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The effect of L-theanine on the oxidant-antioxidant balance in serum and lung tissue in experimentally induced sepsis in rats

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Sepsis induces lung injury and respiratory distress syndrome and is therefore potentially fatal. L-theanine (LT), an amino acid found in tea, is a bioactive compound with an important antioxidant, anti-inflammatory, and antifibrotic properties. The purpose of this study was to evaluates whether, LT exhibits protective effects against lung tissue damage by determining its effect on oxidative stress, inflammation and mineral levels in an experimental model of cecal ligation and perforation (CLP)-induced sepsis in rats. Rats were randomly divided into three groups (n=6): sham, CLP, and CLP+LT. LT was administered intraperitoneally (750 mg/kg) in two equal doses immediately and 12 h after surgery. Malondialdehyde (MDA), advanced protein oxidation product (AOPP), myeloperoxidase (MPO), total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI), and ischemia modified albumin (IMA) values were determined spectrophotometrically. Serum elements (Na, K, Mg, Ca, and Fe), albumin, glucose, triglyceride, and lactate levels were determined using an autoanalyzer. Lung tissues were also examined histopathologically. Treatment of septic rats with LT significantly reduced oxidative stress and inflammation in lung tissues and serum. LT also increased albumin and Na levels and reduced triglyceride levels in serum. In conclusion, LT treatment may exhibit a preventive effect against sepsis-induced lung injury by reducing oxidative stress and inflammation, and by regulating osmotic balance.

Keywords: Cecal ligation and puncture, L-theanine (γ-glutamylethylamide), Sepsis, Oxidative stress, Lung injury

Sepsis is a systemic inflammatory response syndrome with a high mortality rate that causes multiple organ dysfunction, including the lungs (35-40%)[1]. Bacterial, viral, and fungal infections in sepsis can cause fatal complications such as acute lung injury (ALI) and acute respiratory distress syndrome (ARDS)[2]. As the antioxidant-oxidant balance shifts in favor of oxidants, the inflammatory process is triggered in the lungs[3]. The majority of septic patients experience fluid and electrolyte imbalances. In sepsis, glomerular filtration is impaired and water reabsorption from the proximal tubule increases. This, in turn, leads to lower levels of electrolytes such as sodium, potassium, magnesium, and calcium in plasma[4]. Similar to the kidneys, the lungs also play a role in acid-base, fluid, and electrolyte balance[5]. Disruption of balances may be a potential consequence of sepsis-induced lung injury, and *vice-versa*. The use of broad-spectrum antibiotics in sepsis may cause life-threatening complications due to possible antimicrobial resistance[6]. Although studies have investigated this subject, there is still no radical treatment available for septic patients. It is therefore vitally important to identify novel safe and effective agents capable of enhancing the body's defenses against inflammation and oxidative stress in sepsis.

Natural antioxidants play a crucial role in the development of drugs intended for treating various diseases and ailments including viral infections[7]. L-theanine, (γ-glutamylethylamide, LT) is a unique nonproteinic amino acid abundantly present in the leaves of the green tea plant (*Camellia sinensis*) and some mushrooms (Boletus badius). It is regarded as a "safe" and "non-toxic" compound and is licensed for sale as a functional food ingredient in Japan and the USA[8]. Different lines of evidence have indicated that LT posseses antioxidative properties as well as immune-modulating, hepatoprotective, and neuro-protective effects, the ability to bestow mental relaxation and regulate systemic blood pressure, and hypolipidemic and other beneficial activities[9-11]. LT can improve the absorption and utilization efficiency of amino acids and regulates glucose, lipid, and protein metabolism

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via AMP-activated protein kinase (AMPK) and insulin pathways[12]. As an analog of L-glutamic acid (Glu) and L-glutamine, LT is an important modulator for leukocyte, macrophage, and lymphocyte functions. Previous reports have shown that glutamine can attenuate the inflammatory response to lung injury in sepsis by increasing heat shock protein 70 expression, and reducing nuclear factor kappa B (NF-κB) activation and proinflammatory cytokine expression[13]. According to one recent study, LT suppressed airway inflammation in ovalbumin-induced allergic asthma and NF-κB activation in lung carcinoma cells[14]. Our previous study showed that LT exhibited protective effects against sepsis-related liver and kidney injury by reducing inflammation, oxidative stress, and apoptosis[15]. However, it is unclear whether, LT exhibits a similar protective effect against sepsis-induced pathological changes in the lung. Due to its safety and low cost compared to synthetic drugs, LT may exhibit beneficial effects against bacterial and viral lung injuries. This study was therefore performed to investigate whether LT would exert a protective effect against lung damage in septic animals. To the best of our knowledge, there have been no comparable on this subject to date.

