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Anti-inflammatory and anti-oxidant efficacy of origanum species and honey in a rat model of COPD

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A B S T R A C T

Objective(s): Chronic obstructive pulmonary disease (COPD) is a progressive airway condition characterized by chronic inflammation, oxidative stress, and tissue remodeling. Conventional treatments offer limited benefits in halting disease progression, prompting increased interest in natural agents with antioxidant and anti-inflammatory properties. This study aimed to evaluate the therapeutic effects of *Origanum onites* L., *Origanum minutiflorum*, and honey in a rat model of elastase-induced COPD.

Materials and Methods: Thirty-eight female Wistar albino rats were divided into five groups: Control, COPD, *O. onites* L. treatment, *O. minutiflorum* treatment, and honey treatment. COPD was induced via intratracheal porcine pancreatic elastase. Treatments were administered orally for 15 days. Serum levels of IL-1α, IL-33, and HMGB1, and lung homogenate levels of MMP-9 and TIMP-1 were measured by ELISA. T cell subtypes (CD3$^+$, CD4$^+$, CD8$^+$) were analyzed by flow cytometry. Blood gas parameters were evaluated, and histopathological scoring was performed.

Results: *Origanum onites* L., *O. minutiflorum*, and honey exhibited modulatory effects on inflammatory and oxidative markers. O. minutiflorum significantly reduced IL-1α and IL-33 levels ($P<0.05$) and decreased MMP-9 expression. The honey group showed increased TIMP-1 levels and significantly improved blood oxygenation (pO$_2$ and sO$_2$). CD3$^+$ and CD8$^+$ T cell percentages were significantly higher in the treatment groups compared to the COPD group. Emphysema scores were significantly lower in the honey group ($P<0.05$), and lymphoid aggregates were observed only in the COPD group.

Conclusion: The findings indicate that Origanum species and honey may have therapeutic potential for COPD by modulating inflammation, the immune response, and the protease-antiprotease balance. Further molecular and clinical studies are warranted.

Introduction

Chronic Obstructive Pulmonary Disease (COPD) is a heterogeneous lung disorder characterized by persistent respiratory symptoms and airflow restriction, imposing a substantial burden on individuals and healthcare systems (1). Its pathogenesis involves chronic inflammation of the small airways, primarily bronchiolitis, accompanied by structural changes such as mucus hypersecretion, luminal obstruction, and tissue destruction. The accumulation of immune cells, including neutrophils, CD8$^+$ T lymphocytes, and macrophages, initiates an inflammatory cascade that contributes to tissue remodeling and pulmonary vascular alterations, potentially leading to complications such as pulmonary hypertension and right heart failure (2-4).

Current treatment approaches, including bronchodilators, corticosteroids, and phosphodiesterase-4 inhibitors like roflumilast, aim to relieve symptoms and

reduce exacerbations and mortality risk, although they are often insufficient to halt disease progression (5). As a result, there is growing interest in complementary and herbal therapies with anti-inflammatory and bronchodilatory potential (6).

In recent years, thyme species and their derivatives have gained attention as alternative therapeutic options in inflammatory pulmonary diseases such as COPD. *Origanum onites* L. is a steno-endemic species native to the Eastern Mediterranean region and has been widely used in traditional Turkish medicine, primarily due to its high carvacrol content (7, 8). Its essential oil is rich in oxygenated monoterpenes, with carvacrol and thymol being the primary constituents responsible for its potent antioxidant, anti-inflammatory, and antimicrobial effects (9-11). In addition, the plant contains high levels of phenolic acids such as rosmarinic, caffeic, gallic, and ferulic acids, along

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with flavonoids, all of which contribute to its therapeutic potential (12, 13).

Origanum minutiflorum is an endemic species that grows at high altitudes in the Taurus Mountains of southern Turkey. It has been traditionally used to treat respiratory and gastrointestinal ailments (14). The essential oil of *O. minutiflorum* contains up to 90% carvacrol, making it the richest among Origanum species in this compound. It also contains p-cymene and γ-terpinene, which exhibit antimicrobial, antioxidant, anti-inflammatory, and antiproliferative properties (15-17). In prior studies, *Origanum onites* L. and *O. minutiflorum* extracts have been shown to exert anti-inflammatory and antioxidant effects by modulating key cytokines such as IL-1α and HMGB1, as well as proteolytic enzymes, such as MMP-9, thereby reducing tissue destruction in COPD-like models (12, 16, 17). Similarly, natural honey has been demonstrated to attenuate airway inflammation, improve oxidative balance, and enhance immune regulation in experimental models of asthma and lung injury, largely through its polyphenolic content (18, 19). These findings support the potential role of these natural agents as complementary therapies in inflammatory pulmonary diseases. In this context, the present study aimed to investigate the therapeutic effects of *Origanum onites* L., *O. minutiflorum*, and honey in a rat model of elastase-induced COPD.

