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Protective effects of myrtenol against paraquat-induced toxicity in rats

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Abstract

Background Paraquat (PQ) is a widely used pesticide, can cause severe intoxication and respiratory failure. Myrtenol (Mrl), an essential oil derived in various plants, exhibits several biological properties, including anti-inflammatory and antioxidant activities. This study aims to investigate the protective potential of Mrl against oxidative stress and inflammation caused by PQ exposure.

Methods Twenty-five Wistar albino rats were divided into the following groups ($n=5$ in each group): a control group (treated by dimethyl sulfoxide (DMSO)), a PQ group (exposed to 54 mg/m^3 aerosol PQ), and two treatment groups that were exposed to PQ aerosol and administered oral Mrl at doses of 25 mg/kg/day and 50 mg/kg/day, respectively. The final group was exposed to PQ aerosol and treated with oral dexamethasone at a dose of 0.03 mg/kg/day. Various hematological, oxidative, inflammatory, and pathological indices were measured at the conclusion of the treatment period.

Results PQ decreases the levels or activities of superoxide dismutase (SOD), catalase (CAT), and Thiol, while increasing the levels or activities of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and malondialdehyde (MDA). Mrl restored activites of SOD, and CAT, as well as thiol levels to near-control values while reducing TNF-α, IL-6, and MDA levels. Pathological studies further confirmed the therapeutic effects of Mrl.

Conclusion The results of this study demonstrate the promising therapeutic effects of Mrl against inhaled PQ in rats.

Keywords Paraquat, Myrtenol, Inflammation, Oxidative stress, Wistar rats

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Background

Although the use of pesticides to control pests in the agricultural sector is beneficial, direct and indirect contact with them can cause various diseases in humans [1]. Paraquat (PQ) is a green liquid with a very strong and widely used herbicide from the bipyridinium group [2]. It is extremely poisonous to humans and animals. The severe toxicity of PQ in humans and animals has been witnessed by a minimal lethal dose of 30 mg/kg [3]. The lungs are likely to serve as the initial target organ for PQ toxicity [4]. Inhaled PQ is readily absorbed by lung tissues, resulting in pneumonia, pulmonary inflammation and fibrosis, pulmonary hypertension, and numerous

systemic alterations [5]. Multiple studies have been conducted to assess the pulmonary toxicity of PQ. PQ toxicity involves several mechanisms that lead to cellular damage and death such as oxidative stress, inflammatory response, apoptosis, ferroptosis, coagulation irregularities, and autophagy [6, 7]. PQ induces the production of reactive oxygen species (ROS) by interfering with the electron transport chain in cells. This leads to oxidative stress, causing damage to cellular components like lipids, proteins, and DNA [1, 8]. Decreased serum levels of catalase (CAT), superoxide dismutase (SOD), and thiol as well as increased nitric oxide (NO) and malondialdehyde (MDA) levels in animals exposed to PQ have been reported [9]. PQ inhalation has been shown to elevate total and differential white blood cell counts as well as reducing thiol content [10]. Also, PQ disrupts the Keap1/Nrf2 signaling pathway, which is crucial for cellular antioxidant defense. This disruption results in decreased Nrf2 activity and increased oxidative stress, contributing to tissue damage and diseases like pulmonary fibrosis [7]. Additionally, It has been found that PQ exposure can increase inflammatory parameters in lungs, including interleukins and tumor necrosis factor-α (TNF-α) [11, 12]. Treatments for acute poisoning with PQ include prescribing activated charcoal to reduce absorption, using devices to increase excretion such as hemoperfusion and hemodialysis, administering antioxidants, anti-inflammatories, and immunosuppressants [6]. However, the mortality rate of PQ toxicity is as high as 60–80% because of the lack of a specific antidote [13]. Many studies have shown that using agents with antioxidant properties are likely to have beneficial effects in treating PQ poisoning [14].

Myrtenol (Mrl) is a volatile compound belonging to the terpenoid family of monocyclic monoterpenes. It is one of the essential oil constituents found in various plants, including the genera Myrtus, Tanacetum, Artemisia, Hyssopus, and Rhodiola [15–17]. Several reports have demonstrated the different pharmacological properties of Mrl, including its anti-inflammatory [18] and antioxidant activities [19, 20]. Mrl has direct and indirect antioxidant properties by scavenging free radicals and increasing the activity of antioxidant enzymes such as SOD, CAT, and glutathione peroxidase (GPX) while decreasing levels of MDA [19, 21, 22]. Also, Mrl inhibits the production of pro-inflammatory cytokines such as interleukin-1β (IL-1β) and TNF-α and reduces the inflammation in conditions like allergic asthma, gastric ulcers, Gestational diabetes mellitus and orofacial pain [23–26]. Mrl also modulates the p38-MAPK pathway, which plays a crucial role in the inflammatory response [23]. The beneficial effects of Mrl on the lungs have been confirmed in various pathological conditions. Administration of Mrl via inhalation in asthmatic rats reduces the levels of IL-1β, TNF-α, and MDA in lung tissue [27]. Despite its promising properties, the pharmacokinetics of Mrl remain poorly understood. Mrl has poor water solubility and limited bioavailability, which may reduce the therapeutic effect of this monoterpene. However, most studies on this compound utilize oral route administration [28, 29]. While the antioxidant and anti-inflammatory properties of Mrl are well-documented, its protective effects against PQ-induced pulmonary toxicity remain unexplored. Therefore, This study hypothesizes that Mrl can mitigate PQ-induced lung toxicity by modulating oxidative stress and inflammation.

