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Protective effect of *Micromeria congesta* essential oil on lipopolysaccharide-induced acute lung injury in rats

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Abstract

Micromeria congesta (MC) is a medicinal plant traditionally used in the Şanlıurfa, Adıyaman, and Gaziantep provinces of Türkiye for treating respiratory disorders. Despite its ethnobotanical relevance, its pharmacological effects on lung inflammation remain unclear. This study evaluated the protective effects of MC essential oil (MCEO) against lipopolysaccharide (LPS)-induced acute lung injury in rats, with a focus on inflammation and tissue damage. The MC was collected from its natural habitat, and its EO was extracted by hydro-distillation using a Clevenger-type apparatus. The Gas chromatography-mass spectrometry analysis revealed 28 compounds, with piperitenone oxide (33.97%) and pulegone (22.30%) as the main constituents. Thirty-six Wistar albino rats were randomly assigned into six groups, including three MCEO-treated (12.50, 25.00, and 50.00 mg kg^{-1}), dexamethasone, LPS-only, and control groups. The MCEO was administered by gavage for seven days, followed by intravenous LPS on day eight (except for the control group). Blood and lung tissues were collected 6 hr later for biochemical, histopathological, and molecular analyses. The MCEO treatment significantly lowered blood glucose and urea levels compared to the LPS group. It also modulated *NLRP3*, *caspase-3*, and *tumor necrosis factor alpha* genes expressions, as determined by RT-PCR analysis, indicating anti-inflammatory effects. Histopathological findings confirmed a dose-dependent reduction in lung inflammation and tissue damage. The MCEO administration alleviated LPS-induced acute lung injury in rats through anti-inflammatory and protective effects, as confirmed by molecular (*NLRP3*, *caspase-3*, and *tumor necrosis factor alpha*) and histopathological analyses. Additionally, MCEO reduced blood glucose and urea levels, supporting its systemic protective role against endotoxin-induced inflammation.

Introduction

Lung diseases remain a significant global health challenge. Acute lung injury, along with its more severe manifestation, acute respiratory distress syndrome, is a major contributor to morbidity and mortality in critically ill patients.[1] Endotoxins constitute essential structural elements of the bacterial cell wall. Lipopolysaccharide (LPS) is a major endotoxin component located in the outer membrane of Gram-negative bacteria. The LPS acts as a potent immuno-stimulant, primarily activating macrophages and inducing pro-inflammatory cytokines release. It also triggers the secretion of cytokines involved in the inflammation process.[2,3] Inflammation is a protective immune response against harmful infections and pathogens during the initial phase of the healing process. Excessive and persistent inflammation is associated with the pathogenesis of certain chronic illnesses, including cardiovascular and intestinal diseases, diabetes, arthritis, and cancer.[4] Sepsis is a clinical syndrome characterized by severe and abnormal biological processes, developing as a result of impaired inflammatory response to infection.[5]

Micromeria congesta (MC) Boiss. and Hausskn. (*Lamiaceae*; MC) is an endemic plant species naturally distributed in the mountainous regions of southeastern Türkiye, particularly in the provinces of Şanlıurfa, Gaziantep, Adıyaman, and Diyarbakır. Locally, it is known by various Turkish names, such as pünge tehta, gihaye palug, punk, kaya yarpuzu (rock mint), and dağ nanesi (mountain mint).[6,7] The botanical name was verified and confirmed as an accepted name through the Tropicos database, thereby validating its current taxonomic status.[8]

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The aerial parts of the plant are traditionally prepared as an herbal tea by boiling and are commonly used by local communities for the treatment of respiratory tract ailments and persistent coughs. This ethno-medical application has been documented in field-based ethnobotanical studies conducted in the region.[9,10] Additionally, several *Micromeria* species, including MC, have been traditionally employed for conditions related to the heart, lungs, headaches, wounds, colds, and skin infections.[7,11,12] Some species are also used as aromatic herbs for seasoning.[7] Besides its traditional use, the MC essential oil (MCEO) has been reported to possess central nervous system stimulant activity, as well as anti-microbial, anti-oxidant, anti-inflammatory, anti-septic, anti-rheumatic, and analgesic properties, particularly in the treatment of toothaches.[6,11,12] However, these pharmacological effects, while scientifically important, are distinct from and should not be conflated with its traditional use as an herbal remedy for respiratory conditions, and its role in respiratory inflammation remains insufficiently investigated.