Materials and Methods

Experimental design

Thirty male Sprague Dawley rats (weighing 200-300 g and aged 10-12 weeks) obtained from the Karadeniz Technical University Surgical Application Research Center, Turkey, were used in the study. These rats were kept in standard cages under *standard* laboratory conditions (12 h:12 h light: dark cycle, temperature 20±2°C, and relative humidity 55-60%) and were provided with food and water *ad libitum*. All experiments were carried out in accordance with the Karadeniz Technical University Animal Experiments Guide (approval date 24.12.2020, No. 2020/15). Experimental groups were established as described in our previous publication[15].

Thirty male rats were divided randomly into the following three groups:

Group 1 (n = 6, sham): Only midline laparotomy was performed in this group, with neither ligation nor puncture. Cecal ligation puncture was not performed.

Group 2 (n =12, CLP): The animals in this group were first anesthetized with intraperitoneal injection of 50 mg/kg ketamine and 10 mg/kg xylazine. Polymicrobial sepsis was induced surgically using the CLP procedure[16]. Following midline laparotomy and cecum exteriorization, a 5-0 silk ligature was placed 5 mM from the cecal tip. The cecum was punctured twice using a 21 gauge needle. A small quantity of fecal matter (approximately 1 mM^3) was then drained from the colon using slight compression. In the case of sham-operated animals, the cecum was exposed, but not ligated or punctured.

Group 3 (n =12, CLP + LT): LT (Chem-Impex Intl, Inc., Kn: 14293, Chicago, USA) was dissolved in normal saline and injected intraperitoneally (1 mL) immediately and 12 h after completion of surgery (2×750 mg/kg). The sham-operated and the CLP groups received 1 mL of the normal saline instead of LT under the same conditions.

Since the mortality rate in the CLP procedure is very high[16], it was initially started with 24 rats. Of these, 12 were treated with LT. The continuation of the study was carried out with the blood and tissues of animals that survived these applications. Six animals survived in the CLP group and nine animals in the CLP+LT group. Therefore, six randomly selected animals from the CLP+LT group were included so that all groups were equal in number.

Blood and tissue sample parameters

Blood samples taken from the abdominal aorta and placed into tubes with ethylenediaminetetraacetic acid (EDTA) and without anticoagulant were centrifuged at 1800 g for 10 min to yield plasma and serum samples, respectively. The right lobes of the lung tissues were used for histopathological examination, while the other lobes were stored. Serum, plasma and lung tissues were stored in a −80°C freezer until parameter analysis. Levels of lactate, albumin, glucose, triglyceride, Na, K, Mg, Ca, and Fe in sera were analyzed using a Beckman Coulter Autoanalyzer AU5800 with the original reagents (Beckman Coulter, Brea, CA, USA).

Histopathological examination

Right lobe lung tissues were used for histopathological evaluations. These tissues were first fixed in a 10% buffered formaldehyde solution for 48 hours. They were then dehydrated (by passage through alcohol series), made transparent (using xylene), and embedded in paraffin blocks. Sections 5 µM in thickness obtained using a microtome (Leica RM 2255, Leica Instruments, Nussloch, Germany) were subsequently stained with hematoxylin and eosin (H&E) and examined under a light microscope

(Olympus BX51, Tokyo, Japan). Lung injury was graded between 0 (normal) and 4 (severe) based on findings of obstruction, edema, interstitial inflammation, and inflammatory cell infiltration[17].

