and singlet oxygen (Medina-Campos et al. 2007; Colín-González et al. 2012). Therefore, in the present study, we planned to find out if SAC have any anti-inflammatory, antioxidant, and anti-apoptotic activities in LPS-induced septic rats.

LPS-induced hepatic injury is well known by the increased serum liver enzymes demonstrating cellular leakage and loss of functional integrity of hepatocellular membrane. In accordance with this, we found elevated activities of AST, ALT, and

**Table 2** NO content, DNA fragmentation, and MPO activity in lung from experimental groups

	Control	SAC control	Sepsis	Sepsis + SAC
MPO (U/mg protein)	2.21±0.58	2.30±0.53***	11.33±2.11**	6.63±1.22**·***
NO ( $\mu$ mol/mg protein)	1.01±0.37	1.31±0.34***	6.87±1.42**	2.24±0.61*·***
DNA fragmentation (% DNA)	1.41±0.25	1.49±0.31	1.88±0.60	1.89±0.69

Values are shown as ± SD

\* $p < 0.05$  versus control group; \*\* $p < 0.001$  versus control group; \*\*\* $p < 0.001$  versus sepsis



**Table 3** NO content, DNA fragmentation, and MPO activity in liver from experimental groups

	Control	SAC control	Sepsis	Sepsis + SAC
<b>MPO (U/mg protein)</b>	0.0224 (0.0224–0.0411)	0.0224 (0.0224–0.0262)	0.0523 (0.0448–0.0523)	0.0374 (0.0224–0.0374)
<b>NO (<math>\mu\text{mol/mg protein}</math>)</b>	0.085 (0.06–0.12)	0.085 (0.076–0.0935)	0.124 (0.089–0.1615)	0.09 (0.0785–0.1005)
<b>DNA fragmentation (% DNA)</b>	0.94 $\pm$ 0.06	0.94 $\pm$ 0.07	1.49 $\pm$ 0.05*	0.099 $\pm$ 0.04**

Values are shown as  $\pm$  SD and median (interquartile range)

\* $p < 0.001$  versus control group; \*\* $p < 0.001$  versus sepsis group

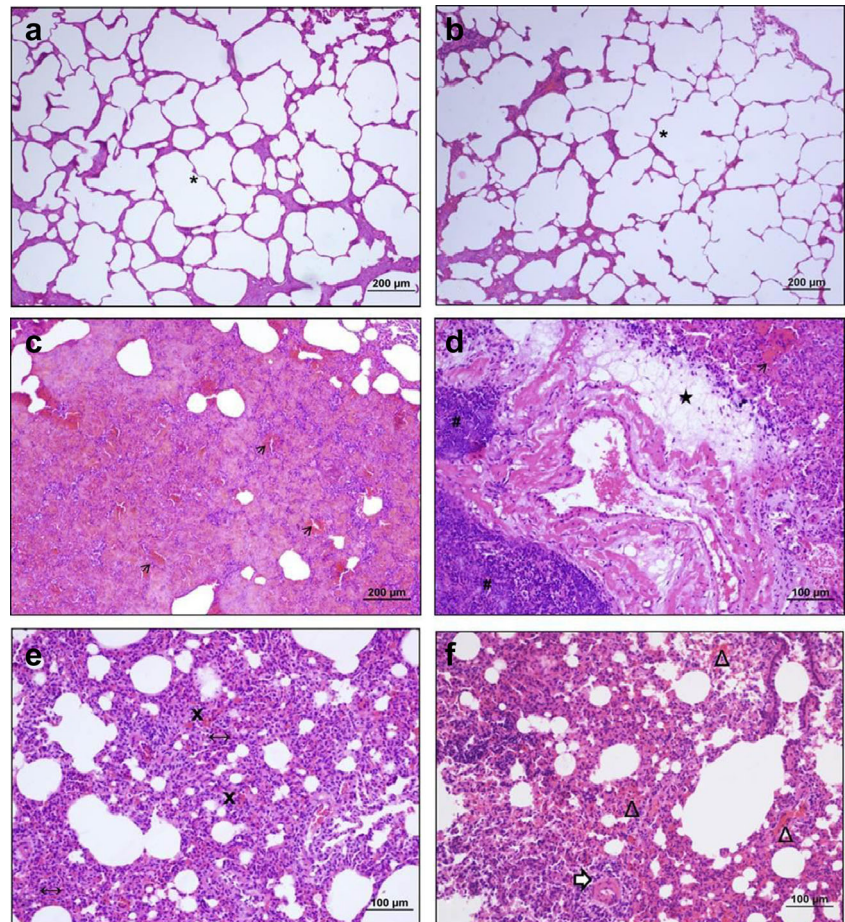
ALP concentration in the serum of the sepsis group. Elevated levels of serum hepatic marker enzymes proposed the extensive liver damage triggered by LPS, depending on its free radical production mechanism, which in turn has the capability to induce hepatic injury resulting in elevated leakage of intracellular enzymes (Mohamadin et al. 2011). Treatment with SAC decreased the levels of the studied parameters in septic rats. This might suggest that SAC can be preventing toxic effect of LPS on liver.