In this study, we identified several major bioactive compounds in MCEO, including piperitenone oxide (33.97%), pulegone (22.30%), N-exadecanoic acid (13.30%), piperitenone (10.71%), spathulenol (5.39%), arvone oxide (3.22%), and isomenthone (2.17%), being thought to contribute to its therapeutic potential. We aimed to investigate the protective effects of MCEO on LPS-induced acute lung injury in rats by evaluating inflammatory responses and tissue damage mechanisms in the lung.

Materials and Methods

Plant materials. The MC plants were collected from Germüş Mountains (Dağeteği Village) and Tektek Mountains (Açıkyazı Village) of Şanlıurfa province between June and August in 2022 and 2023. The collected specimens were identified by Associate Professor Dr. Mustafa Aslan (Department of Biology, Harran University, Şanlıurfa, Türkiye) and deposited under the scientific reference number of Aslan 1427 (HURUB: 4590). The plants were dried outdoors in the shade using the natural drying method and stored in polyethylene bags before content analysis and EO extraction. The MCEO was obtained using the Clevenger apparatus. Storage of the EO was carried out in dark glass vials at 4.00 ˚C, protected from ultra-violet light and heat.[13,14] The chemical components of MCEO were analyzed by Schimadzu Nexis GCMS-QP2020 NX (Shimadzu Corp., Kyoto, Japan) instrument (100% polyethylene glycol column and electron impact detector).[14] Analyses were carried out at GAP Agricultural Research Institute Laboratory.

Experimental design. In this study, 36 male Wistar rats with an average weight of 300 g were used. The rats were sourced from the Harran University Animal Experiment Application and Research Center, Şanlıurfa, Türkiye, and kept there throughout the study. Animals were provided with standard feed and water *ad libitum* and housed in wire cages maintained at room temperature under a 12-hr light/dark cycle. Three rats were placed in each cage and a 1-week adaptation period was applied before the experiment. During the experiment, body temperature, and feed and water consumption were regularly monitored and recorded.[15] Thirty-six rats were divided equally into six groups of six animals each, including 12.50 mg kg^{-1}, 25.00 mg kg^{-1}, and 50.00 mg kg^{-1} of MCEO, dexamethasone (DEX; Topkim, Istanbul, Türkiye), lipopolisaccaride (LPS; Lipopolysaccharide from Escherichia coli O111:B4, Sigma-Aldrich, St. Louis, USA) only, and control groups. Each group was divided into two sub-groups of three rats for a total of 12 cages. All procedures were performed following internationally recognized guidelines for the care and use of laboratory animals, including the European Community Directive 86/609/EEC and the Guide for the Care and Use of Laboratory Animals.[16] In group 1 (MCEO [12.50 mg kg^{-1}] + LPS [5.00 mg kg^{-1}]), MCEO was administered by oral gavage (OG) once daily for seven consecutive days. On day eight, LPS (*Escherichia coli* O111:B4) was administered *via* the tail vein (TV) 6 hr prior to euthanasia. The rats were anesthetized with intraperitoneal injection of 80.00 mg kg^{-1} ketamine (Richter Pharma, Wels, Austria) and 10.00 mg kg^{-1} xylazine (Bayer Türk Kimya San. Ltd. Istanbul, Türkiye), and were subsequently euthanized by decapitation under deep anesthesia).[15,17] In group 2 (MCEO [25.00 mg kg^{-1}] + LPS [5.00 mg kg^{-1}]), MCEO was administered by OG once daily for seven consecutive days. On day eight, LPS was administered *via* the TV 6 hr prior to euthanasia.[15,17] In group 3 (MCEO [50.00 mg kg^{-1}] + LPS [5.00 mg kg^{-1}]), MCEO was administered by OG once daily for seven consecutive days. On day eight, LPS was administered via the TV 6 hr prior to euthanasia.[15,17] In group 4 (DEX [5.00 mg kg^{-1}] + LPS [5.00 mg kg^{-1}]), DEX was administered intraperitoneally as a single dose immediately before LPS injection on day eight. The LPS was administered *via* the TV 6 hr prior to euthanasia.[15,17] In group 5 (LPS [5.00 mg kg^{-1}]), LPS (5.00 mg kg^{-1}) was administered *via* the TV 6 hr prior to euthanasia.[15,17] In group 6 (control), animals received physiological saline (1.00 mL *per* rat daily) by OG at the same time as the treatment groups. On day eight, 6 hr after the corresponding experimental procedures, rats were anesthetized, and blood and lung tissue samples were collected and stored at – 80.00 ˚C until analysis.[15,17-22] This study received ethical approval from the Harran University Local Committee for Animal Experiments, Şanlıurfa, Türkiye (Protocol Number: 2024/001/10).