Materials and methods

Animals

In this study, 25 male Wistar albino rats, each weighing between 200 and 250 g, were obtained from the Animal House of Rafsanjan University of Medical Sciences. The animals were housed in cages under a controlled environment (i.e., at 22 ± 2 °C with 12 h/12 h light/dark cycle) with free access to food and water. All animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals. The Ethics Committee of Rafsanjan University of Medical Sciences approved this study (approval ID: IR.RUMS.AEC.1401.005).

Treatment protocol and PQ aerosol exposure

The animals were randomly divided into five experimental groups, ($n = 5$ in each group) as follows: Ctrl (control, treated by 10% dimethyl sulfoxide (DMSO; Sigma Aldrich Co.; Cat. Number: 67-68-5), PQ (exposure to PQ, no treatment), PQ + Mrl-25 (exposure to PQ, treated with Mrl at a dosage of 25 mg/kg/day), PQ + Mrl-50 (exposure to PQ, treated with Mrl at a dosage of 50 mg/kg/day), PQ + Dexa (exposure to PQ, treated with Dexamethasone at 0.03 mg/kg/day).

Rats were exposed to PQ (Sigma Aldrich Co.; Cat. Number: 75365-73-0) aerosol at a dosage of 54 mg/m^3, 8 times for 16 days (every other day, each time for 30 min) using a compressor nebulizer as described in a previous study (Fig. 1) [30]. PQ aerosols were generated using a compressor nebulizer (Omron CX3, Japan), producing particles sized 3–5 µm at an airflow rate of 8 L/min. For each session, 4.5 ml of PQ solutions at a concentration of 1.33 mg/ml was introduced into the nebulizer's chamber, delivering the aerosol to an animal head box measuring 15 × 18 × 30 cm. The nebulizer had a solution output rate of 0.15 L/min and an air output rate of 3.7 L/min, resulting in PQ aerosol concentration of and 54 mg/m^3, respectively [9].

Mrl (Sigma Aldrich Co.; Cat. Number: 19894-97-4) was dissolved in 10% DMSO and was administered via gavage for 16 days following the PQ exposure period.

Fig. 1 Time line of PQ exposure and treatment with Dexa and Mrl. PQ: paraquat; Mrl: myrtenol; Dexa: dexamethasone

dexamethasone (Dexa; Sigma Aldrich Co.; CAS No: 50-02-2) [30] was administered via gavage for 16 days following the PQ exposure period. The control group received 10% DMSO (in volume of 5 ml/kg/day).

Sampling

The rats were anesthetized at the end of the treatment period with an intraperitoneal injection of xylazine (5 mg/kg) and ketamine (50 mg/kg) on day 32 (Fig. 1).

Blood samples were drawn from animals by cardiac puncture. The whole blood was used for total white blood cell count and serum samples (centrifugation for 6000 rpm for 10 min) were kept at -80 °C for assessment of oxidant and antioxidant biomarkers. The animals were then killed with a guillotine and the lungs were harvested. The left lung was fixed in phosphate-buffered formalin (10%) for histological evaluations; the right one was homogenized (1/10 w/v) in ice-cold buffer solution (100 mM Tris-HCl, pH 7.4) and centrifuged at 6000 rpm for 20 min; then the supernatant was gathered and kept at -80 °C for the evaluation of inflammatory parameters.

Total white blood cell count

To determine the total white blood cell (WBC) count, 0.5 mL of blood was combined with Turk solution. The total WBC count was then performed in duplicate using a Neubauer hemocytometer (Burker chamber) [31].

Oxidant and antioxidant biomarker measurement

The evaluation of oxidant biomarkers like MDA and antioxidant biomarkers including total thiol content, as well as the activities of SOD and CAT in the serum, was conducted in the following manner:

MDA concentration measurement

1 ml of serum was combined with 2 ml of thiobarbituric acid diluted in 2 ml of hydrochloric acid to quantify MDA. Subsequently, 15 g of trichloroacetic acid solution was incorporated and heated for 60 min. After cooling, the solution underwent centrifugation for 10 min at 380 × g. The absorbance was recorded at 535 nm. The concentration of MDA was determined using the formula: C (μM) = Absorbance / 1.65 × 10^5 [32].