Materials and Methods

Plant and honey source

Origanum onites L. was collected from Hadim/Taşkent district of Konya province in Turkey, while *O. minutiflorum* was collected from Hatay/Turkey. The honey samples were obtained from local producers in Muğla/Turkey. Thyme samples were shade-dried and extracted using water. The plant materials used in this study, *O. onites* L. and *O. minutiflorum* O. Schwarz & P.H. Davis, were identified based on their taxonomic and morphological characteristics described in volume 7 of "Flora of Turkey and the East Aegean Islands," on pages 166-167 and 179-180, respectively. Both species are native to Turkey, with *O. minutiflorum* being an endemic species.

Plant extraction

A total of 30 g of each thyme species was extracted with 300 ml of distilled water using a Soxhlet apparatus at 90-95 °C for eight hours. After extraction, solvents were removed using a rotary evaporator, and the extracts were sonicated and transferred to vials. These vials were dried in a water bath for one week.

Chemical analyses

• Volatile oil composition was determined by GC-MS following hydrodistillation.
• Phenolic compounds and organic acids were analyzed using HPLC.
• Antioxidant activity was assessed via the DPPH radical scavenging method.

Animals and experimental groups

Thirty-eight 6-month-old female Wistar Albino rats, weighing approximately 250 g, were used. Female rats were selected for this study due to their more docile nature and lower levels of aggression compared to males. Male rats are known to display territorial and dominance behaviors, which

can lead to increased stress and variability in physiological parameters. These behavioral fluctuations may compromise the consistency of experimental outcomes. In contrast, female rats tend to exhibit more stable social interactions, allowing for a more homogeneous experimental model. For these reasons, female Wistar albino rats were preferred in this study. The sample size was estimated with reference to prior similar studies using elastase-induced COPD models, to ensure sufficient statistical power for detecting differences in biochemical and histopathological parameters. Animals showing signs of infection, injury, or respiratory distress at baseline were excluded. No animals were removed from the study after the experiment began. The primary outcome measure used to justify the sample size was serum IL-1α concentration, based on its reported sensitivity to reflect inflammatory changes in COPD models in previous studies. Animals were housed under a 12-hour light/dark cycle and randomly assigned into five groups (Figure 1). A permanent cage labeling system was used to ensure accurate identification of groups, and each rat was also individually marked with a non-toxic dye for easy distinction.

• Control (n=6)
• COPD group (elastase-induced, untreated) (n=8)
• COPD+*O. onites* L. treatment (n=8)
• COPD+*O. minutiflorum* treatment (n=8)
• COPD+Honey treatment (n=8)

The COPD model was induced via intratracheal administration of porcine pancreatic elastase (PPE)(Sigma Chemical, St. Louis, MO, USA) at a dose of 55 U/100 g (20). Anesthesia was induced with a combination of xylazine (10 mg/kg) and ketamine (80 mg/kg) to minimize pain and distress during procedures. Blood gas analysis and histopathological examination were used to confirm model induction. Dose selection for Origanum extracts was guided by previous studies using aqueous oregano preparations (150-350 mg/kg/day, 14 days) and hydroalcoholic extracts (400 mg/kg/day, 7 days) that showed pulmonary protection (21-23). In addition, Origanum floribundum, with a chemical profile similar to our species, was reported to be safe up to 2000 mg/kg in Wistar rats (24). Based on these data, we administered an aqueous extract at 500 mg/kg/day for 15 days.
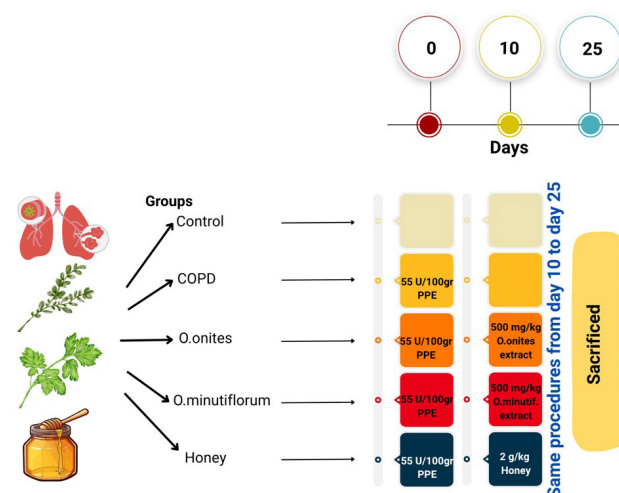

Figure 1. Experimental design and treatment timeline of the study to evaluate the effects of Origanum extracts and honey in a porcine pancreatic elastase (PPE) induced chronic obstructive pulmonary disease (COPD) rat model

Honey has been reported to exert anti-diabetic and antioxidant effects in rodent models at doses of about 1-2.5 g/kg. Therefore, we used 2 g/kg/day for 15 days in our study (18, 25).