C-reactive protein (CRP) is proposed as a reliable biomarker for sepsis suggesting to make possible early detection of critical bacterial infection and to inform prognosis, in a variety of clinical state (Carrol et al. 2009). Increased levels of serum

CRP are associated with an increased risk of organ failure and mortality (Pierrakos and Vincent 2010). In this investigation, septic rats had higher hsCRP levels compared with the control groups. However, the anti-inflammatory possessions of SAC were also showed by the inhibition of LPS-induced increase of serum hsCRP in the sepsis + SAC group.

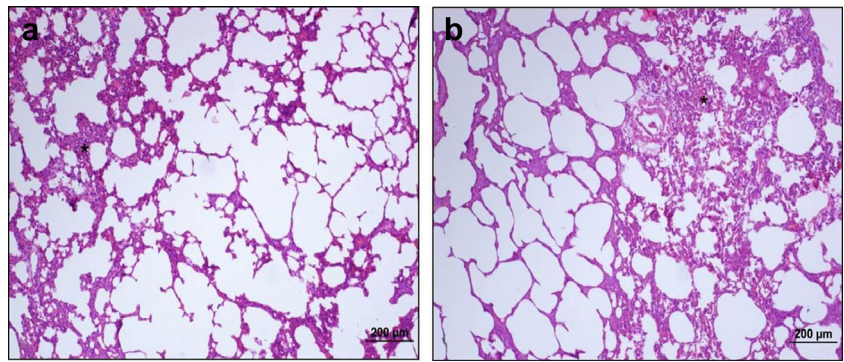
NO is a relatively stable free radical and possesses various promiscuous roles. NO production can be greatly enhanced by LPS. Previous reports have demonstrated a linear relationship between NO levels and inflammation (Alghaithy et al. 2011). NO reacts rapidly with superoxide, converting the peroxynitrite. Additionally, peroxynitrite itself is a highly reactive oxidant which can cause lipid peroxidation, cellular

**Fig. 1** Light microscopy of lung tissue in control group (a), SAC control group (b), and sepsis group (c–f). The lung from control group showing thin alveolar septal wall and normal cellularity (*asterisk*) (a); SAC control group showed almost normal alveoli with thin septum (*asterisk*) (b); lung from a septic rat showing filled alveoli, pulmonary venous congestion (*rightwards arrow*) (c); increase in cellular infiltrates (*number sign*), and edema (*filled star*) (d) (*bar* 200  $\mu\text{m}$ , *bar* 100  $\mu\text{m}$  HE). The sepsis group rats showed intra-alveolar hemorrhage (*x*); the alveoli filled with red blood cells and leukocyte (*left right arrow*) (e); and increase in leukocyte infiltration (*S*) in perivascular area and intra-alveolar hemorrhage (*delta*) (f) (*bar* 200  $\mu\text{m}$ , *bar* 200  $\mu\text{m}$ , *bar* 200  $\mu\text{m}$ , *bar* 100  $\mu\text{m}$ , *bar* 100  $\mu\text{m}$ , *bar* 100  $\mu\text{m}$  HE)





