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The Therapeutic Effect of Flavan-3-Ols from Organic Extracts of *Juniperus drupacea* Fruit Against Elastase-Induced Chronic Obstructive Pulmonary Disease in Rats

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Abstract

BACKGROUND/AIMS: Chronic obstructive pulmonary disease (COPD) is a common chronic airway disease with acute exacerbations of varying frequency that is the main cause of disease morbidity and mortality. The aim of this study was to investigate the utility of extracts rich in flavanol-3-ols (85-92%) from *Juniperus drupacea (J. drupacea)* fruit in the treatment of rats with porcine pancreatic elastase (PPE)-induced COPD.

MATERIALS AND METHODS: Thirty female rats of the Wistar albino breed were randomly divided into four groups: control, PPE, PPE + methanol extract (ME), PPE + water extract. The emphysema in the lung tissues of rats and lymphocyte, [B-cells, cytotoxic T-lymphocyte, natural killer (NK) cells], cytokines [interleukin-8 (IL-8), IL-6, and tumor necrosis factor-alpha (TNF-α)], and blood gas values in blood samples were analyzed.

RESULTS: It was observed that emphysema occurred rats after PPE exposure, and the number of inflammatory cells, except for NK-cells, and IL-6, IL-8, and TNF-α cytokines in their blood increased. Among the blood gas values, $PaCO_2$ increased with emphysema, and PaO_2 decreased. The rats with PPE-induced COPD showed a decrease in the number of B-cells and NK-cells as a result of treatment with *J. drupacea* fruit extracts.

CONCLUSION: Our results showed that PPE application causes COPD, and water and ME as flavan-3-ols-rich *J. drupacea* fruit can protect against the development of elastase-induced lung injuries as an anti-inflammatory and antioxidant factor.

Keywords: COPD, flavan-3-ols, *Juniperus drupacea*, elastase, cytokines, lymphocytes

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a prevalent, avoidable, and manageable disease characterized by persistent respiratory symptoms and restricted airflow due to abnormalities in the respiratory or alveolar tracts, primarily caused by substantial exposure to harmful particles or gases.[1] COPD constitutes a range of advancing lung disorders, notably emphysema and chronic bronchitis, with many individuals experiencing both.[2] Emphysema gradually damages lung air sacs, impeding outward air movement.[3] Chronic bronchitis induces inflammation and constriction of bronchial tubes, resulting in mucus accumulation.[4] COPD is among the leading causes of chronic morbidity and mortality worldwide. COPD is a major and growing global health

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problem, which was estimated to be the third most common cause of death in the world and the fifth most common cause of disability by 2020.[5] The goals of COPD treatment are to improve symptoms, prevent disease progression, improve quality of life, increase exercise tolerance, prevent and treat complications, and reduce mortality. To achieve these goals, it is essential to reduce risk factors that decrease lung function, provide a diagnosis of COPD and educate patients, supported by pharmacological and non-pharmacological treatment.[1]

Worldwide, there may be a large number of herbs for which there is no detailed record of usage in traditional complementary medicine practices to relieve COPD. In the last few years, many herbs have been reported in the scientific literature. *Juniperus drupacea (J. drupacea)* fruits are widely used traditional medicine in Türkiye.[6] For example, *J. drupacea* fruits have been used in the treatment of helminth infections and abdominal pain,[7,8] and against hemorrhoids,[9] while the decoction product of its fresh shoots has been used in the treatment of urinary inflammation, gout, and abdominal pain, and its tar against diarrhea.[8,10] The tar of *J. drupacea* is obtained through the combustion of its stem. It is applied externally for conditions such as alopecia, eczema, and animal wounds. Internally, it is used to treat cough, cold, urinary tract inflammation, and diarrhea.[8,11] Analysis of the extracts of *J. drupacea* fruits suggested that this fruit may be a natural antioxidant supplement for food and beverages.[12] This study aimed to investigate the curative effects of flavan-3-ol rich extracts from *J. drupacea* fruit by assessing the levels of cytokines, blood gases, and lymphocytes in rats with COPD induced by PPE. Some bioactive components found in plants are better dissolved in water, while others are better dissolved in solvents such as methanol. At the same time, methanol is a solvent with a lower density and boiling point than water. With this feature, it penetrates plants faster and allows bioactive components to pass into the solvent in greater amounts. In this study, both water and methanol extracts were used with the idea that they may differ in terms of bioactive components.

The extraction yields of *J. drupacea* fruit used in this study were determined to be 25.35% for the water extract and 27.96% for the methanol extract, with the difference being statistically significant (p=0.012).

MATERIALS AND METHODS

Experimental Procedures

Animals

The protocols presented below are based on animal experiments approved by the Ethics Committee of Selçuk University Experimental Medicine Research and Application Center (approval number: 2020/52, date: 30.11.2020). In this *in vivo* research study, a total of 30 Wistar albino adult female rats aged 4-5 months and weighing 300-350 g were used. PPE (Sigma Chemical, St Louis, MO) was used to construct a model of COPD in rats. Single-sex use aims to minimize anatomical and hormonal differences that may arise from sex.

Animal Treatment and PPE Induction

The rats were randomly selected and divided into four groups. The "draw method" was preferred and used as the randomization method.[13] Six rats were selected for the control group and eight rats for each of the other groups. No procedure was applied to the negative control group. For the other three groups, a COPD model was created with the PPE. The rats were anesthetized by injecting ketamine and xylazine before intubation. The PPE mixture was prepared as 55 U/100 g by mixing with 0.5 mL NaCl following anesthesia and was administered to rats intratracheally.[14] The negative control group received only water by gavage throughout the 21-day treatment period. In the groups, the flavan-3-ols extracts from *J. drupacea* fruit were administered starting 24 h after the PPE treatment. The methanol and water extracts of *J. drupacea* fruits were separately given by gavage at a dosage of 250 mg/kg bw/day to rats for the treatment of PPE-induced COPD over 21 days. All rats in groups were housed at 21-22 °C without any water and food restrictions, under a 12-hour light/dark cycle. Food restriction was applied to all rats 12 h before anesthesia procedures. On the 21st day, the rats were anesthetized with an injection of ketamine and xylazine again before euthanizing them. Intracardiac blood was taken in accordance with euthanasia procedures, followed by the performance of the sacrifice process. Then, various tissues were collected for analysis.

Treatment start date was 04.06.2021; Ethics committee approval date was 30.11.2020. The 250 mg/kg bw/day concentration used for therapeutic purposes in this study, was determined based on the procedures described by Laouar et al.[15]

Number of Animals to be Included in the Study

The number of animals required for the animal experiment model (4 groups: group-1, control group; group-2, PPE; group-3, PPE + WE; group-4, PPE + ME), created to evaluate the effect of methanol and water extracts of *J. drupacea* fruit applied in the COPD animal model, on blood gas, histopathology (emphysema), and inflammatory markers [pH, PaO_2, $PaCO_2$, interleukin-6 (IL-6), IL-8, tumor necrosis factor-alpha (TNF-α), cytotoxic T-cells, B-cells, natural killer (NK)-cells], was determined by the resource equalitymethod.[16-18] According to this method, the number of animals to be included in each group for an experimental design consisting of 4 study groups was determined by calculating (20/4)+1=6. Considering the possible animal losses during the experimental phase, the study was planned to be conducted with 8 animals in each experimental arm, and 6 animals in the control group.

Plant Materials

Specimens of *J. drupacea* Labill., a member of the *Cupressaceae* family, were collected from the Sebil forest area in Çamlıyayla, Mersin Province, Türkiye, during July. These botanical samples were accurately identified by Prof. Dr. Osman Tugay from the Department of Pharmaceutical Botany, Faculty of Pharmacy at Selçuk University (Konya, Türkiye) and were cataloged under the herbarium record number (KNYA Herb. No: 30.115).

The Extraction Procedures

In November 2020, the fruits of *J. drupacea* at optimal maturity were collected from a forest located at an altitude of 1400 meters in the Çamlıyayla district of Mersin. After transportation to the laboratory, the fruits were thoroughly cleaned to remove any contaminants. Once broken, the fruits were ground using a hammer mill (Arzum, model AR1034, Türkiye) to produce a fine powder for extraction. For the extraction process, fifteen grams of the ground fruits were separately treated with methanol (150 mL) and distilled water (150 mL) using a Soxhlet apparatus (Electro-mag MX 425, Türkiye). The obtained extracts were collected, then the solvents were removed using a rotary evaporator (Scilogex RE100-Pro) under vacuum at 40 °C. The extracts

were then frozen at -80 °C and lyophilized. The final powdered extracts were stored at -18 °C for further analysis.

