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Original research article

Effect of *Usnea longissima* ethyl acetate extract on acute oxidative and inflammatory lung damage from *Staphylococcus aureus* infection in rats

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Abstract

The role of oxidants and proinflammatory cytokines in the pathogenesis of pneumonia caused by *Staphylococcus aureus* (*S. aureus*) has been demonstrated. The present study aims to investigate the protective effect of ethyl acetate extract (EtOAc) obtained from *Usnea longissima* (UL) against acute oxidative and inflammatory lung damage due to *S. aureus* infection in rats. Albino Wistar-type male rats were divided into three groups: Healthy (HG), *S. aureus* inoculated (SaG), and *S. aureus* inoculated + ULEtOAc administered (SUL). SaG ($n = 6$) and SUL ($n = 6$) group rats' left nostrils (excluding HG) were inoculated with 0.1 ml bacterial mixture. After 24 hours, ULEtOAc (50 mg/kg) was administered orally to the SUL group, and the same volume of normal saline was administered orally to the HG ($n = 6$) and SaG groups. This procedure was performed once a day for seven days. Levels of oxidant and antioxidant parameters such as malondialdehyde (MDA) and total glutathione (tGSH), as well as pro-inflammatory cytokine levels such as nuclear factor-kappa B (NF-κB), tumor necrosis factor-alpha (TNF-α), interleukin-one beta (IL-1β), were measured in removed lung tissues. Tissues were also examined histopathologically. Biochemical results showed that ULEtOAc significantly suppressed the increase of MDA, NF-κB, TNF-α, and IL-1β levels and the decrease of tGSH caused by *S. aureus* in lung tissue. *S. aureus* inoculation caused severe mononuclear cell infiltration in interstitial areas, severe lymphoid hyperplasia in bronchial-associated lymphoid tissue and severe alveolar edema, histopathologically. Treatment with ULEtOAc had an attenuating effect on these histopathological findings. Experimental results from this study suggest that ULEtOAc may be beneficial in treating *S. aureus*-induced oxidative and inflammatory lung damage.

Keywords: Ethyl acetate extract; Lung; Oxidative and inflammatory; Rats; *Staphylococcus aureus*; *Usnea longissima*

Highlights:
- *Staphylococcus aureus* caused oxidative stress and inflammation in lung tissue.
- Oxidative stress and increased pro-inflammatory cytokines are the basis of lung damage.
- *Usnea longissima* (UL) is a type of filamentous lichen present in foggy areas.
- UL significantly suppressed the increase in MDA, NF-κB, TNF-α, and IL-1β levels, and the decrease in tGSH.
- UL also alleviated *S. aureus*-induced oxidative and inflammatory lung damage histopathologically.

Introduction

Staphylococcus aureus (*S. aureus*) is one of the pathogens that play an important role in nosocomial and community-acquired diseases (Kimmig et al., 2020). *S. aureus* causes various organ and tissue diseases (Prasad et al., 2011) and is considered the primary cause of pneumonia (Schreiber et al., 2011). Severe pneumonia is also known as the primary cause of acute lung injury (ALI) (Tsushima et al., 2009). Acute lung injury is a severe clinical condition characterized by diffuse lung inflammation and non-cardiogenic pulmonary edema (Johnson and Matthay, 2010). *S. aureus* infection leads to an uncontrolled inflammatory response by inducing the infiltration of inflammatory cells (neutrophils and macrophages) and the expression of cytokines (Lowy, 1998). The role of tumor necrosis

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factor-alpha (TNF-α), interleukin one beta (IL-1β), interleukin six (IL-6), and nuclear factor kappa B (NF-κB) expression in the pathogenesis of inflammatory damage induced by *S. aureus* has been shown (Wang et al., 2018). However, *S. aureus* and Gram (+) microorganisms trigger different systems and induce the production and release of cytokines, according to an *in vitro* study (Bone, 1991). Also, in previous studies, it has been reported that *S. aureus* has the ability to induce reactive oxygen species (ROSs) in animals (Chakraborty et al., 2012).

In this study, *Usnea longissima* (UL), a type of filamentous lichen present in foggy areas (Wang et al., 2021), is investigated for its protective effect against acute oxidative and inflammatory lung damage due to *S. aureus* infection. UL is used as an expectorant, pain reliever, and antipyretic among people (Halici et al., 2005; Wang et al., 2021). They have become important natural medicinal resources due to the production of depsids, depsidones, dibenzofurans, pulvinic acid derivatives, anthraquinones, naphthoquinones, and xanthones in lichens (Reddy et al., 2019). Most of these components are hypothesized to be responsible for their pharmacological activities (Sun et al., 2016). Previous studies show that UL extracts have several biological activities, including anticancer, anti-inflammatory, analgesic, antipyretic, anti-cholesterol, and nematocidal effects (Azizuddin et al., 2017; Halici et al., 2005; Mammadov et al., 2019; Maulidiyah et al., 2020). In the studies of Kinalioğlu et al. (2019), UL extract has been reported to be effective against microorganisms such as *S. aureus, Bacillus subtilis* (*B. subtilis*), *Bacillus cereus* (*B. cereus*), *Enterobacter aerogenes* (*E. aerogenes*), *Proteus vulgaris* (*P. vulgaris*), *Enterococcus faecalis* (*E. faecalis*), *Escherichia coli* (*E. coli*), *Candida tropicalis* (*C. tropicalis*), *Candida albicans* (*C. albicans*), and *Saccharomyces cerevisiae* (*S. cerevisiae*).