**Fig. 2** Light microscopy of lung tissue in sepsis + SAC group (**a**, **b**). Although damage (*asterisk*) was still observed in some regions of lung tissue, it was significantly reduced with SAC treatment in the sepsis + SAC group (**a**, **b**) (*bar* 200  $\mu$ m, *bar* 200  $\mu$ m)

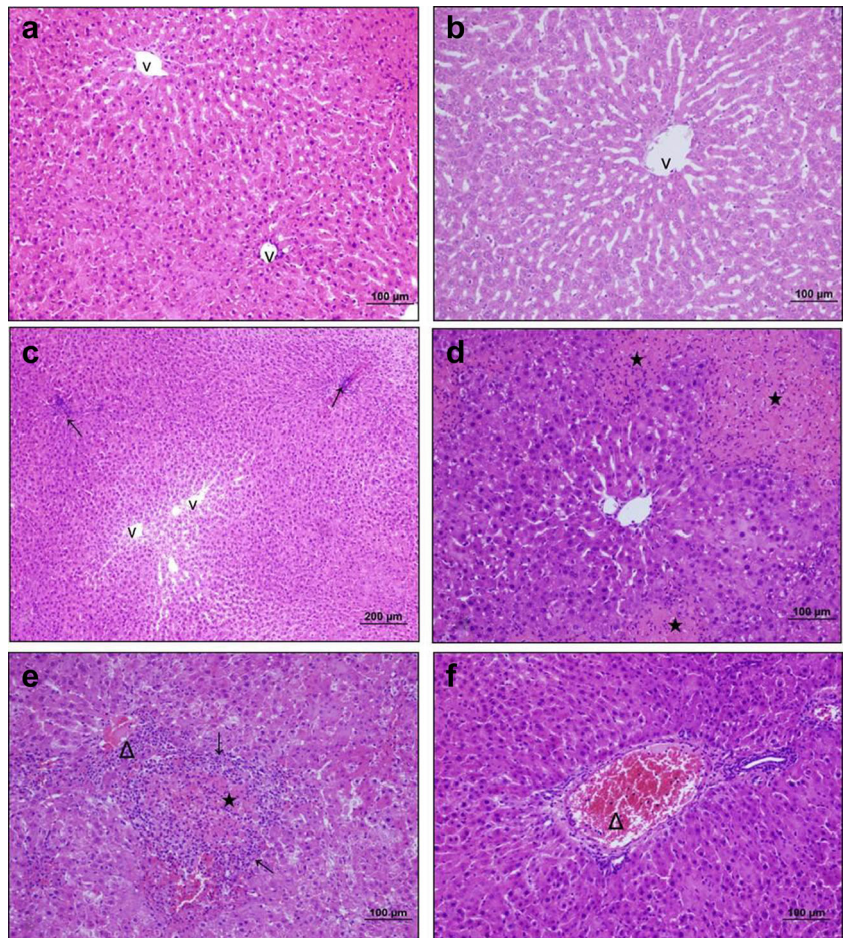


injury, and cell death (Ozdemir et al. 2007). SAC acts to prevent NO generation in LPS/cytokine-induced macrophages and hepatocytes by blocking iNOS induction. SAC stimulated suppression of NO production may be associated with its capability to reduce NF- $\kappa$ B signaling, thus leading to cell protection (Colín-González et al. 2012). In the present study, the elevation of NO levels in lung was observed in the LPS-induced septic rats. An important finding from our study was that application of SAC reduced NO levels in lung tissue of septic rats. The preventive effects of SAC on NO levels may reveal its role as a powerful scavenger of ROS and a