Akbulut and Akbulut[19] analyzed the phenolic compounds of water and methanol extracts of *J. drupacea* fruit used in this study and published the results. The high-performance liquid chromatography analysis revealed that flavan-3-ols, including catechin, epicatechin, epicatechin gallate, and procyanidin A2, constituted approximately 92% of the total phenolic compounds in the aqueous extract and 85% in the methanol extract.[19]

Histopathological Evaluation

The lung tissues were fixed in 10% buffered formaldehyde for 24 hours for pathological evaluation. For macroscopic sampling, 1x1 cm samples were taken from both lungs of each animal. The pieces were embedded in paraffin and 4-micron sections were taken onto slides with the help of a microtome. The slides stained with hematoxylin-eosin were evaluated under the Olympus BX53 model light microscope for the development of emphysema. The presence and extent of emphysema were evaluated by scanning the entire lung parenchyma at 40x magnification using the microscope. In the evaluation, the ratio of lung parenchyma developing emphysema to normal lung parenchyma was determined as a percentage. Accordingly, the affected area was scored as <25%, score 1; 25-50%, score 2; 50-75%, score 3; >75%, score 4. The mean of emphysema scores of each group was calculated, and the results were compared using statistical methods.[20,21]

Blood Gas Analysis (pH, pCO_2, pO_2)

For blood gas analysis, the blood was carefully drawn from the heart with a 2 mL heparin injector in the experimental rats under anesthesia. Blood gas analyses were performed on the day the study was completed. After the rats were anesthetized, blood was taken from the heart using blood gas injectors and analyzed without delay by the ABL9 blood gas analyzer (Dadiometer, Denmark). pH, arterial oxygen partial pressure (PaO_2), and arterial carbon dioxide partial pressure ($PaCO_2$) values were determined in their analyses.

Cytokine Analyzes (IL-6, IL-8, TNF-α) by ELISA Test Kit

For the analysis of cytokines, the blood taken from the heart of the rats under anesthesia, was collected in purple capped EDTA tubes and transferred to the laboratory without waiting. This blood was then centrifuged, and the plasma was kept at -80 °C until analysis. IL-6, IL-8, and TNF-α analyses were carried out using Elabscience Rat IL-6, Bioassay Technology Laboratory Rat IL-8, and Elabscience Rat TNF-α ELISA test kits, following the test procedures provided with them.

Cytotoxic T-Lymphocyte, B and NK-Cell Analysis by Flow Cytometry

The surfaces of cells were stained with monoclonal antibodies targeting anti-Rat CD8a, CD45RA, and CD161a (BD Bioscience) to assess the proportions of B-cells, cytotoxic T-cells, and NK-cells through flow cytometry. Three sample tubes were prepared: one control, one containing CD3 (APC)/CD4 (PE)/CD8 (FITC), and another with CD3 (APC)/CD45RA (FITC)/CD161a (PE). A 100 µL aliquot of rat peripheral blood was added to each tube. According to the kit instructions, the appropriate amounts of monoclonal antibodies were added to all tubes except the control. After vortexing, the tubes were incubated for ten minutes at room temperature in the dark. Next, two mL of 10X lysing solution, diluted 1:10 with distilled water, was added to lyse red blood cells. The samples were incubated for fifteen minutes in the dark at room temperature. The tubes were centrifuged at 1500 rpm for five minutes to isolate white blood cells, and the supernatant was discarded. After washing the pellet with 2 mL of phosphate-buffered saline (PBS) and centrifuging again, the supernatant was removed. The remaining cell pellet was resuspended in 500 µL of PBS. Finally, the samples were analyzed using the FACS Aria III flow cytometer (BD Bioscience), with data processed using FACS Diva version 6.1.3 software. In the generated dot plots, the cells were categorized as CD3 (+) CD8 (+) cytotoxic T-cells, CD45RA (+) B-cells, and CD161a (+) NK-cells and their respective percentages were recorded.[22]

Statistical Analysis

To statistically evaluate the histopathological, flow cytometry, blood gas, and ELISA test results obtained at the end of the study, the results were subjected to analysis of variance using the MINITAB release 16.0 (Minitab Inc., PA, USA) program. Duncan's Multiple Range Test was used to see whether the differences between group means were significant. The significance level was accepted as $p<0.05$. To differentiate between the experimental groups: control, PPE, PPE + WE, and PPE + ME, methods such as principal component analysis (PCA), hierarchical cluster analysis (HCA), and heatmap clustering analysis were employed. To estimate the effect sizes, the partial eta squared (η^2) was chosen for this study, because it allows the calculation of variation for more than one variable.[23] In One-Way ANOVA analyses, the partial eta-squared effect size was used to determine the effect size for results that were significant between groups. Partial eta-squared tells us how large an effect the independent variable(s) has on the dependent variable. For partial eta-squared, if $\eta^2 \geq 0.14$, the effects are large; between 0.06 and 0.14, the effects are considered moderate; and when $\eta^2 \leq 0.06$, the effects are considered small.[24]

RESULTS

Lung Histopathological Evaluation

Histopathological tests are the most important indicators of whether COPD occurs in rats after PPE induction. The photomicrographs of general lung histology are presented in Figure 1. The rats without PPE comprised the control group (Figure 1A); the rats with PPE-induced COPD comprised the PPE group (Figure 1B); the rats with COPD exposed to PPE and treated by gavage with the water extract (250 mg/kg body weight/day) of *J. drupacea* fruit for 21 days comprised the PPE-WE group (Figure 1C); the rats with COPD exposed to PPE and treated by gavage with the methanol extract (250 mg/kg body weight/day) of *J. drupacea* fruit for 21 days comprised the PPE-ME group (Figure 1D).

Development of emphysema in the lung tissues of the rats with PPE-induced COPD and treated with the water and methanol extracts of *J. drupacea* fruit is shown in Figure 1E. The emphysema scores in the histopathological evaluation were determined to be 0.667±0.516, 2.400±0.548, 1.286±0.488, and 1.000±0.001 in the control group rats, the PPE group rats, the PPE + WE group rats, and the PPE + ME group rats, respectively. In the method section, detailed information is given about the development of emphysema. In this determination method, the ratio of the lung parenchyma developing emphysema to the normal lung parenchyma was determined as a percentage. Accordingly, the affected area was scored as <25%: score 1, 25-50%: score 2, 50-75%: score 3, >75%: score 4. According to the results of this evaluation, the highest emphysema development was detected in the rats with the

PPE-induced COPD (between 50-75%), while the lowest emphysema development was determined in the control group (<25%). Emphysema in the rats in the PPE + WE, PPE + ME groups decreased compared to the rats in the PPE group. This indicated that both water and methanol extracts of *J. drupacea* fruits were effective in the treatment of COPD. Emphysema in the rats treated with the methanol extracts of *J. drupacea* fruit was lower than that in the group of rats treated with its water extracts. It has been determined that the methanol extracts of *J. drupacea* fruit are more effective in the regression of emphysema. It seems that the methanol extracts of *J. drupacea* fruit may be more effective than the water extracts in the treatment of COPD.

Arterial Blood Gas Analyses in the Rat Bloods

The pH, $PaCO_2$, and PaO_2 results determined in the rats in our study are given in Table 1. As seen in Table 1, the pH was determined as 7.3950±0.0152, 7.3520±0.0045, 2.3714±0.605, 7.3529±0.340, and 7.3400±0.0185 for the rats of the control group, the PPE group, the PPE + WE group, and the PPE + ME group, respectively. The distinction in pH levels between the groups was determined statistically significant ($p<0.05$). The highest pH was determined in the rats in the Control group, followed by the PPE + WE, the PPE, and the PPE + ME groups, respectively. The pH values of the rats, to which the COPD model was applied and which either received treatment or did not, were found to be lower than those of the rats that did not receive the COPD model or any treatment. $PaCO_2$ was determined as 45.317±1.903, 51.520±2.960, 49.757±4.170, and 49.350±2.677 mmHg for the control, the PPE, the PPE + WE and the PPE + ME, respectively (Table 1). While the highest $PaCO_2$ was found in the rats in the PPE group with COPD but no treatment, the lowest $PaCO_2$ was determined in the rats in the control group. $PaCO_2$ in the rats with COPD treated with water and methanol extracts of *J. drupacea* fruits decreased with the treatment process compared to the PPE group and was found to be close to the values in the control group rats. It is seen that $PaCO_2$ values are close to each other in the rats in the PPE + WE and the PPE + ME, treated with the water and methanol extracts. The PaO^2 determined in the rats in this study was 63.800±11.52, 48.750±5.37, 51.833±6.43 and 50.857±5.18 mmHg for the control, the PPE, the PPE + WE and the PPE + ME, respectively (Table 1). The highest PaO_2 values were detected in the control group rats; the lowest PaO_2 values were observed in the PPE group rats, in which COPD was formed but no treatment was applied. In the rats with COPD, an increase in PaO_2 was observed after 21 days of treatment with the water and methanol extracts of *J. drupacea* fruits. It was determined that the increase in PaO_2 between these two groups was higher in the PPE + WE group rats than in the PPE + ME rats and statistically significant ($p<0.05$).