All this information in the literature signals that ethyl acetate extract (EtOAc) obtained from UL may be effective against acute oxidative and inflammatory lung damage due to *S. aureus* infection. The present study aimed to investigate the protective effect of EtOAc obtained from UL against acute oxidative and inflammatory lung damage due to *S. aureus* infection in rats.

Materials and methods

Animals

18 albino Wistar-type male rats weighing between 255–270 grams were obtained from Atatürk University Medical Experimental Application and Research Center for the experiment. Before the experiment, the animals were housed and fed in an appropriate laboratory environment at room temperature (22 °C).

Preparation of bacterial isolate and inoculum

The standard *Staphylococcus aureus* ATCC 29213 strain was from the Medical Microbiology Laboratory of Erzincan Mengucek Gazi Training and Research Hospital, Turkey. The standard strain stored at –80 °C was inoculated in a 5% sheep blood medium (BioMérieux, France) and incubated at 37 °C for 16–18 hours. Afterward, this procedure was repeated for the second time, and suspended in pure colonies from the sheep blood medium were transferred (BioMérieux, France) to 0.9% saline. The turbidity was adjusted to 3 MacFarland (900×10^6 CFU) in a DensiCHEK Plus (BioMérieux, France) densitometer device.

Chemicals

The thiopental sodium used in the study was obtained from IE Ulagay (Turkey). The ULEtOAc extract was produced in the central laboratory of Erzincan Binali Yildirim University.

Lichen material

UL lichen species were collected from forests around Trabzon and Rize in April 2021. The material was classified by Recep Tayyip Erdogan University Pazar Vocational School Medical Aromatic Plants Program lecturer, Huseyin Baykal, and is being kept in the herbarium of the Faculty of Arts and Sciences (No: HB 1029).

Preparation of Lichen extract

For this purpose, 100 grams of ground lichen sample was placed into a brown bottle, and 1,000 ml of ethyl acetate was poured on it and subjected to extraction in an ultrasonic bath for 2 h. The extract was removed by filtration and the remainder was extracted once more with 1,000 ml ethyl acetate in the same way and filtered. The extracts were collected, and the solvent evaporated in an evaporator at 40 °C.

Around 9 grams of the dried extract was obtained (yield 9%).

Semi-preparative separation of the EtOAc extract

Semi-preparative separation was performed to identify the main compounds in the EtOAc extract of the lichen. The chromatographic analyses were performed using a Dionex (Thermo Scientific, Germering, Germany) Ultimate 3000 High-Performance liquid chromatography system (HPLC), equipped with an Ultimate 3000 diode array detector (DAD) and fraction collector. Diffractaic acid (CAS Registry Number: 436-32-8) and usnic acid (CAS Registry Number: 7562-61-0) were supplied from Sigma-Aldrich. They were used as standards to set the chromatographic separation conditions. A Thermo Scientific reverse phase column (250 mm, 4,6 mm, 5μ, USA) was used with gradient elution in phase A: 2% acetic acid and phase B: 70% acetonitrile (ACN) using the following time-set; 0–5 min 75% B; 5–8 min 75–90% B; 8–15 min 90–100% B; 15–50 min 100% B; 50–58 min 75–100% B; 58–68 min 75% B. The injection volume was 170 μl, the column temperature was 30 °C, and the flow rate was 1.0 ml.min-1. HPLC-DAD chromatograms were recorded at 260 nm in the range of 200–400 nm to create the spectra of the lichen compounds. Each peak was collected in a different fraction tube. Repeated runs were carried out to accumulate sufficient amounts of each fraction. All fractions were dried under vacuum via an evaporator at 40 °C. They were identified according to their retention times, UV (ultraviolet), and MS/MS (mass) spectra.

Experimental groups

The rats were separated into three groups: Healthy control (HG); *S. aureus* inoculated (SaG); *S. aureus* inoculated + ULEtOAc applied (SUL).