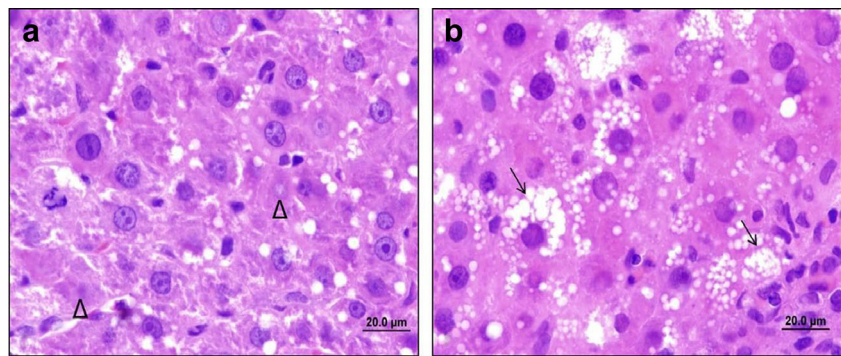
strong antioxidant. On the other hand, liver NO levels did not statistically differ between the groups. This might suggest that NO can react with superoxide to form peroxynitrite in the sepsis group.

Recent studies suggest that ROS play a critical role in the recruitment of neutrophils into the septic tissue, but activated neutrophils also produce ROS. ROS can produce hypochlorous acid (HOCl) in the presence of neutrophil-derived MPO which leads to tissue injury. MPO activity is used to explain the role of neutrophils in septic tissue injury (Mohamadin et al. 2011). We have found increased lung MPO

**Fig. 3** Light microscopy of liver tissue in control group (**a**), SAC control group (**b**), sepsis + SAC group (**c**), and sepsis group (**d–f**). In controls, normal liver architecture was seen. Sections from control rats showing a central vein (*V*) surrounded by hepatocytes and sinusoids (**a**); SAC control group exhibited normal architecture of the hepatocytes (**b**); the sepsis + SAC group showed slight cellular infiltration (*rightwards arrow*) in portal area of parenchymal cells with almost normal architecture (**c**); the sepsis group rats showed necrotic lesions (*filled star*) (**d**); mononuclear cell infiltration (*rightwards arrow*), cell degeneration, necrotic lesions (*filled star*) (**e**), and congestion (*delta*) of the portal veins in portal areas (**e**, **f**) (*bar* 100  $\mu$ m, *bar* 100  $\mu$ m, *bar* 200  $\mu$ m, *bar* 100  $\mu$ m, *bar* 100  $\mu$ m, *bar* 100  $\mu$ m HE)



**Fig. 4** Light microscopy of liver tissue in sepsis group (a, b). Liver from a septic rat showing karyolysis (*delta*) (a) and microvesicular steatosis (*rightwards arrow*) (b) in hepatocytes (*bar* 20.0  $\mu\text{m}$ , *bar* 20.0  $\mu\text{m}$  HE)



activity in the sepsis group compared to the control group. Moreover, our results are similar to other studies (Yang et al. 2005; Liu et al. 2002). Furthermore, our data provided evidence that treatment of rats with SAC decreased the MPO activity, a marker for neutrophil infiltration. From our data, it is proposed that sepsis-related oxidative damages in lung tissues are neutrophil-dependent with free-radical-mediated mechanisms and are returnable by SAC application (Mohamadin et al. 2011). This improvement can be a result of anti-inflammatory effect of SAC. On the other hand, there was no statistical alteration in liver MPO activity among the control group and the other studied groups.