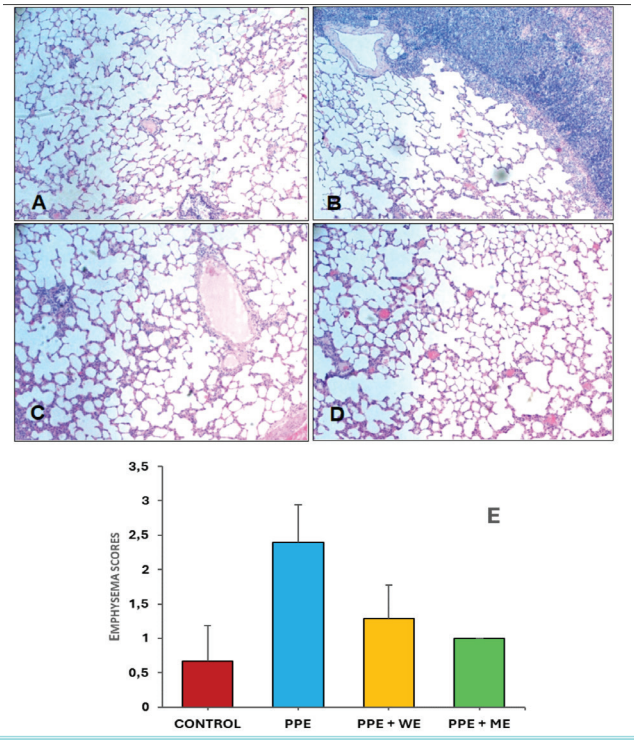

Figure 1. Photomicrographs of general lung histology. (A) Rats without PPE, (B) Rats with PPE, (C) Rats induced COPD with PPE and treated for 21 days by gavage with WE of *Juniperus drupacea* fruit, (D) Rats induced COPD with PPE and treated for 21 days by gavage with ME of *Juniperus drupacea* fruit, (E): Emphysema scores of all rat groups [the effect size (η^2)=0.698, p=0.001).

PPE: Porcine pancreatic elastase, COPD: Chronic obstructive pulmonary disease, WE: Water extract, ME: Methanol extract.

The Effects of Flavan-3-Ols Rich Extracts from *J. drupacea* Fruit on the Cytokine Production in the Rat Bloods

In the present study, IL-8, IL-6, and TNF-α cytokines were also determined, in addition to other markers, to observe the COPD status created with PPE in the rats. The treatment process involved the water and methanol extracts of *J. drupacea* fruits. IL-8, IL-6, and TNF-α were analyzed by the ELISA method. The changes between the groups are also shown in Figure 2. According to these results, IL-6 was determined as 20.633±2.53, 22.563±2.58, 20.753±9.52, and 20.598±2.235 pg/mL for the control, PPE, PPE + WE, and PPE + ME groups of rats, respectively. The highest IL-6 levels were observed in the rats with PPE-induced COPD. It is observed that IL-6 values obtained in the rats of PPE + WE and PPE + ME groups, were quite close to those of the control group. The IL-6 values of rats with COPD, which were treated using *J. drupacea* fruit extracts, decreased to the levels of normal rats without COPD. This indicates that the rats responded positively to the treatment process.

When the IL-8 measured in this study was investigated (Figure 2), it was observed that a similar situation occurred with the IL-6. However, the differences in IL-8 levels were statistically significant ($p<0.05$). The IL-8 in the control, PPE, PPE + WE and PPE + ME groups of rats was

Table 1. The changes in arterial blood5 gas results of the rats with PPE exposure and *J. drupacea* fruit extracts treatment

Groups	pH	$PaCO_2$ (mmHg)[3]	PaO_2 (mmHg)[4]
Control	7.3950±0.0152[a]	45.317±1.903[b]	63.800±11.52[a]
PPE	7.3520±0.0045[b]	51.520±2.960[a]	48.750±5.37[b]
PPE + WE	7.3529±0.0340[b]	49.757±4.170[a,b]	51.833±6.43[b]
PPE + ME	7.3400±0.0185[b]	49.350±2.677[a,b]	50.857±5.18[b]
Effect sizes (η^2)	**0.514**	**0.363**	**0.411**
p-value	**0.009**	**0.026**	**0.016**

[1]Values are expressed as "mean ± standard deviation". [2]There is no statistical difference between the values indicated with the same letter. [3]PaO_2: Arterial oxygen partial pressure, [4]$PaCO_2$: Arterial carbon dioxide partial pressure, [5]Blood gas analyses were performed on the day the study was completed, after the rats were anesthetized and blood was taken from the heart using blood gas injectors and analyzed without delay by the ABL9 blood gas analyzer (Dadiometer, Denmark) in the room at that time. PPE: Porcine pancreatic elastase, WE: Water extract, ME: Methanol extract.

determined to be 174.37±3.71, 222.05±12.54, 210.52±23.49, and 169.71±22.12 ng/mL. The highest levels of IL-8, similar to those of IL-6, were found in the group of rats with PPE-induced COPD. The IL-8 of the control and PPE + ME groups of rats was found to be close to each other. It was seen that IL-8 levels in rats with COPD treated with *J. drupacea* fruit water extract (PPE + WE) were higher than the IL-8 levels in those treated with methanol extract (PPE + ME). This situation gives the result that the methanol extract of *J. drupacea* fruit is more effective at decreasing IL-8.

TNF-α for the control, PPE, PPE + WE, and PPE + ME group rats was determined to be 255.12±25.4, 417.97±108.6, 267.44±36.1, and 259.57±39.5 pg/mL, respectively. The differences in TNF-α were determined to have strong statistical significance ($p<0.05$). As with IL-6 and IL-8, the highest TNF-α was observed in the group of rats with PPE-induced COPD. It was found that the TNF-α of the control group was close to those in the PPE + WE and PPE + ME group rats. TNF-α of the rats with PPE-induced COPD increased compared to the control group, but the values decreased following treatment with *J. drupacea* extracts. These results show that both extracts of *J. drupacea* fruit are effective in decreasing TNF-α.

Effects of Flavan-3-Ols Rich Extracts from *J. drupacea* Fruit on the B, Cytotoxic T- and NK-Cells in the Rat Bloods

The results of B-cells, cytotoxic T-lymphocyte (CTLs), and NK-cells are exhibited in Figure 3. B-cells (%) for the control, PPE, PPE + WE, and PPE + ME groups were detected as 23.940±5.91, 31.980±5.12, 16.800±3.08, and 22.575±4.43, respectively. The differences between the groups in terms of B-cell counts were statistically significant ($p<0.05$). The highest number of B-cells was determined in the rats in the PPE-induced COPD group (31,980), while the lowest was detected in the rats in the PPE + WE group (16,800). B-lymphocyte counts decreased in the rats with PPE-induced COPD, treated by the water and methanol extracts.

The number of NK-cells was determined as 2.8167±1.910, 1.3200±0.286, 2.3714±0.605 and 2.3714±0.605 for the control, PPE, PPE + WE and PPE + ME groups of rats, respectively. The highest number of NK-cells was determined in the rats in the control group, followed by the PPE + WE, PPE + ME, and PPE group rats, respectively. The NK count was the lowest in the rats with PPE-induced COPD.

CTL counts were determined as 11.250±4.57, 16.720±4.52, 17.357±2.88, and 19.400±4.45 (%) for the control, PPE, PPE + WE, and PPE + ME group rats, respectively. The lowest number of CTLs was determined in the control group without COPD, while the highest number was found in the PPE + ME group, rats, (Figure 3). Although there was an increase in CTLs in the rats with PPE-induced COPD compared to the control group, no change was observed as a result of 21-day treatment, with both water and methanol extracts of *J. drupacea* fruit.

PCA, HCA and Heatmap Analyzes Regarding Inflammation Biomarker Values

In this study, statistical methods such as PCA, HCA, and Heatmap analysis were employed to visually and comprehensively evaluate the analyses from different perspectives. PCA mitigates correlations among numerous variables under investigation by reducing them to linear combinations of principal components. In our study, a technique was utilized to visualize differences in inflammation biomarkers such as IL-6, IL-8, TNF-α, B-cells, cytotoxic T-cells, and NK-cells. Figure 4 illustrates a scatter plot (Figure 2A) where points represent the control, PPE,

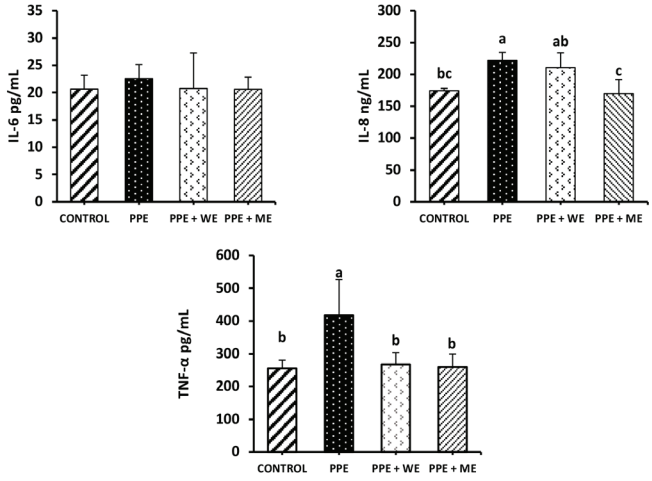

Figure 2. Effect of *Juniperus drupacea* fruit extract on the cytokines in the blood of rats. The levels of IL-8, IL-6 and TNF-α in the rat blood were detected by ELISA. Control: No procedure was applied to the control group, PPE treated, PPE + WE of *Juniperus drupacea*, PPE + ME of *Juniperus drupacea* fruit, Values are expressed as mean ± standard deviation, there is no statistical difference between the values indicated with the same letter ($p<0.05$) [IL-8, the effect size (η^2)=0.632, p=0.001; TNF-α, the effect size (η^2)=0.591, p=0.001].