Biochemical analysis of blood serum. Biochemical analysis of blood serum was performed using an autoanalyzer device (Integra 800; Roche, Mannheim,

Germany). Total anti-oxidant status and total oxidant status were measured using commercially available colorimetric assay kits (Rel Assay Diagnostics, Gaziantep, Türkiye), according to the manufacturer's instructions. The intra-assay coefficient of variation for both assays was below 3.00%.[23]

Histopathological examination. Lung tissue samples obtained through necropsy were fixed in 10.00% buffered formalin and then processed through routine tissue procedures (dehydration, clearing, and impregnation). Subsequently, the tissues were embedded in paraffin blocks, and 5.00-µm thick sections were cut from each block using a rotary microtome (RM2125; Leica Microsystems GmbH, Wetzlar, Germany) and placed onto slides. The sections were stained using Hematoxylin and Eosin for histopathological analysis. The stained slides were examined using a light microscope (BX53; Olympus, Tokyo, Japan), and the lesions were scored based on their severity as none (−/0), mild (+/1), moderate (++/2), and severe (+++/3). For statistical analysis, these scores were treated as numerical values ranging from 0 to 3.[24]

Tissue homogenization and RNA isolation. For gene expression analysis, rat lung tissues were harvested and stored at – 80.00 ˚C until the day of the experiment. The gene expression levels of *NLRP3*, *caspase-3*, and *TNF-α* were analyzed by RT-PCR as key regulators of inflammation, apoptosis, and cytokine response in acute lung injury (Table 1). The tissues were homogenized in lysis buffer supplied with the RNeasy Mini Kit (Qiagen, Hilden, Germany) using a refrigerated homogenizer. The samples were incubated for 5 to 10 min to achieve complete lysis. Then, 0.20 mL of chloroform was added, the mixture was vortexed for 30 sec, and centrifuged at 12,000 × *g* for 10 min at 4.00 ˚C. About 540 µL of the supernatant was transferred into a 1.50 mL RNase-free centrifuge tube. To the supernatant, 810 µL of 100% absolute ethanol was added, and the solution was mixed by repeated pipetting. Total RNA was isolated using High Pure RNA Isolation Kit (Roche), and micro-RNA was isolated by Sanprep Column Micro-RNA Mini-Preps Kit (Bio Basic, Markham, Canada), following manufacturers' protocols. The purity and concentration of the RNA preparations were assessed at 260/280 nm using NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, USA).

Table 1. Primers designed from selected base sequences of target and reference genes.

Genes	Primers
NLRP3	F: 5'GCTGAAGTGGATCGAAGTGAA3' R: 5'CTT GGGTGAGTTGTGGAGAAA3'
Caspase-3	F: 3'CCTGTGATGGAGAGAAGATGG 5' R: 3'GGTTCAGTCTCATGTGGTAACT5'
TNF-α	F: 5'GGCTTT CGG AAC TCA CTG GA3' R: 5'CCCGTAGGGCGATTACAGTC3'
GAPDH	F: 5'-CCTGGAGAAACCTGCCAAGTAT-3' R: 5'-AGCCCAGGATGCCCTTTAGT-3'

Synthesis of cDNA from mRNA. The cDNA synthesis was performed using the Transcriptor First Strand cDNA Synthesis Kit (Roche) according to the manufacturer's instructions. One µg RNA was added to each well and incubated at 25.00 ˚C for 10 min, followed by incubation at 42.00 ˚C for 60 min. The reaction was terminated by heating at 70.00 ˚C for 5 min in a final volume of 20.00 µL, and the cDNA samples were stored at – 20.00 ˚C.

Real-time PCR. The RT-PCR assays were carried out in 20.00 µL reaction volumes using the FastStart Universal SYBR Green Master (Roche). The appropriate amount of cDNA was added to each well, and primers were designed and synthesized based on the NCBI GenBank database. Thermal cycling was performed on a Roche LightCycler 96 system under the following conditions: 95.00 ˚C for 10 min, followed by 40 cycles of 95.00 ˚C for 15 sec, 60.00 ˚C for 30 sec, and 72.00 ˚C for 30 sec, and a final melting curve analysis (72.00 - 95.00 ˚C) to confirm specificity. Relative mRNA expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method, with *GAPDH* serving as the internal control gene.