Thiol concentration measurement

50 ml of serum was mixed with 1 ml of ethylenediaminetetraacetic acid (EDTA), and the absorbance of the sample was assessed against the Tris-EDTA buffer alone (A1) at 412 nm. Subsequently, 20 μl of DTNB reagent was administered, and absorbance was recorded after 15 min (A2). The absorbance of the DTNB reagent was documented as the blank (B) [33]. The thiol concentration (μM) was determined using the equation: C (μM) = (A2−A1−B)×1.07/0.05 × 13.6.

SOD enzyme activity

Madesh and Balasubramanian colorimetric approach was used to calculate the SOD activity. Pyrogallol, MTT, and serum were injected into wells of 96-microliter plates. After 15 min incubation by adding DMSO, the reaction was terminated and enzyme activity (U/ml) was assessed at 570 nm [33].

CAT enzyme activity

The Aebi technique was employed to assess CAT activity. In summary, 30 mM hydrogen peroxide and 50 mM phosphate buffer were combined with the appropriate volume of serum. The reduction in absorbance at 240 nm can be attributed to the consumption of hydrogen peroxide, which is measured as CAT activity (U/ml) [32].

Cytokine measurement

The concentrations of cytokines, such as interleukine-6 (IL-6; Karmania Pars, Kerman, Iran, Cat. Number: KPG-IL-6) and TNF-α (Karmania Pars, Kerman, Iran, Cat. Number: KPG-RTNF), in the lung tissue, were measured using enzyme-linked immunosorbent assay (ELISA) kits. The measurement procedure followed the technique recommended by the manufacturer. Lung tissue is homogenized in RIPA buffer. Then Fifty μl of standards and samples are added to the wells of a 96-well microplate. After 2 h of incubation at 37 °C, the plate is washed three times. Then, 50 μl of conjugated antibody is added to the wells, and after 1 h of incubation at 37 °C, the plate is washed three times. Next, Avidin-HRP is added, and after 30 min of incubation in room temperature, the plate is washed five times. Fifty microliters of substrate are then added to the wells, and the plate is incubated at room temperature for 15 min. Finally, 25 μl of stop

solution is added, and the absorbance is measured at a wavelength of 450 nm [10].

Pathological studies

The lung tissue samples were dehydrated using a series of progressively higher concentrations of alcohol and then made transparent with a series of xylene solutions. Afterward, the samples were embedded in paraffin. A microtome was used to cut thin sections with a thickness of 5 µm from the paraffin blocks. These sections were subsequently stained with hematoxylin and eosin (H&E).

Three features of pulmonary injury resulting from PQ exposure were evaluated: hemorrhage, alveolar congestion, and the infiltration or aggregation of neutrophils in the airspace or vessel wall. Based on these features, the following scoring was applied: minimal (0), mild (1), moderate (2), severe (3), and maximal damage (4) 34].

Statistical analysis

GraphPad Prism software version 8 (GraphPad Software, USA) was used for statistical analysis. Differences between groups were evaluated using ANOVA followed by Tukey's post hoc analysis. Non-parametric variables were analyzed by the Kruskal-Wallis test followed by Dunn's post hoc analysis. The results are presented as the mean ± SEM. Statistical significance was considered at a p-value of < 0.05.

Results

Total WBCs count

Treatment with PQ significantly increased the number of WBCs (10^3/µl) ($p < 0.001$) (Fig. 2). Administration of Mrl at both doses did not have a significant effect on reducing the number of WBCs. Furthermore, treatment of PQ-treated animals with Dexa did not affect the number of WBCs.

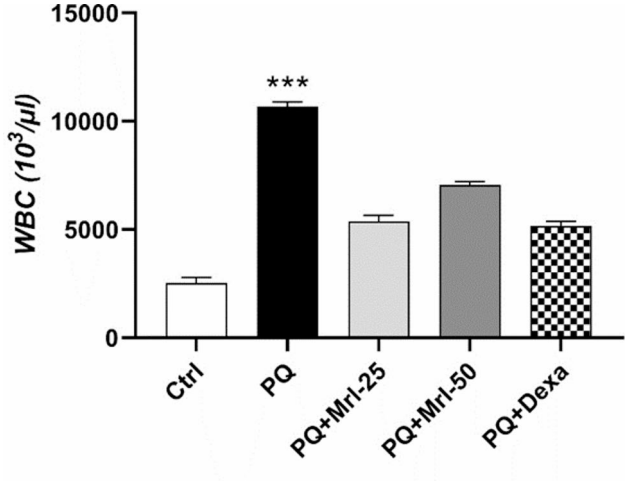

Fig. 2 The effect of Mrl and Dexa on WBC count in rats exposed to PQ aerosol. Values are presented as means ± SEM ($n = 5$). ***$p < 0.001$ indicates a significant difference compared to Ctrl group. Ctrl: control; PQ: paraquat; Mrl: myrtenol; Dexa: dexamethasone

In addition, there are no significant differences between Dexa group and both groups of Mrl in terms of this index.