IL: Interleukin, TNF-α: Tumor necrosis factor-alpha, PPE: Porcine pancreatic elastase, WE: Water extract, ME: Methanol extract.

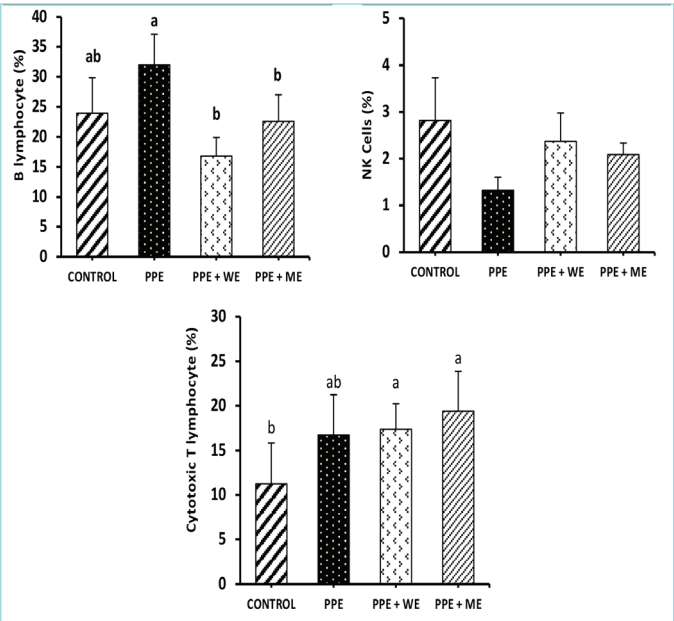

Figure 3. The effect of water and methanol extract treatment of *Juniperus drupacea* fruit on B-cells, CTLs, and NK-cells in the blood of rats. Control: No procedure was applied to the control group, PPE treated, PPE + WE of *Juniperus drupacea* fruit, PPE + ME of *Juniperus drupacea* fruit, values are expressed as mean ± standard deviation, there is no statistical difference between the values indicated with the same letter ($p<0.05$) [B-cells, the effect size (η^2)=0.314, p=0.001; TNF-α, the effect size (η^2)=0.389, p=0.032].

CTLs: Cytotoxic T-lymphocyte, NK: Natural killer, PPE: Porcine pancreatic elastase, WE: Water extract, ME: Methanol extract.

PPE + WE, and PPE + ME groups, while the vectors represent tested inflammation biomarkers. PC1 explains 61.8% of the variance, and PC2 explains 31.6%, as shown in Figure 4.

The control, PPE + WE, and PPE + ME groups showed close relationships with NK-cells, pH, and pO_2 clustering on the negative side of PC1. In contrast, the PPE group, which exhibits strong association with B-lymphocyte, IL-6, IL-8, emphysema, TNF-α, pCO_2, and CTL, were clustered on the positive right side of PC1 (Figure 4). In Figure 5, it is observed that the control, PPE, PPE + WE, and PPE + ME groups were divided into two clusters based on inflammation biomarkers tested. The first cluster consisted of the control, PPE + WE, and PPE + ME, along with IL-6, TNF-α, emphysema, IL-8, pCO_2, and CTLs. The second cluster consisted of the PPE group along with B-cells, inflammation, NK-cells, pH, and pO_2. This situation indicated that all groups could be clearly differentiated based on the inflammation biomarkers tested. It was understood that PC1 explains 61.8% and PC_2 explains 31.6% of the variance, and that PC1 and PC2 together explain 93.4% of the variance (Table 2). PC1, PC2, and PC3 vectors that contribute to this separation are seen in Table 2.

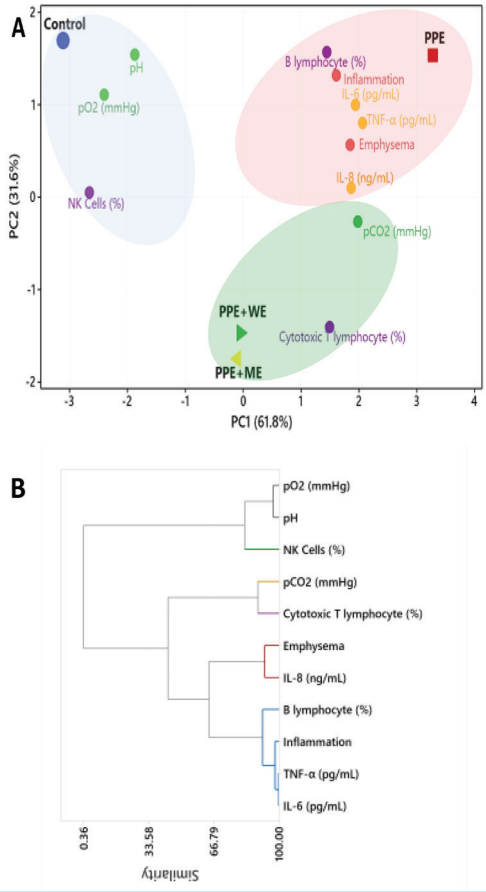

Figure 4. (A) The loading and score plot of PC1 and PC2 describing the changes among the pro-inflammatory cytokines, lymphocytes and blood gas values of the control, PPE, PPE + WE and PPE + ME group rats, (B) Dendrogram obtained through hierarchical cluster analysis.

PPE: Porcine pancreatic elastase, WE: Water extract, ME: Methanol extract, NK: Natural killer, IL: Interleukin, TNF-α: Tumor necrosis factor-alpha, PC1: The first principal component, PC2: The second principal component.

Table 2. PCA results regarding the evaluation of the effects of water and methanol extracts of *Juniperus drupacea* fruits on inflammation biomarkers in the rats applied with a COPD model

Items	PC1*	PC2	PC3
Eigenvalue	6.80	3.48	0.72
Variance percentage (%)	61.8	31.6	6.6
Cumulative variance (%)	61.8	93.4	100
Eigenvectors			
IL-6 (pg/mL)	**0.324**	0.286	-0.031
IL-8 (ng/mL)	0.291	0.103	**-0.734**
TNF-α (pg/mL)	**0.331**	0.272	-0.003
B-lymphocyte (%)	0.210	**0.386**	**0.503**
NK-cells (%)	**-0.374**	-0.055	-0.234
Cytotoxic T-lymphocyte (%)	0.235	**-0.410**	0.233
pH	-0.270	**0.368**	-0.215
pCO_2 (mmHg)	**0.367**	-0.150	-0.086
pO_2 (mmHg)	**-0.341**	0.243	-0.045
Emphysema	**0.365**	0.148	-0.154
Inflammation	-0.065	**0.525**	0.141

PCA: Principal component analysis, COPD: Chronic obstructive pulmonary disease, IL: Interleukin, TNF-α: Tumor necrosis factor-alpha, NK: Natural killer,PC1*: The first principal component, PC2: The second principal component, PC3: The third principal component.

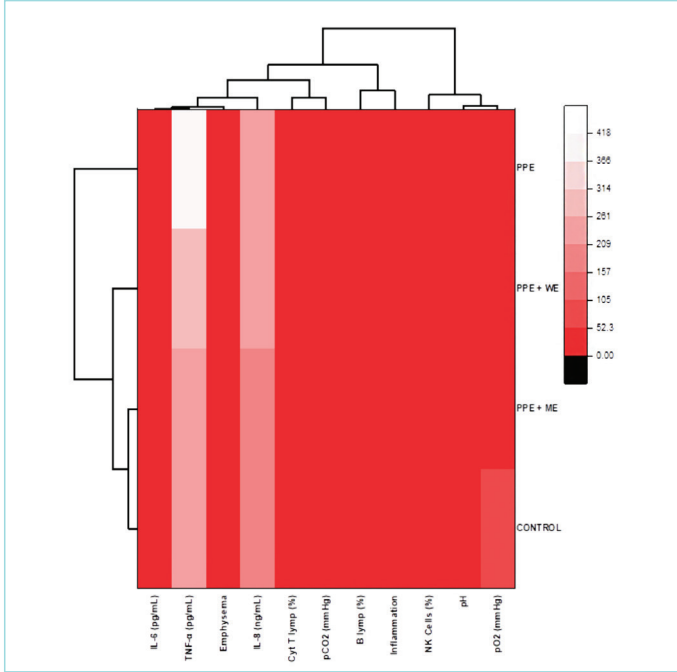

Figure 5. Heatmap obtained through the evaluation of the effects of water and methanol extracts of *Juniperus drupacea* fruits on inflammation biomarkers in rats applied with a COPD model. Heatmap shows the changes and contribution levels of the biomarkers evaluated in the monitoring of the treatment process with the water and methanol extracts of *Juniperus drupacea* fruit to the treatment process of COPD rats.