Histopathological evaluation

Lung tissues were fixed in 10% formalin, stained with hematoxylin and eosin (H&E)(Merck, Germany), and examined under an Olympus BX53 light microscope (Olympus Corporation, Tokyo, Japan). In histopathological evaluation, findings such as emphysema, inflammation, lymphoid accumulation, fibrosis, necrosis, and granuloma formation were scored semiquantitatively, graded from 0 (absent) to 3 (severe), based on their severity and prevalence. The semi-quantitative scoring system for histopathological findings is shown in Table 1.

Biochemical and immunological measurements

COPD was induced in rats via intratracheal administration of PPE. On the 3rd day after induction, venous blood gas analysis was performed in all rats using the tail vein, serving as a pre-treatment reference point. On day 10, one randomly selected rat from the COPD group and one from the control group were sacrificed; cardiac blood was collected for blood gas analysis, and lung tissues were harvested for histopathological confirmation of the COPD model. Typical signs of emphysema and inflammation were observed in the COPD rat, while control lungs appeared normal. Following confirmation, the remaining rats in the treatment groups received *Origanum onites* L., *O. minutiflorum*, or honey by oral gavage for 15 consecutive days. At the end of the treatment period, blood samples were collected from the heart under anesthesia for the final blood gas analysis. Venous blood gas analysis was performed using an ABL9 blood gas analyzer (Radiometer Medical ApS, Denmark). The parameters measured included pH, partial carbon dioxide pressure (pCO_2), partial oxygen pressure (pO_2), bicarbonate (HCO_3^-), and oxygen saturation percentage ($sO_2\%$). Due to expected individual variability among animals, pre- and post-treatment values were statistically compared within each group using the paired samples t-test, and results were considered significant at $P < 0.05$.

$CD3^+$, $CD4^+$, and $CD8^+$ cell percentages were determined by flow cytometry. EDTA blood samples taken from rats were stained with Anti-Rat $CD3^+$, $CD4^+$, and CD8+ monoclonal antibodies (Elabscience, manufacturer country not specified), and $CD3^+$, $CD4^+$, and $CD8^+$ cell percentages were determined using the Beckman Coulter CytoFLEX flow cytometry device.

Serum IL-1, IL-33, HMGB1, and lung homogenate MMP-9 and TIMP-1 levels were measured using ELISA kits (BT LAB, China). Samples and standards were prepared as specified in the kit. After incubation and washing, the reactions were stopped, and absorbance values were recorded.

The researchers performing the histopathological, biochemical, and immunological evaluations were blinded to the group allocations to minimize assessment bias.

Statistical analysis

All analyses were performed using SPSS version 29. The Shapiro-Wilk test was used to assess normality, and the Levene test was used to assess homogeneity of variance. Parametric data were analyzed using ANOVA and Tukey's HSD post hoc test, while non-parametric data were evaluated using the Kruskal-Wallis H test. A *P*-value <0.05 was considered statistically significant. R Statistical Language version 4.5.0 (www.r-project.org) was used to visualize blood gas results.

Results

Chemical and phenolic composition of O. onites L. and O. minutiflorum

As a result of the analysis of the essential oil of *O. onites* L., the highest amount of carvacrol (66.75%) was detected. Thymoquinone was second with 29.46%. As a result of the analysis of the essential oil of *O. minutiflorum*, the highest amount of carvacrol (77.56%) was detected, followed by thymoquinone with 11.32%. Thymol was detected at 7.69%. Other components found in the essential oil of *O. onites* L. and *O. minutiflorum* are shown in Table 2.

As a result of the phenolic substance analysis of *O. onites* L., the highest levels of rosmarinic acid (2295.4 ppm), hesperidin (180.3 ppm), luteolin (174.8 ppm), and catechin (95.5 ppm) were detected. As a result of the phenolic substance analysis of *O. minutiflorum*, the highest levels of rosmarinic acid (885.8 ppm) and kaempferol (148.6 ppm) were detected. In addition, as shown in Table 3, substances with known antioxidant activity, such as gallic acid, epicatechin, and benzoic acid, were detected in both origanum species.

