

Ameliorative Effects of Oleuropein on Lipopolysaccharide-induced Acute Lung Injury Model in Rats

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Abstract

Acute lung injury (ALI) is one of the most common causes of death in diseases with septic shock. Oleuropein, one of the important components of olive leaf, has antioxidant and anti-inflammatory effects. The objective of this study was to investigate the effects of oleuropein on lipopolysaccharide (LPS)-induced ALI in rats. Oleuropein was administered to rats at a dose of 200 mg/kg for 20 days and LPS was given through intratracheal administration to induce ALI. The study was terminated after 12 hours. The results showed that in the group treated with oleuropein; inflammatory cytokines and oxidative stress decreased in serum, bronchoalveolar lavage fluid (BALF), and lung tissue, and there were significant improvements in the picture of acute interstitial pneumonia (AIP) caused by LPS in histopathological examination.

Based on the findings of the present study, oleuropein showed protective effects against LPS-induced ALI.

Introduction

Acute lung injury (ALI) is a serious complication caused by stress situations such as trauma, burns, or sepsis, and is associated with high morbidity and mortality rates. There is no specific and effective treatment for ALI[1]. This condition is an acute progressive hypoxic respiratory failure caused by various factors and is an early stage of acute respiratory distress syndrome (ARDS). It is mainly characterized by increased pulmonary vascular permeability, exudation of tissue fluid, accumulation of inflammatory cells, and dysfunction of gas exchange caused by various intrapulmonary and extrapulmonary pathogenic factors[2, 3]. Inflammation and apoptosis are the main mechanisms during the development of ALI[4]. The pathogenesis of ALI includes various factors, such as coagulation, fibrinolysis, redox imbalance, cell apoptosis, and genetic factors that have been reported to cause excessive inflammatory responses[5]. Pulmonary and systemic bacterial infections are the main cause of ALI. Here, bacterial cell components have a very important role[6]. Lipopolysaccharide (LPS) is an important pathogenic component contributing to the development of ALI, and its intratracheal administration is widely used in animal models[7]. Nuclear factor- κ B (NF- κ B) and mitogen-activated protein kinases (MAPKs) are recognized as target molecular pathways for ALI and ARDS[8, 9]. The NF- κ B is an important transcription factor for M1 macrophages and induces inflammatory genes, including tumor necrosis factor- α (TNF- α), interleukin IL-1 β (IL-1 β), interleukin-6 (IL-6), and cyclooxygenase-2 (COX-2)[10, 11]. The activation of NF- κ B, which plays a critical role in modulating the immune response against infection, is reported to be induced by LPS[12]. Lipopolysaccharide, the primary endotoxin of gram-negative bacteria, increases TNF- α and IL-6 secretion and supports the inflammatory reaction to accelerate cell infiltration in lung tissues[13]. Proinflammatory cytokines such as TNF- α , IL-1 β , and IL-6 are produced by these inflammatory cells and are abundant in the lung. During the onset of ALI, many types of damaging factors promote apoptosis of pulmonary vascular endothelial cells and alveolar epithelial cells, increasing lung tissue damage and contributing to the inflammatory response in ALI[14, 15]. The inflammatory reaction accelerates the release of protease and the formation of reactive oxygen species (ROS). This is closely associated with the aggravation of

lipid peroxidation and decreased antioxidant enzyme activity[16]. The primary treatment of ALI aims at reducing inflammation and preventing respiratory failure. Anti-inflammatory drugs such as corticosteroids, aspirin, salbutamol, and ketoconazole are widely used in clinical practice. Respiratory support is provided by ventilators to improve hypoxemia[17]. There is no specific treatment strategy for ALI. Therefore, there is a need to discover new and effective plant-based anti-inflammatory drugs that are more advantageous for the treatment of ALI[18].

The major compound of olive leaves is oleuropein, which modulates inflammatory parameters and reduces oxidative stress[19]. It has been shown to have antioxidant[20], anti-inflammatory[21], antihypertensive[22], cardioprotective[23], antidiabetic[24], anticancer[25], and hypolipidemic[26] effects.

The literature review has shown that there are no studies on the effects of oleuropein on LPS-induced ALI in rats. Therefore, this study aimed to investigate the effects of oleuropein on proinflammatory cytokines, oxidative damage, and morphometric changes in lung tissue in the LPS-induced ALI model.

Materials And Methods

This study was approved by Hatay Mustafa Kemal University Animal Experiments Local Ethics Committee (Approval no. 2020/02-17) The male Wistar albino rats weighing 180–250 g were used for the study These experimental administrations were conducted in accordance with the Conditions for the Care and Use of Laboratory Animals (12 hours of light-12 hours of dark and at $21\pm 1^{\circ}\text{C}$). Throughout the experimental practices, the rats were provided with standard commercial feed (pellet feed) and tap water ad-libitum. Rats were divided into four groups with eight animals in each group. The control group rats received no treatment. **Group 1** (Control Group): In this group, 1 mL of saline was administered to the rats once a day for 20 days via oral gavage technique. One hour after the last saline administration on day 20, rats were anesthetized with ketamine (60 mg/kg, IM)+xylazine (10 mg/kg, IM), and phosphate-buffered saline (PBS) solution of 0.2 mL/100 g was administered intratracheally by endotracheal intubation using a laryngoscope. **Group 2** (LPS): The rats in this group were administered LPS only once at a dose of 5 mg/kg intratracheally by endotracheal intubation using a laryngoscope under anaesthesia. **Group 3** (Oleuropein): In this group, the rats were given 200 mg/kg oleuropein in 1 mL normal saline by oral gavage technique once a day for 20 days. One hour after the last saline administration on day 20, rats were anesthetized with ketamine (60 mg/kg, IM)+xylazine (10 mg/kg, IM), and phosphate-buffered saline (PBS) solution of 0.2 mL/100 g was administered intratracheally by endotracheal intubation using a laryngoscope. **Group 4** (LPS + Oleuropein): In this group, the rats were given 200 mg/kg oleuropein in 1 mL normal saline by oral gavage technique once a day for 20 days. The rats in this group were additionally given LPS only once at a dose of 5 mg/kg intratracheally by endotracheal intubation using a laryngoscope under anaesthesia one hour after the last oleuropein administration. The studies by Qin et al.[27] and Andreadou et al.[28] were referenced for the experimental ALI model created with LPS in rats and for the use dose of oleuropein, respectively.

On day 20, blood samples were collected from the tail vein of anesthetized rats into tubes containing and not containing anticoagulants 12 hours after LPS application. Samples were centrifuged at 3000 rpm for 15 minutes to obtain blood serum. The prepared serum samples were placed in Eppendorf tubes and kept in a freezer at -80 °C until the time of biochemical analyses. Half of the lung tissue samples were stored in 10% formaldehyde for histopathological analysis and the other half were kept at -80°C for analyses.