Statistical analysis. All analyses were performed using Jamovi Software (version 2.5; Jamovi Project, Sydney, Australia). Data normality was evaluated using the Shapiro-Wilk test, while variance homogeneity was assessed by Levene's test. For data meeting the assumptions, one-way analysis of variance (with Welch's test if necessary) was used, while the Kruskal-Wallis H test was applied for data did not meet the assumptions. Post-hoc comparisons were performed using Tukey's, Least Significant Difference (LSD), and Dunn's tests.[25]

Results

Chemical composition of MCEO. The gas chromatography-mass spectrometry (GC-MS) results of MCEO revealed the presence of 28 components. Among these components, seven main constituents were observed to have higher concentrations. The constituent with the highest concentration was identified as piperitenone oxide (33.97%), followed by pulegone (22.30%), N-hexadecanoic acid (13.30%), piperitenone (10.71%), spathulenol (5.39%), carvone oxide (3.22%), and isomenthone (2.17%) in increasing concentrations. The representative GC–MS chromatogram of MCEO is shown in Figure 1, and the detailed chemical composition is presented in Table 2.

Biochemical results. There was a significant variation in blood glucose levels among the groups ($p < 0.01$). According to the results, it was determined that the highest mean for glucose parameter was in the 6th group and the lowest mean was in the 3rd group. This shows that the glucose levels between the 6th and 3rd groups are significantly different at the $p < 0.01$ level (Table 3).

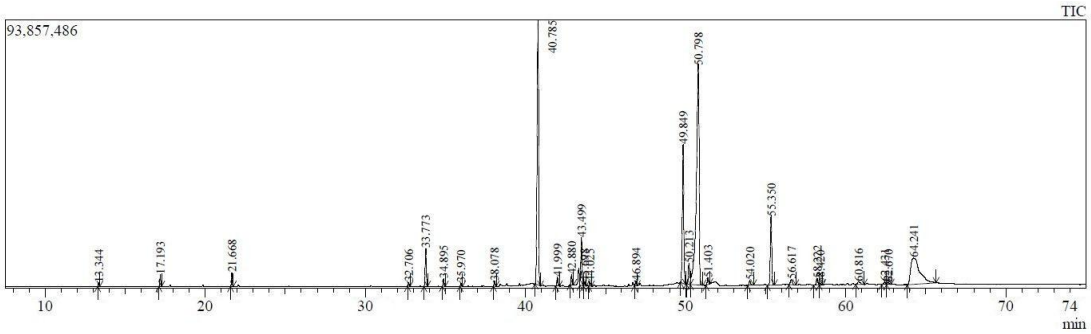

Fig. 1. The GC-MS chromatogram of the essential oil of *Micromeria congesta*.

Table 2. Chemical composition of the essential oil of *Micromeria congesta* determined by GC-MS.

Compounds	Retention time (min)	Peak area	Area (%)
Alpha-pinene	13.344	4,387,337	0.16
Beta-pinene	17.193	15,967,366	0.58
l-Limonene	21.668	18,967,666	0.69
1-Octen-3-ol	32.706	5,437,027	0.20
Isomenthone	33.773	59,883,116	2.17
P-menthone	34.895	12,684,479	0.46
Beta-bourbonene	35.970	4,657,654	0.17
Cis-isopulegone	38.078	8,113,966	0.29
Pulegone	40.785	616,442,501	22.30
3-Cyclohexene-1-methanol, alpha, alpha-4-trimethyl	41.999	14,998,779	0.54
Cis-sesquisabinene hydrate	42.880	16,275,096	0.59
Carvone oxide	43.499	89,027,910	3.22
Bicyclogermacrene	43.698	6,108,570	0.22
Trans-isopiperitenol	44.025	6,787,465	0.25
Benzenemethanol, 4-(1-methylethyl)	46.894	9,020,740	0.33
Piperitenone	49.849	296,091,860	10.71
Cis-jasmone	50.213	49,131,903	1.78
Piperitenone oxide	50.798	938,920,555	33.97
8,9-Dehydrothymol	51.403	13,783,650	0.50
2,5-Cyclohexadien-1-one, 2,6-bis(1,1-dimethylethyl	54.020	10,689,066	0.39
Spathulenol	55.350	148,880,628	5.39
Naphthalene, 1, 2, 3, 4, 4a, 5, 6, 8a-octahydro-7-methyl	56.617	14,139,070	0.51
Isospathulenol	58.222	15,139,240	0.55
Geranyl-alpha-terpinene	58.420	3,247,693	0.12
Dotriacontane	60.816	11,693,170	0.42
1-Naphthalenamine, 4-bromo-	62.431	7,419,099	0.27
Diethyl Phthalate	62.670	5,649,983	0.20
N-Hexadecanoic acid	64.241	360,252,952	13.03
Total	-	2,763,798,541	100

Table 3. Statistical analysis results for biochemical parameters. Data are presented as mean ± standard error.