The overwhelming oxidative and nitrosative stresses such as superoxide anion and NO lead to DNA damage and apoptosis (Bhavsar et al. 2010; von Dessauer et al. 2011). The cytotoxic effects of NO and superoxide have been explained by the mechanism in which they were converted to peroxynitrite (Sandoval et al. 1997). Apoptosis is fundamentally significant in the biological process that is included in inflammation, cell differentiation, and cell proliferation. Apoptosis is a distinctive form of cell death characterized by morphological and biochemical alterations, involving plasma membrane blebbing, phosphatidylserine exposure, nuclear condensation, and DNA fragmentation (Kitazumi and Tsukahara 2011). Recently, Jesmin and his colleagues (2012) showed that levels of proapoptotic proteins were up-regulated, whereas those of anti-apoptotic protein were down-regulated in lung tissues following LPS induction. Recently, it was declared that LPS-induced hepatic injury is caused by hepatocyte apoptosis, mediated mainly by TNF- $\alpha$  (Morikawa et al. 1999). Recent reports demonstrated that LPS triggers cytokine-mediated DNA fragmentation in liver, lung, and kidney; and LPS causes the apoptosis-mediated tissue injury (Bohlinger et al. 1996; Medan et al. 2002). We evaluated the levels of DNA fragmentation in lung and liver tissues of studied groups to determine the apoptosis. In the present study, there were no any considerable differences in the control group compared to other groups concerning lung DNA fragmentation levels. Sepsis group demonstrated a net increase in DNA fragmentation levels in liver. However, we determined that the administration of SAC caused an

important reduction in the levels of liver DNA fragmentation in septic rats. The defensive properties of SAC on DNA fragmentation could be associated with its capability to quench reactive oxygen and nitrogen species.

Lipopolysaccharide induces morphological changes in many tissues (González-Renovato et al. 2013). The lung is the most frequently identified organ damaged in sepsis. Cellular infiltration together with the release of proinflammatory mediators leads to the development of acute lung injury, characterized by edema, hemorrhage, and destruction of alveolar wall with severe infiltration of inflammatory cells in septic condition (Al-Saidya and Ismail 2013). In this study, lung tissue in SAC-treated control group had almost normal histological structure. We observed lung degeneration, focal necrosis, infiltration of mononuclear leukocytes, and blood vessel congestions in the sepsis group. In addition, alveolar hemorrhage was observed in lung tissue of septic rats. This may be a reason for organ dysfunction upon excessive inflammatory response mediated by LPS. The lungs of septic rats treated with SAC showed nearly normal alveolar structure, although some damaged areas were seen. In our study, we demonstrated that SAC significantly reduced lung injury in septic rats.

The liver is one of the most important organs, and it is well-known that it is also the main target of lipopolysaccharides, especially Kupffer cells; these hepatic macrophages engulf lipopolysaccharides by phagocytosis, leading to possible physiological as well as morphological changes (González-Renovato et al. 2013). In this study, hepatocytes in SAC-treated control group had almost normal histological structure. All sepsis group samples exhibited liver degeneration, focal necrosis, infiltration of mononuclear leukocytes, and blood vessel congestions. The hepatocytes showed microvesicular steatosis and karyolysis in the sepsis group. We suggest that these histological changes may be due to the effect of LPS because LPS administration results in excessive production of cytokines and ROS, which leads to endothelial cell injury, recruitment of neutrophils, lipid peroxidation and oxidation, and DNA damage (Galley 2011). The sepsis + SAC group showed less inflammatory cell infiltration and cell degeneration in the portal area. In addition, hepatocytes and sinusoidal



structures showed nearly normal histology. SAC treatment significantly decreased these pathological changes in the sepsis + SAC group. Therefore, SAC can also be used for liver protection. This was clearly evident in the histological examination of the liver and demonstrated by the decrease in serum hepatic marker enzymes.

The data presented here is the first report that a novel free-radical scavenger, SAC, have some beneficial effects on histological, oxidative, and enzymatic changes in LPS-induced sepsis model. In conclusion, the results of the current study showed that treatment with SAC can inhibit the NO and MPO overproduction in lung and improve DNA fragmentation in the liver of septic rats. SAC also minimized the degenerative effects of LPS in lung and liver. Therefore, our data suggest that SAC may be a useful agent for the treatment of LPS-induced sepsis.