Oxidative studies

PQ induces its deleterious effects by reducing antioxidant levels and increasing oxidative biomarkers.

Treatment with PQ significantly increased the serum levels of MDA ($p < 0.001$) (Fig. 3A). Mrl at both doses (25 and 50 mg/kg) significantly reduced the elevated levels of MDA induced by the PQ treatment (all $p < 0.001$). Furthermore, treatment of PQ-treated animals with Dexa decreased the serum levels of MDA ($p < 0.001$).

The administration of PQ reduced the activity of CAT ($p < 0.001$) (Fig. 3B). The administration of Mrl at a dose of 25 mg/kg increased CAT activity ($p < 0.05$) However, the administration of Mrl (50 mg/kg) to PQ-treated animals did not have a significant effect on reducing CAT activity induced by PQ. Furthermore, treatment of PQ-treated animals with Dexa increased the activity of CAT in lung tissues ($p < 0.05$).

Treatment with PQ significantly reduced the activity of SOD ($p < 0.05$) (Fig. 3C). Administration of Mrl at a dose of 25 mg/kg did not have a significant effect on reducing PQ- induced SOD activity. However, administering Mrl at a dose of 50 mg/kg to PQ-treated animals significantly increased SOD activity ($p < 0.05$). Furthermore, treatment of PQ-treated animals with Dexa does not affect the activity of SOD in lung tissues.

The PQ administration reduced the level of thiol ($p < 0.01$) (Fig. 3D). Mrl at both doses of 25 and 50 mg/kg increased the thiol levels in lung tissues of PQ-treated animals ($p < 0.05$ and $p < 0.01$, respectively). Furthermore, treatment of PQ-treated animals with Dexa increased the thiol levels in lung tissues ($p < 0.01$).

In addition, there are no differences between the Dexa group and the groups of Mrl in terms of these indices, except for MDA levels, in which both doses of Mrl have significant differences with the Dexa group ($p < 0.05$ for PQ + Mrl-25, and $p < 0.001$ for PQ + Mrl-50).

Inflammatory studies

The PQ administration led to increased levels of IL-6 and TNF-α in lung tissue ($p < 0.05$ and $p < 0.01$, respectively) (Fig. 4A and B). Mrl at a dose of 25 mg/kg significantly reduced the level of IL-6 and TNF-α in PQ-treated animals compared to the PQ group ($p < 0.01$ and $p < 0.05$, respectively). Treatment of PQ-treated animals with Mrl at a dose of 100 mg/kg decreased the levels of IL-6 and TNF-α compared to the animals treated with PQ ($p < 0.01$ and $p < 0.05$, respectively). Furthermore, treatment of PQ-treated animals with Dexa significantly reduced