Free radical scavenging activity of the honey

According to Table 4; radical scavenging activity was determined as 40.98% in the honey sample at a

Table 1. Semi-quantitative scoring system for histopathological findings rat lung tissues

Score	Emphysema	Inflammation	Lymphoid aggregation	Fibrosis, necrosis, granuloma
0	None: Normal alveolar structure	None: No signs of inflammation	None: No lymphoid accumulation	None: No pathological findings
1	Mild: Minimal alveolar enlargement	Mild: Slight inflammatory cell infiltration	Mild: Small and irregular clusters	Mild: Limited and focal pathological changes
2	Moderate: Noticeable alveolar destruction	Moderate: Moderate infiltration and disruption	Moderate: Localized lymphoid aggregations	Moderate: More extensive but confined changes
3	Severe: Widespread alveolar destruction	Severe: Diffuse infiltration and tissue damage	Severe: Dense and organized lymphoid nodules	Severe: Extensive or structurally disruptive changes

Table 2. Chemical composition of the essential oils of *Origanum onites* L. and *Origanum minutiflorum*

Chemical Component	*Origanum onites* Area (%)	*Origanum minutiflorum* Area (%)
Benzeneacetaldehyde	0.09	
4-Terpineol	0.08	0.70
Thymoquinone	29.46	11.32
2-Acetyl-4,4-dimethyl-cyclopent-2-enone	0.49	
Thymol	0.83	7.69
Carvacrol	66.75	77.56
2,4-Di-tert-butylphenol	0.13	0.13
Heptadecane	0.25	0.20
Nonadecane	1.80	1.20
Pentacosane	0.13	0.07
Octen-3-ol		0.53
Octan-3-ol		0.18
Simol 47804		0.05
1,2,3-Trimethyl-cyclopent-2-enecarboxaldehyde		0.03
Borneol		0.04
Dihydrocarvone		0.27
Heptadecanol		0.03

Table 3. Phenolic compound content of *Origanum onites* L. and *Origanum minutiflorum* (ppm)

Phenolic Compound	*Origanum minutiflorum* (ppm)	*Origanum onites* L. (ppm)
Gallic acid	26.2	16.9
Protocatechuic acid	10.9	7.6
Catechin	36.3	95.5
p-Hydroxybenzoic acid	3.1	–
Chlorogenic acid	2.9	14.3
Caffeic acid	41.8	52.8
Epicatechin	6.3	22.3
Ferulic acid	10.1	26.3
Benzoic acid	28.9	8.5
Hesperidin	115.0	180.3
Rosmarinic acid	885.8	2295.4
Cinnamic acid	2.6	–
Quercetin	–	50.4
Luteolin	20.3	174.8
Kaempferol	148.6	56.5

Table 4. Radical scavenging activity (%RSA) of honey samples at different

| Concentration (mg/ml)	% Radical Scavenging Activity (Mean ± SD)	IC$_{50}$ (=SC$_{50}$) (mg/ml)
5	40.98 ± 0.38	
10	47.90 ± 0.06	15.301
20	52.00 ± 0.09	
40	59.55 ± 0.16	

concentration of 5 mg/ml, 47.90% in the honey sample at a concentration of 10 mg/ml, 52% in the honey sample at a concentration of 20 mg/ml, and 59.55% in the honey sample at a concentration of 40 mg/mL. According to the results, the IC50 (SC50) value was 15.301, as determined from the graph in Figure 2.

Flow cytometry analyses (CD3$^+$, CD4$^+$, CD8$^+$)

CD3$^+$ T cell percentages differed significantly among groups (P=0.017), with the highest expression in the *O. minutiflorum* group and the lowest in the COPD group. CD8$^+$ levels also showed significant group differences (P=0.03), while CD4$^+$ levels did not differ significantly (P=0.33)(Table 5).

Blood gas parameters

Blood gas parameters were evaluated before and after the experimental period in each group. Notably, although
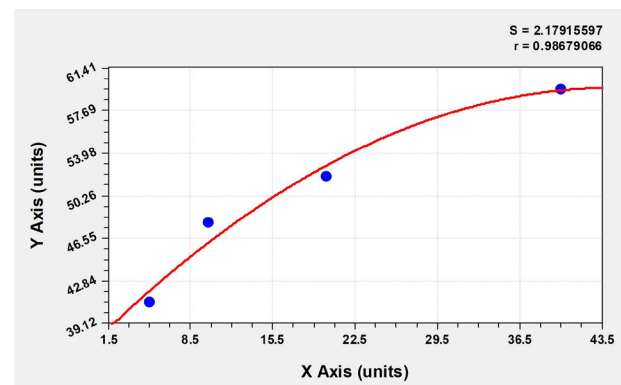
Figure 2. Dose-response curve of the radical scavenging activity of honey (DPPH assay)