COPD: Chronic obstructive pulmonary disease, PPE: Porcine pancreatic elastase, WE: Water extract, ME: Methanol extract, IL: Interleukin, TNF-α: Tumor necrosis factor-alpha, NK: Natural killer.

DISCUSSION

In our study, the number of B-cells and CTLs increased, and NK-cells decreased due to inflammation/emphysema in the rats with PPE-induced COPD. At the same time, the levels of IL-6, IL-8, and TNF-α increased with COPD. As a result of the 21-day treatment of the rats with PPE-induced COPD using the water and methanol extracts of *J. drupacea* fruit, the numbers of B-cells and NK-cells, as well as the levels of IL-6, IL-8, and TNF-α, returned to the levels of the control group rats. This study found that both extracts of *J. drupacea* fruit showed a protective effect against lung inflammation and alveolar deterioration caused by exposure to the PPE in rats. It was estimated that this might be due to the high levels of catechins contained in *J. drupacea* fruit extracts, and phenolic acids such as gallic acid (GA), which have been shown to have a positive effect on COPD. The *J. drupacea* fruit extracts are rich in catechins such as catechin, epicatechin, and epicatechin gallate, contain significant amounts of protocatechuic and GA.[19,25,26] Akbulut and Akbulut[19] stated that among the phenolic compounds, catechins such as catechin, epicatechin, and epicatechin gallate, appears to be the most abundant phenolic compound isolated in all extracts and the total of these catechins constitute approximately 79.7-81.5% of all phenolics in *J. drupacea* fruit extracts. In some studies, it was stated that catechins were dominant among phenolic compounds in *J. drupacea* fruit extracts,[19] and in some studies, it was stated that protocatechuic acid was the most abundant phenolic compound.[25,26] It is important to note that different distributions and concentrations of phenolic compounds in *J. drupacea* fruit may vary depending on factors such as geographical location, climatic conditions, and growth and harvest stages of the plant.[19]

Sohn et al.[27] reported that the levels of IL-1β and IL-6, known as pro-inflammatory cytokines, were reduced in the lung BAL fluids of the mice, treated with extracts from a mixture of sixteen medicinal plants (Gamijinhae-tang extract) for acute lung injury. Ahmed[28] observed that epigallocatechin 3-gallate, a catechin group phenolic, suppressed the expression of IL-6 and IL-8 *in vitro.* In the present study, the findings obtained regarding the changes in the levels of pro-inflammatory cytokines IL-6 and IL-8 in the blood of COPD rats treated with *J. drupacea* fruit extracts were consistent with the results obtained from the study of Sohn et al.[27] and Ahmed[28].

In light of the existing body of literature, the assessment of arterial blood gas (ABG) exchange is a primary measure to determine the damage to the alveolar wall and confirm the efficiency of gas exchange in the lungs, which indicates lung injury in rats.[29,30] ABG analysis is a crucial diagnostic tool for assessing the severity of emphysema.[31] Jiang et al.[31] stated that at the end of the treatment process of rats with lipopolysaccharide (LPS)-induced COPD with pyrrolidine dithiocarbamate pH and PaO_2, which are blood gas values, decreased compared to the rats in the control group, $PaCO_2$ increased. In the present study, the severity of emphysema increased significantly rats with COPD due to PPE exposure, and this severity decreased again at the end of the 21-day treatment period with the water and methanol extracts of *J. drupacea* fruit. A similar situation was seen in the blood gas analysis results of rats, as $PaCO_2$ increased and PaO_2 decreased of COPD rats exposed to PPE. However, at the end of 21-days of treatment with both extracts of *J. drupacea* fruit at a dose of 250 mg/kg body weight/day, $PaCO_2$ decreased, and PaO_2 increased, approaching the blood gas values in the control group rats. This shows that the treatment of inflammation/emphysema in the rats with *J. drupacea* fruit extracts helps alleviate the PPE-induced COPD (Table 1).

COPD is a progressive respiratory disorder characterized by permanent airflow limitation, inflammation of the airways, and respiratory symptoms such as shortness of breath, chronic cough, and sputum production. COPD primarily includes two main conditions: chronic bronchitis and emphysema.[27,32]

COPD is a long-term lung condition in which the flow of air in the respiratory system is consistently restricted. This restriction tends to worsen over time and is linked to a heightened, ongoing inflammatory reaction in the air passages and lungs, triggered primarily by harmful substances like cigarette smoke (CS). Inflammation plays a critical role in the pathogenesis and progression of COPD, leading to structural changes in the airways and lung tissue. Chronic inflammation in COPD primarily involves the small airways and lung parenchyma. In response to inhaled irritants like CS, there is an influx of inflammatory cells such as neutrophils, macrophages, and lymphocytes into the airways and lung tissue. These cells release various inflammatory mediators, including cytokines, chemokines, proteases, and reactive oxygen species (ROS), contributing to tissue damage and inflammation.[33,34]

Cytokines such as IL-1, IL-6, and TNF-α are elevated in COPD patients and contribute to the inflammatory response, tissue destruction, and recruitment of immune cells. Chemokines such as IL-8 play a crucial role in recruiting neutrophils to the airways, contributing to airway inflammation and obstruction. Matrix metalloproteinases (MMPs) and neutrophil elastase are proteases released by inflammatory cells that lead to tissue destruction and remodeling in COPD. The chronic inflammatory process in COPD also results in oxidative stress due to an imbalance between ROS and antioxidants. Oxidative stress further exacerbates inflammation and tissue damage in the lungs. Chronic inflammation in COPD triggers structural changes in the airways, including thickening of the airway wall, mucus hypersecretion, and destruction of lung parenchyma (emphysema). This remodeling further exacerbates airflow limitation and respiratory symptoms. Inflammatory responses are amplified during acute exacerbations of COPD, leading to worsened symptoms, increased airway inflammation, and accelerated decline in lung function.[33,34]

IL-8, IL-6, and TNF-α are pro-inflammatory cytokines associated with COPD. These cytokines play a significant role in the inflammatory process and contribute to the pathogenesis and progression of COPD. IL-6 is a pro-inflammatory cytokine that is elevated in COPD patients. It is produced by a variety of cells, including macrophages, T-cells, and endothelial cells. Elevated levels of IL-6 are associated with the severity of COPD and its related comorbidities, including muscle wasting and cardiovascular disease.[35] IL-8 is a chemokine that plays a crucial role in recruiting neutrophils to the airways and promoting inflammation. Increased levels of IL-8 are found in COPD patients and are associated with increased airway neutrophilia, exacerbations, and disease severity.[36] TNF-α is a pro-inflammatory cytokine involved in the regulation of immune cells and inflammation. In patients with COPD, TNF-α levels increase depending on the severity of the disease and are associated with airway inflammation, disease progression, and systemic symptoms.[37] Understanding the role of these cytokines is essential for developing targeted therapies and interventions to manage COPD and improve the quality of life for individuals with this chronic respiratory condition.

B-cells, CTLs, NK-cells, and their involvement in COPD are important aspects of the immune response and its impact on respiratory health.

However, it's important to note that the exact role and mechanisms of these immune cells in COPD may be complex and not fully understood. B-cells play a role in COPD through antibody production and involvement in the inflammatory response. They can produce autoantibodies, such as rheumatoid factor and anti-elastin antibodies, which may contribute to tissue damage in COPD. B-cell activation and antibody production may contribute to chronic inflammation and lung tissue destruction in COPD.[38] CTLs play a crucial role in cell-mediated immune responses, including targeting infected or damaged cells. In COPD, CTLs are involved in targeting and eliminating infected or damaged lung cells, but an excessive CTL response may also contribute to tissue damage and inflammation in the lungs.[39] NK-cells are part of the innate immune response and are involved in early defense against viral infections and tumor cells. In COPD, altered NK-cell activity has been observed, which may contribute to impaired antiviral defense and increased susceptibility to respiratory infections.[40]

Monitoring specific parameters like pH (acidity), PaO_2 and $PaCO_2$ is crucial in managing and assessing the severity of COPD. The pH of blood is an important indicator of the body's acid-base balance. In COPD, the blood pH can be affected due to respiratory acidosis, where there is an accumulation of carbon dioxide in the blood.[41] PaO_2 represents the pressure of oxygen dissolved in the blood. In COPD, impaired lung function often leads to decreased PaO_2 levels, resulting in hypoxemia, which can further worsen the symptoms and prognosis of COPD patients.[42] $PaCO_2$ measures the pressure of carbon dioxide dissolved in the blood. In COPD, the retention of carbon dioxide due to impaired lung function can lead to respiratory acidosis and affect overall blood gas levels.[1] Regular monitoring of these parameters through ABG analysis is critical in managing and adjusting treatment plans for individuals with COPD. It helps healthcare professionals assess the severity of the disease, optimize oxygen therapy, and make appropriate adjustments to ventilation strategies.