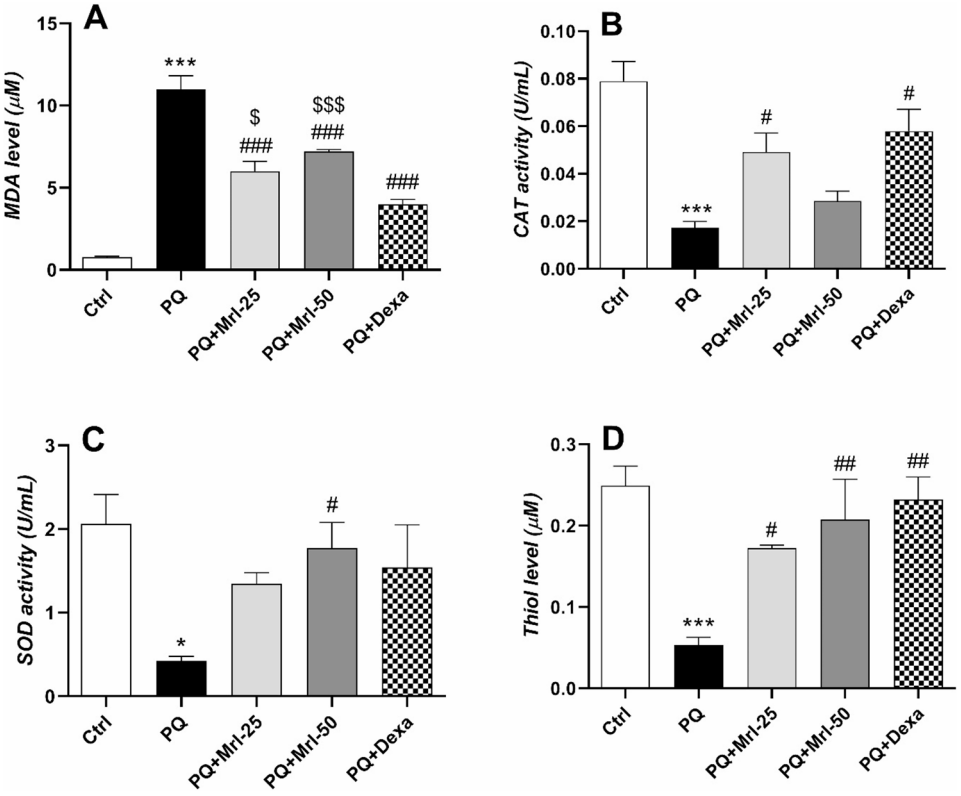

Fig. 3 The effects of Mrl and Dexa on serum levels of MDA and total thiol, as well as the activities of CAT and SOD in rats exposed to PQ aerosol. Values are presented as means ± SEM ($n = 5$). *$p < 0.05$ and ***$p < 0.001$ indicates a significant difference compared to Ctrl group. #$p < 0.05$, ##$p < 0.01$, and ###$p < 0.001$ indicates a significant difference compared to the PQ group. $$p < 0.05$ and $$$$p < 0.001$ indicates a significant difference compared to the Dexa group. Ctrl: control; PQ: paraquat; Mrl: myrtenol; Dexa: dexamethasone

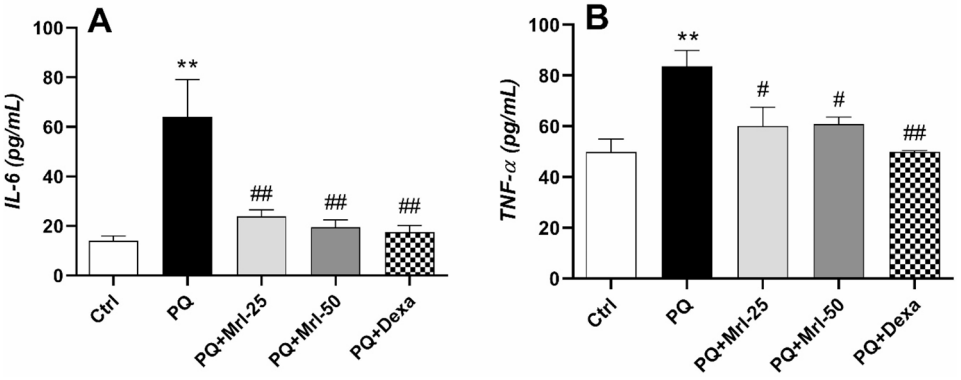

Fig. 4 The effect of Mrl and Dexa on serum levels of of IL-6 and TNF-α in rats exposed to PQ aerosol. Values are presented as means ± SEM ($n = 5$). **$p < 0.01$ indicates a significant difference compared to the Ctrl group. #$p < 0.05$ and ##$p < 0.01$ indicates a significant difference compared to the PQ group. Ctrl: control; PQ: paraquat; Mrl: myrtenol; Dexa: dexamethasone

these indices in lung tissue compared to the PQ group (all $p < 0.01$).

In addition, there are no differences between Dexa group and both groups of Mrl in terms of these indices.

Pathological studies

Histological evaluations of the Ctrl group showed a natural structure of lung tissues (Fig. 5A). All the measured histological indices of lung are within the normal range in this group (Table 1). In the PQ group, we observed significant congestion, hemorrhaging, and infiltration of neutrophils (Fig. 5B). All the measured histological indices of the testes have significantly increased compared to that of the Ctrl group (all $p < 0.001$) (Table 1).

Administration of 25 mg/kg Mrl to PQ-treated animals attenuated hemorrhage and infiltration of inflammatory