Table 5. Distribution of CD3$^+$ and CD8$^+$ T cell percentages among experimental groups of rats

|	Control (n=6) Median IQR [Q1-Q3]	COPD (n=7) Median IQR [Q1-Q3]	*Origanum onites* (n=8) Median IQR [Q1-Q3]	*Origanum minutiflorum* (n=7) Median IQR [Q1-Q3]	Honey (n=7) Median IQR [Q1-Q3]	*P*-value
CD3$^+$ (%)	50,86 [0 65,86]^a	74,87 [66,06 83,42]^b	59,36 [49,83 61,07]^a	57,87 [41,88 62,38]^a	60,52 [52,03 68,23]^a	*0,017**
CD4$^+$ (%)	31,28 [0 46,82]	42,12 [38,91 51,45]	41,18 [35,82 43,96]	40,68 [28,79 43,31]	40,37 [35,91 46,35]	*0,334*
CD8$^+$ (%)	17,11 [0 18,61]^a	21,69 [19,34 42,52]^b	14,83 [13,24 15,71]^a	13,83 [10,53 18,95]^a	17,38 [15,09 22,19]^a	*0,033**
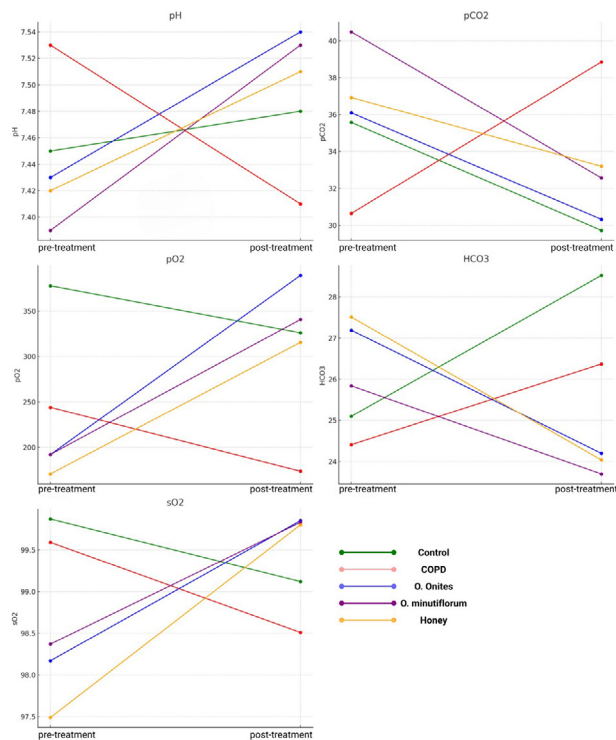
Figure 3. Line graphs showing group means of pH, pCO$_2$, pO$_2$, HCO$_3^-$, and sO$_2$ values measured at baseline (3 days after COPD induction) and after the 15-day treatment period

COPD was induced using the same protocol across all groups, baseline (pre-treatment) values of pCO$_2$ and pO$_2$ varied slightly between groups, possibly due to individual physiological differences. In the COPD group, disease induction led to a significant decrease in pH (7.53±0.04 to 7.41±0.05, P=0.005) and an increase in pCO$_2$ (30.64±6.25 to 38.86±5.27 mmHg, P=0.018). Treatments with *O. onites* L., *O. minutiflorum*, and honey significantly improved pH levels (7.43±0.04 to 7.54±0.08, P=0.015; 7.39±0.03 to 7.53±0.07, P=0.012; 7.42±0.05 to 7.51±0.05, P=0.007, respectively). pCO$_2$ levels significantly decreased in the *O. onites* L. (36.1±4.48 to 30.32±7.9 mmHg, P=0.048) and *O. minutiflorum* (40.49±5.39 to 32.57±4.32 mmHg, P=0.001)

groups. pO$_2$ levels significantly increased after treatment in the *O. onites* L. (192.12±90.86 to 389±76.95 mmHg, P=0.012) and honey (170.86±53.32 to 315.43±56.29 mmHg, P=0.013) groups. sO$_2$ levels were significantly elevated only in the honey group (97.49±1.79 to 99.8±0.13, P=0.024) (Figure 3). All statistical comparisons were performed using a paired samples t-test (P<0.05 considered significant). It is important to note that all animals were exposed to identical procedures; however, minor baseline variations in blood gas parameters were observed, possibly due to individual physiological differences. Therefore, pre- and post-treatment comparisons were statistically evaluated within each group, rather than between groups, to assess treatment effects accurately.