Determination of malondialdehyde levels

The levels of MDA, a lipid peroxidation product, in lung tissues were determined using spectrophotometric thiobarbituric acid reagent (TBARS) assay[18]. Briefly, 100 mg of tissue was homogenized (OMNI-Tissue Master 125, USA) in 1 mL of ice-cold 1.15% KCl solution containing 0.05% Triton X-100. Next, 0.5 mL of the supernatant obtained by centrifugation at 3200 g for 10 min (Eppendorf Centrifuge 5810, USA) was mixed with 3 mL of 1% H_3PO_4 and 1 mL of 0.67% thiobarbituric acid. This mixture was incubated in boiling water for 45 min and then centrifuged. The absorbances of the obtained supernatants and standard (tetramethoxypropane) were read at 532 nm. Tissue MDA levels were expressed as nmol/mg protein.

Plasma MDA levels were determined using the TBARS method described by Yagi[19]. Briefly, a mixture containing 0.3 mL of plasma, 2.4 mL of 0.08 N H_2SO_4, and 0.3 mL of 10% phosphotungstic acid was incubated at room temperature for 5 min, and then centrifuged at 1600 g for 10 min. The resulting precipitate was then suspended in 4 mL of distilled water by vortexing. Next, 1 mL of 0.67% thiobarbituric acid was added and heated in boiling water for 60 min. The absorbance of the organic layer obtained by centrifugation at 1600 g for 10 min after the addition of n-butanol was read at 532 nm. Tetramethoxypropane was used as standard, and plasma MDA levels were calculated as nmol/L.

Determination of advanced oxidation protein product levels

Plasma and lung tissue AOPP levels as a marker of protein oxidation (dityrosine containing cross-linking product) were determined using the method described by Witko-Sarsat[20]. First, 100 mg lung tissue was homogenized with 1 mL of 10 mM phosphate buffer (pH: 7.4). These homogenates were then centrifuged at 3200 g for 10 min. Samples were prepared by diluting 200 µL of the supernatant fraction of the homogenate or plasma at a 1:5 concentration with PBS. To each tube was then added 10 µL 1.16 M potassium iodide, followed by 20 µL acetic acid 2 min later. The absorbance at 340 nm was determined immediately on the microplate reader. Chloramine-Thydrate (Cas No: 149358-73-6; Sigma) was used as a standard. AOPP levels were expressed as mmol/mg protein in tissue and as mmol/mL in plasma.

Determination of myeloperoxidase levels

For the MPO (a marker that reflects neutrophil infiltration) enzyme activity assay[21]in lung tissues, 100 mg of tissue was first placed into 1 mL of phosphate buffer (50 mM, pH 6.0) and homogenized using a glass homogenizer for 30 sec. The precipitate obtained by centrifugation at 19,000 g for 15 min was suspended in a phosphate buffer (50 mM, pH 6.0) containing 0.5% hexadecyltrimethyl-ammonium bromide (HETAB). These suspensions were centrifuged for 20 min at 15,000 g, followed by sonication in triplicate for 5 sec, freeze-thawing in triplicate, and incubation at 4°C for 20 min. Next, 0.02 mL of supernatant or plasma was added to 0.28 mL of phosphate buffer (50 mM, pH 6.0, containing 0.167 mg/mL o-dianicidine dihydrochloride and 0.0005% H_2O_2) to monitor absorbances at 460 nm for 5 min. Finally, 1 mmol H_2O_2 degradation per min at 25°C was regarded as one unit of MPO enzyme activity. MPO activity was expressed as U/mg protein in tissue and U/mL in plasma.

Determination of tissue protein levels

Following the tissue homogenization process performed using different methods for different parameters, the results were expressed by dividing protein values to ensure standardization. Total protein concentrations in lung tissues were thus estimated using bovine serum albumin (Sigma, Lot 117K7415, USA) as a standard. In this method, Coomassie Brilliant Blue G-250 interacts with acidic and basic protein groups, and its color changes from reddish-brown to blue[22]. The reading was performed at 595 nm, the peak absorbance of the acidic dye solution when binding to the protein. The within-analysis coefficient of variation value is 6.35%.