Collection of Bronchoalveolar Lavage Fluid (BALF):

Rats were euthanized under anaesthesia and their rib cages were opened. Then, a cannula was inserted into the trachea and the right lung lobe was washed with the help of a tracheal cannula for a total of five times, with 1 mL of saline each time. The collected BALF was centrifuged at 4°C at 2500 rpm for 10 minutes and separated into supernatants. Protein analyses in the supernatants obtained were made using bicinchoninic acid (BCA) assay[29]. Neutrophil count was performed in accordance with the literature[30]. The remaining preparations were stored at -80°C until the time of biochemical analysis.

Calculation of the lung wet-to-dry (W/D) weight ratio

After the rats were euthanized under anaesthesia, right lung lobes were collected and weighed to measure wet weight. Then, the same tissues were kept in the incubator at 60°C for 72 hours and the dry weights of the tissues were measured. After that, the lung W/D weight ratio was calculated.

Biochemical Analyses

For lipid peroxidation and antioxidant activity analysis, samples were analysed using a spectrophotometer. The level of lipid peroxidation was measured according to the concentration of thiobarbituric acid reactive substances. The amount of synthesized MDA was used as an index of lipid peroxidation. The MDA concentrations were expressed in nanomolar units per gram of the protein at 532 nm[31]. The method described by Sedlak and Lindsay was used to measure GSH concentrations[32]. The GSH level at 412 nm was expressed in nanomoles per gram of total protein. The method described by Lawrence and Burk[33] was used to determine glutathione peroxidase (GSH-Px, EC 1.11. 1.9) activity. The GSH-Px activity at 340 nm was expressed in international units per protein gram. Hydrogen peroxide (H₂O₂) decomposition at 240 nm was measured to determine catalase (CAT, EC 1.11.1.6) activity, which was expressed in kg/protein[34]. The ELISA kit [Bioassay Technology Laboratory (BT Lab)] was used to measure TNF- α , NF- κ B, IL-1 β , IL-6, and myeloperoxidase (MPO) levels in tissue, serum, and BALF. The results were expressed in protein concentrations. The Lowry[35] method was used for protein analyses.

Histological Investigation

At the end of the experiment, the lobe of the left lung taken after necropsies were fixated in a 10% buffered formaldehyde solution for 48 hours. Within the scope of routine tissue follow-up, the tissue samples were blocked in paraffin after they were dehydrated by passing through a series of alcohol (70, 80, 90, 100 %) and became transparent by passing through a series of xylol. Serial sections of 4-5

microns thick were taken from these paraffin blocks by microtome (Leica RM 2135). Hematoxylin and eosin (H&E) staining was performed according to a standard protocol. Pathological changes in lung tissue were observed under a light microscope. The lung injury scores were based on categories: inflammation, edema, and congestion, haemorrhage, interalveolar septum thickening, alveolar macrophage infiltration etc. according to the Smith et al. method with minor changes[36]. Those score indexes were graded as follows semi- quantitatively: no injury=score of 0; injury in 25% of the field=score of 1 (slight); injury in 50% of the field=score of 2 (medium); injury in 75% of the field=score of 3 (medium-severe); and injury throughout the field=score of 4 (severe). Each sample was investigated in ten microscopic fields, and the severity of lung injury was evaluated by the average score.

Statistical Analysis

Shapiro-Wilk normality analysis was performed to determine whether the data obtained showed normal distribution and the analysis showed that all parameters followed a normal distribution. One-Way Analysis of Variance (ANOVA) was used to compare the group averages and Tukey test to determine differences between groups. Statistical analysis was performed using IBM SPSS version 23.0 software and a p value of <0.05 was considered statistically significant.

Oleuropein Isolation

Isolation of oleuropein was carried out in the Central Research Laboratory of Agri Ibrahim Cecen University. Isolation procedure was performed as proposed by Comez et al.[23]. The purity of oleuropein obtained was found to be 97.35%.

Results

Neutrophil Count and Total Protein Amount

Figure 1 presents the results of neutrophil count and total protein amount in BALF. There was a significant increase in the neutrophil count and total protein amount in BALF values of the group with LPS-induced ALI compared to the control group. However, these levels were observed to be the same in the group treated with oleuropein and the control group.

Biochemical Analyses

The data of oxidative stress and antioxidant markers obtained as a result of the assay are given in Figures 2 and 3. Serum and tissue MDA levels of the group with LPS-induced ALI were observed to increase significantly whereas these levels decreased and reached the control group levels in the group treated with oleuropein. Tissue and serum GSH levels, GSH-Px, and CAT activities decreased significantly in the LPS group. In the group treated with oleuropein; tissue GSH levels, GSH.Px and CAT activities, and serum GSH-Px activities increased to the same level as the control group.

ELISA Parameters

Data related to ELISA parameters are given in Figures 4, 5, and 6. In the group with LPS-induced ALI, there was a significant increase in the BALF, tissue, and serum levels of IL-6, MPO, NF- κ B, and TNF- α , whereas these levels in the group treated with oleuropein were found to be the same as the control group.

Histopathological Analysis

Inflammation scores and histopathological findings of lung tissues of rats in each group are given in Figures 7 and 8, respectively. All histopathological parameters showed that there was a statistically significant difference between the groups in terms of mean histopathological scores. The mean histopathological score was 0 in the control group and oleuropein group, 4 in the LPS group, and 1 in the LPS+Oleuropein group. Considering histopathological findings, the normal histological structure was observed in the control and oleuropein groups (Figure 8a and f). Very pronounced morphological alterations were observed histopathological in the LPS group. Inter-alveolar septum was observed to be enlarged due to pronounced capillary hyperaemia, edema, and leukocyte infiltration in this group. Furthermore; edema, congestion, and intense inflammatory cell infiltration were observed in perivascular and peribronchial interstitial regions. A focal bleeding in the lung parenchyma and emphysematous changes in certain alveoli were observed (Figure 8b and c). Necrosis in Type-I epithelial cells and proliferation (epithelization) in type-II epithelial cells were observed in some alveoli. A small number of macrophages (Figure 8d) and locally pink-coloured plasmatic fluid accumulation were observed in the alveolar lumens. Inflammatory cell infiltration and peribronchiolar follicle-like lymphoid cell hyperplasia were noted in the lamina propria mucosa of bronchi and bronchioles. These morphological changes were evaluated as acute interstitial pneumonia (Figure 8b). In the LPS+oleuropein group, negligible mild histopathological findings were identified. There was a mild enlargement of the inter-alveolar septum and hyperaemia of the capillaries in the interstitial area due to the inflammatory cell infiltration observed locally in the interstitium (Figure 8e). Data on histopathological findings are given in Table 1.

Discussion

This study investigated the effects of oleuropein on the LPS-induced ALI model through the histopathological examination of proinflammatory cytokines, oxidative stress, and antioxidant parameters. The literature review showed that this is the first study demonstrating the protective effects of oleuropein against ALI.