**Acknowledgments** The authors declare no conflicts of interest in this study.

## References

- Alghaithy AA, El-Beshbishy HA, Abdel-Naim AB, Nagy AA, Abdel-Sattar EM (2011) Anti-inflammatory effects of the chloroform extract of *Pulicaria guestii* ameliorated the neutrophil infiltration and nitric oxide generation in rats. *Toxicol Ind Health* 27:899–910
- Al-Saidya AM, Ismail HK (2013) Histopathological study of sepsis experimentally induced by cecal ligation and puncture in rats. *Bas J Vet Res* 12:104–115
- Atif F, Yousuf S, Agrawal SK (2009) S-allyl L-cysteine diminishes cerebral ischemia-induced mitochondrial dysfunctions in hippocampus. *Brain Res* 10:128–137
- Atroshi F, Rizzo A, Biese I, Vejjalainen P, Saloniemi H, Sankari S, Andersson K (1999) Fumonisin B-1-induced DNA damage in rat liver and spleen: effects of pretreatment with coenzyme Q(10), L-carnitine, alpha-tocopherol and selenium. *Pharmacol Res* 40:459–467
- Bashir A, Banday MZ, Haq E (2011) Lipopolysaccharide, mediator of sepsis enigma: recognition and signalling. *Int J Biochem Res Rev* 1: 1–13
- Bhavsar TM, Patel SN, Lau-Cam CA (2010) Protective action of taurine, given as a pretreatment or as a post-treatment, against endotoxin-induced acute lung inflammation in hamsters. *J Biomed Sci* 17:S19
- Bohlinger I, Leist M, Gantner F, Angermüller S, Tiegs G, Wendel A (1996) DNA fragmentation in mouse organs during endotoxic shock. *Am J Pathol* 149:1381–1393
- Burton K (1968) Determination of DNA concentration with diphenylamine. *Methods Enzymol* 12:163–166
- Carrol ED, Mankambo LA, Jeffers G, Parker D, Guiver M, Newland P, Banda DL, IPD Study Group, Molyneux EM, Heyderman RS, Molyneux ME, Hart CA (2009) The diagnostic and prognostic accuracy of five markers of serious bacterial infection in Malawian children with signs of severe infection. *PLoS One* 4:e6621
- Colín-González AL, Santana RA, Silva-Islas CA, Cháñez-Cárdenas ME, Santamaría A, Maldonado PD (2012) The antioxidant mechanisms underlying the aged garlic extract and S-allylcysteine induced protection. *Oxid Med Cell Longev* 2012:907162
- Cortas NK, Wakid NW (1990) Determination of inorganic nitrate in serum and urine by a kinetic cadmium-reduction method. *Clin Chem* 36:1440–1443
- Ekingen G, Ceran C, Demirtola A, Demirogullari B, Sancak B, Poyraz A, Sonmez K, Basaklar AC, Kale N (2006) The effect of reperfusion duration in intestinal ischemia reperfusion on bio-chemical parameters and small intestinal anastomosis healing. *J Inonu Univ Med Fac* 13:7–12
- Escames G, Acuña-Castroviejo D, López LC, Tan DX, Maldonado MD, Sánchez-Hidalgo M, León J, Reiter RJ (2006) Pharmacological utility of melatonin in the treatment of septic shock: experimental and clinical evidence. *J Pharm Pharmacol* 58:1153–1165
- Galley HF (2011) Oxidative stress and mitochondrial dysfunction in sepsis. *Br J Anaesth* 107:57–64
