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Research article

The interactive effect of seven weeks aerobic exercise training and piperine against paraquat-induced lung damage in male Wistar rats: Investigating role of oxidative and inflammatory indices

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ABSTRACT

We aimed to evaluate the effects of seven weeks of aerobic exercise training and piperine on paraquat-induced lung damage. Forty-eight male Wistar rats (230 g, six-eight weeks old) were randomly divided into six groups (n = 8): sham, paraquat (5 mg/kg three times a week; intraperitoneally), paraquat + piperine (10 mg/kg/day; orally), paraquat + aerobic exercise training, paraquat + piperine + aerobic exercise training; and paraquat + vitamin E (20 mg/kg/day; orally) as a positive control. Rats were sacrificed on day 50, and both lung tissues were isolated to measure oxidative (MDA), anti-oxidative (GSH), inflammatory (TNF-α), anti-inflammatory (IL-10) markers, and histological evaluations (hematoxylin-eosin staining). The results of the present study revealed that paraquat significantly decreased body weight, GSH, GSH/MDA ratio, IL-10, and IL-10/TNF-α ratio while increasing MDA, TNF-α, and histopathological damage in lung tissue ($P < 0.01$ to 0.001). In contrast, treatment with all four interventions meaningfully diminished oxidative, inflammatory markers, and histopathological damage while propagating body weight, anti-oxidative and anti-inflammatory markers following the paraquat-induced lung damage ($P < 0.05$ to $P < 0.001$). Interestingly, piperine and piperine + exercise training possessed stronger protective effects against paraquat-induced lung damage than exercise training alone ($P < 0.01$ to 0.001). Treatment with piperine, exercise training, piperine + exercise training, and vitamin E significantly ameliorated paraquat-induced lung damage. Interestingly, the piperine and piperine + exercise training had more protective effects than other groups. Therefore, piperine and the combination of piperine and exercise training may be valuable candidates for preventing lung injuries.

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1. Introduction

Herbicide paraquat is a bipyridine heterocyclic compound that is non-selective and highly water-soluble. The use of this chemical in agriculture has been widespread throughout the world for a long time [1]. Although its efficiency in agriculture, paraquat is highly toxic to humans and animals. Paraquat intoxication leads to multiple organ failure and is linked to a high mortality rate [2]. In particular, the lung tissue is considered the major target of paraquat accumulation and toxicity, and plasma concentrations of this substance are about six to ten times greater than those in the lungs [3,4]. Oxidative stress and excessive inflammatory responses due to immune system imbalance are responsible for the pathogenesis of paraquat-induced lung damage [5,6].

Piperine is an alkaloid found in numerous piper species belonging to the Piperaceae family, particularly *Piper nigrum* and *Piper longum* [7]. Numerous pharmacological properties have been found to be associated with piperine, including anti-proliferative, anti-allergic, anti-oxidant, anti-microbial, anti-inflammatory, and immunomodulatory effects [8]. Lu and coworkers showed that piperine has promising protective effects on acute lung injury caused by lipopolysaccharide through anti-inflammatory mechanisms [9].

Regular physical exercise training is a feasible, safe, easy, and nonpharmacological intervention for ameliorating multiple disorders [10]. Several pieces of evidence reported that regular physical exercise training is associated with anti-oxidative and anti-inflammatory impacts. It also had promising impacts on cardiovascular diseases, diabetes, obesity, and metabolic adaptation [11,12]. Therefore, this study hypothesized that aerobic exercise training protocol and piperine supplementation could attenuate paraquat-induced lung damage in Wistar rats. The present examination aimed to determine the effects of seven weeks of aerobic exercise training protocol and piperine supplementation on paraquat-induced lung damage in Wistar rats.

2. Material and methods

2.1. Drugs and chemicals

Paraquat, piperine, and vitamin E were provided by Sigma®, USA. Moreover, xylazine and ketamine were prepared by ChemiDaru Company (Iran). Furthermore, tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-10 ELISA kits were obtained from MyBioSource Company (San Diego, CA, USA). Additionally, kits were provided by ZellBio Company (Germany) for malonaldehyde (MDA) and glutathione (GSH). The rest of the reagents were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) and are of analytical grade.

2.2. Animals

In the current examination, 48 Wistar-Albino male rats were obtained from the animal laboratory of the Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. We maintained rats under standard laboratory conditions, including housing them in separate standard cages, providing 12/12 h of light and darkness in rooms with good ventilation, and a temperature of 22 ± 2 °C. Furthermore, a free supply of food and water was available to them, along with keeping cages clean and removing feces and spilled feed on a daily basis. Hakim Sabzevari University's Research Ethics Committee approved the study in accordance with its ethical guidelines (No. IR.HSU.REC.1400.017).

2.3. Experimental groups and interventions

There were 48 healthy male Wistar rats (230 g, six-eight weeks old) randomly divided into six groups (n = 8), including:
Group one: Sham;
Group two: Control; receiving paraquat and the vehicle of piperine (corn oil; oral gavage);
Group three: receiving paraquat + piperine (10 mg/kg/day; oral gavage) [13];
Group four: receiving paraquat + aerobic exercise training;
Group five: receiving paraquat + piperine + aerobic exercise training;
Group six: receiving paraquat + vitamin E as a positive control (20 mg/kg/day; oral gavage) [14].
During the experiment, paraquat was intraperitoneally injected at a dosage of 5 mg per kilogram three times a week (every other day) for seven weeks [15,16].

Table 1
The speed and duration of the treadmill exercise training during seven weeks of exercise training.