Lipopolysaccharide is a glycolipid that can bind to the membrane receptor of macrophages and endothelial cells, can activate the signal transduction system, and forms the main part of the outer membrane in gram-negative bacteria[37]. As a gram-negative bacterial component, LPS is a common cause of pneumonia. Lipopolysaccharide is reported to induce pulmonary inflammations, such as ALI, with its pathogenic effect lasting four to 48 hours[38, 39]. Therefore, studies have reported that LPS stimulation is a standard model for inducing experimental ALI.[40, 41]

Irregular inflammation and excessive leukocyte infiltration play an important role in the ethology of ALI[42]. Activated neutrophils can directly cause endothelial damage and destroy the basement

membrane, causing vascular leakage and ultimately lung edema[43]. Decreased neutrophils can be accepted as a clear indicator of the alleviation of the severity of ALI in animal models[44]. In the present study, while BALF neutrophil count was observed to increase with LPS administration, these values were found to be at normal levels following the oleuropein treatment. The lung W/D ratio, another marker of ALI, is an important indicator of both systemic and local inflammation[45]. Studies have reported that the W/D ratio reaches high levels at the 12th hour in the LPS-induced ALI model[46]. Similarly, the W/D ratio was found to be high 12 hours after LPS application in the present study; however, this ratio was at normal levels in the group treated with oleuropein. An increase in BALF protein concentration due to protein extravasation is one of the indicators of pulmonary edema and inflammation[47]. In the present study, the amount of BALF protein concentration increased with LPS administration returned to normal levels with oleuropein treatment.

The production of reactive oxygen compounds, an important defence mechanism against bacterial infections, increases due to sepsis[48]. In the present study, there was an increase in MDA levels, which is the end product of lipid peroxidation due to oxidative stress, in the presence of sepsis. Previous studies reported that MDA levels increased[49, 50], while antioxidant activity decreased[51] in LPS-induced ALI. Increased ROS levels in sepsis affect proteins, lipids, and nucleic acids, causing cellular damage and accordingly, endothelial dysfunction. Therefore, treatment protocols with antioxidant compounds in sepsis are being investigated both experimentally and clinically. In a study using the LPS-induced sepsis model, MDA and MPO levels were reported to increase whereas CAT and superoxide dismutase (SOD) levels decreased[52]. In a study by Cinar et al. using the LPS-induced sepsis model, the authors reported that MDA levels increased while GSH and SOD levels decreased[53]. Various studies have reported that oleuropein has anti-lipid peroxidation effects and antioxidant activity-enhancing effects [23, 54, 55]. Similar findings were obtained in this study in terms of MDA, GSH-Px, and CAT. Antioxidant activity levels were increased in the group treated with oleuropein compared to the group treated with LPS. Furthermore, oleuropein administration decreased serum and lung MDA levels in septic rats, suggesting that oleuropein exerts antioxidant capacity in lung tissue damaged by sepsis. These findings may be associated with the anti-inflammatory effects of oleuropein, which reduces oxidative damage, and its own antioxidant properties.

An association between inflammation and oxidative stress has been reported in various studies[56]. Oxidative stress can be triggered by the inflammatory process and thus, it can increase inflammation. Numerous studies have reported that oxidative stress and inflammatory responses are important in the development of lung injury[57]. Increased proinflammatory cytokine-assisted inflammation is thought to play a key role in the pathogenesis of ALI/ARDS. Previous studies have shown that the increased BALF levels of TNF- α , IL-1, and IL-6 in patients with ARDS and the persistent increase of these proinflammatory cytokines in patients with ALI or sepsis are indicators of poor prognosis[58]. Both TNF- α and IL-1 β cause inflammatory damage by strengthening the inflammatory cascade, and transport neutrophils to the lungs[42]. Plasma IL-6 is known to be an important morbidity and mortality predictor in patients with ARDS[59]. As some proinflammatory cytokines, such as TNF- α , IL-1 β , and IL-6, are potent mediators of potentially damaging tissue responses, limiting the effects of such cytokines plays an important role in

changing the course of the disease. In the present study, LPS administration was observed to increase the levels of TNF- α and IL-6 in BALF, serum, and lung tissue homogenate. The expression of proinflammatory genes is regulated by a transcriptional mechanism. NF- κ B is a critical transcription factor that regulates the expression of numerous pro-inflammatory cytokines, including TNF- α , IL-1 β , and IL-6, which play a role in the pathogenesis of ALI[60]. The NF- κ B proteins are found in normal cells as an inactive structure bound to the cytoplasmic inhibitory I κ B protein. When the cell is exposed to various activation signals such as LPS, NF- κ B is displaced from its inhibitor and increases the transcription of cytokines that could potentially intensify the inflammatory process of ALI. Studies have reported a significantly increased NF- κ B activity in alveolar macrophages of patients with septic lung injury. On the other hand, inhibition of NF- κ B activation reduces LPS-associated lung injury[61, 62]. Results of the present study showed that NF- κ B activities significantly increased in BALF, serum, and lung tissue 12 hours after exposure to LPS. Herbal ingredients with anti-inflammatory effects are known to be effective in the inhibition of the NF- κ B cascade and the cytokines released by it. Various studies have reported that oleuropein, which is a compound present in olive that is one of the main components of the Mediterranean diet, has a protective effect in multiple organ injury models[19, 63, 64]. In the present study; NF- κ B, TNF- α , and IL-6 levels were found to be significantly decreased in the group treated with oleuropein. These findings showed that oleuropein exerted anti-inflammatory effects by inhibiting the NF- κ B pathway.

The lung tissues of rats exposed to LPS-induced ALI were examined histopathologically to confirm the therapeutic efficacy of oleuropein. In the literature, histopathological examinations showed that there was an inflammatory response characterized by the desquamation of alveolar epithelial cells, thickening of the interalveolar septum due to bleeding and inflammation, fibroblast formation, and excessive leukocyte infiltration in LPS-induced ALI[53, 65]. Asbaugh et al.[66] reported edema, atelectasis, vascular congestion, bleeding, and hyaline membrane formation as histopathological findings. In the present study, findings in the LPS group were observed to be compatible with the literature; however, no hyaline membrane formation was present. Ichikado et al. [67] and Pelosi et al. [68] reported that macrophages were stimulated by proinflammatory cytokines following neutrophil infiltration and increased vascular permeability in the lungs in the acute phase. Meng et al. [65] and Cinar et al. [53] did not report findings similar to those in the literature; however, they reported that dense interalveolar macrophage infiltration and proliferation in type II epithelial cells were identified in the group with ALI and that plant-derived compounds prevented the destruction of lung tissue and had curative effects[69, 70]. In the present study, oleuropein was observed to significantly reduce findings of LPS-induced ALI with its anti-inflammatory effect.

The results obtained from the present study showed that oleuropein demonstrated a protective effect against ALI, which was induced by the intratracheal administration of LPS, by exerting its antioxidant activity and tissue destruction preventive effects through the inhibition of inflammatory cytokines and lipid peroxidation. Future studies may contribute to the evaluation of oleuropein as a potential therapeutic agent for the treatment of diseases with ALI/ARDS.