The primary approach for treating COPD involves using a combination of medications and non-drug-based strategies. Pharmacological treatments aim to relieve symptoms, improve exercise tolerance, reduce exacerbations, and improve overall quality of life. There are some common classes of medications such as bronchodilators, inhaled corticosteroids (ICS), phosphodiesterase-4 (PDE-4) inhibitors, methylxanthines, antibiotics, oxygen therapy, and vaccinations used in the pharmacological treatment of COPD.[43] In the treatment of inflammatory lung diseases, classical drugs, in combination with different drugs, include inhaled glucocorticosteroids, β2-adrenoceptor agonists, leukotriene receptor antagonists, methylxanthines, theophylline, and others.[27,44-46] However, bronchodilators, ICSs, and PDE-4 inhibitors, which are the classes of drugs used in this treatment, may cause side effects such as tachycardia, tremor, headache, palpitations, increased heart rate, muscle cramps, thrush (oral yeast infection), hoarseness, increased risk of pneumonia, bone density loss with long-term use, nausea, diarrhea, weight loss, depression, and insomnia.[27,47-51] That's why there is a need to develop treatment methods that are more effective, safer, and with little or no side effects. In our study, we observed that exposure to PPE induced structural and functional changes that were typical of COPD, including airway remodeling, alveolar expansion, emphysema, lung inflammation, and increased numbers of lymphocytes (B-cells, CTLs, NK-cells), and the levels of IL-8, IL-6, TNF-α in the rat blood. At the same time, there were changes in the blood gas values of pH, PaO_2, and $PaCO_2$, and as in typical COPD, a decrease in PaO_2 and an increase in $PaCO_2$ occurred. On the other hand, during the treatment process with the water and methanol extracts of *J. drupacea* fruits, the structural and functional changes in the lung caused by the PPE exposure model began to normalize. These results suggest that both extracts of *J. drupacea* fruit, especially methanol extracts, are useful therapeutic agents in preventing these structural and functional changes related to COPD.

Medicinal plants are used in traditional and complementary medicine as an alternative to modern methods, or in combination with them for addressing health problems. Many studies have reported that various extracts of some medicinal plants, such as *Nigella sativa L*. seed extracts,[52] *Myristica fragrans*, *Cinnamomum cassia*, *Camellia sinensis*, and *Curcuma longa* have anti-elastase activity. This is mainly because plant extracts are rich in bioactive compounds, especially phenolic compounds. Green tea (*Camellia sinensis*) is rich in flavonoids such as catechin and epigallocatechin, and these phenolic compounds are known to be elastase inhibitors.[53]

Bioactive phytochemicals with high antioxidant and anti-inflammatory activities may have a healing effect on impaired lung functions. It has been observed that the use of fruits, vegetables, and plants as rich sources of bioactive phytochemicals, can reduce the risk of COPD.[3,54,55] Bioactive compounds found in plants, like phenolic compounds, carotenoids, and alkaloids, inhibit DNA methylation by preventing oxidative stress and inflammation, thereby preventing the progression of COPD.[3,56]

J. drupacea, commonly known as the Syrian juniper, is a species of juniper native to the eastern Mediterranean region, including parts of Türkiye, Syria, Lebanon, Israel, and North Cyprus.[19] It is often used in traditional medicine for various purposes, including respiratory ailments.[6] The phenolic compounds present in *J. drupacea* fruits may include various classes of polyphenols, such as flavonoids, phenolic acids, lignans, and tannins. *J. drupacea* fruits include phenolic acids (GA, protocatechuic acid, p-hydroxybenzoic acid, and p-coumaric acid) and flavonoids (catechin, epicatechin, epicatechin gallate, and procyanidin A2).[19,25,26] Yaglioglu and Eser[57] determined four different phenolic compounds in the cones of four different *Juniperus* species, *J. communis*, *J. excelsa*, *J. foetidissima*, and *J. oxycedrus*, and reported that the most abundant phenolic compound was catechin. Akbulut and Akbulut[19] stated that among the phenolic compounds, catechins such as catechin, epicatechin, and epicatechin gallate, appears to be the most abundant phenolic compound isolated in all extracts and the total of these catechins constitute approximately 79.7-81.5% of all phenolics in *J. drupacea* fruit extracts. In some studies, it has been stated that catechins are dominant among phenolic compounds in *J. drupacea* fruit extracts,[19] and in some studies, it has been stated that protocatechuic acid is the most abundant phenolic compound.[25,26] It is important to note that different distributions and concentrations of the phenolic compounds in *J. drupacea* fruit may vary depending on factors such as geographical location, climatic conditions, and growth and harvest stages of the plant.[19]

Oxidative stress significantly contributes to the development and progression of COPD. Antioxidants, including those found in medicinal plants, can help combat oxidative stress and potentially mitigate the progression of COPD. (-)-epigallocatechin-3-gallate, a flavanol polyphenolic compound, is a potent natural leukocyte elastase inhibitor that can be used to reduce elastase-mediated emphysema.

This flavanol is abundant in green tea and exhibits a dose-dependent, non-competitive inhibition of leukocyte elastase at a non-cytotoxic concentration, being effective in neutrophil culture.[19,58] In a study, the use of (-)-epigallocatechin-3-gallate showed promise in reducing the severity of acute lung injury caused by LPS in mice. This was evident through several positive outcomes: improved lung injury scores; reduced total cell, neutrophil, and macrophage counts; suppressed myeloperoxidase activity; lower wet-to-dry weight ratio of lung tissues; and a decrease in the release of inflammatory cytokines TNF-α, IL-1β, and IL-6.[59] Some flavonoids exert anti-inflammatory effects through blockade of inflammasomes, such as nuclear factor kappa B and NLRP3, suppression of production of pro-inflammatory cytokines such as IL-1β, IL-2, IL-6, TNF-α, and IL-17A, down-regulation of chemokines, and reduction of reactive nitrogen species and ROS.[60] In a study, BAL cellularity, neutrophil recruitment, and BAL MCP-1, IL-6, and TNF-α expressions, lung histological parameters, and platelet uptake increased in rats in which lung inflammation was induced by applying the intratracheal elastase model. However, on the 7th day of treatment with pomegranate peel extract (250 mg/kg body weight), it was determined that MCP-1, MMP-2, and IL-6 levels in the animals decreased to the levels of the animals in the control group, and lung TNF-α and MCP-1 expression decreased significantly.[61] GA is a naturally occurring and abundant phenolic compound in plants that is known to have antioxidant/anti-inflammatory activities. Singla et al.[62] stated that elastase and IL-6 and TNF-α cytokine levels in CS-induced COPD mice were significantly increased compared to control group mice, but after GA treatment, these factors returned to the control group level.

In our study, treatment with water and methanol extracts of *J. drupacea* fruit significantly decreased the levels of IL-6, IL-8, TNF-α, and B-cells in the blood and increased the levels of NK-cells. In particular, treatment with methanol extracts of the plant was more effective in reducing IL-6, IL-8, and TNF-α levels. This suggests that the anti-inflammatory effect of *J. drupacea* fruit methanol extracts can be attributed to the suppression of proinflammatory cytokine production in the lung. It is thought that both extracts of *J. drupacea* fruit may provide beneficial clinical effects in the treatment of COPD, but methanol extract may be more effective in this treatment due to its greater impact on inflammation biomarkers. Although in the present study, the power calculation result showed that the number of animals used was sufficient to convey statistical significance, the sample size of this study was relatively small (n=6), which might have led to misleading data interpretation. To overcome such limitations, additional studies are needed to investigate the effects of *J. drupacea* fruit extracts on lung inflammation.

J. drupacea trees are endemic plants and are widespread in high altitude forests along the Mediterranean coast of Türkiye. From ancient times to the present day, the cones (fruits) of this endemic tree have been collected by local people and used in traditional folk medicine to treat respiratory diseases such as asthma.[6,19] At the same time, its fruits are boiled for a long time to obtain a thick syrup called pekmez (molasses) and are considered a healthful food.[63] The reason why these fruits, which grow widely in forests, are widely used by the public is that they are easily accessible, sustainable and cheap.