	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
1st week	5 min; 10 m/min	3 min; 14 m/min	12 min; 18 m/min	3 min; 14 m/min	5 min; 10 m/min
2nd week	5 min; 10 m/min	3 min; 14 m/min	12 min; 18 m/min	3 min; 14 m/min	5 min; 10 m/min
3rd week	4 min; 10 m/min	3 min; 14 m/min	12 min; 18 m/min	3 min; 14 m/min	4 min; 10 m/min
4th week	4 min; 10 m/min	4 min; 14 m/min	10 min; 18 m/min	4 min; 14 m/min	4 min; 10 m/min
5th week	3 min; 10 m/min	4 min; 14 m/min	10 min; 18 m/min	4 min; 14 m/min	3 min; 10 m/min
6th week	3 min; 10 m/min	4 min; 14 m/min	10 min; 18 m/min	4 min; 14 m/min	3 min; 10 m/min
7th week	3 min; 10 m/min	4 min; 14 m/min	10 min; 18 m/min	4 min; 14 m/min	4 min; 10 m/min

2.4. Aerobic exercise training protocol

Before starting the aerobic exercise training protocol, rats were accustomed to the treadmill for one week, with 7–10 m/min speed and 5–10 min/day. After acclimation to the treadmill, the physical exercise training was performed for seven consecutive weeks, with five sessions per week (from Saturday to Wednesday, while resting on Thursday and Friday) between 9 and 12 a.m. The speed and duration of the treadmill exercise training were summarized in Table 1.

2.5. Sampling

After 48 h of the last exercise training session, rats were sacrificed using deep anesthetized utilizing ketamine (100 mg/kg) and xylazine (10 mg/kg), and both lung tissues were isolated. Lung tissue was used to measure oxidative and inflammatory parameters and histological evaluations. After immediate fixation in 10 % neutral buffered formalin, the lung tissue was histopathologically evaluated and stained with hematoxylin-eosin (H&E). Furthermore, homogenates of the lung (10 % w/v) were prepared using 5 % potassium chloride, 0.5 mM phenylmethylsulfonyl fluoride (PMSF), as well as a cocktail of protease inhibitors. After that, separated supernatants were stored at −80 °C for further investigation after centrifuging at 4 °C for 10 min at 3000 rpm for 10 min [17]. In addition, measurement of total protein concentrations was performed using Bradford's method [18].

2.6. Evaluation of oxidative and anti-oxidative markers in lung tissue

MDA, which is a marker for oxidative stress, and GSH, which is an antioxidative factor, were assessed in lung tissue. Following the manufacturer's instructions, measurements were carried out with commercially available ELISA kits [19].

2.7. Assessment of inflammatory and anti-inflammatory cytokines in lung tissue

The levels of TNF-α, as an inflammatory cytokine, and a cytokine that inhibits inflammation, IL-10, were measured in lung tissue. The evaluations were performed using commercially available ELISA kits in accordance with the instructions provided by the manufacturer [20].

2.8. Data analysis

Data analysis was carried out using Graph Pad Prism software (version 8.01). Typically, the mean ± standard deviation (SD) or median and interquartile range (IQR) are statistical terms that are used to express parametric or non-parametric data., respectively. First, an evaluation of normality was performed using the Shapiro-Wilk test, and the significant differences between SDs were checked using Welch's ANOVA test. One-way analysis of variance (ANOVA) with Dunnett's post-test was figured out for parametric data. In addition, the Brown-Forsythe ANOVA test with Dunnett's T3 multiple comparisons test was performed for parametric data with

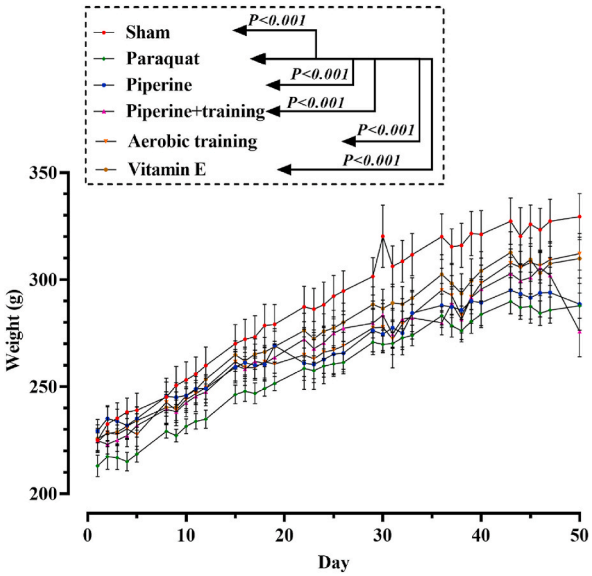

Fig. 1. The effects of piperine (10 mg/kg/day) and aerobic exercise training on body weight following paraquat (5 mg/kg three times a week) induced lung damage. Data were presented as mean ± SEM. ***P < 0.001 compared to the paraquat group. Data were analyzed using Two-way ANOVA with Dunnett's multiple comparisons test.

significant SD differences. On the other hand, the Kruskal-Wallis test with a two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli post hoc test for the non-parametric data. The significance level of statistical analysis was considered for two-tailed. A significant level of statistical significance was considered the levels of P values (P) lower than 0.05, 0.01, and 0.001.

3. Findings

3.1. The effects of piperine and exercise training on body weight

Two-way ANOVA test showed significant changes in body weight during seven weeks of treatment between the studied groups ($F_{Interaction\ (175,\ 1260)} = 0.44$, $P > 0.99$, $F_{Time\ (35,\ 1260)} = 59.31$, $P < 0.001$, $F_{Intervention\ (5,\ 1260)} = 52.29$, $P < 0.001$). Following seven weeks of treatment, the body weight was significantly decreased in the paraquat group compared to the sham group ($P < 0.001$, Fig. 1). However, treatment with all four interventions notably increased body weight compared to the paraquat group ($P < 0.001$, Fig. 1).