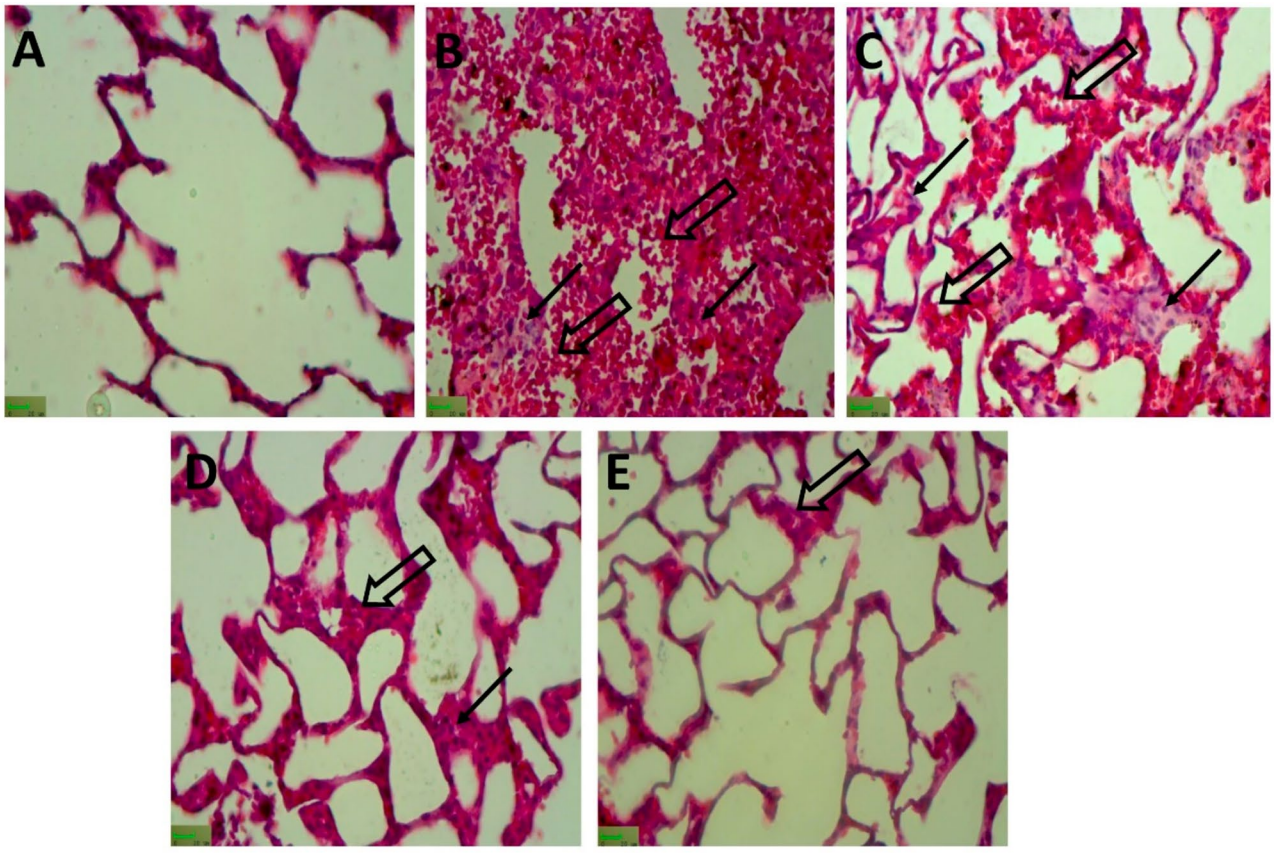

Fig. 5 Effect of Mrl and dexa on histological evaluations (H&E, X 40) of PQ aerosol-exposed rats. (**A**) Ctrl group: showing natural structure of the lung tissue; (**B**) PQ group: showing significant congestion, hemorrhaging, and infiltration of neutrophils; (**C**) PQ + Mrl-25 group: showing attenuation in hemorrhage and inflammatory cell infiltration; (**D**) PQ + Mrl-50 group: showing attenuation in alveolar congestion and inflammatory cell infiltration; (**E**) PQ + Dexa: showing signs of recovery in all measured indices. Line arrow: inflammatory cell infiltration; arrow: hemorrhage; Ctrl: control; PQ: paraquat; Mrl: myrtenol; Dexa: dexamethasone

Table 1 Effect of Mrl and dexa on histological indices of PQ aerosol-exposed rats

	Pathological indices		
	Hemorrhage	**Alveolar congestion**	**Neutrophils infiltration**
Ctrl	0.28 ± 0.18	0.28 ± 0.18	0.57 ± 0.20
PQ	3.71 ± 0.18***	3.85 ± 0.14***	3.85 ± 0.14***
PQ + Mrl-25	1.14 ± 0.34#	1.28 ± 0.18	1.28 ± 0.18#
PQ + Mrl-50	1.42 ± 0.36	1.14 ± 0.26#	1.28 ± 0.28#
PQ + Dexa	0.85 ± 0.26##	0.85 ± 0.26##	1.00 ± 0.21##

Values are presented as means ± SEM ($n = 5$)

***$p < 0.001$ indicates a significant difference compared to the Ctrl group

#$p < 0.05$ and ##$p < 0.01$ indicates a significant difference compared to the PQ group

cells (all $p < 0.05$) (Fig. 5C; Table 1). Furthermore, alveolar congestion and infiltration of neutrophils were mitigated at the dose of 50 mg/kg Mrl, showing significant recovery (all $p < 0.05$) (Fig. 5D; Table 1). The administration of Dexa attenuated these pathological lesions in PQ-treated animals (all $p < 0.01$) (Fig. 5E; Table 1).

In addition, there are no differences between Dexa group and both groups of Mrl in terms of these pathological indices (Table 1).

Discussion

This investigation aimed to identify the beneficial effects of Mrl on lung damage induced by inhaled PQ in rats. We demonstrated that 16 days of PQ administration causes lung injuries via decreasing the levels or activities of SOD, CAT, and thiol yet increasing the levels of TNF-α, IL-6, and MDA in lung tissue. Furthermore, these disorders are confirmed by pathological studies. We found that the administration of Mrl at both doses attenuated the lung injuries induced by PQ. This protective effect was demonstrated by restoring and improving all the measured oxidative, antioxidant, and inflammatory biomarkers, although not entirely restoring them to Ctrl levels (unexposed to PQ). Furthermore, we also discovered that these advantageous effects of Mrl are consistent with the histological assessments of lung tissue.