Although herbal remedies are among traditional supportive methods used in the treatment of COPD, their natural origin does not guarantee they are safe. Herbal products contain active ingredients, and these can have both beneficial and harmful effects. The use of extracts from the fruits of *J. drupacea* in the treatment of COPD should be closely monitored in future clinical studies; particularly regarding potential side effects such as allergic reactions, including skin rashes, itching, swelling, or respiratory distress; drug interactions; gastrointestinal issues such as nausea, diarrhea, vomiting, or stomach discomfort; hormonal effects; respiratory impacts; and other related adverse effects. Therefore, the possible toxicity and side effects of herbal treatments that may carry risks in terms of individual health status and interactions with other medications should be kept in mind.[64]

The fact that no negative effects have been observed in the use of the fruits of this tree for a long time indicates that the fruits of this plant can be used in the treatment of COPD together with modern drugs. The use of plant extracts in the treatment of COPD should be considered with caution due to the potential for interactions with modern drugs. Herbal products may affect the efficacy and safety of prescription drugs through pharmacokinetic and pharmacodynamic interactions. In this respect, advanced research should be conducted to evaluate the components found in *J. drupacea* fruit extracts from a broader perspective. It is essential to study and reveal the interactions of bioactive components that may be present in the structure of *J. drupacea* fruits with modern drugs used in the treatment of COPD through *in vitro* and *in vivo* studies.

Study Limitations

The findings from animal research do not completely represent the conditions in humans. Therefore, more extensive and detailed studies are needed to assess the therapeutic effects of water and methanol extracts of *J. drupacea* fruits on COPD biomarkers.

CONCLUSION

In this study, the therapeutic effect of water and methanol extracts of *J. drupacea* fruit on PPE-induced COPD was investigated. This study sought to determine with inflammatory mediators whether COPD occurs with PPE, and whether both extracts of *J. drupacea* fruit are effective in the treatment of COPD. For this purpose, the changes in the histopathological tests (emphysema), ABG values (pH, PaO_2 and $PaCO_2$), IL-6, IL-8, TNF-α cytokines, and B-cells, CTLs, NK-cells were observed. In rats with PPE-induced COPD, emphysema increased significantly, PaO_2 decreased, and $PaCO_2$ increased. In addition, with PPE exposure, an increase in B-cells and CTLs, but a decrease in NK-cells, was observed in the blood of rats. At the same time, IL-6, IL-8, and TNF-α levels, which are among the cytokines examined in the current study, increased. This strongly indicates that COPD occurred in PPE-induced mice. In rats with COPD that were treated with water and methanol extracts (250 mg/kg bw/day) of *J. drupacea* fruit for 21 days, via gavage, there is a decrease in IL-6, IL-8, and TNF-α cytokines compared to PPE group rats. These decreases were close to those of the control group rats without COPD. The methanol extracts of *J. drupacea* fruit were determined to be more effective in decreasing IL-6, IL-8, and TNF-α cytokines. In terms of blood gas values, the water extract might be more effective, but the difference between the values is low, and both water and methanol extract affect the result to a similar extent. In addition, with PPE exposure, an increase in B-cells and CTLs, but a decrease in NK-cells, was observed in the blood of rats. According to the results of flow cytometric analysis, the water extracts of *J. drupacea* fruit were more effective on B-lymphocytes than on other cell types. In conclusion, treatment of rats with COPD using *J. drupacea* extracts, which have been determined by studies to be rich in bioactive components, shows that *J. drupacea* extracts can reduce the negative effects of COPD. This shows that *J. drupacea* fruit extracts can be used in the treatment of COPD, but more research is needed.

MAIN POINTS

- In all rats with porcine pancreatic elastase (PPE)-induced chronic obstructive pulmonary disease (COPD), emphysema in the lungs was observed from histopathological evaluation.
- As a result of treatment with water and methanol extracts of *Juniperus drupacea (J. drupacea)* fruit given to PPE-induced COPD rats, emphysema decreased to the control sample levels.
- After PPE-induced COPD, levels of IL-6, IL-8, and TNF-α in the blood, as well as B-cells and NK-cells, returned to levels similar to those in rats without COPD after extract treatment.
- Both extracts of *J. drupacea* fruit responded positively to COPD treatment.

ETHICS

Ethics Committee Approval: The protocols are based on animal experiments approved by the Ethics Committee of Selçuk University Experimental Medicine Research and Application Center (approval number: 2020/52, date: 30.11.2020).

Informed Consent: Patient approval has not been obtained as it is performed on animals.

FOOTNOTES

Authorship Contributions

Surgical and Medical Practices: B.Ç., Concept: H.F.A., H.V., Design: H.F.A., H.V., B.Ç., Data Collection and/or Processing: H.F.A., H.V., M.A., Analysis and/or Interpretation: H.F.A., H.V., H.Ö., Z.E.Ç., M.A., Literature Search: H.F.A., M.A., Writing: H.F.A., M.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Global Initiative for Chronic Obstructive Lung Disease (GOLD). Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease 2024 Report. 2024.
2. Turner MC, Chen Y, Krewski D, Calle EE, Thun MJ. Chronic obstructive pulmonary disease is associated with lung cancer mortality in a prospective study of never smokers. Am J Respir Crit Care Med. 2007; 176(3): 285-90.
3. Jasemi SV, Khazaei H, Momtaz S, Farzaei MH, Echeverría J. Natural products in the treatment of pulmonary emphysema: therapeutic effects and mechanisms of action. Phytomedicine. 2022; 99; 153988.
4. Yoshida T, Tuder RM. Pathobiology of cigarette smoke-induced chronic obstructive pulmonary disease. Physiol Rev. 2007; 87(3): 1047-82.
5. World Health Organization (WHO). The top 10 causes of death. (Accessed: 27 July 2024). Available from: https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death
6. Akkol EK, Güvenç A, Yesilada E. A comparative study on the antinociceptive and anti-inflammatory activities of five Juniperus taxa. J Ethnopharmacol. 2009; 125(2): 330-6.
7. Honda G, Yeşilada E, Tabata M, Sezik E, Fujita T, Takeda Y, et al. Traditional medicine in Turkey. VI. Folk medicine in west Anatolia: Afyon, Kütahya, Denizli, Muğla, Aydin provinces. J Ethnopharmacol. 1996; 53(2): 75-87.
8. Yeşilada E, Honda G, Sezik E, Tabata M, Goto K, Ikeshiro Y. Traditional medicine in Turkey. IV. Folk medicine in the Mediterranean subdivision. J Ethnopharmacol. 1993; 39(1): 31-8.
9. Başer KHC, Honda G, Miki W. Herb drugs and herbalists in Turkey. Tokyo : Institute for the Study of Languages and Cultures of Asia and Africa, 1986.
10. Yeşilada E, Honda G, Sezik E, Tabata M, Fujita T, Tanaka T, et al Traditional medicine in Turkey. V. Folk medicine in the inner Taurus Mountains. J Ethnopharmacol. 1995; 46(3): 133-52.
11. Baytop T. Therapy with medicinal plants in Turkey (past and present). İstanbul University; 1999.
12. El-Ghorab A, Shaaban HA, El-Massry KF, Shibamoto T. Chemical composition of volatile extract and biological activities of volatile and less-volatile extracts of juniper berry (Juniperus drupacea L.) fruit. J Agric Food Chem. 2008; 56(13): 5021-5.
13. Keskin İ, Başpınar E, Altay Y, Mikail N. Experimental Statistical Methods (RStudio Applied). NEU Press, 2025.
14. Borzone G, Liberona L, Olmos P, Sáez C, Meneses M, Reyes T, et al. Rat and hamster species differences in susceptibility to elastase-induced pulmonary emphysema relate to differences in elastase inhibitory capacity. Am J Physiol Regul Integr Comp Physiol. 2007; 293(3): R1342-9.
15. Laouar A, Klibet F, Bourogaa E, Benamara A, Boumendjel A, Chefrour A, et al. Potential antioxidant properties and hepatoprotective effects of Juniperus phoenicea berries against CCl4 induced hepatic damage in rats. Asian Pac J Trop Med. 2017; 10(3) 263-9.
16. Mead R. The design of experiments : statistical principles for practical applications. Cambridge England ; New York : Cambridge University Press; 1988.
17. Mead R, Gilmour SG, Mead A. Statistical principles for the design of experiments: Applications to Real Experiments. Cambridge, UK: Cambridge University Press; 2012.
18. Arifin WN, Zahiruddin WM. Sample size calculation in animal studies using resource equation approach. Malays J Med Sci. 2017; 24(5):101-5.
19. Akbulut HF, Akbulut M. Mineral composition, the profile of phenolic compounds, organic acids, sugar and in vitro antioxidant capacity, and antimicrobial activity of organic extracts of Juniperus drupacea fruits. Food Sci Nutr. 2023; 11(10): 6435-46.
20. Germann PG, Häfner D. A rat model of acute respiratory distress syndrome (ARDS): Part 1. Time dependency of histological and pathological changes. J Pharmacol Toxicol Methods. 1998; 40(2): 101-7.
21. Yilmaz S, Daglioglu K, Yildizdas D, Bayram I, Gumurdulu D, Polat S. The effectiveness of heliox in acute respiratory distress syndrome. Ann Thorac Med. 2013; 8(1): 46-52.
22. Baltaci SB, Mogulkoc R, Baltaci AK, Emsen A, Artac H. The effect of zinc and melatonin supplementation on immunity parameters in breast cancer induced by DMBA in rats. Arch Physiol Biochem. 2018; 124(3): 247-52.
23. Richardson JTE. Eta squared and partial eta squared as measures of effect size in educational research. Educational Research Review. 2011; 6(2): 135-47.
24. Cohen J. Statistical power analysis for the behavioral sciences, 2nd ed. Hillsdale, NJ: Lawrence Erlbaum Associates Inc; 1988.
25. Miceli N, Trovato A, Marino A, Bellinghieri V, Melchini A, Dugo P. et al. Phenolic composition and biological activities of Juniperus drupacea Labill. berries from Turkey. Food Chem Toxicol. 2011; 49(10): 2600-8.
26. Özkan K, Karadağ A, Sağdıç O. Determination of the in vitro bioaccessibility of phenolic compounds and antioxidant capacity of Juniper berry (Juniperus drupacea Labill.) pekmez. Turk J Agric For. 2021; 45(3): 290-300.