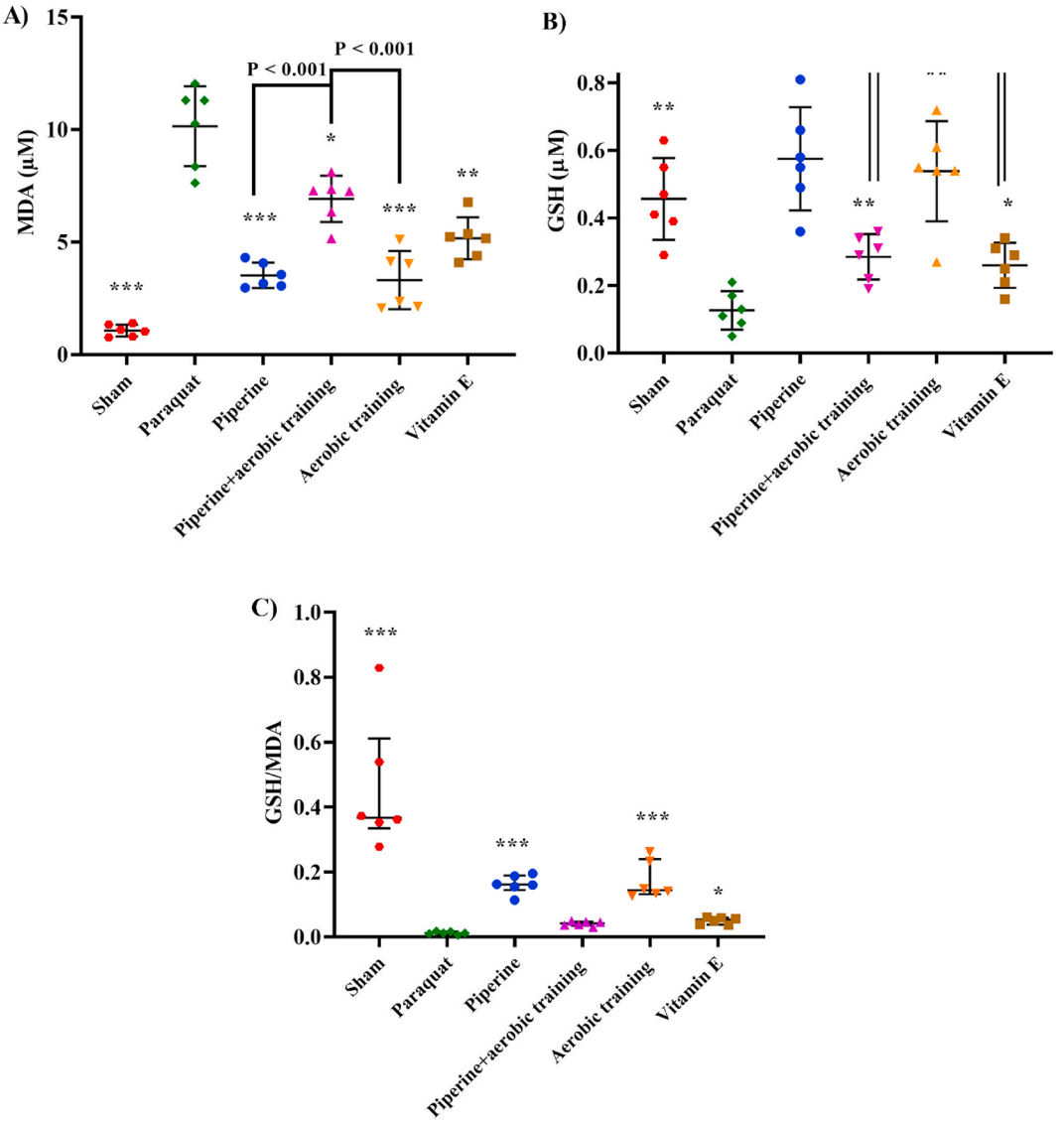

Fig. 2. The effects of piperine (10 mg/kg/day) and aerobic exercise training on A) MDA, B) GSH, and C) GSH/MDA levels following paraquat (5 mg/kg three times a week) induced lung damage. *P < 0.05, **P < 0.01, and ***P < 0.001 compared to the paraquat group.
A) Data were presented as mean ± SD and analyzed using the Brown-Forsythe ANOVA test with Dunnett's T3 multiple comparisons test; B) Data were presented as mean ± SD and analyzed using a one-way ANOVA and Dunnett's multiple comparisons test; C) Data were presented as median and interquartile range and analyzed using the Kruskal-Wallis test with a two-stage linear step-up procedure of the Benjamini, Krieger, and Yekutieli comparisons test.

3.2. The effects of piperine and exercise training on MDA level

Brown-Forsythe ANOVA test revealed significant differences in MDA levels among studied groups ($F_{(5, 17)} = 51.31$, $P < 0.001$). Furthermore, seven weeks of treatment with paraquat markedly enhanced MDA level in lung tissue compared to the sham group ($P < 0.001$). In contrast, piperine, exercise training, piperine + exercise training, and vitamin E groups meaningfully diminished MDA level in comparison with the control group ($P < 0.05$ to $P < 0.001$, Fig. 2A). Interestingly, the result of piperine and piperine + exercise training on the MDA level was significantly greater than the exercise training group ($P < 0.001$, Fig. 2A).

3.3. The effects of piperine and exercise training on GSH level

We found significant differences in GSH levels among groups using ordinary one-way ANOVA ($F_{(5, 30)} = 15.57$, $P < 0.001$). As compared with the sham group, paraquat remarkably alleviated GSH levels ($P < 0.01$). On the other hand, a seven-week treatment period with all four interventions strikingly elevated the GSH level comparatively to the control group ($P < 0.05$ and $P < 0.01$).