Experimental procedure

The animals were anesthetized with thiopental sodium. To experiment, 0.1 ml of the bacterial mixture (per rat: 900×10^6 CFU was inoculated into the left nostril of the SaG (n = 6) and SUL (n = 6) group rats (except HG) (*S. aureus* strain ATCC 29213 was suspended at 900×10^6 CFU [colony-forming units] per ml). The animals were kept upright for 1 min after inoculation. 24 h after *S. aureus* administration, UL extract

(50 mg/kg) obtained by ethyl acetate extraction was dissolved in distilled water as the solvent and administered orally to the SUL group. The determination of the ULEtOAc dose of 50 mg/kg is based on this being a dose administered in a previous study (Mammadov et al., 2019). HG (n = 6) and SaG groups were also given the same volume of distilled water orally into the stomach. This procedure was performed once a day for seven days. At the end of this period, the animals were sacrificed with high-dose anesthesia (50 mg/kg thiopental sodium), and lung tissues were removed. Malondialdehyde (MDA), total glutathione (tGSH), NF-κB, TNF-α, and IL-1β levels were measured in the removed tissues. Lung tissues were also examined histopathologically. All biochemical and histopathological results from the HG and SUL groups were evaluated by comparing them with the results from the SaG group.

Biochemical analysis

Preparation of samples

Before dissection, all tissues were rinsed with a phosphate-buffered saline solution. The tissues were homogenized in ice-cold phosphate buffers (50 mM, pH 7.4) that were appropriate for the variable to be measured. The tissue homogenates were centrifuged at 5,000 rpm for 20 min at 4 °C, and the supernatants were extracted to analyze tGSH and MDA. All tissue results were expressed by dividing them into gram of protein. All spectrophotometric measurements were performed on a microplate reader (Bio-Tek, USA).

Tissue MDA and tGSH determination

MDA measurements are based on the method by Ohkawa et al. (1979), including spectrophotometric measurement of the absorbance of the pink complex created by thiobarbituric acid (TBA) and MDA. tGSH measurement was performed according to the method defined by Sedlak and Lindsay (1968).

Tissue NF-κB, TNF-α, and IL-1β determination

The tissue homogenate NF-κB, TNF-α, and IL-1β concentrations were measured using a rat-specific sandwich enzyme-linked immunosorbent assay (ELISA): Rat NF-κB ELISA kit (Cat. no: 201-11-0288; Shanghai SunRed Biological Technology Co. Ltd., Shanghai, PR, China); Rat TNF-α ELISA kit (Cat no: YHB1098Ra; Shanghai SunRed Biological Technology Co. Ltd., Shanghai, PR, China); Rat IL-1β ELISA kit (Cat no: YHB0616Ra; Shanghai SunRed Biological Technology Co. Ltd., Shanghai, PR, China). The analyses were performed according to the manufacturer's instructions.

Histopathological analysis

Hematoxylin-eosin method

Necropsy of the rats was performed, and lung tissues were placed into a 10% buffered formalin solution. The samples were then subjected to routine follow-up procedures and embedded in paraffin blocks. The five μm sections taken from the blocks on the slides were stained with Haematoxylin-Eosin and examined under a light microscope for histopathological findings. The evaluation was performed semi-quantitatively as none (0), mild (1), moderate (2), and severe (3) in terms of mononuclear cell infiltration in interstitial areas, hyperplasia in the bronchus-associated lymphoid tissue (BALT), and edema.

Statistical analysis

The biochemical test results were expressed as the "mean value ± standard error" (x ± SEM). The normality of the distribution for continuous variables in the biochemical test results was checked by the Shapiro–Wilk test. The significance level of the difference between the groups was determined using one-way ANOVA. Levene's test was performed to determine whether the homogeneity of variances was achieved. Tukey HSD (honest significant differences) *post-hoc* test was performed because the homogeneity of the variances for NF-κB and IL-1β was achieved, and the Games-Howell *post-hoc* test was performed because the homogeneity of the variances for MDA, tGSH, and TNF-α was not achieved. All biochemical statistical procedures were performed in the SPSS for Windows 22.0 (IBM Corp., released 2013, Armonk, NY, USA) statistical program, and a $p < 0.05$ value was the threshold for the statistical significance. Histopathological data were analyzed with the SPSS for Windows, 20.00 (IBM Corp., released 2011, Armonk, NY, USA) program. The difference between the groups was determined by the Kruskal–Wallis test, one of the nonparametric tests, and the group that created the difference were determined by the Mann–Whitney U test ($p < 0.05$).

Results

Identification of major compounds in the ULEtOAc extract

Major compounds of ULEtOAc extract were separated using the optimized prep-HPLC-DAD method. Diffractaic acid, evernic acid, and usnic acid were found as major compounds in the EtOAc extract as shown in Fig. 1.