- García E, Villeda-Hernández J, Pedraza-Chaverri J, Maldonado PD, Santamaría A (2010) S-allylcysteine reduces the MPTP-induced striatal cell damage via inhibition of pro-inflammatory cytokine tumor necrosis factor- $\alpha$  and inducible nitric oxide synthase expressions in mice. *Phytomedicine* 15:65–73
- González-Renovato ED, Alatorre-Jiménez M, Bitzer-Quintero OK, Sánchez-Luna S, Flores-Alvarado LJ, Romero-Dávalos R, Romero L, Hernández-Andalón JJ, Ortiz GG, Pacheco-Moisés FP (2013) Effect of Nutrisim© on endotoxic shock induced by lipopolysaccharide from *Escherichia coli*: 0111:b4 in rats: structural study of liver, kidney and lung. *J Clin Exp Pathol* 4:1
- Ho SE, Ide N, Lau BH (2001) S-allyl cysteine reduces oxidant load in cells involved in the atherogenic process. *Phytomedicine* 8:39–46
- Ippoushi K, Itou H, Azuma K, Higashio H (2002) Effect of naturally occurring organosulfur compounds on nitric oxide production in lipopolysaccharide-activated macrophages. *Life Sci* 71:411–419
- Javed H, Khan MM, Khan A, Vaibhav K, Ahmad A, Khuwaja G, Ahmed ME, Raza SS, Ashafaq M, Tabassum R, Siddiqui MS, El-Agnaf OM, Saffhi MM, Islam F (2011) S-allyl cysteine attenuates oxidative stress associated cognitive impairment and neurodegeneration in mouse model of streptozotocin-induced experimental dementia of Alzheimer's type. *Brain Res* 1389:133–142
- Jesmin S, Zaedi S, Islam AM, Sultana SN, Iwashima Y, Wada T, Yamaguchi N, Hiroe M, Gando S (2012) Time-dependent alterations of VEGF and its signalling molecules in acute lung injury in a rat model of sepsis. *Inflammation* 35:484–500
- Kim KM, Chun SB, Koo MS, Choi WJ, Kim TW, Kwon YG, Chung HT, Billiar TR, Kim YM (2001) Differential regulation of NO availability from macrophages and endothelial cells by the garlic component S-allyl cysteine. *Free Radic Biol Med* 30:747–756
- Kingsley GR (1942) The direct biuret method for the determination of serum proteins as applied to photoelectric and visual colorimetry. *J Lab Clin Med* 27:840–846
- Kitazumi I, Tsukahara M (2011) Regulation of DNA fragmentation: the role of caspases and phosphorylation. *FEBS J* 278:427–441
- Kodera Y, Suzuki A, Imada O, Kasuga S, Sumioka I, Kanezawa A, Taru N, Fujikawa M, Nagae S, Masamoto K, Maeshige K, Ono K (2002) Physical, chemical, and biological properties of s-allyl cysteine, an amino acid derived from garlic. *J Agric Food Chem* 30:622–632
- Li A, Dong L, Duan ML, Sun K, Liu YY, Wang MX, Deng JN, Fan JY, Wang BE, Han JY (2013) Emodin improves lipopolysaccharide-induced microcirculatory disturbance in rat mesentery. *Microcirculation* 20:617–628
- Liu Z, Yu Y, Jiang Y, Li J (2002) Growth hormone increases lung NF- $\kappa$ B activation and lung microvascular injury induced by lipopolysaccharide in rats. *Ann Clin Lab Sci* 32:164–170
- Liu YC, Chang AY, Tsai YC, Chan JY (2009) Differential protection against oxidative stress and nitric oxide overproduction in cardiovascular and pulmonary systems by propofol during endotoxemia. *J Biomed Sci* 16:8
- Lowes DA, Webster NR, Murphy MP, Galley HF (2013) Antioxidants that protect mitochondria reduce interleukin-6 and oxidative stress,