Declarations

Ethics approval and consent to participate

This study was approved by Hatay Mustafa Kemal University Animal Experiments Local Ethics Committee (Approval no. 2020/02-17)

Consent for publication

Not Applicable

Availability of data and materials

The data used to support the findings of this study are available from the corresponding author on reasonable request.

Competing interests

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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Author Contributions

ND: Project administration, Funding acquisition, Methodology, Conceptualization, Writing - Review & Editing, Supervision. MG: Methodology, Formal analysis, Investigation, Writing - Original Draft. AU: Methodology, Formal analysis, Resources, Investigation. MC: Validation, Resources, Investigation. CTI: Formal analysis, Resources, Investigation. TA: Formal analysis, Validation, Investigation. ME: Methodology, Visualization, Investigation.

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Tables

Table 1. Scoring the effect of Oleuropein on histopathological changes on lung injury induced by LPS.

Changes/Lesions		Control	LPS	OLE	LPS+OLE
Haemorrhage	<i>None</i>	8	0	8	6
	<i>Slight</i>	-	-	-	2
	<i>Moderate</i>	-	2	-	-
	<i>Severe</i>	-	6	-	-
Edema	<i>None</i>	8	-	8	6
	<i>Slight</i>	-	1	-	2
	<i>Moderate</i>	-	1	-	-
	<i>Severe</i>	-	6	-	-
Hyperaemia/Congestion	<i>None</i>	8	-	8	5
	<i>Slight</i>	-	1	-	3
	<i>Moderate</i>	-	3	-	-
	<i>Severe</i>	-	4	-	-
Alveolar macrophage infiltration	<i>None</i>				
	<i>Slight</i>	8	-	8	7
	<i>Moderate</i>	-	2	-	1
	<i>Severe</i>	-	1	-	-
Thickened interalveolar septa	<i>None</i>	8	-	8	6
	<i>Slight</i>	-	2	-	1
	<i>Moderate</i>	-	3	-	1
	<i>Severe</i>	-	3	-	-
Epithelization					

	<i>None</i>	8	-	8	7
<i>Slight</i>		-	-	-	1
<i>Moderate</i>		-	5	-	-
<i>Severe</i>		-	3	-	-
Lymphoid cell hyperplasia					
	<i>None</i>	8	-	8	7
	<i>Slight</i>	-	1	-	1
<i>Moderate</i>		-	4	-	-
<i>Severe</i>		-	3	-	-

Data are numbers of rats showing changes/number of rats examined for each treatment group. All groups, n = 8. No injury=score of 0; injury in 25% of the field=score of 1 (slight); injury in 50% of the field=score of 2 (medium); injury in 75% of the field=score of 3 (medium-severe); and injury throughout the field=score of 4 (severe).

Figures

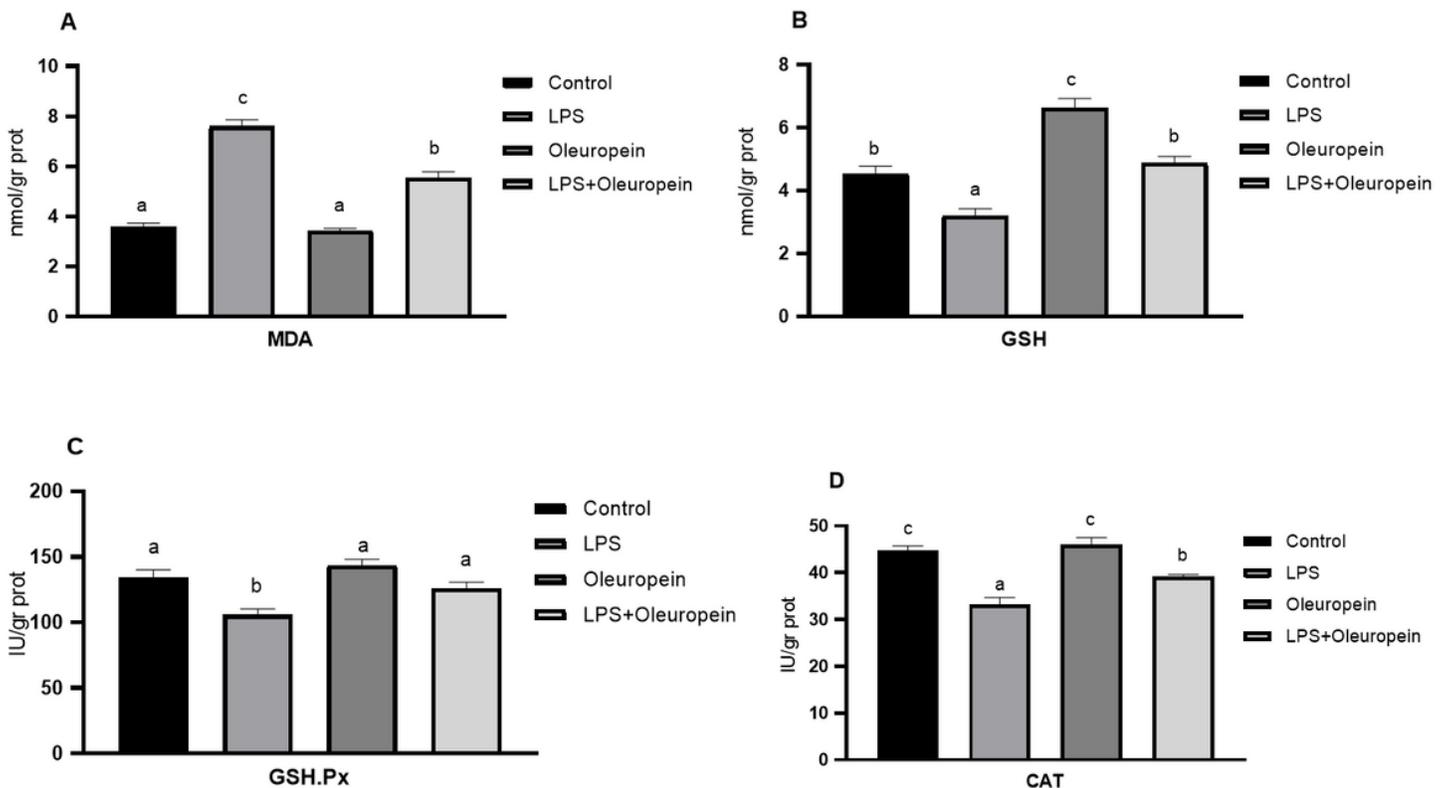


Figure 1

Effect of LPS and Oleuropein on Biochemical Markers in the lung tissue of control and experimental rats. A. MDA(Malondialdehyde) Levels in Lung. Data are expressed as mean \pm SEM. Different superscript letters (a, b) within the same column show statistically significant differences between the groups (P:0.004). B. GSH(Glutathione) Levels in Lung. Data are expressed as mean \pm SEM. Different superscript letters (a, b, c) within the same column show statistically significant differences between the groups (P:0.001). C. GSH.Px (Glutathione Peroxidase) Activities in Lung. Data are expressed as mean \pm SEM. Different superscript letters (a, b, c) within the same column show statistically significant differences between the groups (P:0.002). D. CAT (Catalase) Activities in Lung. Data are expressed as mean \pm SEM. Different superscript letters (a, b, c) within the same column show statistically significant differences between the groups (P:0.000).