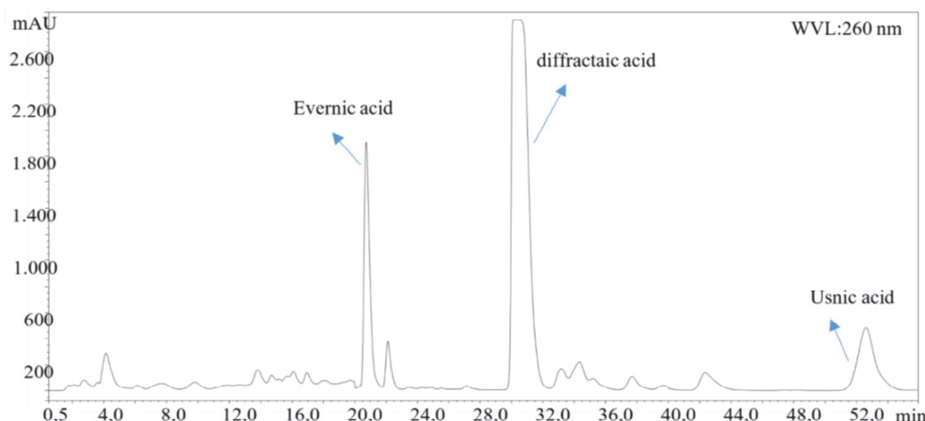

Fig. 1. HPLC-DAD chromatogram of ULEtOAc extract at 260 nm. The two peaks at 30.3 and 52.6 minutes were compatible with the standards for diffractaic acid and usnic acid respectively based on their retention times and UV spectra obtained by the DAD detector. Therefore, it was ensured that these two peaks were diffractaic acid and usnic acid. They are two of the main compounds of lichen species as well. The other major compound eluted at 20.8 min was identified as evernic acid, based on its UV and MS/MS spectra (as shown in Fig. 2).

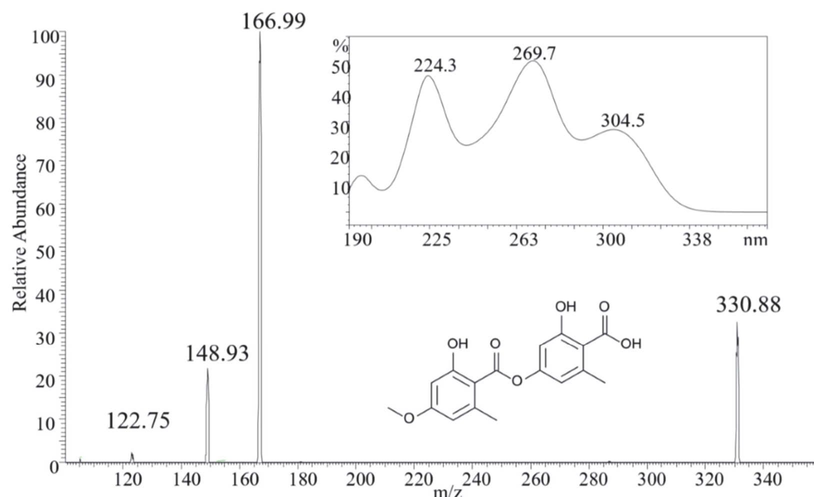

Fig. 2. MS/MS and UV spectrums of the evernic acid. The molecular weight of evernic acid is 332.31 amu and its negative ion has m/z 331. There are also three known fragments of this compound which are at 167, 149, and 123 m/z.

Biochemical results

MDA and tGSH analysis of lung tissue

As in Fig. 3 and Table 1, the amount of MDA in the lung tissue of *S. aureus* inoculated animals was significantly higher than in the healthy ($p < 0.001$) and ULEtOAc-treated ($p < 0.001$) groups. The difference in the amount of MDA between the healthy group and the ULEtOAc-treated group was statistically insignificant ($p = 0.269$).

In addition, the amount of tGSH in the lung tissue of *S. aureus* inoculated animals was significantly lower than in the healthy ($p < 0.001$) and ULEtOAc-treated ($p < 0.001$) groups. The difference in the amount of tGSH between the healthy group and the ULEtOAc-treated group was statistically insignificant ($p = 0.260$; Fig. 3; Table 1).

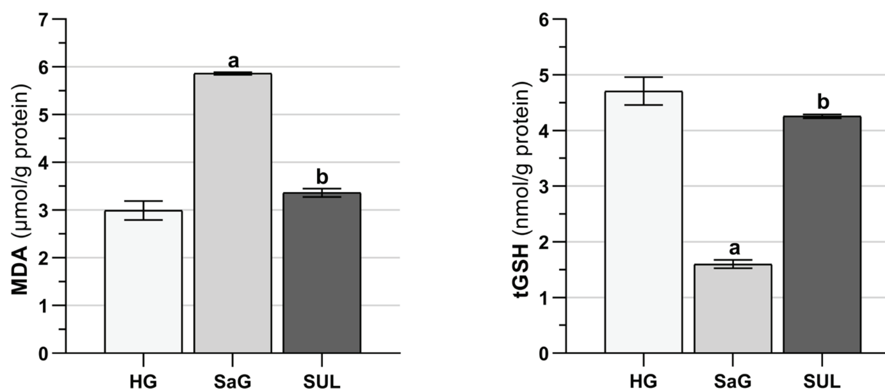

Fig. 3. MDA and tGSH levels in the lung tissue of experimental groups. Bars are mean ± SEM. [a] means $p < 0.001$ according to the HG group, and [b] means $p < 0.001$ according to the SaG group, $n = 6$. MDA: malondialdehyde; tGSH: total glutathione; HG: healthy group; SaG: *Staphylococcus aureus* inoculated group; SUL: *Staphylococcus aureus* inoculated + ULEtOAc applied group.