Cytokines and protein markers

The amount of IL-1α was significantly elevated in the COPD group (46,94±5,01 ng/l), while treatment with *O. minutiflorum* resulted in the lowest IL-1α amount (33,86±4,35 ng/l)(P<0.001). IL-33 amounts were also highest in the COPD group (142,81±18,71 ng/l), and significantly lower in the *O. minutiflorum* (96,96±28,38 ng/l) and honey groups (100,42±28,57 ng/l)(P=0.004).

The highest HMGB1 amount was observed in the COPD group (6,05±0,45 ng/ml), while all treatment groups showed a reduction, especially *O. minutiflorum* (4,36±0,69 ng/ml)(P=0.003). MMP-9 amounts increased in the COPD group (2,4±0,28 ng/ml) and significantly decreased in the treatment groups, with the lowest amount again in *O. minutiflorum* (1,50±0,23 ng/ml)(P<0.001).

TIMP-1 amounts were significantly higher in the honey group (1,93±0,25 ng/ml) and lowest in the COPD group (1,41±0,14 ng/ml), with moderate increases observed in the other treatment groups (P=0.021)(Table 6). These findings suggest that *O. minutiflorum* notably reduced inflammatory and tissue-damage markers, while honey appeared to enhance anti-proteolytic response.

Pearson correlation analysis was performed to evaluate the relationships between HMGB1, IL-1α, IL-33, MMP-9, and TIMP-1 levels in all groups. The results revealed a positive correlation between HMGB1 and MMP-9, as well as between IL-1α and both IL-33 and MMP-9.

Table 6. Distribution of serum HMGB1, IL-1α, IL-33, MMP-9 and TIMP-1 levels among experimental groups of rats

	Control (n–6)	COPD (n–7)	*Origanum onites* (n=8)	*Origanum minutiflorum* (n=7)	Honey (n–7)	*P*-value
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
HMGB1 (ng/ml)	4,52±0,75 [a]	6,05±0,45 [b]	4,49±0,84 [a]	4,36±0,69 [a]	4,68±0,9 [a]	*0,003**
IL-1α (ng/l)	42,9±5,66 [ab]	46,94±5,01 [a]	37,62±2,95 [bc]	33,86±4,35 [c]	38,41±4,32 [bc]	*<0,001**
IL-33 (ng/l)	130,99±20,56 [ab]	142,81±18,71 [b]	113,68±8,58 [ab]	96,96±28,38 [a]	100,42±28,57 [a]	*0,004**
MMP-9 (ng/ml)	1,52±0,3 [a]	2,4±0,28 [b]	1,58±0,3 [a]	1,50±0,23 [a]	1,61±0,2 [a]	*<0,001**
TIMP-1 (ng/ml)	1,49±0,33 [ab]	1,41±0,14 [a]	1,64±0,29 [ab]	1,59±0,22 [ab]	1,93±0,25 [b]	*0,021**

[1] One-way ANOVA
[2] *P*<0.05 was considered statistically significant
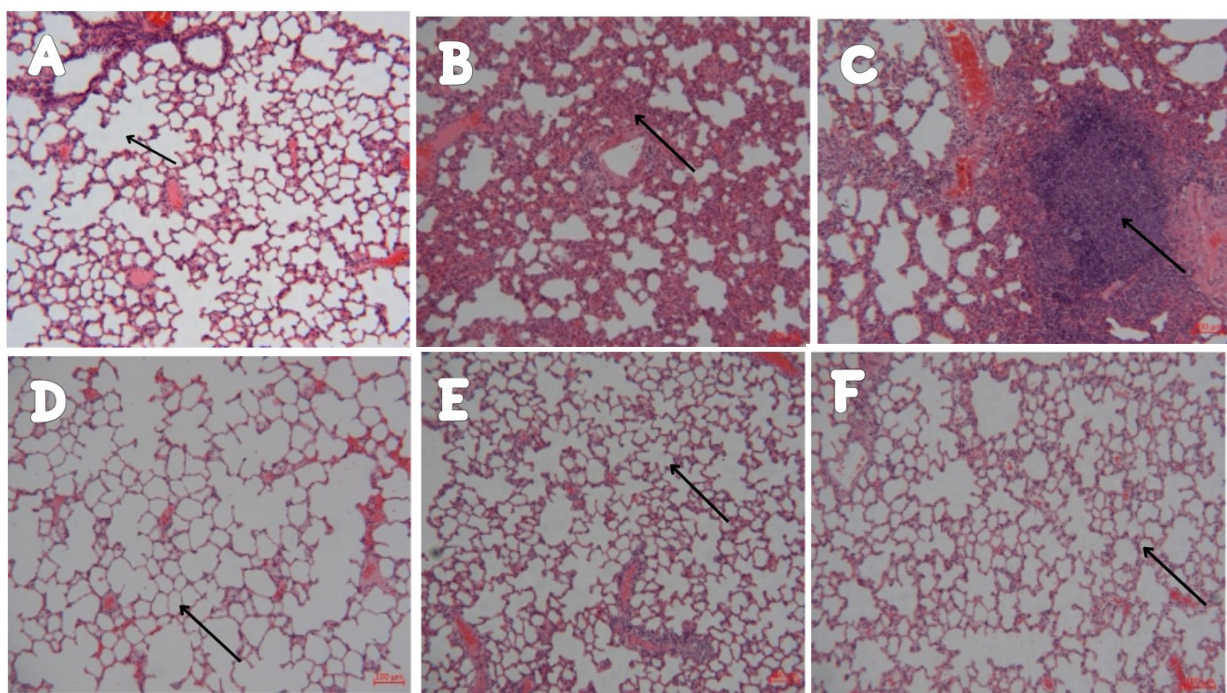[3] Values sharing the same letter are not significantly different