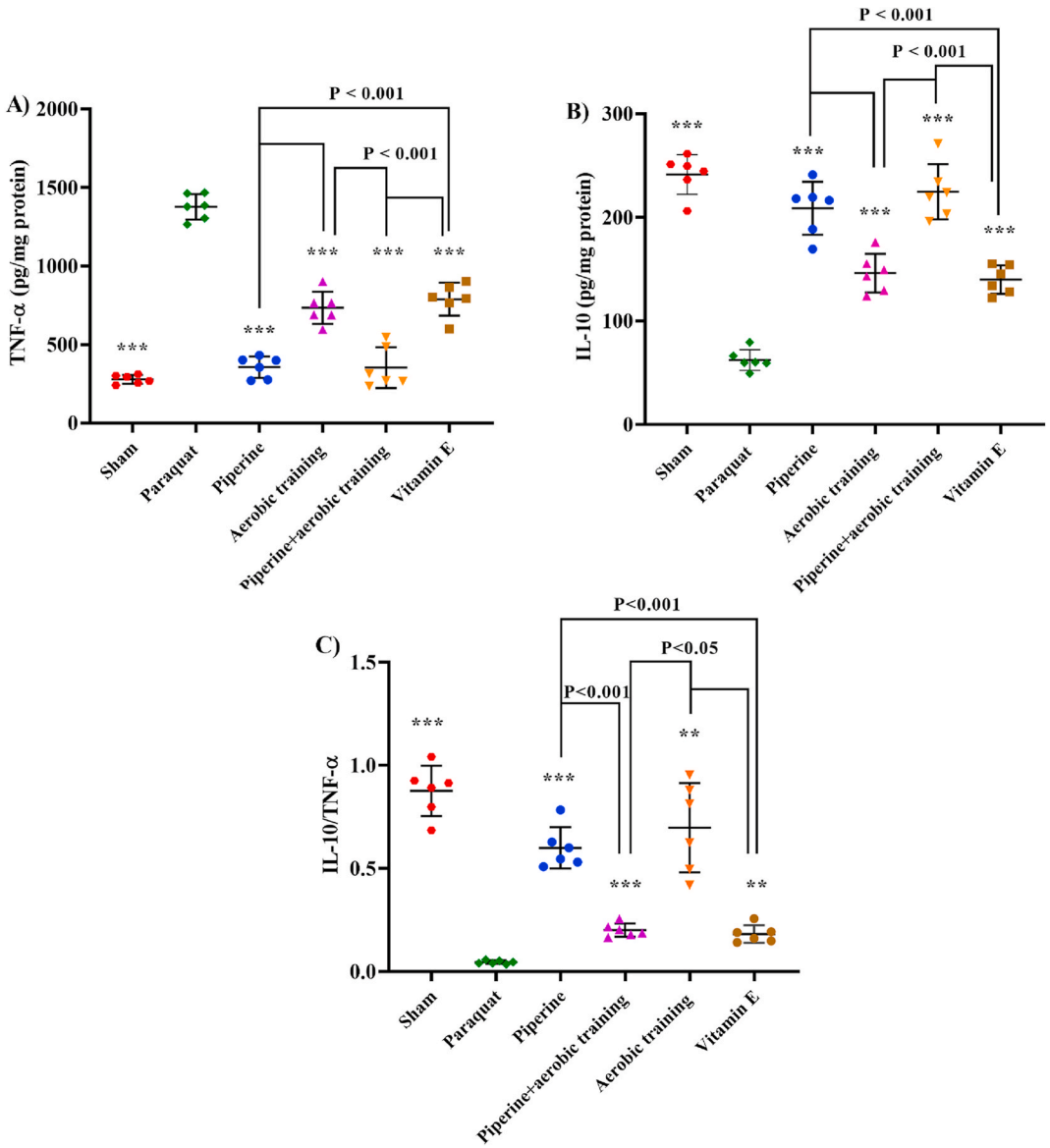

Fig. 3. The effects of piperine (10 mg/kg/day) and aerobic exercise training on A) TNF-α, B) IL-10, and C) IL-10/TNF-α levels following paraquat (5 mg/kg three times a week) induced lung damage. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ compared to the paraquat group.
A and B) Data were presented as mean ± SD and analyzed using a one-way ANOVA and Dunnett's multiple comparisons test; C) Data were presented as mean ± SD and analyzed using the Brown-Forsythe ANOVA test with Dunnett's T3 multiple comparisons test.