Determination of total oxidant and antioxidant status

Commercial colorimetric kits (Rel Assay Diagnostics, Gaziantep, Turkey) were used according to the manufacturer's instructions in order to determine serum total oxidant and antioxidant status, TOS and TAS, respectively. The units were expressed as µmol H_2O_2 equivalent/L for TOS and mmol Trolox equivalent/L for TOS. Oxidative stress index (OSI) values were calculated according using the formula

OSI= [(TOS, µmol H_2O_2 equivalent/L)/(TAS, µmol Trolox equivalent/L)×100]

Determination of ischemia-modified albumin levels

Serum IMA levels were determined by the previously described rapid and colorimetric method[23], based on measuring the binding capacity of cobalt to

albumin at 470 nm spectrophotometrically (Shimadzu UV1601, Ausburn, Australia). The color of the dichlorodiphenyltrichloroethane containing specimens was compared with that of the colorimetric sham tubes. The results were expressed as absorbance units (ABSUs). IMA ratio (IMAR) values were calculated as a ratio of IMA to albumin, determined by using a Cobas 6000 automated analyzer (Roche, Germany), and were expressed as ABSU/per g albumin.

Statistical analysis

Statistical analysis was carried out using SPSS (IBM SPSS 23) software. The results were expressed as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by the Tukey post-hoc test was used to compare multiple groups. In all cases, $P< 0.05$ was regarded as statistically significant.

Results

Mortality rates

Twenty-four hours after the operation, the mortality rate was 50% (six out of 12) in the CLP group, 25% (nine out of 12) in the CLP+LT group, while no death was observed in the sham group. The mortality rate was significantly higher in the CLP group than the sham group ($P< 0.05$). The results indicated that LT was effective in increasing CLP-induced sepsis survival rates compared with the CLP group ($P< 0.05$). Six randomized animals from each group (sham, CLP, CLP+LT) were therefore selected for use in serum /plasma examination and biochemistry experiments.

Histopathological results

The histopathological scores and photomicrographs of the lung tissues shown in (Fig. 1) were evaluated by a blinded expert histologist. While a normal morphological structure was observed in the sham group (Fig. 1A), significant morphological changes such as edema, and perihilar and interstitial inflammation and occlusions were detected in the CLP group (Fig. 1B). Lower levels of interstitial edema and neutrophil infiltration were observed in the lung tissues treated with LT (Fig. 1C) compared to the CLP group. Similarly, tissue damage scores were