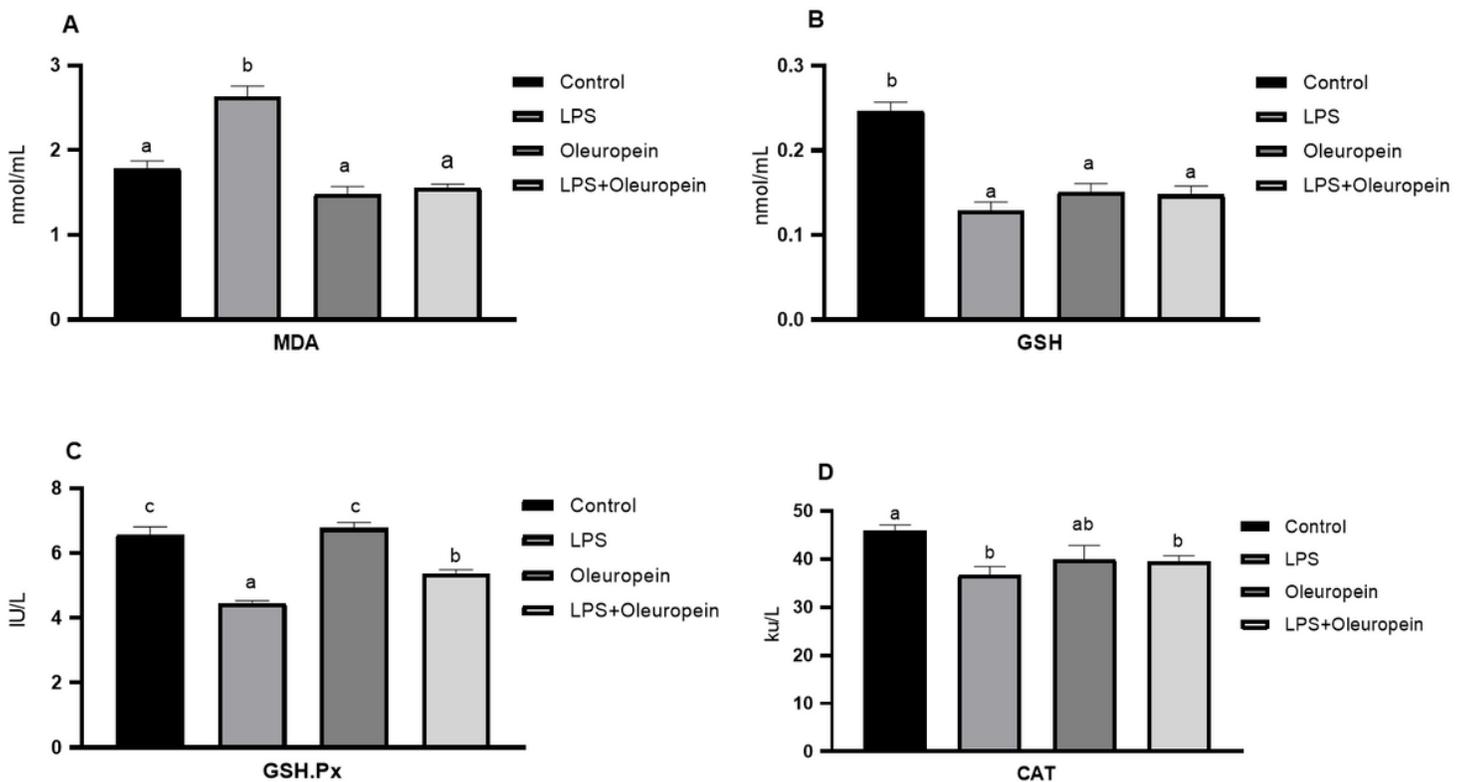


Figure 2

Effect of LPS and Oleuropein on Biochemical Markers in the serum of control and experimental rats. A. MDA(Malondialdehyde) Levels in Serum. Data are expressed as mean \pm SEM. Different superscript letters (a, b) within the same column show statistically significant differences between the groups (P:0.000). B. GSH(Glutathione) Levels in Serum. Data are expressed as mean \pm SEM. Different superscript letters (a, b) within the same column show statistically significant differences between the groups (P:0.000). C. GSH.Px (Glutathione Peroxidase) Activities in Lung. Data are expressed as mean \pm SEM. Different superscript letters (a, b, c) within the same column show statistically significant differences between the groups (P:0.000). D. CAT (Catalase) Activities in serum. Data are expressed as mean \pm SEM. Different superscript letters (a, b) within the same column show statistically significant differences between the groups (P:0.004).

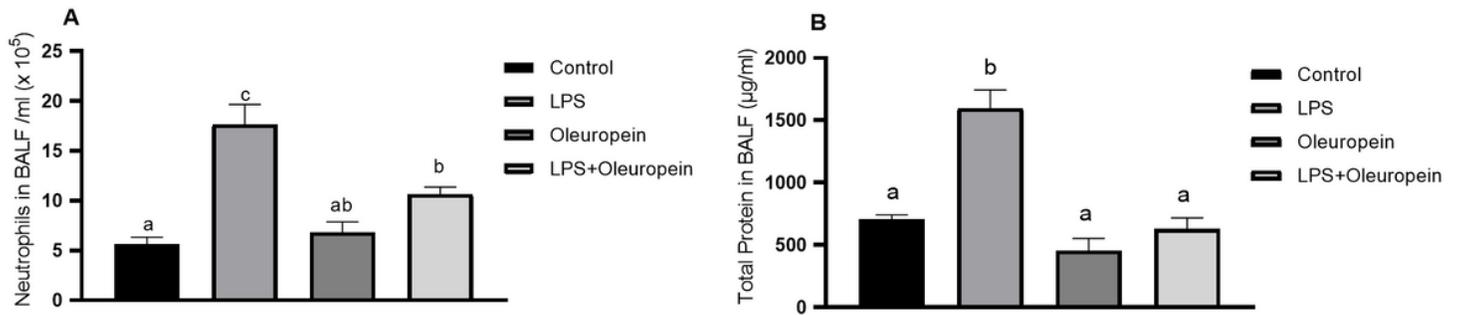


Figure 3

Effect of LPS and Oleuropein on neutrophils and total protein in the BALF of control and experimental rats. A. Neutrophils in the BALF. Data are expressed as mean \pm SEM. Different superscript letters (a, b, c) within the same column show statistically significant differences between the groups (P: 0.000). B. Total protein in the BALF. Data are expressed as mean \pm SEM. Different superscript letters (a, b) within the same column show statistically significant differences between the groups (P: 0.000).