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	*p*-value
Glucose (mg dL^{-1})	245.17 ± 14.59 ^{a}	171.33 ± 24.14 ^{b}	142.50 ± 14.54 ^{b}	211.00 ± 31.02 ^{ab}	157.50 ± 24.98 ^{b}	280.00 ± 28.60 ^{a}	$p < 0.01$
Urea (mg dL^{-1})	43.00 ± 3.56 ^{a}	45.67 ± 7.55 ^{a}	65.67 ± 8.22 ^{b}	43.67 ± 3.19 ^{a}	81.50 ± 11.69 ^{c}	41.17 ± 3.60 ^{a}	$p < 0.05$

^{abc} Different letters indicate statistically significant differences between groups ($p < 0.05$; some comparisons $p < 0.01$).

When the blood urea parameter was examined, it was observed that only the 5th group treated with LPS had a significantly higher mean compared to the 1st, 4th, and 6th groups ($p < 0.05$). However, no significant differences were detected among the 1st, 4th, and 6th groups, which showed lower mean values. This result indicates that LPS significantly increases blood urea levels, while the MC and control groups remained at similarly low levels (Table 3). No statistically significant differences were observed in the C-reactive protein, alanine transaminase, aspartate aminotransferase, high-density lipoprotein, low-density lipoprotein, total anti-oxidant status, total oxidant status, and oxidative stress index parameters.

Gene expression results. Gene expression levels of *NLRP3*, *caspase-3*, and *TNF-α* were evaluated using RT-PCR and analyzed by the $2^{-\Delta\Delta CT}$ method with *GAPDH* as a reference gene. The results are summarized in Table 4.

Table 4. Statistical analysis results of *NLRP3*, *caspase-3*, and *tumor necrosis factor alpha* (*TNF-α)* genes expressions in lung tissue. Data are presented as mean ± standard error.

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	*p*-value
NLRP3	0.00051 ± 0.00013^{c}	0.00091 ± 0.00012^{a}	0.00078 ± 0.00014^{b}	0.00091 ± 0.00017^{a}	0.00094 ± 0.00015^{a}	0.00034 ± 0.00008^{d}	$p < 0.001$
Caspase-3	0.00115 ± 0.00016^{b}	0.00088 ± 0.00027^{c}	0.00076 ± 0.00014^{c}	0.00087 ± 0.00007^{c}	0.00196 ± 0.00019^{a}	0.00148 ± 0.00023^{ab}	$p < 0.05$
TNF-α	0.00033 ± 0.00043^{ab}	0.00022 ± 0.00052^{b}	0.00021 ± 0.00035^{b}	0.00038 ± 0.00032^{ab}	0.00056 ± 0.00054^{a}	0.00046 ± 0.00061^{a}	$p < 0.05$

$^{a–d}$ Different letters indicate the statistical differences between groups ($p < 0.05$; some comparisons $p < 0.001$).

Histopathological examination of tissues. Histopathological examination of lung tissue samples of group 1 (12.50 mg kg^{-1}) revealed degenerative and necrotic changes in the bronchiolar and alveolar epithelia. There was a hyperemia in the vessels and marked hemorrhages in the interstitial tissue. Eosinophilic exudation, containing leukocytes, was observed in the inter-alveolar spaces. The pathological findings in this group were noted to be similar to those observed in group 5, which received LPS alone; however, the severity of the lesions was reduced (Fig. 2A). In the group 2 (25.00 mg kg^{-1}) lung tissue samples, degenerative and necrotic changes were observed in the cells. Inflammation, cellular infiltration, hemorrhage, and mild exudation were detected in the inter-alveolar spaces. The severity of vascular changes was found to be minimal. The pathological findings in this group were found to be less severe than those in group 1 (Fig. 2B). In the lung tissue samples from group 3 (50.00 mg kg^{-1}), a few degenerative changes and necrosis were observed in the bronchiolar and alveolar epithelia. Exudation and vascular changes were significantly reduced. A few leukocyte infiltrations and hemorrhagic foci were observed in the inter-alveolar spaces. The pathological findings in this group were found to be less severe than those in group 2 (Fig. 2C). Lung tissue samples from group 4 (DEX + LPS) exhibited no degenerative or necrotic alterations. Exudation and vascular changes were virtually negligible. Very few leukocyte infiltrations were observed in the interstitial tissue. The histopathological findings in this group were quite similar to the control group (Fig. 2D). Lung tissue samples from group 5 (LPS only) exhibited markedly severe inflammation, especially in the interstitial region. The inflammation was primarily exudative in nature. Degeneration and necrosis-induced tissue damage was observed in the bronchiolar and alveolar epithelia. Significant hyperemia and focal thrombosis were detected in the vessels. In addition to these vascular changes, hemorrhagic foci were observed in some areas. Intense inflammatory cell infiltration, predominantly composed of polymorphonuclear leukocytes, was detected in the inter-alveolar spaces (Fig. 2E). No pathological findings were observed in the lung tissue samples from group 6 (control). It was observed that the cells maintained their regular structure. As a result of the examination, it was determined that the tissues in this group retained their normal histological structure, with no changes observed, and displayed a healthy appearance (Fig. 2F).