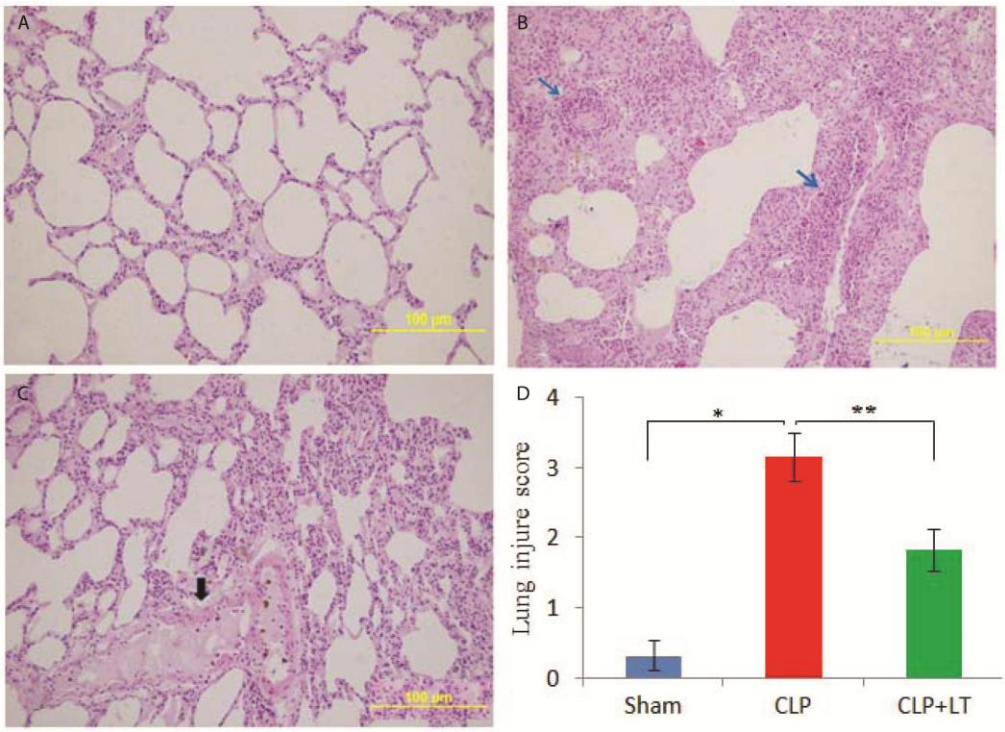

Fig. 1— Histopathological results from lung tissues from rats from each group (n=6), (hematoxylin and eosin; magnification 200×). The scale bar represents 100 µmoL. (A) Normal lung parenchyma histopathology in the sham group; (B) The tissue specimens were examined for the presence of increased perihilar and interstitial inflammation, edema, and congestion in the sepsis group (Blue arrow); (C) Mild interstitial inflammation and congenital vascular structures were observed in the rats treated with LT (black arrow); and (D) The extent of lung injury was estimated by means of scores for neutrophil infiltration, necrosis, congestion, and edema in different sections. The relationships between groups were evaluated using one-way ANOVA followed by the Tukey's post-hoc test.*: $P< 0.05$ compared with Sham (Control); $^{**}P< 0.05$ compared with Cecal ligation and perforation (CLP)

significantly higher in the CLP group compared to the sham group ($P< 0.05$). However, in the CLP + LT group, these scores were significantly lower than in the CLP group (Fig. 1D).

Serum minerals and lactate levels

As shown in Table 1, albumin, glucose, Na, and Ca levels decreased significantly, while lactate and triglyceride levels increased significantly, in the CLP group compared to the sham group ($P< 0.05$). Mg, K and Fe levels decreased, although not significantly in the CLP group ($P> 0.05$). LT treatment significantly increased albumin and Na levels in serum, while reducing triglyceride levels, compared to the CLP group ($P< 0.05$). No significant elevation was found in Ca, K, Mg, and Fe levels in the LT group compared with the CLP group ($P> 0.05$). Lactate levels in the LT group were relatively lower than in the CLP group, although the difference was not statistically significant ($P> 0.05$).

Oxidative stress and inflammatory parameter levels

In terms of oxidative stress markers, while serum TOS, OSI, IMA, and IMAR values increased significantly in the CLP group compared to the sham group, TAS levels decreased (Table 2) ($P< 0.05$). LT treatment significantly reduced these parameters compared to the CLP group ($P< 0.05$). TAS levels were significantly higher in the CLP+LT group as a result of LT treatment compared to the CLP group.

As shown in Table 3, MDA, AOPP levels and MPO activity in plasma and lung tissue were significantly higher in the CLP group than in the sham group ($P< 0.05$). LT treatment significantly lowered

Table 1— Effect of LT treatment on some ions and biochemical parameters in serum

	Sham	CLP	CLP+LT
Lactate (mg/dL)	5.54 ± 0.23	11.73 ± 2.35*	10.23 ± 0.86*
Albumin (g/dL)	2.50±0.02	2.31±0.03*	2.45±0.05**
Glucose (mg/dL	182.00±4.34	135.00±3.54*	134.83±8.68
Triglyceride (mg/dL)	46.00±2.58	69.00±5.55*	49.83±1.62**
Na^+ (mEq/L)	140.66±0.21	136.50±1.23*	141.00±0.44**
K^+ (mEq/L)	4.30±0.08	4.11±0.03	4.13±0.06
Ca^{2+} (mg/dL)	9.45±0.03	9.20±0.04*	9.23±0.10
Mg^{2+} (mg/dL)	2.28±0.17	1.93±0.04	1.95±0.05

Sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}). Control (Sham), cecal ligation and perforation (CLP) and cecal ligation and perforation+L-theanine treatment group (CLP+LT). Data were expressed as means ± SEM (n=6). The relationships between groups were evaluated using one-way ANOVA followed by the Tukey's post-hoc test.*: $P< 0.05$ compared with Sham, **: $P< 0.05$ compared with CLP

Table 2 — Effect of L-theanine on oxidative stress biomarker values in plasma

	Sham	CLP	CLP+LT
TOS (μmol H_2O_2 equivalent/L)	8.61±0.13	10.70±0.45*	8.81±0.07**
TAS (μmol Trolox equivalent/L)	0.99±0.03	0.65±0.05*	1.05±0.08**
OSI	0.81±0.03	1.47±0.09*	0.77±0.04**
IMA (ABSU)	0.77±0.02	0.89±0.02*	0.79±0.03**
IMAR (ABSU/per g albumin)	0.29±0.01	0.37±0.01*	0.31±0.01**