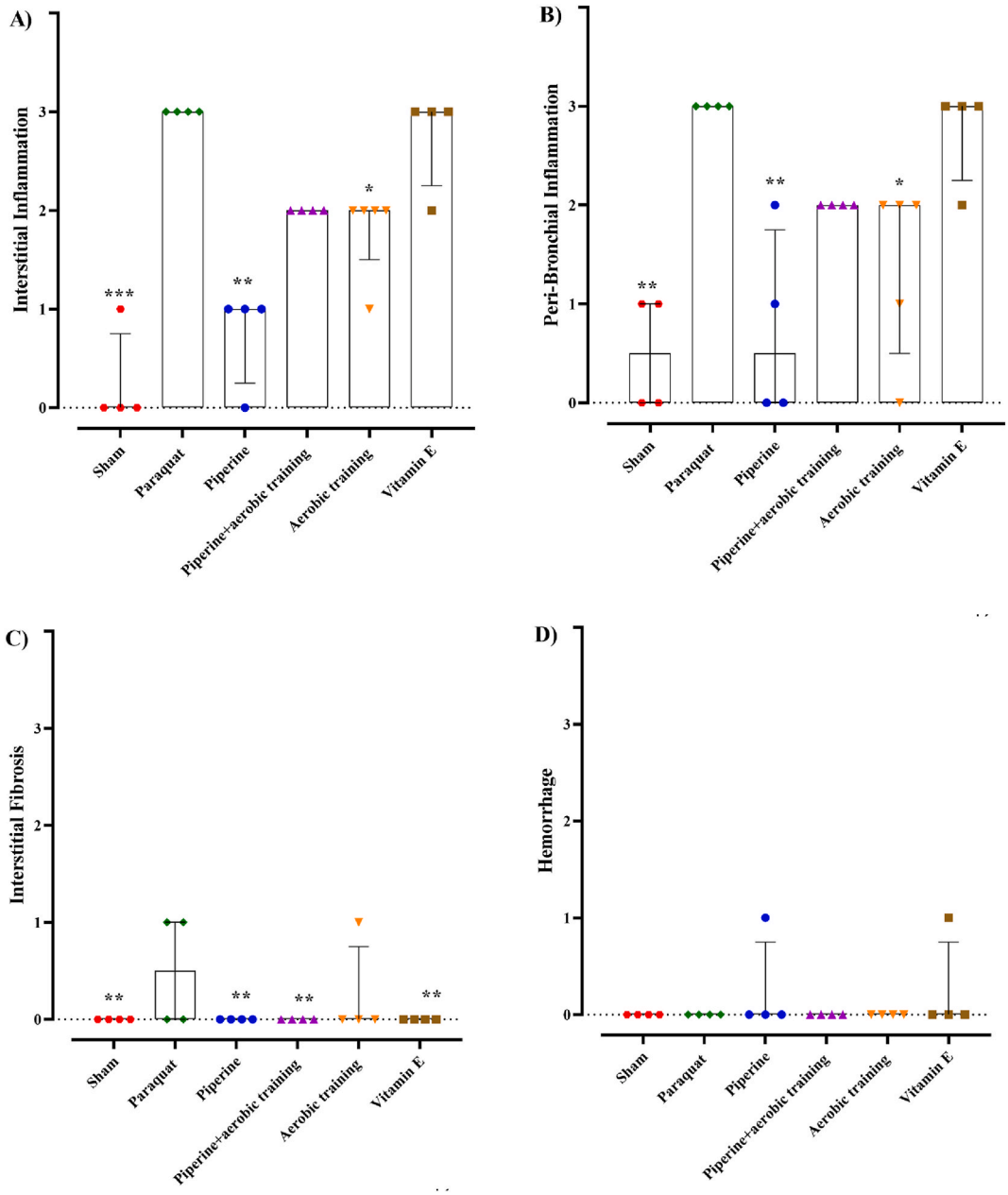

Fig. 4. The effects of piperine (10 mg/kg/day) and aerobic exercise training on histopathological changes following paraquat (5 mg/kg three times a week) induced lung damage. A) Interstitial inflammation, B) Peri-Bronchial Inflammation, C) Interstitial fibrosis, D) Hemorrhage, E-I) Histopathological H&E staining of rat lung tissue. E) Sham group with normal histological structure of the alveolar wall and bronchial epithelial lining, F) Paraquat group with interstitial pneumonia induced by infiltration of severe numbers of leukocytes including neutrophils, lymphocytes, macrophages, and eosinophils associated with minimal fibrosis (asterisks, H&E, × 400), G) Piperine group with interstitial pneumonia induced by infiltration of moderate numbers of leukocytes (asterisks, H&E, × 400), H). Aerobic exercise training group with interstitial pneumonia induced by infiltration of moderate numbers of leukocytes including neutrophils, lymphocytes and macrophages (asterisks, H&E, × 400), I) Piperine + aerobic exercise training group with interstitial pneumonia induced by infiltration of mild numbers of leukocytes including neutrophils, lymphocytes and macrophages (asterisks, H&E, × 400), J) Vitamin E group with interstitial pneumonia induced by infiltration of severe numbers of leukocytes (asterisks, H&E, × 400). Data were presented as median and Interquartile range. *P < 0.05, **P < 0.01, and ***P < 0.001 compared to the paraquat group. Data were analyzed using the Kruskal-Wallis test with a two-stage linear step-up procedure of the Benjamini, Krieger, and Yekutieli comparisons test.

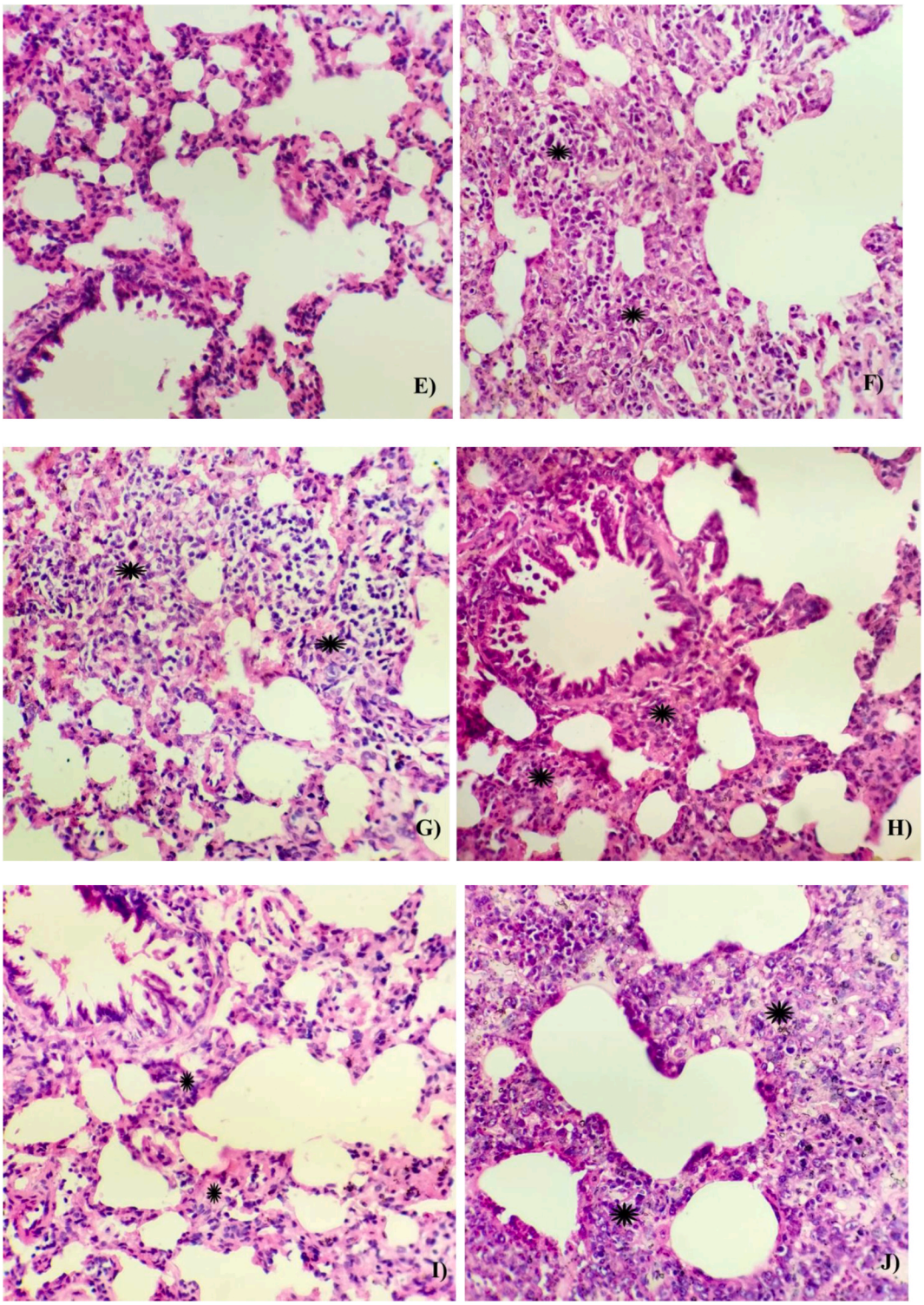

Fig. 4. (*continued*).