Table 1. Mean and standard error values of the groups in terms of biochemical test results and, for biochemical variables, the *p*-values of *post-hoc* comparisons between experimental groups

					p values	
Biochemical parameters	HG	SaG	SUL	HG vs. SaG	HG vs. SUL	SaG vs. SUL
MDA*	2.99 ± 0.20	5.86 ± 0.03	3.36 ± 0.09	<0.001	0.269	<0.001
tGSH*	4.71 ± 0.25	1.60 ± 0.07	4.26 ± 0.03	<0.001	0.260	<0.001
NF-κB**	3.06 ± 0.11	5.84 ± 0.22	3.36 ± 0.05	<0.001	0.346	<0.001
TNF-α*	2.19 ± 0.05	6.13 ± 0.03	2.65 ± 0.20	<0.001	0.150	<0.001
IL-1β**	1.91 ± 0.25	4.44 ± 0.11	2.30 ± 0.12	<0.001	0.280	<0.001

Note: HG: healthy group; SaG: *Staphylococcus aureus* inoculated group; SUL: *Staphylococcus aureus* inoculated + ULEtOAc applied group; MDA: malondialdehyde; tGSH: total glutathione; NF-κB: nuclear factor kappa B; TNF-α: tumor necrosis factor-alpha; IL-1β: interleukin one beta.
Footnotes: * Games-Howell *post-hoc* test was performed after one-way ANOVA for pairwise comparisons. ** Tukey HSD (Honest Significant Difference) *post-hoc* test was performed after one-way ANOVA for pairwise comparisons.

NF-κB, TNF-α, and IL-1β analysis of lung tissue

As in Fig. 4 and Table 1, *S. aureus* inoculation has increased the production of NF-κB, and proinflammatory cytokines such as TNF-α, IL-1β in the lung tissue of animals. The NF-κB, TNF-α, and IL-1β values in the lung tissue of the group inoculated with *S. aureus* alone were significantly higher than the healthy (respectively, $p < 0.001$; $p < 0.001$; $p < 0.001$) and ULEtOAc-treated (respectively, $p < 0.001$; $p < 0.001$; $p < 0.001$) groups. The difference between the healthy group and the ULEtOAc-treated group was not statistically significant (respectively, $p = 0.346$; $p = 0.150$; $p = 0.280$).

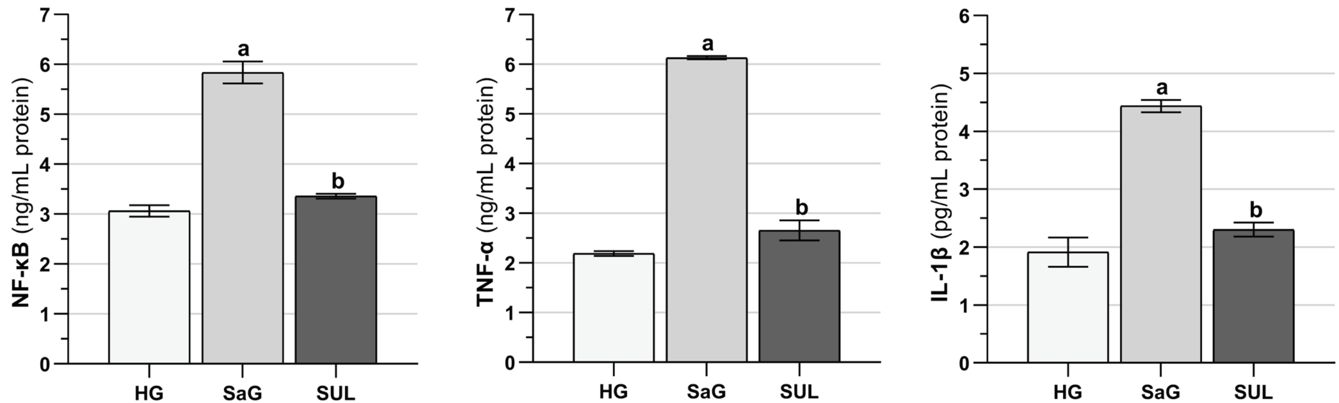

Fig. 4. NF-κB, TNF-α and IL-1β levels in the lung tissue of experimental groups. Bars are mean ± SEM. [a] means $p < 0.001$ according to the HG group, and [b] means $p < 0.001$ according to the SaG group, $n = 6$. NF-κB: nuclear factor kappa B; TNF-α: tumor necrosis factor-alpha; IL-1β: interleukin one beta; HG: healthy group; SaG: *Staphylococcus aureus* inoculated group; SUL: *Staphylococcus aureus* inoculated + ULEtOAc applied group.