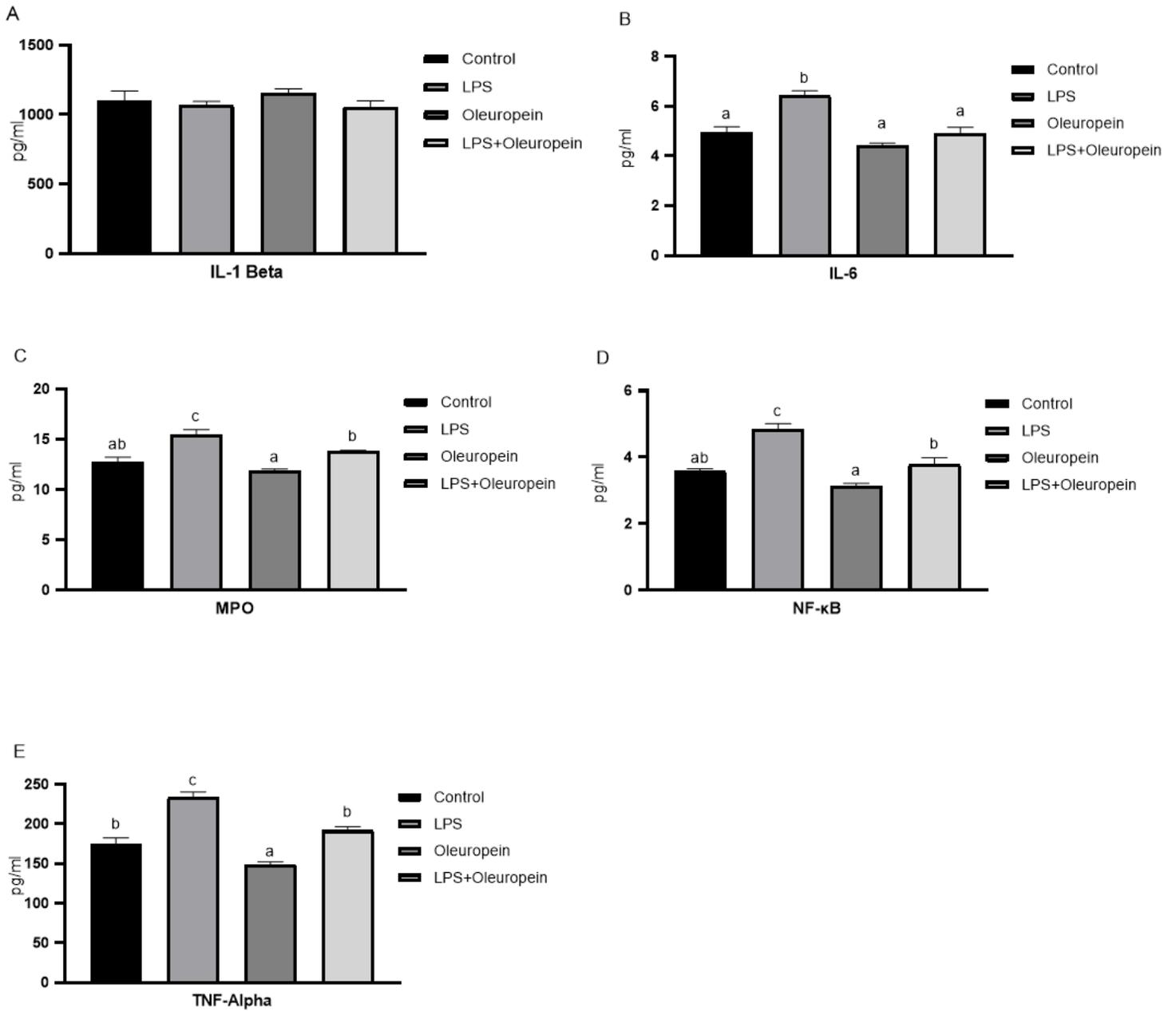


Figure 4

Effect of Oleuropein on Inflammatory Markers in the lung tissue of control and experimental rats. A. IL-1 Beta Levels p:0.430, B. IL-6 Levels p:0.000. C. MPO Levels p:0.000 D. NF-kB Levels p:0.000. E. TNF-Alpha Levels p:0.016 Data are expressed as mean \pm SEM. Different superscript letters (a, b, c) within the same column show statistically significant differences between the groups.