It is well-established that PQ has a deleterious effect on oxidative stress and antioxidant biomarkers [1]. The over-generation of free radicals plays an important role in PQ-induced toxicity through two mechanisms : (i) elevating the levels or activities of oxidative stress markers such as MDA, and (ii) reducing the levels or activities of antioxidant biomarkers such as CAT, SOD, and thiol [35]. Memarzia et al., reported that serum levels of interleukine-10 (IL-10), CAT, SOD, and thiol reduced and TNF-α, MDA, and total and differential WBC elevated in animals exposed to PQ [36]. In another study, Shakeri et al.,. demonstrated that inhaled PQ diminished serum levels of SOD, CAT, and thiol yet elevated WBC count, MDA, NO2, and NO3 in rats [37]. Elevated blood and bronchoalveolar-lavage fluid (BALF) levels of MDA and WBC as well as decreased thiol concentration and SOD and CAT activities due to PQ inhalation were shown by Ghasemi et al., [38]. The results of the mentioned studies confirmed the findings of our results. Moreover, it has been found that Mrl has a potent direct antioxidant effect by suppressing ROS production and scavenging free radicals [20, 39]. Xuemei et al., demonstrated that administering Mrl (50 mg/kg) to diabetic rats reduced the level of MDA, and increased the activity of CAT and SOD in liver tissues [26]. Furthermore, Mrl exerts its protective effects against myocardial ischemia-reperfusion injury by preventing the generation of ROS [39]. Moreover, Mrl ameliorates acute cisplatin-induced kidney injury by reducing the levels of renal MDA and increasing renal SOD, and CAT activities [40]. In another study, Bejeshk et al. demonstrated that Mrl reduced MDA levels and increased SOD levels in the BALF of asthmatic rats [27]. Also, It has been reported that inhaled Mrl reduces the MDA level in BALF and lung tissue of asthmatic rats [41]. Therefore, the mitigating effects of Mrl against PQ toxicity may be attributed to its direct and/or indirect antioxidant properties. On the other hand, the role of CAT and SOD as the primary enzymatic antioxidants in the lungs in human studies is demonstrated. Decreased serum level of CAT in asthmatic patients is reported [42]. Also, studies reported the elevated plasma activity of CAT in chronic obstructive pulmonary disease (COPD) patients [43, 44]. Previous studies have shown that SOD activity in the serum and BALF was decreased in patients experiencing acute exacerbations of COPD compared to healthy individuals and those with stable COPD [43, 45, 46]. This reduction in SOD activity indicates an imbalance between oxidants and antioxidants. In COPD, the production of transforming growth factor beta (TGF-β) by epithelial cells is significantly elevated which inhibits the activities of the enzymes including CAT and SOD in the lung tissues of humans [47]. SOD activity is markedly reduced in the epithelial lining fluid and airway epithelial cells of asthma patients compared to healthy controls which demonstrated an inverse relationship between airway reactivity and SOD activity [48].

In addition to the direct harmful impact of free radicals in PQ toxicity, these active compounds can also induce inflammatory responses in the lungs by activating various of different transcription factors that regulate the expression of other inflammatory mediators such as TNF-α and IL-6 [49]. Nuclear factor kappa B (NF-κB) is a transcription factor playing a significant role in the regulation of genes involved in inflammation and immune responses. It stimulates the production of various pro-inflammatory cytokines, including TNF-α, IL-1β, COX-2, and IL-6, which are all associated with severe inflammation and diseases [50, 51]. Our results disclosed elevated TNF-α and IL-6 levels in the PQ group. In this regard, Ijaz et al., indicated that PQ administration significantly increased inflammatory markers including TNF-α, IL-6, NF-κB and IL-1β [50]. Also, Mohammadi Mahjoob et al., showed that PQ inhalation increased TNF-α in serum [52]. We found that administration of Mrl to PQ–treated animals decreases the levels of inflammatory cytokines such as TNF-α and IL-6 in lung tissues. It is well established that Mrl has inhibitory effects on inflammatory processes. For example, Oliveira et al., showed that Mrl attenuates orofacial inflammation by reducing the levels of IL-1β in the masseter muscle [23]. Moreover, in diabetic pregnant rats, Mrl could attenuate the increased levels of inflammatory markers such as IL-1β and TNF-α in liver tissue [26]. Furthermore, Bejeshket al., demonstrated that Mrl mitigated the asthma-induced behavioral dysfunctions by decreasing the levels of IL-6, Interleukine-17 (IL-17), and TNF-α in the hippocampal tissue of rats [41]. In another study, Mrl reduced the levels of inflammatory biomarkers such as IL-10, IL-1β and TNF-α in lungs and serum of asthmatic rats [24]. Also, our results were in line with those of Rajizadeh et al., indicating that inhalation of niosomal form of myrtenol declined TNF-α and IL-6 in lung tissues of asthmatic rats [53]. Indeed, by inhibiting the activation of NF-κB and p38-MAPK pathways, Mrl can reduce the expression of pro-inflammatory cytokines such as IL-1β and TNF-α, thereby mitigating inflammation [23, 26]. IL-6 is involved in the inflammatory cascade that leads to airway inflammation and hyperresponsiveness in asthma patients. Increased IL-6 levels in BALF are associated with more severe asthma symptoms and frequent exacerbations [54, 55]. Moreover, elevated peripheral blood level of IL-6 and IL-4 has been reported in pediatric asthma [56]. Also, researches have shown that IL-6 is actively involved in the development of asthma, rather than just being an inflammatory byproduct. IL-6 regulates the immune responses of CD4 + Th2 and Th17 cells by facilitating T cell differentiation. These cells are important to the immune mechanisms involved in asthmatic conditions [57, 58]. Also elevated level of