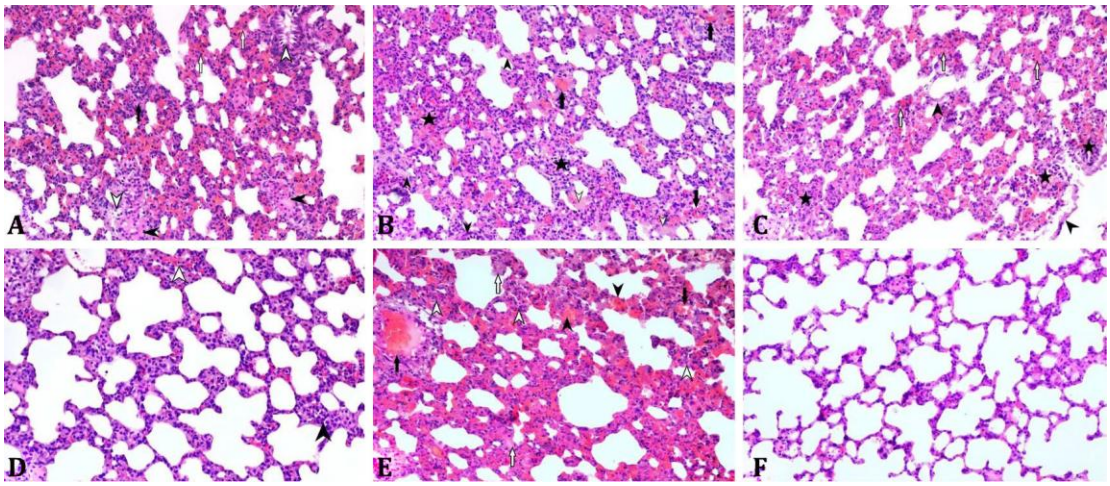

Fig. 2. Histopathological findings of lung tissue, demonstrating the effects of the test compound. **A)** Group 1 (12.50 mg kg^{-1} *Micromeria congesta* essential oil) showed degenerative and necrotic changes (open arrowheads), hemorrhagic foci (open arrows), leukocyte infiltration (arrow), and eosinophilic exudate (arrowheads); **B)** Group 2 (25.00 mg kg^{-1} *M. congesta* essential oil) showed degenerative and necrotic changes (arrowheads), leukocyte infiltration (asterisks), hemorrhagic foci (arrows), and exudative areas (open arrowheads); **C)** Group 3 (50.00 mg kg^{-1} *M. congesta* essential oil) showed degenerative and necrotic changes (arrowheads), hemorrhagic areas (open arrows), and leukocyte infiltration (asterisks); **D)** Group 4 (dexamethasone + lipopolysaccharide [LPS]) showed mild leukocyte infiltration (arrowhead) and hemorrhage (open arrowhead); **E)** Group 5 (LPS only) showed degenerative and necrotic changes (open arrows), leukocyte infiltration (open arrowheads), vascular hyperemia (arrows), and hemorrhagic areas (arrowheads); **F)** Group 6 (control) showed normal histological appearance (Hematoxylin-Eosin staining; 100 ×).

The stained slides were examined and the lesions were scored according to their severity as none (−/0), mild (+/1), moderate (++/2), and severe (+++/3). For statistical analysis, these scores were treated as numerical values ranging from 0 to 3. According to the Kruskal-Wallis H test, significant differences were observed among the groups for degenerative and necrotic lesions, inflammatory cell infiltrations, vascular changes, and exudations ($p < 0.05$; Fig. 3).