Figure 4. Histopathological images of lung tissues stained with hematoxylin and eosin (H&E) from the sham group and treatment groups following PPE-induced COPD in rats. In the sham group, representative images show emphysematous changes (A), inflammatory cell infiltration (B), and lymphoid accumulation (C) under light microscopy at 100× magnification. Panels (D), (E), and (F) represent lung tissue sections from rats treated with *Origanum onites* L., *Origanum minutiflorum*, and honey, respectively, all visualized at 100× magnification

Histopathological findings

Histopathological analysis demonstrated (Figure 4):
• The honey group exhibited the lowest emphysema score, whereas the COPD group had the highest (*P*<0.05)(Table 7).
• Inflammation scores were decreased in all treatment groups, although the change was not statistically significant (*P*>0.05)(Table 7).
• Lymphoid aggregates were observed only in the COPD group (Table 7).
• No fibrosis or granuloma formations were detected in any group.

Discussion

In the PPE-induced COPD model, characteristic alterations such as hypoxemia, hypercapnia, and acidosis are typically observed due to impaired gas exchange and ventilatory dysfunction. Consistent with these features, our study demonstrated significantly reduced pO_2 and pH levels, and increased pCO_2 in the COPD group (*P*=0.005) (26). These findings mirror previous reports indicating that elastase exposure leads to hypoventilation and respiratory acidosis.

Therapeutic interventions such as dexmedetomidine (27), low molecular weight Heparin (28), baicalin (29), gallic acid (30), and heliox therapy (31) have been shown to reverse these changes. In alignment, our study found significant increases in pO_2 in the *O. onites* (*P*=0.012) and honey (*P*=0.013) treated groups, along with significant

Table 7. Histopathological evaluation scores of inflammation, lymphoid aggregation, and emphysema in the experimental groups of rats

	Inflammation	Emphysema	Lymphoid aggregation
	Mean (%95 CI)	Mean (%95 CI)	Mean (%95 CI)
Control (n=6)	1,29 (0,83-1,73)	0,71 (0,26-1,17) [ab]	0 (0-0)[a]
COPD (n=7)	1,57 (1,08-2,07)	0,86 (0,22-1,5) [a]	1,57 (0,17-2,97)[b]
Origanum onites (n=8)	1,12 (0,43-1,82)	0,5 (0,05-0,95) [ab]	0 (0-0) [a]
Origanum Minutiflorum (n=7)	1,14 (0,5-1,78)	0,29 (0-0,73) [ab]	0 (0-0) [a]
Honey (n=7)	1 (0,24-1,76)	0 (0-0) [b]	0 (0-0) [a]
P-value	0,597	0,024*	0,000*

* One-way ANOVA
[1] Values are presented as mean (95% confidence interval, lower–upper limits)
[2] Values sharing the same letter are not significantly different
[3] *P*<0.05 was considered statistically significant

decreases in pCO_2 in the *O. onites* (*P*=0.048) and *O. minutiflorum* (*P*=0.001) groups. Although pCO_2 reduction in the honey group did not reach statistical significance (*P*=0.314), all treatment groups demonstrated a significant pH increase (*P*<0.05), indicating partial correction of acidosis. Moreover, sO_2 improvement in the honey group (*P*=0.024) is consistent with prior evidence of enhanced oxygenation following antioxidant interventions.

T cell analysis revealed significantly elevated CD3$^+$ and CD8$^+$ T cell levels in the COPD group, indicating heightened immune activation. CD8$^+$ T cells have been extensively implicated in COPD pathogenesis, contributing to cytotoxic damage and tissue remodeling (32,33). Treatment with *O. onites, O. minutiflorum*, and honey significantly reduced CD8$^+$ T cell levels, while CD4$^+$ levels remained unchanged, highlighting the specific immunomodulatory effects targeting cytotoxic lymphocyte populations.