TOS :Total oxidative status, TAS: Total anti-oxidative status; OSI: Oxidative stres index; IMA: Ischemia-modified albumin; IMAR:IMA/albumin ratio; Sham (Control), cecal ligation and perforation (CLP) and cecal ligation and perforation+L-theanine treatment group (CLP+LT). Data were expressed as means ± SEM (n=6). The relationships between groups were evaluated using one-way ANOVA followed by the Tukey's post-hoc test. *: $P< 0.05$ compared with Sham, **: $P< 0.05$ compared with CLP

Table 3— Effects of LT treatment on MDA, AOPP levels and MPO activity in lung tissue and plasma

	Sham	CLP	CLP+LT
Lung Tissue			
MDA (nmol/mg protein)	0.88±0.03	1.62±0.10*	1.07±0.07**
AOPP (nmol/mg protein)	46.58±1.72	81.07±2.64*	71.81±1.01*
MPO (U/mg protein)	3.76± 0.90	26.77±2.12*	5.84±1.78**
Plasma			
MDA (nmol/mg protein)	1.31±0.06	2.53±0.24*	2.04±0.05**
AOPP (nmol/mg protein)	294.42±52.99	556.96±24.43*	0374.48±59.88**
MPO (U/mg protein)	26.84±1.70	66.17±8.64*	24.37±4.32**

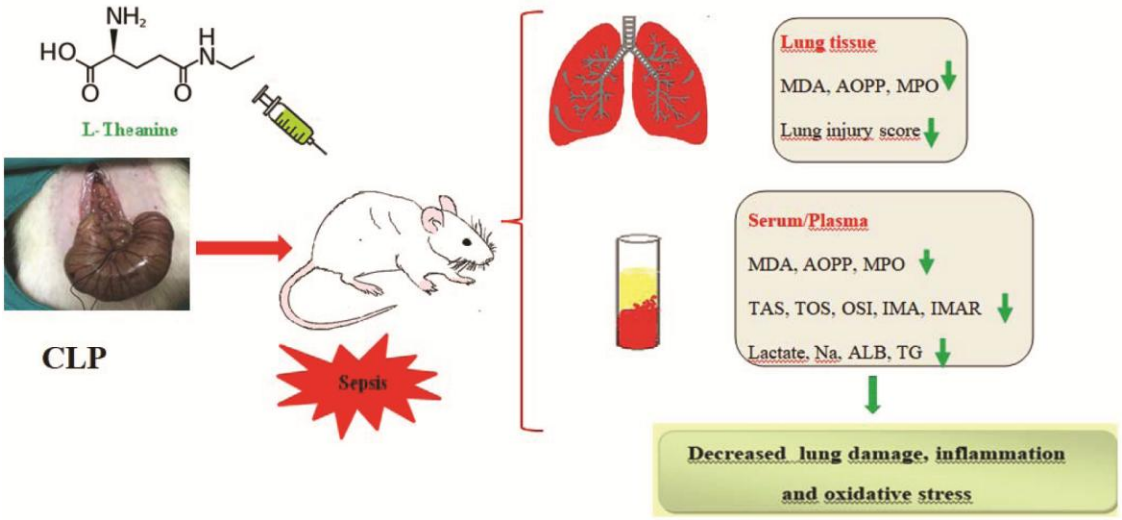

Fig. 2 — The effects of LT on lung tissues and serum in rats in a CLP-induced sepsis model

MDA levels in lung tissue and plasma compared to the CLP group ($P< 0.05$). LT treatment significantly lowered MDA levels and MPO activity in plasma and lung tissue ($P< 0.05$), although this decrease in AOPP levels in lung tissue was not significant ($P> 0.05$) compared to the CLP group (Fig. 2).

Discussion

Sepsis results from an uncontrolled immune response to infection[1]. Regulation of this inflammatory response is one of the most important treatment strategies in sepsis. Treatment with antibiotics alone is not sufficient to achieve an improvement in septic patients. Antioxidant supplementation may therefore help improve the outcomes in such patients[24]. The findings of the present study showed that LT prevented lung injury by CLP-induced sepsis in rats by reducing inflammation, oxidative stress and mortality, by strengthening the antioxidant defense system, and by improving levels of some serum minerals.