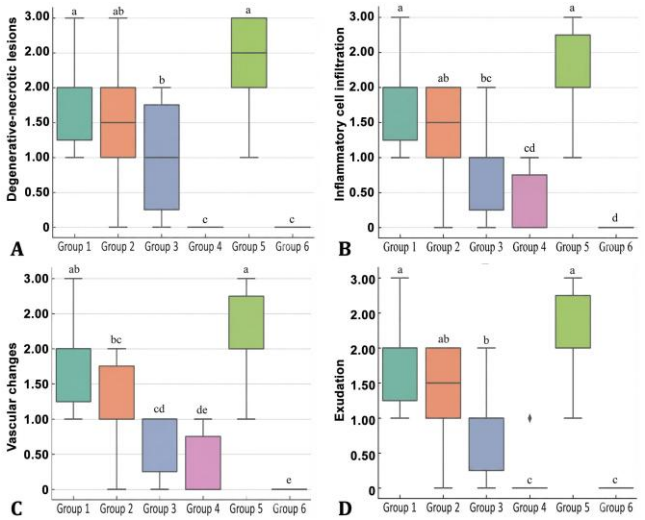

Fig. 3. Statistical analysis of histopathological changes in lung tissue induced by the test compound. Score of **A)** degenerative and necrotic lesions, **B)** inflammatory cell infiltration, **C)** vascular changes, and **D)** exudation. Data are presented as mean ± standard error.
a–d Different letters indicate the statistical differences between groups ($p < 0.05$; some comparisons $p < 0.001$).

Discussion

Acute lung injury develops under the influence of inflammatory factors. In this study, intravenous administration of LPS was used to induce systemic inflammation, subsequently triggering pulmonary inflammation. Circulating LPS activates immune cells, such as macrophages and neutrophils, leading to the release of inflammatory cytokines that can damage the alveolar-capillary barrier. This increased vascular permeability allows inflammatory cells and mediators to accumulate in lung tissue, resulting in infiltration, edema, and systemic inflammation. Excessive cytokine accumulation can further lead to severe complications, such as septicemia and multiple organ failure.[26-28] Although direct intra-tracheal or aerosol delivery of LPS is often used to induce localized acute respiratory injury, systemic intravenous LPS administration is a well-established model for mimicking sepsis-induced acute lung injury in rats.[27] Acute lung inflammation is characterized by inflammatory cell infiltration, excessive production of mediators, and widespread inflammation of lung tissue.[29]

Sepsis, resulting from an exaggerated inflammatory response following host-pathogen interactions, leads to widespread dysfunction in multiple organ systems and is often fatal. As a major global health concern, sepsis not only poses significant risks to human health but also carries a substantial economic burden. Despite recent advancements in medical research, effective solutions for sepsis remain elusive. Consequently, improving survival rates and developing novel therapeutic approaches have become central objectives in scientific research. In this context, numerous natural plant extracts, known for their minimal side effects and ease of use, are being explored as promising alternatives for addressing various health conditions, making them the focus of extensive research efforts.[30-32]

Dexamethasone, a potent synthetic glucocorticoid with well-documented anti-inflammatory and immuno-suppressive properties, was used as a positive control in this study. It exerts its effects primarily by inhibiting nuclear factor-kappa B signaling, suppressing the NLRP3 inflammasome, and reducing pro-inflammatory cytokines production, such as TNF-α. Therefore, DEX serves as a standard reference to validate the anti-inflammatory efficacy of novel therapeutic agents in acute lung injury models.[33,34]

In our study, LPS administration decreased blood glucose while increasing blood urea levels. This finding is consistent with the literature, reporting that LPS enhances nitric oxide production in splenocytes, thereby reducing hepatic gluconeogenesis and contributing to sepsis-induced hypoglycemia.[35] In another study, LPS administration was shown to impair renal function, leading to elevated plasma urea and creatinine levels, as well as renal inflammation and oxidative stress.[36] The increase observed in blood urea parameters in our study may similarly reflect the effects of LPS on renal function. Furthermore, rosemary (*Rosmarinus officinalis*) extract has been reported to exert anti-diabetic effects by lowering blood glucose in alloxan-induced diabetic rabbits.[37] In line with this, in our study, blood glucose levels were found to be reduced in MCEO-treated groups in a dose-dependent manner.

The MCEO contains numerous active compounds. It has been reported that the EO of the plant exhibits central nervous system stimulant effects, as well as anti-microbial, anti-oxidant, anti-inflammatory, anti-septic, and anti-rheumatic properties, and is also used as an analgesic in the treatment of dental pain.[6,10,14] The GC-MS analysis revealed that MCEO contains 28 bioactive compounds. Among these, seven major constituents were identified in higher concentrations, namely piperitenone oxide (33.97%), pulegone (22.30%), N-hexadecanoic acid (13.30%), piperitenone (10.71%), spathulenol (5.39%), carvone oxide (3.22%), and isomenthone (2.17%). In our study, the potential protective effects of MCEO against

LPS-induced lung injury were investigated through biochemical, genetic, and histopathological methods.