Histopathological results

As shown in Table 2, there were statistically significant differences between the groups ($p < 0.05$). The lung tissues of the rats in the healthy control group (HG) had a normal histological appearance (Fig. 5). Grade-3 intensity mononuclear cell infiltration in the interstitial areas (Fig. 6A), grade-3 lymphoid hyperplasia in BALT (Fig. 6B), and grade-3 edema in the alveoli were present in the SaG group inoculated with *S. aureus* alone (Fig. 6C). Grade-2 intensity mononuclear cell infiltration (Fig. 6D) was present in the interstitial areas of the ULEtOAc-treated group, while grade-1 hyperplasia was present in the BALT (Fig. 6E). Again, there was grade-2 severity of edema in the lung alveoli of the SUL group (Fig. 6F).

Table 2. Histopathological evaluation results

Groups	MNC infiltration in interstitial areas	Hyperplasia in BALT	Edema
HG	0.33 ± 0.51^a	0.33 ± 0.40^a	0.00 ± 0.00^a
SaG	2.83 ± 0.40^b	2.66 ± 0.51^b	2.83 ± 0.40^b
SUL	2.16 ± 0.40^c	1.16 ± 0.40^c	2.16 ± 0.40^c

Note: HG: healthy group; SaG: *Staphylococcus aureus* inoculated group; SUL: *Staphylococcus aureus* inoculated + ULEtOAc applied group; MNC: Mononuclear cell; BALT: bronchial-associated lymphoid tissue. *Footnotes:* Kruskal–Wallis test was performed for statistical analysis, and the Mann–Whitney *U* test was used as a *post-hoc* test. Results are presented as median ± standard deviation. Groups marked with the same letter in the same column are statistically similar ($p > 0.05$), while there is a statistical difference in groups with different letters ($p < 0.05$).

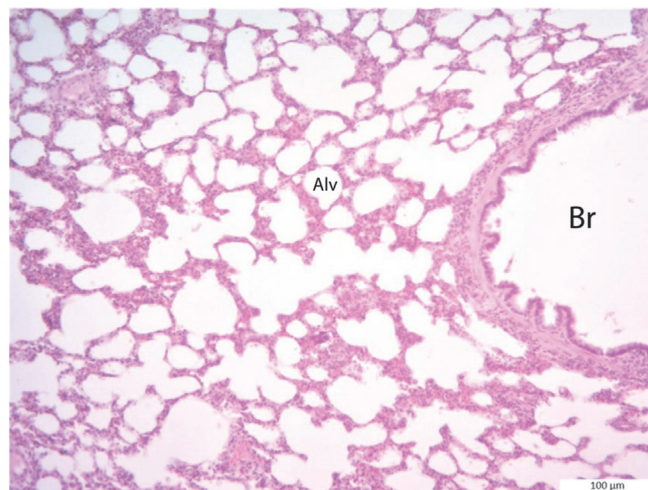

Fig. 5. Lung tissue of the healthy control group (HG). In the lung tissue of the healthy control group (HG), normal histological appearance. Br: bronchiole; alv: alveoli.