- improve mitochondrial function, and reduce biochemical markers of organ dysfunction in a rat model of acute sepsis. *Br J Anaesth* 110: 472–480
- Medan D, Wang L, Yang X, Dokka S, Castranova V, Rojanasakul Y (2002) Induction of neutrophil apoptosis and secondary necrosis during endotoxin-induced pulmonary inflammation in mice. *J Cell Physiol* 191:320–326
- Medina-Campos ON, Barrera D, Segoviano-Murillo S, Rocha D, Maldonado PD, Mendoza-Patiño N, Pedraza-Chaverri J (2007) S-Allylcysteine scavenges singlet oxygen and hypochlorous acid and protects LLC-PK1 cells of potassium dichromate-induced toxicity. *Food Chem Toxicol* 4:2030–2039
- Mohamadin AM, Elberry AA, Elkablawy MA, Gawad HS, Al-Abbasi FA (2011) Montelukast, a leukotriene receptor antagonist abrogates lipopolysaccharide-induced toxicity and oxidative stress in rat liver. *Pathophysiology* 18:235–242
- Morikawa A, Kato Y, Sugiyama T, Koide N, Chakravorty D, Yoshida T, Yokochi T (1999) Role of nitric oxide in lipopolysaccharide-induced hepatic injury in D-galactosamine-sensitized mice as an experimental endotoxic shock model. *Infect Immun* 67:1018–1024
- Ozdemir D, Uysal N, Tugyan K, Gonenc S, Acikgoz O, Aksu I, Ozkan H (2007) The effect of melatonin on endotoxemia-induced intestinal apoptosis and oxidative stress in infant rats. *Intensive Care Med* 33: 511–516
- Pérez-Severiano F, Salvatierra-Sánchez R, Rodríguez-Pérez M, Cuevas-Martínez EY, Guevara J, Limón D, Maldonado PD, Medina-Campos ON, Pedraza-Chaverri J, Santamaría A (2004) S-Allylcysteine prevents amyloid-peptide-induced oxidative stress in rat hippocampus and ameliorates learning deficits. *Eur J Pharmacol* 12:197–202
- Pierrakos C, Vincent JL (2010) Sepsis biomarkers: a review. *Crit Care* 14: R15
- Ren Y, Xie Y, Jiang G, Fan J, Yeung J, Li W, Tam PK, Savill J (2008) Apoptotic cells protect mice against lipopolysaccharide-induced shock. *J Immunol* 180:4978–4985
- Sandoval M, Zhang XJ, Liu X, Mannick EE, Clark DA, Miller MJ (1997) Peroxynitrite-induced apoptosis in T84 and RAW 264.7 cells: attenuation by L-ascorbic acid. *Free Radic Biol Med* 22:489–495
- Sepehr R, Audi SH, Maleki S, Staniszewski EAL, Konduri GG, Ranji M (2013) Optical imaging of lipopolysaccharide induced oxidative stress in acute lung injury from hyperoxia and sepsis. *J Innov Opt Health Sci* 6:1350017
- Tiruppathi C, Naqvi T, Wu Y, Vogel SM, Minshall RD, Malik AB (2004) Albumin mediates the transcytosis of myeloperoxidase by means of caveolae in endothelial cells. *Proc Natl Acad Sci U S A* 101:7699–7704
- von Dessauer B, Bongain J, Molina V, Quilodrán J, Castillo R, Rodrigo R (2011) Oxidative stress as a novel target in pediatric sepsis management. *J Crit Care* 26:103.e1–7
- Werner J, Saghier M, Warshaw AL, Lewandrowski KB, Laposata M, Lozzo RV, Carter EA, Schatz RJ, Ferna C, Castillo ND (2002) Alcoholic pancreatitis in rats: injury from nonoxidative metabolites of ethanol. *Jens Am J Physiol Gastrointest Liver Physiol* 283:65–73
- Yamashita T, Kawashima S, Ohashi Y, Ozaki M, Ueyama T, Ishida T, Inoue N, Hirata K, Akita H, Yokoyama M (2000) Resistance to endotoxin shock in transgenic mice overexpressing endothelial nitric oxide synthase. *Circulation* 101:931–937
- Yan CK, Zeng FD (2005) Pharmacokinetics and tissue distribution of S-allylcysteine in rats. *Asian J Drug Metab Pharmacokinet* 5:61–69
- Yang J, Li W, Duan M, Zhou Z, Lin N, Wang Z, Sun J, Xu J (2005) Large dose ketamine inhibits lipopolysaccharide-induced acute lung injury in rats. *Inflamm Res* 54:133–137