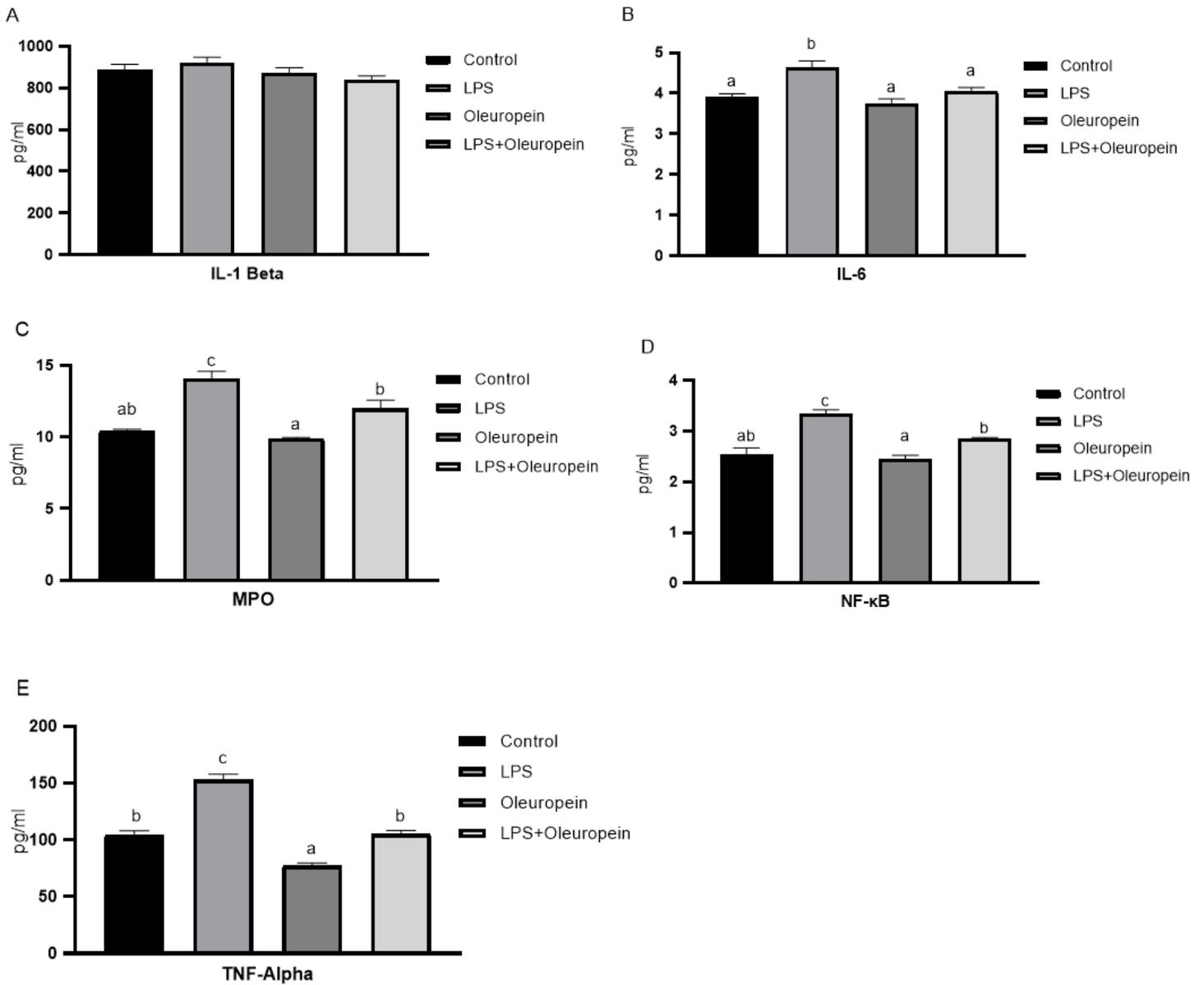


Figure 5

LPS and Effect of Oleuropein on Inflammatory Markers in the serum of control and experimental rats. A. IL-1 Beta Levels p:0.132, B. IL-6 Levels p:0.000. C. MPO Levels p:0.000 D. NF-κB Levels p:0.008. E. TNF-Alpha Levels p:0.000 Data are expressed as mean ± SEM. Different superscript letters (a, b, c) within the same column show statistically significant differences between the groups.

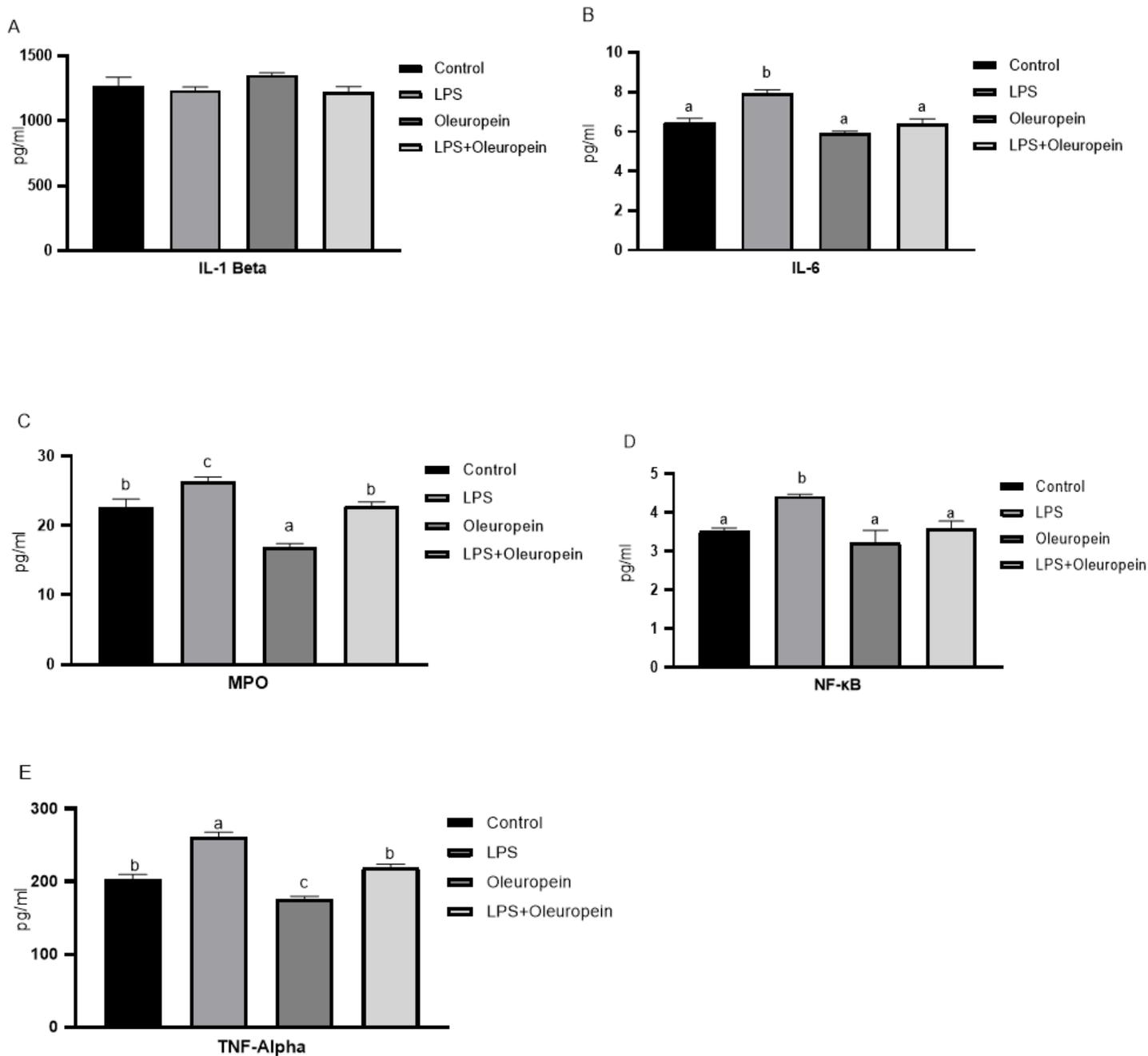


Figure 6

Effect of LPS and Oleuropein on Inflammatory Markers in the BALF of control and experimental rats. A. IL-1 Beta Levels p:0.159, B. IL-6 Levels p:0.000. C. MPO Levels p:0.000 D. NF-kB Levels p:0.003. E. TNF-Alpha Levels p:0.000 Data are expressed as mean \pm SEM. Different superscript letters (a, b, c) within the same column show statistically significant differences between the groups.