In this study, edema, perihilar and interstitial inflammation, and congestion were determined in the CLP group as indicators of pathological conditions in lung tissues (Fig. 1B). Pulmonary edema and inflammatory cell infiltration are the main features of ALI. Lung injury due to sepsis may result from microvascular permeability, endothelial cell damage in alveoli, and neutrophil accumulation in pulmonary tissue[17]. LT treatment was seen to be capable of alleviating inflammatory cell infiltration and pulmonary interstitial hyperemia and edema (Fig. 1C). It may therefore be concluded that it can effectively improve the degree of the local inflammatory response, restore the pathological morphology of lung tissues, and therefore reduce the damage scores in these tissues (Fig. 1D). The anti-inflammatory properties of LT, which protects against lung tissue, were reflected in the increase in the animals' survival rates.

A disturbance of the oxidant-antioxidant balance in favor of oxidants in sepsis is one of the first pathological events responsible for inflammatory reactions in the lung[26]. Levels of other oxidative stress parameters, TOS, OSI, IMA and IMAR, increased in the serum from the CLP group, while those of antioxidants such as TAS and albumin decreased (Tables 1 & 2). While TAS reflects the total antioxidant level and TOS reflects the total oxidant level, OSI values are used as an indicator of oxidative stress[29]. IMA elevation is specifically associated with the presence of bacterial infection, as a result of the acute change occurring in the systemic redox state. An increase in IMAR levels may occur in association with the severity of the disease, indicating the modification of albumin due to oxidative stress[30].This is because, albumin, which is mostly present in serum and which exhibits antioxidant properties, undergoes modifications within minutes under ischemic-hypoxic conditions. Low albumin concentrations are correlated with longer intensive care unit ventilator requirements and the development of new infections[31].

Oxidative stress is an essential component of the pathogenesis of sepsis. Excessive production of reactive oxygen species (ROS) in sepsis causes dysfunctions such as ARDS and ALI, which are

characterized by the accumulation of numerous neutrophils in the lungs[25]. In this study, CLP-induced hypoxia and oxidative stress increased MDA and AOPP levels and MPO activity in both lung tissue and plasma (Table 3). Oxidative stress increases the product of lipid peroxidation (MDA), disrupts plasma membrane integrity, and ultimately, results in cell death. MPO is an important marker showing neutrophil migration and aggregation. MPO activity in lung tissues of rats with CLP-induced sepsis therefore reflects neutrophil and macrophage diapedesis and histological findings[26]. Uncontrolled migration of neutrophils into the lungs causes severe damage to pulmonary tissues by causing excess production of cytokines, chemokines, and myeloperoxidase[27]. AOPP, a marker of protein oxidation, occurs under conditions of oxidative and carbonyl stress and increased global inflammatory activity. It potentially reflects various aspects of ALI/ARDS pathogenesis and pathophysiology. It has also been suggested that AOPP can be used as a marker for detecting early-onset neonatal sepsis[28].

In this study, serum glucose levels decreased, while lactate and triglyceride levels increased 24 h after CLP (Table 1). A previous study described blood sugar as biphasic in CLP-induced sepsis, rising in the first 12 h and decreasing after 24 h. Ischemia and hypoxia can activate the glycolytic pathway in the early phase of sepsis, resulting in significant glucose consumption, followed by low blood glucose levels. This is because increased blood lactate levels may be attributed to tissue hypoxia and to hypoperfusion resulting from oxygen consumption in sepsis[32]. Hyperlipidemia in sepsis is another biomarker that indicates severity in the critical patient. In septic conditions, suppression of lipoprotein lipase enzyme activity, adipose tissue lipolysis, increased hepatic fatty acid synthesis, and decreased very low-density lipoprotein clearance are the main causes of hypertriglyceridemia[33]. Low levels of Na, K, Ca, Mg, and Fe were also observed in the CLP group in the present study (Table 1). An excess or deficiency of these elements suppresses immune cell functions and increases the risk of morbidity and mortality due to infectious disease. Na^+ and K^+ homeostasis are vital for high-energy phosphate metabolism, cardiac muscle activity, cellular volume, osmolarity, and organ functions[34]. Hyponatremia is a common electrolyte imbalance seen in inflammatory disease and infection in clinical practice. Sepsis increases the absorption of Na^+ and water in the proximal tubule associated with excess and low plasma Na^+ and K^+ levels[4]. Evidence from in vitro experiments has shown that *in vivo* hypoxic exposure disrupts transalveolar fluid transport, and that this deterioration is associated with a decrease in alveolar epithelial Na-K-ATPase hydrolytic activity[35]. Ca^{2+} and Mg^{2+} are two main elements with important roles in lung functions. Experimental and clinical studies involving both humans and animals have associated hypocalcemia and hypomagnesemia with greater disease severity and a two- to three-fold increase in mortality[36,37]. Iron is an essential element for all living organisms and constitutes part of the normal defense against infections. Serum iron levels decrease during inflammatory disease, often resulting in the development of anemia or inflammation/ infection[38].