HMGB1, IL-1α, and IL-33 are damage-associated molecular pattern (DAMP) molecules and alarmin cytokines that initiate and sustain pulmonary inflammation. Elevated HMGB1 levels, often associated with IL-1β and epithelial injury, have been reported in COPD patient sputum and serum (34,35). Our findings showed that HMGB1 was significantly elevated in the COPD group but markedly reduced in all treatment groups, suggesting that honey and Origanum extracts effectively suppress inflammation at a molecular level.

Similarly, IL-1α and IL-33, both epithelial-derived alarmins, were significantly elevated in the COPD group and most strongly suppressed in the *O. minutiflorum* and honey-treated groups. These results suggest that these treatments interfere with the upstream signaling events that drive chronic airway inflammation, potentially via NF-κB and TLR4 inhibition and Nrf2 pathway activation. Phytochemicals such as carvacrol, thymol, and thymoquinone, abundantly present in Origanum species, likely contribute to these anti-inflammatory effects.

MMP-9, a protease responsible for extracellular matrix degradation, was significantly elevated in the COPD group, consistent with prior studies (36, 37). All treatment groups exhibited a significant reduction in MMP-9 levels, with *O. minutiflorum* producing the most pronounced decrease. TIMP-1, an endogenous inhibitor of MMPs, was lowest in the COPD group but significantly increased only in the honey group, indicating honey's unique potential to restore protease-antiprotease balance. This restoration is critical in preventing alveolar destruction and tissue remodeling, hallmarks of advanced COPD.

Histopathological analysis further supported these findings. The COPD group exhibited the most severe emphysema, alveolar wall destruction, and inflammatory cell infiltration. In contrast, the honey-treated group showed the most marked histological improvement, with significantly lower emphysema scores and minimal inflammation. Notably, lymphoid aggregates were detected only in the COPD group, suggesting that treatment mitigated chronic immune activation. Absence of fibrosis or granulomas across all groups may reflect the relatively short experimental duration or limitations of the elastase model in mimicking late-stage COPD.

The phytochemical profiles of *O. onites* and *O. minutiflorum* provide a plausible explanation for the observed therapeutic effects. Both species are rich in carvacrol, though levels were higher in *O. minutiflorum*. *O. onites* also contains high levels of rosmarinic acid, hesperidin, and luteolin—compounds known for anti-inflammatory and antioxidant activities. Organic acids such as malic and citric acid found in these plants may also contribute to mucus modulation and metabolic support. Honey, on the other hand, contains flavonoids and phenolic acids with established roles in immune modulation and tissue repair (38, 39).

Taken together, our findings demonstrate that *O. onites, O. minutiflorum*, and honey possess significant therapeutic potential in the management of COPD. These agents reduced inflammatory cytokine levels, restored T cell balance, improved gas exchange, and mitigated tissue damage. The effects are likely mediated by multifaceted mechanisms, including inhibition of pro-inflammatory pathways (NF-κB, TLR4), activation of antioxidant responses (Nrf2), and modulation of protease activity (MMP-9/TIMP-1). These results support the potential use of Origanum species and honey as complementary therapeutic strategies in COPD management, warranting further clinical investigation to assess their long-term efficacy and safety.

Conclusion

In this study, the therapeutic effects of *O. onites* L., *O. minutiflorum*, and honey were investigated in an elastase-induced COPD model. The findings suggest that these natural products may exert protective effects against COPD pathophysiology by suppressing inflammation, modulating immune balance, and restoring the protease-antiprotease equilibrium. Notably, *O. minutiflorum* was particularly effective in reducing IL-1α and MMP-9 levels, while honey stood out by increasing TIMP-1 levels and lowering emphysema scores. These results indicate that thyme species and honey may hold potential as complementary or supportive agents in COPD treatment. Further experimental and clinical studies are needed to validate these findings and advance their therapeutic application.

Ethical Approval

Ethical approval was granted by the Selçuk University Experimental Medicine Application and Research Center Animal Experiments Local Ethics Committee with decision number 2023-26.

Availability of Data and Materials

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

Authors' Contributions

F O contributed to conceptualization, methodology, investigation, data curation, formal analysis, visualization, writing the original draft, review and editing, and project administration. Hu V contributed to supervision, validation, resources, and review and editing of the manuscript. Ha V was responsible for investigation, data curation, software, and formal analysis. B C contributed to methodology, project administration, and resources. ıh handled histopathological

analysis, visualization, and validation.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Declaration

Artificial intelligence (ChatGPT, OpenAI) was used solely for language editing purposes. All scientific content was written and verified by the authors.

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