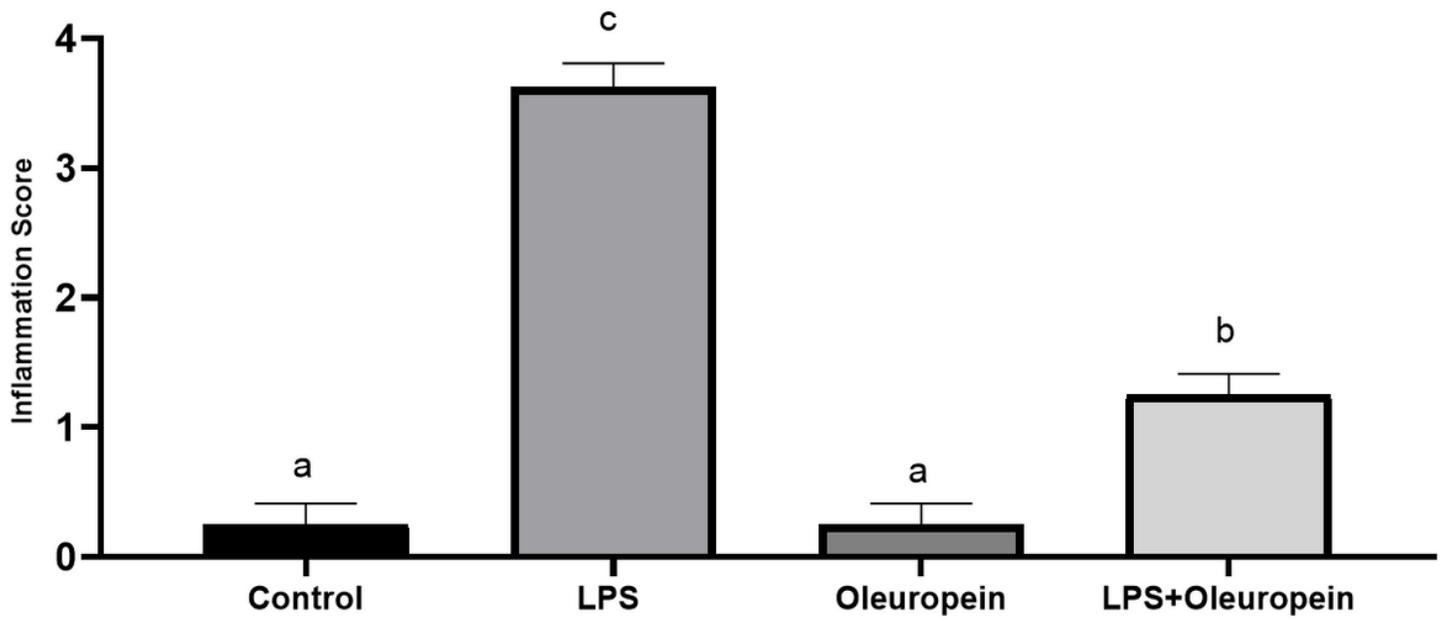


Figure 7

Effects of LPS and Oleuropein on the inflammation score of pulmonary tissues. P:0.000 Data are expressed as mean \pm SEM. Different superscript letters (a, b, c) within the same column show statistically significant differences between the groups.

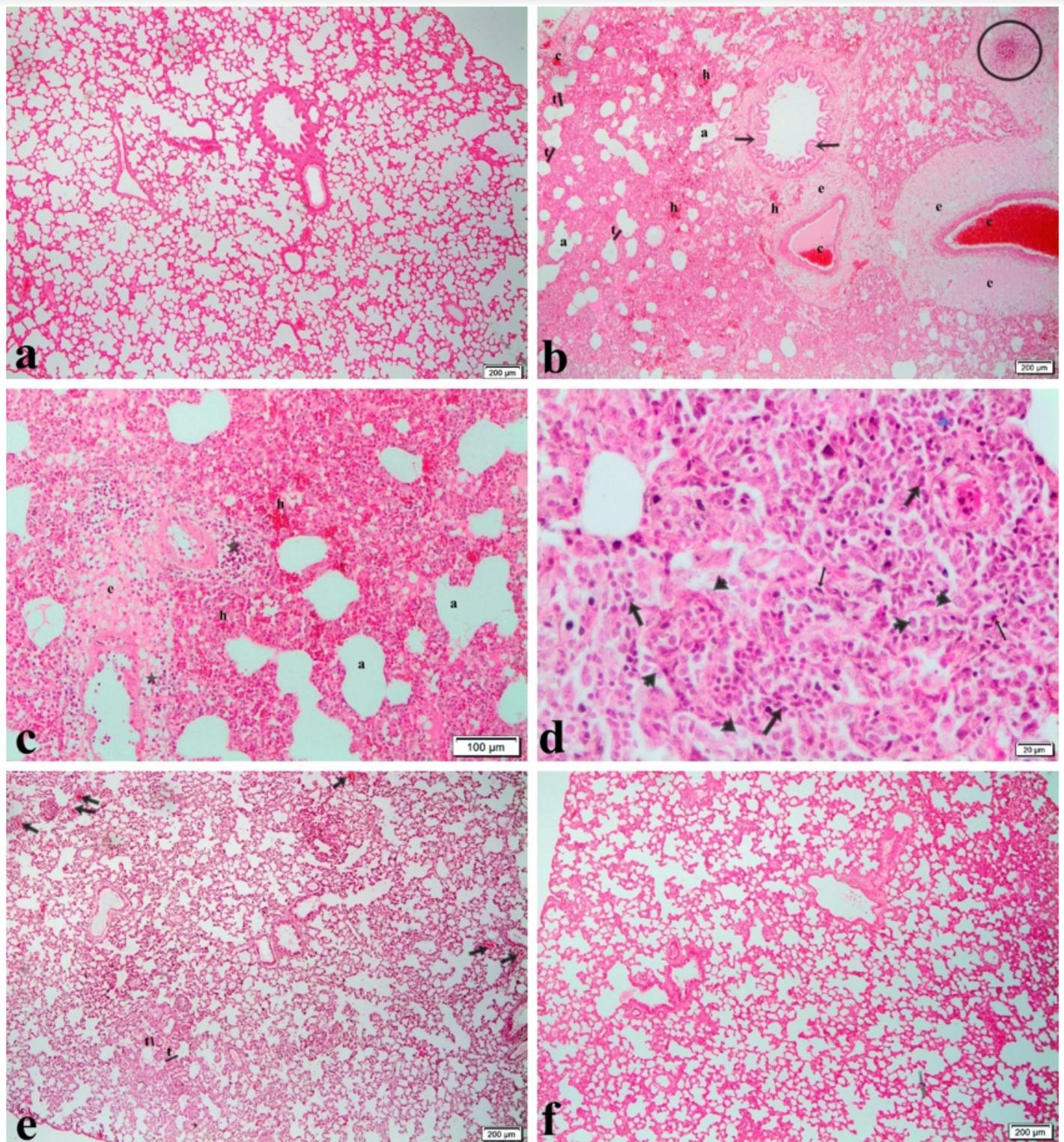


Figure 8

Representative lung histological image stained with haematoxylin and eosin. Control: a) Normal appearance of lung. LPS: b) Interalveolar septum enlargement (t), edema (e), haemorrhage (h), emphysema in alveoli (a), congestion (c), inflammatory cell infiltration in bronchiolar propria mucosa (arrow), follicular lymphoid cell hyperplasia (circle). c) perivasculitis (star), edema (e), haemorrhage (h), the emphysema, alveoli (a). d) Necrosis (thin arrow) in Type-I epithelial cells, proliferation (epithelization) (thick arrow).

in type-II epithelial cells (thick arrow), alveolar macrophages (arrowhead). LPS+Oleuropein: e) Hyperaemia in interalveolar capillaries (arrow), enlargement of interalveolar septum (t) near normal histological appearance of lung except mild findings. Oleuropein: f) Normal appearance of lung.