In the present study, LT treatment affected lung histopathology, inflammation and oxidative stress, and also serum oxidant-antioxidant balance. It also effectively lowered interstitial edema, neutrophil infiltration, and lung injury scores (Fig. 1C & D). Although no studies have investigated the effect of LT on lung tissue damage in sepsis, LT has been reported to reduce NF-kB activation resulting from oxidative stress, thereby alleviating airway inflammation in the lung[14]. LT may affect antioxidant potential by reducing oxidative stress. The present study results showed a significant decrease in MDA, AOPP, MPO, TOS, OSI, IMA, and IMAR values in lung tissue and plasma/serum of septic rats receiving LT, and an increase in antioxidant parameters such as TAS and albumin (Fig 2). This improvement can be attributed to LT metabolites exhibiting antioxidant effects by reducing inflammatory and oxidant conditions in sepsis. Glutamine is an LT metabolite involved in various biological processes that are capable of increasing the expression of heat shock protein and of reducing NF-κB, oxidative stress, and neutrophil accumulation[13]. Glutamine is also a glutamate donor for glutathione that represents one of the most important celluler antioxidant defenses against oxidative stress. This has been attributed to increased levels of glycine and glutamic acid after LT administration in rats, one of the constituent components of glutathione[39]. In addition, glycine inhibits the release of Ca, reduces cytokine production, and plays a protective role against oxidative damage[40]. The other metabolite, ethylene amine, induces the γδ T cell antigen isopentenyl

pyrophosphate by inhibiting the mevalonate pathway, which may be partly responsible for ameliorating ALI induced by sepsis[41].

In the present study, serum albumin and Na levels increased following the administration of LT to septic rats. A previous study showing the binding capacity of LT to human serum albumin using the molecular docking method suggested that LT may be transported in circulation by albumin[42].This transportation is important for the direct effect of LT on vital organs such as the lung. These findings show that LT treatment may regulate the osmotic balance and be beneficial against edema by regulating albumin and Na levels, as observed in lung tissue histopathology (Fig. 1D).

LT application reduced lactate and triglyceride levels, but did not restore glucose, K, Ca, Mg, or Fe levels in septic rats to sham group levels. In the light of the significant reducing effects of LT on plasma lactate levels in the present study, it may be concluded that LT may exhibit beneficial effects by correcting pathological conditions such as hypoxia. LT may affect the absorption of the nutrients by regulating the expression of glucose, fatty acid, and amino acid transporters in the gut[12]. Unchanged cation and glucose levels may be the result of unknown compensatory factors. Although serum levels exhibited insignificant changes, lung minerals may be affected by LT treatment without reflecting serum levels.

There are also a number of limitations to this research. First, we were unable to investigate the effects of LT on bronchoalveolar lavage fluid. Second, the number of parameters investigated was restricted by budgetary constraints. This study is descriptive in nature, and further studies involving LT are now needed for a better understanding of the pathophysiological mechanisms underlying sepsis, and thus to permit the development of more effective therapeutic measures.

Conclusion

In conclusion, LT contributed to the survival of CLP rats, reduced sepsis-induced inflammation and oxidative stress, and improved serum albumin and sodium levels (Fig. 2). LT may exert these effects either directly or indirectly on metabolites such as glutamine, ethyleneamine and glycine. It may therefore be concluded that LT may protect against lung injury deriving from various diseases, including virus-induced sepsis, such as COVID-19. However, further studies are now needed to elucidate the mechanism by which LT exhibits its therapeutic efficacy.

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Conflict of interest

All authors declare no conflict of interest.

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