Interestingly, the effects of piperine and piperine + exercise training on GSH levels were significantly more significant than exercise training and vitamin E groups ($P < 0.001$ and $P < 0.01$, as depicted in Fig. 2B).

3.4. The effects of piperine and exercise training on the GSH/MDA ratio

Kruskal-Wallis test showed significant differences in the GSH/MDA ratio among studied groups ($H = 32.42$, $N_{group} = 6$, $N_{total} = 36$, $P < 0.001$). As compared to the sham group, the GSH/MDA ratio was firmly mitigated in the control group ($P < 0.001$, Fig. 2C). Contrary to this, treatment with piperine and piperine + exercise training notably propagated the GSH/MDA ratio compared to the control group ($P < 0.01$ regarding both cases, Fig. 2C).

3.5. The impacts of piperine and exercise training on TNF-α level

Our results revealed significant differences in TNF-α levels among groups using Ordinary one-way ANOVA ($F_{(5, 30)} = 123.4$, $P < 0.001$). Seven weeks of treatment with paraquat markedly enhanced TNF-α levels in lung tissue compared to the sham group ($P < 0.001$). However, treatment with piperine, exercise training, piperine + exercise training, and vitamin E groups remarkably attenuated TNF-α levels compared to the control group ($P < 0.001$ in every case, Fig. 3A). Interestingly, piperine and piperine + exercise training notably reduced TNF-α levels compared to exercise training and vitamin E groups ($P < 0.001$, Fig. 3A).

3.6. The effects of piperine and exercise training on IL-10 level

Ordinary one-way ANOVA test showed significant differences in IL-10 levels among studied groups ($F_{(5, 30)} = 69.06$, $P < 0.001$). Moreover, paraquat firmly decreased IL-10 level compared to the sham group ($P < 0.01$, Fig. 3B). Contrary to that, seven weeks of treatment with all four interventions markedly stimulated the IL-10 level compared to the control group ($P < 0.01$ for all cases, Fig. 3B). Interestingly, the impacts of piperine and piperine + exercise training on IL-10 level were remarkably more robust than exercise training and vitamin E groups ($P < 0.001$ applies to all cases, as illustrated in Fig. 3B).

3.7. The effects of piperine and exercise training on IL-10/TNF-α ratio

Brown-Forsythe ANOVA test reported remarkable differences in IL-10/TNF-α ratio among studied groups ($F_{(5, 11.03)} = 54.01$, $P < 0.001$. Seven weeks of treatment with paraquat strikingly mitigated IL-10/TNF-α ratio compared to the sham group ($P < 0.001$, Fig. 3C). However, all four interventions significantly propagated the IL-10/TNF-α ratio comparatively to the control group ($P < 0.01$ and $P < 0.001$). Interestingly, piperine ($P < 0.001$) and piperine + exercise training ($P < 0.05$) notably enhanced IL-10/TNF-α ratio compared to exercise training and vitamin E groups (presented in Fig. 3C).

3.8. The effects of piperine and exercise training on histopathological changes

The histopathological H&E staining of rat lung tissue showed that interstitial inflammation, peri-bronchial inflammation, and interstitial fibrosis were notably stimulated in the paraquat group compared to the sham group ($P < 0.001$ and $P < 0.01$, Fig. 4A–D). Furthermore, piperine and piperine + exercise training treatment markedly diminished interstitial inflammation, peri-bronchial inflammation, and interstitial fibrosis when compared to those exposed to paraquat ($P < 0.01$ and $P < 0.05$, shown in Fig. 4A–D).

As illustrated in Fig. 4E, observations of the lung sections from the sham group revealed the normal histological structure of the alveolar wall and bronchial epithelium. In contrast, the paraquat group demonstrated severe peri-bronchial leukocytic infiltration associated with interstitial pneumonia induced by infiltration of severe numbers of leukocytes, including neutrophils, lymphocytes, and macrophages associated with minimal fibrosis (Fig. 4F). However, piperine and piperine + exercise training administration meaningfully alleviated paraquat-induced lung damage and inflammation, resulting in mild peri-bronchial leukocytic infiltration associated with interstitial pneumonia induced by infiltration of mild numbers of leukocytes including neutrophils, lymphocytes, and macrophages (Fig. 4G and I). The exercise training group also showed moderate peri-bronchial leukocytic infiltration associated with interstitial pneumonia induced by infiltration of moderate numbers of leukocytes, including neutrophils, lymphocytes, and macrophages (Fig. 4H). The vitamin E group also reduced interstitial fibrosis compared to the paraquat group (Fig. 4J).

4. Discussion

To the best of our knowledge, this is the first investigation determining the impacts of aerobic exercise training and piperine supplementation against paraquat-induced lung damage in male Wistar rats. **The findings of the present study revealed that** paraquat remarkably decreased body weight, GSH, GSH/MDA ratio, IL-10, and IL-10/TNF-α ratio while increasing MDA, TNF-α, and histopathological damage in lung tissue. In contrast, treatment with piperine, aerobic exercise training, piperine + aerobic exercise training, and vitamin E groups meaningfully diminished oxidative, inflammatory markers, and histopathological damage while propagating body weight, anti-oxidative and anti-inflammatory markers following the paraquat-induced lung damage.

Paraquat, a lethal quaternary ammonium herbicide, accumulates in the body and causes several organ failures [21]. It is uptake by alveolar epithelial cells and accumulates in the lung tissue, possibly leading to severe lung injuries. Paraquat intoxication is associated with acute pulmonary edema, alveolar epithelial damage, hemorrhage, and interstitial inflammation, leading to respiratory failure

[22]. We found that paraquat significantly diminished body weight and anti-oxidative and anti-inflammatory markers while enhancing oxidative and inflammatory parameters in the lung tissue. In addition, paraquat causes histopathological damage to the lung tissue. In accordance with our findings, Zhang and colleagues indicated that paraquat notably elevated MDA levels and caspase-3 activity while reducing superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) activities, and GSH levels in lung tissue. Paraquat also causes diffuse alveolar collapse and thickening in H&E staining of lung tissue [23].