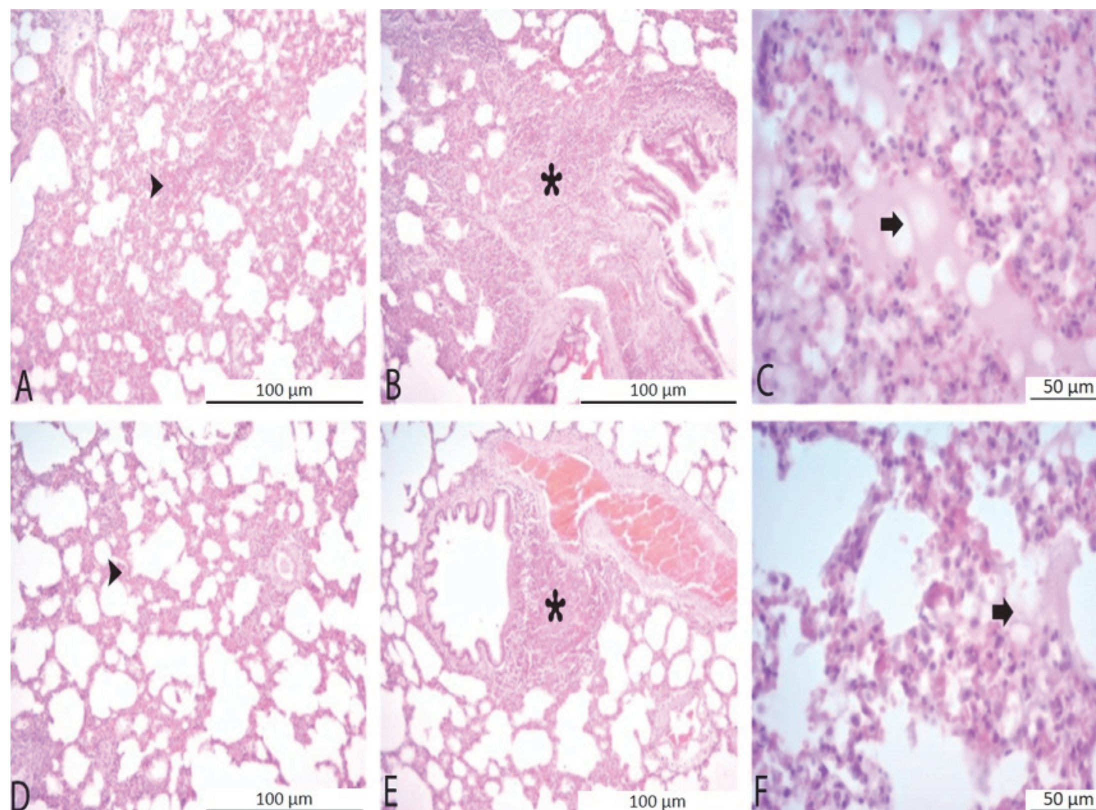

Fig. 6. Lung tissue of the SaG and SUL groups. In the lung tissue of the SaG group: severe MNC infiltration in interstitial areas (arrowhead; **A**); severe hyperplasia in BALT (*; **B**); and severe edema in alveoli (arrow; **C**) appearance. In the lung tissue of the SUL group: moderate MNC infiltration in interstitial areas (arrowhead; **D**); mild hyperplasia in BALT (*; **E**); and moderate edema in alveoli (arrow; **F**) appearance.
SaG: *Staphylococcus aureus* inoculated group; SUL: *Staphylococcus aureus* inoculated + ULEtOAc applied group; MNC: mononuclear cell; BALT: bronchial-associated lymphoid tissue.

Discussion

In this study, the protective effect of EtOAc obtained from UL against oxidative and inflammatory lung injury by intranasal inoculation of *S. aureus* in rats was under a biochemical and histopathological investigation. The results of the biochemical experiment showed that intranasally inoculated *S. aureus* leads to an increase in malondialdehyde (MDA), NF-κB, TNF-α, and IL-1β, and a decrease in total glutathione (tGSH) in lung tissue. In a recent study, MDA, NF-κB, TNF-α, IL-1β, and cyclooxygenase-2 increased, and tGSH decreased in tissue inoculated with *S. aureus* (Dilber et al., 2022). MDA, which increased in the lung tissue of the animal groups we inoculated with *S. aureus*, is the final cytotoxic product of lipid peroxidation (LPO) (Gaschler and Stockwell, 2017). Factors inducing LPO chain reactions and causing cell deaths are based on ROSs (Su et al., 2019). According to previous studies, it has been stated that *S. aureus* has the ability to induce ROSs in animals (Chakraborty et al., 2012). The results of this study indicate that *S. aureus* causes oxidative damage in the tissue.

Another parameter used to determine oxidative damage in living organs and tissues is tGSH. In the present study, the tGSH level in the lung tissue of the *S. aureus* group decreased. GSH is an endogenous antioxidant found in body fluids and tissue. It is known that GSH acts as a ROS scavenger and inhibitor of LPO in living tissues and protects tissues against oxidative damage (Owen and Butterfield, 2010). According to a study that coincides with our results, *S. aureus* increased oxidant parameters in lung tissue, and decreased GSH levels were reported (Wang et al., 2019a). This information reveals that *S. aureus* alters the oxidant-antioxidant balance in favor of oxidants in lung tissue. This situation is considered as oxidative damage in the literature (Kisaoglu et al., 2013).

In the present study, proinflammatory cytokine levels, such as NF-κB, TNF-α, and IL-1β, were found to be high in the lung tissues of the *S. aureus* group, where the oxidant-antioxidant balance changed in favor of oxidants. Our findings are supported by information showing the role of cytokines such as NF-κB, TNF-α, and IL-1β in the pathogenesis of inflammatory damage induced by *S. aureus* (Wang et al., 2018). It is also known that oxidative stress activates the transcription factor NF-κB, which regulates the expression of proinflammatory cytokines (Kumari et al., 2016). In another study, it was also reported that NF-κB activation could be induced by ROSs (Ding et al., 2012). In the clinic, information has been documented that increased production of NF-κB is associated with arthritis, septic shock, pulmonary fibrosis, diabetes, cancer, arteriosclerosis, and various inflammatory diseases (Baldwin, 2001). TNF-α, another proinflammatory cytokine, has been shown to induce oxidative stress and cause cell dysfunction (Wang et al., 2019b). IL-1βs are known to have many functions such as an oxidative burst of neutrophils and release of free radicals (Dinarello, 2000; Hasturk et al., 2009). Information obtained from the literature suggests that *S. aureus* causes oxidative and

inflammatory damage in lung tissue. It also signs that there is a link between oxidative stress and inflammation.