The anti-inflammatory effects of MCEO, containing phenolic compounds and volatile oils, are consistent with previous findings showing that secondary metabolites can suppress nuclear factor-kappa B and NLRP3 inflammasome pathways, thereby reducing pro-inflammatory genes expression. This mechanism may explain the down-regulation of TNF-α and NLRP3 observed in our study.[33,38]

In this study, it was found that LPS administration resulted in significant pathological lesions in lung tissue, whereas the administration of increasing doses of MCEO reduced the severity of these findings. Similarly, molecular analyses revealed dose-dependent decreases in the expression levels of the genetic markers (*NLRP3*, *caspase-3*, and *TNF-α*) following MCEO administration.

Previous studies have demonstrated that molecular changes in inflammatory pathways closely parallel histopathological damage in LPS-induced lung injury. Activation of the NLRP3 inflammasome has been reported to correlate with alveolar wall thickening, edema, and leukocyte infiltration, while its inhibition improves both gene expression and tissue structure.[39] Similarly, resveratrol treatment attenuated LPS-induced lung injury by activating the Nuclear factor erythroid 2-related factor 2/heme oxygenase-1 (Nrf2/HO-1) pathway and suppressing NLRP3, resulting in reduced TNF-α levels and improved histology.[40] Mechanistic studies have also highlighted mitochondria as key regulators of NLRP3-driven tissue injury.[41] These findings support our results, showing that the down-regulation of *NLRP3*, *caspase-3*, and *TNF-α* after MCEO treatment is consistent with the observed reduction in lung pathology.

Peripheral administration of LPS has been reported to induce severe systemic inflammation and is considered an effective method for triggering neuro-inflammation.[42] The LPS administration triggers an inflammatory response, leading to elevated levels of cytokines, such as interleukin (IL)-6 and TNF-α. This is associated with the activation of nuclear factor-kappa B signaling pathways and can result in a cytokine storm, potentially leading to endotoxin-induced shock.[43] To date, no studies have been found in the literature investigating the potential effects of MCEO on LPS-induced lung tissue damage.

The pulegone compound in the EO of *Schizonepeta* demonstrated anti-inflammatory properties and reduced neutrophil production in lung tissues.[44] Pulegone exhibited anti-inflammatory effects by inhibiting *NLRP3* expression in LPS + adenosine triphosphate /nigericin-stimulated THP-1 cells (human acute monocytic leukemia cell line).[45] *Mentha arvensis* EO inhibited *NLRP3* and *caspase-1* expressions in BMDM cells, thereby suppressing LPS + adenosine triphosphate -induced NLRP3 inflammasome activation and IL-1β production.[46] The EO extracted from the leaves of *Blumea balsamifera* exhibited anti-inflammatory effects in LPS-induced inflammation in RAW264.7 cells (mouse macrophage cell line). This effect was associated with significant inhibition of inflammatory mediators, such as TNF-α, IL-1β, IL-6, and cyclooxygenase-2, as well as the expression of *NLRP3*.[47] The effects of *Chimonanthus nitens* EO on LPS-induced acute lung injury were also investigated. The study revealed an increase in white blood cell count, a decrease in immune organ index, and histopathological improvement. Additionally, IL-1β levels were reduced, anti-oxidant enzyme levels increased, and inflammation was regulated.[48] Pulegone significantly inhibited the production of inflammatory mediators and enhanced the expression of anti-oxidant proteins in LPS-stimulated RAW 264.7 cells.[49]

This study revealed that the expression levels of *NLRP3*, *caspase-3*, and *TNF-α*, key bio-markers of inflammation, were markedly elevated in lung tissues following LPS administration. However, these expression levels were significantly reduced with increasing doses of MCEO. In addition, pronounced histopathological alterations were observed in the lung tissue during LPS-induced inflammation, being notably alleviated by MCEO treatment in a dose-dependent manner. Biochemical analysis of serum showed that blood glucose and urea levels were significantly lower in the MCEO-treated groups. Consequently, these findings suggest that MCEO may be effective in mitigating sepsis-induced organ damage. Nevertheless, further studies, including quantitative analyses, are required to validate the therapeutic potential of MCEO as a potential therapeutic agent for sepsis.

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Conflict of interest

The authors state that they have no competing interests to disclose.

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