Similarly, paraquat markedly stimulated the amount of inflammatory cytokines, IL-6 and IL-1β, in the broncho-alveolar lavage fluid (BALF) of C57BL/6 mice. Additionally, paraquat provided a significant increment in lung injury score through inflammatory cell infiltration, penetration in the alveolar cavity, alveolar septum thickening, and hemorrhage [24]. Li and coworkers also supported that paraquat propagated MDA, MPO, and IL-6 levels and lung injury score in H&E staining while decreasing SOD activity in lung tissue of C57BL/6J mice [25]. The findings of these studies may confirm our hypothesis that paraquat induces lung injury by increasing oxidative stress and inflammation in the lungs.

Our results also revealed that piperine meaningfully increased body weight, GSH and IL-10 level, GSH/MDA ratio, and IL-10/TNF-α ratio while attenuating MDA and TNF-α level and histopathological damage in lung tissue. Our results are in line with Saha and co-workers, which emphasized that piperine remarkably alleviated MDA, nitric oxide (NO), TNF-α, IL-6, IL-1β, and TGF-β levels in cigarette smoke-stimulated lung injury in mice. Moreover, they also found that piperine notably elevated the levels GSH and the expression of nuclear factor erythroid 2–related factor 2 (Nrf2) mRNA following lung injury in mice. Piperine also improved lung histology and inflammation scores in mice [26].

Another investigation showed that piperine reduced MDA level, TNF-α, and intercellular adhesion molecule 1 (ICAM-1) mRNA expression while enhancing total anti-oxidant capacity following renal ischemia-reperfusion stimulated acute kidney damage in rats [27]. Aside from that, Yan et al. noticed that piperine significantly diminished TNF-α, IL-6, monocyte-chemoattractant protein 1 (MCP-1), nuclear factor kappa-B (NF-κB), and MDA levels while elevating SOD activity and Nrf2 expression following doxorubicin-induced cardiotoxicity in mice [28]. In terms of the anti-oxidative and anti-inflammatory properties of piperine, these studies support our findings.

We found that seven weeks of aerobic exercise training protocol strikingly reduced oxidative stress, inflammatory markers, as well as histopathological damage while ascending body weight, anti-oxidative, and anti-inflammatory markers following the paraquat-induced lung damage. In line with our results, Fredrickson and colleagues indicated that aerobic exercise training exercise training notably diminished IL-1β, TNF-α, ICAM-1, MCP-1, and TGF-β while elevated IL-10 expression in liver tissue following non-alcoholic steatohepatitis (NASH) model in mice [29]. Another study investigated the effects of aerobic exercise training on patients with Parkinson's disease. They noticed that 12 weeks of aerobic exercise training markedly alleviated TNF-α and IL-6 while enhancing IL-10 and IL-10/TNF-α ratio in the serum of patients with Parkinson's disease [30].

Similarly, Wang and coworkers supported that aerobic exercise training significantly mitigated ROS levels while propagating GSH level, SOD, CAT, and GPx activities, and Nrf2 expression in skeletal muscle of ApoE KO mice receiving a high-fat diet regimen [31]. Zhang et al. also showed that aerobic exercise training attenuated MDA and NO levels and TGF-β1/Smad Signaling while elevating GSH level and SOD activity in liver tissue of type 2 diabetic mice [32]. We have observed the anti-oxidative and anti-inflammatory impacts of aerobic exercise during training, which is in accordance with these studies.

Vitamin E is a natural supplementary factor that is well known for its anti-oxidant impacts [33]. Therefore, as a positive control, vitamin E was utilized in this study. Based on our results, we found that vitamin E meaningfully diminished MDA and TNF-α levels and histopathological damage while propagating body weight, GSH, and IL-10 levels following the paraquat-induced lung damage. Similar to our findings, Jiang et al. suggested that vitamin E notably alleviated IL-4, IL5, IL-13, IL-25, and IL-33 amounts in mice models of asthma and rhinitis [34].

Similarly, Abdelrazik and coworkers noticed that vitamin E remarkably diminished MDA, NO, and iNOS levels while enhanced GSH levels following bisphenol A-induced rat nephrotoxicity in rats. Vitamin E also mitigated inflammation through decreasing NF-κB while propagating IL-4, and Nrf2 expression levels in rats [35]. Furthermore, meta-analysis research evaluating in vitro and in vivo evidence emphasized that vitamin E meaningfully diminished oxidative stress (ROS and MDA) as well as markers of inflammation (TNF-α, IL-6, and C-reactive protein) while elevating anti-oxidative markers (GPx) in nanomaterial-induced oxidative stress and inflammation [36]. Interestingly, we revealed that the effects of piperine and a combination of piperine and exercise training on GSH, TNF-α, and IL-10 levels were significantly greater than vitamin E alone.

Finally, we hypothesized that a combination of piperine supplementation and aerobic exercise training could potentiate their protective impacts against paraquat-induced lung damage. In fact, the effects of the two combined interventions on MDA, GSH, TNF-α, and IL-10 levels were more potent than the aerobic exercise training group. Similarly, Groussard et al. found that linseed oil supplementation and aerobic exercise training significantly potentiate their anti-oxidative effects against obesity-stimulated oxidative stress in rats fed with high-fat diet regimens [37]. Similarly, the combination of *Camelina sativa* oil supplementation and aerobic exercise training firmly attenuated TNF-α, MDA, and histopathological damage than *Camelina sativa* oil and aerobic exercise training alone in streptozotocin-induced diabetic rats [38]. Taken together, combined use of piperine and aerobic exercise may possess more protective effects against paraquat-induced lung damage than each one alone.