IL-6 in COPD has been reported. In this regard, another research revealed that both IL-6 and IL-10 levels were significantly higher in patients with COPD [59]. Macrophages, along with epithelial cells, eosinophils, and mast cells, can release TNF-α in the lungs. Elevated TNF-α levels are linked to various pulmonary inflammatory diseases, such as asthma, COPD, and interstitial pulmonary fibrosis. TNF-α exacerbates these diseases by attracting inflammatory cells, promoting the production of inflammatory mediators, enhancing oxidative and nitrosative stress, and causing airway hyperresponsiveness [60, 61]. Accordingly, the results may suggest that the effects of Mrl could be mediated by reducing the inflammatory responses.

The current study revealed that administering PQ to animals resulted in lung toxicity, as evidenced by histological lesions in lung tissues such as hemorrhage, alveolar congestion, and infiltration of neutrophils; the results are thus in line with those of the previous studies [3, 10, 13]. It is well-documented that inflammatory and oxidative biomarkers significantly contribute to tissue injuries in lung tissues [24, 62]. Moreover, histological studies of lungs revealed the beneficial effects of Mrl in preserving lung integrity after being exposed to PQ. These protective effects of Mrl have been demonstrated in previous studies. Bejeshk et al., confirmed that Mrl could mitigate the histological lesions such as smooth muscle thickness of the bronchial wall and epithelial thickness in animals with induced asthma [27]. In another study, it has been shown that intraperitoneal injection of Mrl mitigated the pathologic changes in the bronchial epithelium, inflammatory cell infiltration, and thickening of the subepithelial smooth muscle layer in the rats with allergic asthma [24]. Furthermore, it has been demonstrated that Mrl has protective effects on lung structure parameters of lung ischemia-reperfusion injury in rats such as severe edema, capillary congestion, neutrophil infiltration, and bleeding in the alveolar region [62]. It appears that Mrl exerted these protective effects on lung tissue by its potent antioxidant and anti-inflammatory properties.

Since both administration doses had the same beneficial effects, it is recommended that a lower dose be used. This will minimize substance exposure and reduce potential side effects. Future research should attempt to unveil the precise molecular mechanism of Mrl in this pathological condition to determine the optimum dose. Another limitation, examine the effects of inhaled Mrl on PQ toxicity and compare it with oral administration to determine the best route of administration. Moreover, The excessive generation of ROS caused by paraquat can trigger the expression of NF-κB which is a key transcription factor involved in inflammation, stress response, cell growth and survival. Furthuemore, ROS can alter the activities of antioxidant defense system such as glutathione. The effects of PQ and Mrl on these factors (NF-κB and glutathione) should be explained in future study.

Conclusions

In summary, this study represents the first evaluation of the beneficial effect of Mrl against injuries induced by inhaled PQ exposure. The current study demonstrated the antioxidant and anti-inflammatory effects of Mrl against molecular and histopathological adverse effects of PQ.

Abbreviations

PQ	Paraquat
TNF-α	Tumor necrosis factor-α
Mrl	Myrtenol
SOD	Superoxide dismutase
CAT	Catalase
IL-1β	Interleukin-1β
MDA	Malondialdehyde
Ctrl	Control
Dexa	Dexamethasone
DMSO	Dimethyl sulfoxide
WBC	white blood cells
IL-6	Interleukine-6
ELISA	Enzyme-linked immunosorbent assay
H&E	Hematoxylin and eosin
IL-17	Interleukine-17
IL-10	Interleukine-10
COPD	Obstructive pulmonary disease
BALF	Bronchoalveolar-lavage fluid
TGF-β	Transforming growth factor beta
NF-κB	Nuclear factor kappa B

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Author contributions

FA: Conceptualization, Writing – original draft, Formal analysis. HB and NP: Methodology. M Kh and EH: Data curation. IF: Writing – review and editing, Supervision.

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Data availability

The data is not publicly accessible, but it can be obtained from the corresponding author upon a reasonable request.

Declarations

Ethical approval

This study was conducted following the ARRIVE guidelines and was approved by the Local Ethical Review Committee of the University of Rafsanjan (Ethics code: IR.RUMS.AEC.1401.005).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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