27. Sohn SH, Jang H, Kim Y, Jang YP, Cho SH, Jung H, et al. The effects of Gamijinhae-tang on elastase/lipopolysaccharide-induced lung inflammation in an animal model of acute lung injury. BMC Complement Altern Med. Med. 2013; 13: 176.

28. Ahmed S. Green tea polyphenol epigallocatechin 3-gallate in arthritis: progress and promise. Arthritis Res Ther. 2010; 12(2): 208.

29. Cukic V. The changes of arterial blood gases in COPD during four-year period. Med Arch. 2014; 68(1): 14-8.

30. Sohrabi F, Dianat M, Badavi M, Radan M, Mard SA. Gallic acid suppresses inflammation and oxidative stress through modulating Nrf2-HO-1-NF-κB signaling pathways in elastase-induced emphysema in rats. Environ Sci Pollut Res Int. 2021; 28(40): 56822-34.

31. Jiang H, Zhu Y, Xu H, Sun Y, Li Q. Activation of hypoxia-inducible factor-1alpha via nuclear factor-kappa B in rats with chronic obstructive pulmonary disease. Acta Biochim Biophys Sin (Shanghai). 2010; 42(7): 483-8.

32. Tamimi A, Serdarevic D, Hanania NA. The effects of cigarette smoke on airway inflammation in asthma and COPD: therapeutic implications. Respir Med. 2012; 106(3): 319-28.

33. Barnes PJ. Inflammatory mechanisms in patients with chronic obstructive pulmonary disease. J Allergy Clin Immunol. 2016; 138(1): 16-27.

34. Vestbo J, Hurd SS, Agustí AG, Jones PW, Vogelmeier C, Anzueto A, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med. 2013; 187(4): 347-65.

35. Hurst JR, Vestbo J, Anzueto A, Locantore N, Müllerova H, Tal-Singer R, et al.; Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) Investigators. Susceptibility to exacerbation in chronic obstructive pulmonary disease. N Engl J Med. 2010; 363(12): 1128-38.

36. Keatings VM, Collins PD, Scott DM, Barnes PJ. Differences in interleukin-8 and tumor necrosis factor-alpha in induced sputum from patients with chronic obstructive pulmonary disease or asthma. Am J Respir Crit Care Med. 1996; 153(2): 530-4.

37. Pauwels RA, Buist AS, Calverley PM, Jenkins CR, Hurd SS; GOLD Scientific Committee. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) Workshop summary. Am J Respir Crit Care Med. 2001; 163(5): 1256-76.

38. Barnes PJ. Immunology of asthma and chronic obstructive pulmonary disease. Nat Rev Immunol. 2008; 8(3): 183-92.

39. Freeman CM, Curtis JL, Chensue SW. CC chemokine receptor 5 and CXC chemokine receptor 6 expression by lung CD8+ cells correlates with chronic obstructive pulmonary disease severity. Am J Pathol. 2007; 171(3): 767-76.

40. Freeman CM, Martinez FJ, Han MK, Washko GR Jr, McCubbrey AL, Chensue SW, et al. Lung CD8+ T cells in COPD have increased expression of bacterial TLRs. Respir Res. 2013; 14(1): 13.

41. Celli BR, MacNee W; ATS/ERS Task Force. Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. Eur Respir J. 2004; 23(6): 932-46.

42. Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, et al.; Global Initiative for Chronic Obstructive Lung Disease. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med. 2007; 176(6): 532-55.

43. Rehman AU, Hassali MAA, Abbas S, Ali IABH, Harun SN, Muneswarao J, et al Pharmacological and non-pharmacological management of COPD; limitations and future prospects: a review of current literature. J Public Health. 2020; 28(21): 357-66.

44. Burge PS, Lewis SA. So inhaled steroids slow the rate of decline of FEV1 in patients with COPD after all? Thorax. 2003; 58(11): 911-3.

45. Calverley PM, Boonsawat W, Cseke Z, Zhong N, Peterson S, Olsson H. Maintenance therapy with budesonide and formoterol in chronic obstructive pulmonary disease. Eur Respir J. 2003; 22(6): 912-9.

46. Bousquet J, Clark TJ, Hurd S, Khaltaev N, Lenfant C, O'byrne P, Sheffer A. GINA guidelines on asthma and beyond. Allergy. 2007; 62(2): 102-12.

47. Field SK. Roflumilast: an oral, once-daily selective PDE-4 inhibitor for the management of COPD and asthma. Expert Opin Investig Drugs. 2008; 17(5): 811-8.

48. Crespo MI, Gràcia J, Puig C, Vega A, Bou J, Beleta J, et al. Synthesis and biological evaluation of 2,5-dihydropyrazol. Bioorg Med Chem Lett. 2000; 10(23): 2661-4.

49. Dahl R. Systemic side effects of inhaled corticosteroids in patients with asthma. Respir Med. 2006; 100(8): 1307-17.

50. Paulin MV, Caney SM, Cosford KL. Online survey to determine client perceptions of feline chronic lower airway disease management: Response to therapy, side effects and challenges encountered. J Feline Med Surg. 2022; 24(12): 1219-27.

51. Bodum KS, Hjerrild BE, Dalsgaard S, Rubak SLM. Behavioural side effects of inhaled corticosteroids among children and adolescents with asthma. Respir Res. 2022; 23(1): 192.

52. Kacem R. Phenolic compounds from medicinal plants as Natural anti-elastase products for the therapy of pulmonary emphysema. J Med Plants Res. 2013; 7(44): 3499-507.

53. Kim YJ, Uyama H, Kobayashi S. Inhibition effects of (+)-catechin-aldehyde polycondensates on proteinases causing proteolytic degradation of extracellular matrix. Biochem Biophys Res Commun. 2004; 320(1): 256-61.

54. Baines KJ, Backer V, Gibson PG, Powell H, Porsbjerg CM. Investigating the effects of arctic dietary intake on lung health. Eur J Clin Nutr. 2015; 69(11): 1262-6.

55. Siedlinski M, Boer JM, Smit HA, Postma DS, Boezen HM. Dietary factors and lung function in the general population: wine and resveratrol intake. Eur Respir J. 2012; 39(2): 385-91.

56. Zhai T, Li S, Hu W, Li D, Leng S. Potential micronutrients and phytochemicals against the pathogenesis of chronic obstructive pulmonary disease and lung cancer. Nutrients. 2018; 10(7): 813.

57. Yaglioglu AS, Eser F. Screening of some Juniperus extracts for the phenolic compounds and their antiproliferative activities. S Afr J Bot. 2017; 113: 29-33.

58. Sartor L, Pezzato E, Garbisa S. (-)Epigallocatechin-3-gallate inhibits leukocyte elastase: potential of the phyto-factor in hindering inflammation, emphysema, and invasion. J Leukoc Biol. 2002; 71(1): 73-9.

59. Wang M, Zhong H, Zhang X, Huang X, Wang J, Li Z, et al. EGCG promotes PRKCA expression to alleviate LPS-induced acute lung injury and inflammatory response. Sci Rep. 2021; 11(1): 11014.

60. Fu YS, Kang N, Yu Y, Mi Y, Guo J, Wu J, et al. Polyphenols, flavonoids and inflammasomes: the role of cigarette smoke in COPD. Eur Respir Rev. 2022; 31(164): 220028.

61. Fatma Z, Luis MBJ, Antonio EDLMYZ, Laura SR, Pherraez HT, María GMJ, et al. An aqueous pomegranate peel extract (Punica granatum) protect against Elastase-induced pulmonary emphysema in Sprague Dawley rats model. Braz J Pharm Sci. 2021; 57(5): e18972.

62. Singla E, Dharwal V, Naura AS. Gallic acid protects against the COPD-linked lung inflammation and emphysema in mice. Inflamm Res. 2020; 69(4): 423-34.

63. Akbulut M, Çoklar H, Özen G. Rheological characteristics of Juniperus drupacea fruit juice (pekmez) concentrated by boiling. Food Sci Technol Int. 2008; 14(4): 321-8.

64. Shah S, Kejariwal C, Mahawar K, Kumawat VK, Singh, L, Shringirishi M, et al. COPD diseases management with herbal drugs: a review. Int J Cur Pharm Rev Res. 2023; 15(7): 208-11.