In the present study, it was observed that our biochemical experiment results coincided with histopathological findings. Biochemically, MDA, NF-κB, TNF-α, and IL-1β levels were high, and tGSH levels were low in the lungs of the *S. aureus* inoculated group. Severe (grade-3) mononuclear cell infiltration was also observed in interstitial areas on histopathological examination in the same group. Moreover, severe lymphoid hyperplasia in the BALT and severe edema in the alveoli were present. Histopathological findings reveal that inflammation develops in the lungs. In a previous study, intranasal inoculation of *S. aureus* caused acute lung inflammation (Ma et al., 2019). In addition, inflammatory cell infiltration and edema were detected in the studies of Shaukat et al. (2021). It has been reported that *S. aureus*-induced lung injury is not limited to increased inflammatory cell release and cytokine production but is also associated with neutrophilic infiltration and macrophage activation, which also increases ROS production (Grommes and Soehnlein, 2011).

In this study, ULEtOAc significantly inhibited the increase of MDA, NF-κB, TNF-α, and IL-1β in the lung tissues of animals inoculated with *S. aureus* and the decrease of tGSH. ULEtOAc, which significantly inhibits oxidant and proinflammatory cytokine increase, also alleviated the above-mentioned histopathological damage. As is known, UL extract is effective against *S. aureus* and many pathogenic microorganisms (Kinalioğlu et al., 2019). No information was present in the literature investigating the antioxidant effect of ULEtOAc against *S. aureus*-induced oxidative lung injury. However, there are studies reporting that it protects stomach tissue from damage by oxidants (Halici et al., 2005). Methanol extracts of UL have been reported to show potent antioxidant activities *in vitro* (Odabasoglu et al., 2004). A polysaccharide isolated from UL has been reported to exhibit both antioxidant activity and modulate cytokine increase (Wang et al., 2021). It is also stated in the literature that UL has ROS scavenging effect (Bian et al., 2002). It is advocated that the antioxidant effect of UL is due to its major components such as usnic acid, diffractaic acid, and evernic acid (Odabasoglu et al. 2006, 2012; Shcherbakova et al., 2021). Usnic acid has also been explained to have an inhibitory effect on proinflammatory cytokines (Vanga et al., 2017). In our previous study, death did not occur in any of the rats administered with ULEtOAc extract orally at doses of 500, 1,000, and 2,000 mg/kg (Mammadov et al., 2019).

To the best of our knowledge, no study has examined the protective effects of ULEtOAc against acute oxidative and inflammatory lung damage induced by an *S. aureus* infection. In this respect, it will be the first study to provide valuable information about the potential therapeutic effects of ULEtOAc treatment against acute oxidative and inflammatory lung injury caused by *S. aureus* infection. Although the easy availability of ULEtOAc, its low cost and the absence of toxic effects are the strengths of this study, some limitations should be recognized. To elucidate the current issue in more detail, it may be useful to examine total oxidants and antioxidants as well as anti-inflammatory cytokines, and to perform histopathological examination at the molecular level.

Conclusion

Intranasal inoculation of *S. aureus* led to oxidative and inflammatory damage in the lung tissue of animals. *S. aureus*-induced oxidative and inflammatory lung injury has been shown biochemically and histopathologically. ULEtOAc significantly reduced the damage caused by *S. aureus* on the lung by inhibiting the increase in oxidant and proinflammatory parameters and the decrease in antioxidant parameters. As ULEtOAc extract is non-toxic, it is thought that it may be more advantageous than other antibiotics. Experimental results of this study suggest that ULEtOAc may be beneficial in treating oxidative and inflammatory lung injury associated with *S. aureus* infection.

Ethics declaration

All animal experiments were performed in accordance with the ARRIVE guidelines and in accordance with the UK Animals (Scientific Procedures) Act 1986 and related guidelines, EU Directive 2010/63/EU on animal experiments, or the National Institutes of Health guidelines for the care and use of laboratory animals (NIH Publication No. 8023, revised 1978). The authors declare that these guidelines have been followed. The protocols and procedures were approved by the Local Animal Experimentation Ethics Committee (Meeting Date: 30.07.2021; Meeting No: 6; Decision No: 179).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Author contributions

All authors were financially involved in the research and/or preparation of the manuscript. BD and HS conceived and designed the study. BS, RM, BY, SA, OA, EAT, DA, and TAC performed the experiments. SA, OA, BM, EAT and TAC acquired the data. BD, SA, OA, EAT, DA, and HS analyzed the data. BD and HS wrote the paper. BD, BS, RM, BY, SA, OA, BM, EAT, DA, TAC, and HS drafted and critically revised the article. BD and BY were responsible for polishing the manuscript. All the authors have discussed the results and approved the final manuscript.

Conflict of interest

The authors have no conflict of interest to declare.

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