As the strengths of the present study, we evaluated oxidative, anti-oxidative, inflammatory, and anti-inflammatory markers in homogenates of the lung tissues. In fact, the tissue homogenates are more accurate than the lavage fluids since the lavage collected the surface indicators but the tissue homogenates represent wholes. However, as the limitations of the present study, we did not evaluate oxidative and inflammatory markers in bronchoalveolar lavage fluid (BALF), which is also an important inclusion in any lung toxicology studies. In addition, we determined MDA as oxidative, GSH as anti-oxidative, TNF-α as inflammatory, and IL-10 as anti-inflammatory markers. However, adding further confirmatory parameters could have further strengthened the study. In this

regard, other anti-oxidants in addition to GSH (superoxide dismutase, glutathione reductase, and catalase), other oxidative damage parameters in addition to MDA (reactive oxygen species), and other cytokines in addition to TNF-α and IL10 (IL-6, IL-17) may be evaluated in future studies. Furthermore, more mechanistic evaluations may also help us better understand the exact mechanism of action responsible for the protective effects of aerobic exercise training and piperine against paraquat-induced lung damage. In addition, future clinical investigations may be necessary to determine these promising impacts in clinical practice.

5. Conclusion

In conclusion, treatment with piperine, aerobic exercise training, aerobic exercise program combined with piperine, and vitamin E significantly mitigated oxidative, inflammatory markers, and histopathological damage while propagating body weight, anti-oxidative and anti-inflammatory markers following the paraquat-induced lung damage. Interestingly, piperine and aerobic exercise training combined with piperine possessed more substantial protective effects against paraquat-induced lung damage than training alone with aerobic exercise. Therefore, piperine and combined use of piperine and aerobic exercise may be considered valuable for preventing lung injuries and conducting future clinical evaluations.

Ethical approval

Hakim Sabzevari University's Research Ethics Committee approved the study in accordance with its ethical guidelines (No. IR.HSU.REC.1400.017).

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A statement of data availability

Upon a reasonable request, the corresponding author will provide data.

CRediT authorship contribution statement

Reyhane Ariyanasab: Investigation, Data curation. **Vahid Reza Askari:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition. **Roya Askari:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Vafa Baradaran Rahimi:** Writing – review & editing, Writing – original draft, Supervision, Formal analysis. **Keyvan Hejazi:** Methodology, Investigation. **Milad Asadi:** Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] H. Guo, L. Li, L. Gao, Paraquat and diquat: recent updates on their pretreatment and analysis methods since 2010 in biological samples, Molecules 28 (2) (2023) 684.
[2] S.H. Zyoud, Investigating global trends in paraquat intoxication research from 1962 to 2015 using bibliometric analysis, Am. J. Ind. Med. 61 (6) (2018) 462–470.
[3] R. Jain, A.S. Bhalla, P. Naranje, S. Vyas, V. Rewari, I.A. Banday, et al., Ingestion poisoning related lung injury- a pictorial review, Emerg. Radiol. 29 (4) (2022) 757–767.
[4] V. Baradaran Rahimi, H. Rakhshandeh, F. Raucci, B. Buono, R. Shirazinia, A. Samzadeh Kermani, et al., Anti-inflammatory and anti-oxidant activity of portulaca oleracea extract on LPS-induced rat lung injury, Molecules 24 (1) (2019).
[5] Y. Zhang, D. Yuan, Y. Li, F. Yang, L. Hou, Y. Yu, et al., Paraquat promotes acute lung injury in rats by regulating alveolar macrophage polarization through glycolysis, Ecotoxicol. Environ. Saf. 223 (2021) 112571.
[6] V. Baradaran Rahimi, V.R. Askari, M. Hosseini, B.S. Yousefsani, H.R. Sadeghnia, Anticonvulsant activity of viola tricolor against seizures induced by pentylenetetrazol and maximal electroshock in mice, Iran. J. Med. Sci. 44 (3) (2019) 220–226.
[7] C.R. Quijia, V.H. Araujo, M. Chorilli, Piperine: chemical, biological and nanotechnological applications, Acta Pharm. 71 (2) (2021) 185–213.
[8] I.U. Haq, M. Imran, M. Nadeem, T. Tufail, T.A. Gondal, M.S. Mubarak, Piperine: a review of its biological effects, Phytother Res. 35 (2) (2021) 680–700.
[9] Y. Lu, J. Liu, H. Li, L. Gu, Piperine ameliorates lipopolysaccharide-induced acute lung injury via modulating NF-κB signaling pathways, Inflammation 39 (1) (2016) 303–308.
[10] E.T. de Lemos, J. Oliveira, J.P. Pinheiro, F. Reis, Regular physical exercise as a strategy to improve antioxidant and anti-inflammatory status: benefits in type 2 diabetes mellitus, Oxid. Med. Cell. Longev. 2012 (2012) 741545.

[11] L. Luo, M. Liu, H. Xie, Y. Fan, J. Zhang, L. Liu, et al., High-Intensity interval training improves physical function, prevents muscle loss, and modulates macrophage-mediated inflammation in skeletal muscle of cerebral ischemic mice, Mediat. Inflamm. 2021 (2021) 1849428.
[12] Y. Özçatal, F. Akat, Y. Tatar, H. Fıçıcılar, B. Serdaroğlu, F. Topal Çelikkan, et al., Effects of high-intensity interval training (HIIT) on skeletal muscle atrophy, function, and myokine profile in diabetic myopathy, Cytokine 169 (2023) 156279.
[13] H. Roshanbakhsh, M. Elahdadi Salmani, S. Dehghan, A. Nazari, M. Javan, F. Pourabdolhossein, Piperine ameliorated memory impairment and myelin damage in lysolecethin induced hippocampal demyelination, Life Sci. 253 (2020) 117671.
[14] H. Zeinvand-Lorestani, A. Nili-Ahmadabadi, F. Balak, G. Hasanzadeh, O. Sabzevari, Protective role of thymoquinone against paraquat-induced hepatotoxicity in mice, Pestic. Biochem. Physiol. 148 (2018) 16–21.
[15] T. Pangestiningsih, W. Wendo, Y. Selan, F. Amalo, N. Ndaong, V. Lenda, Histological features of catecholaminergic neuron in substantia nigra induced by paraquat dichloride (1,1-dimethyl-4,4 bipyridinium) in wistar rat as A model of Parkinson disease, Indonesian J. Biotechnol. 19 (2015) 91.
[16] M. Toygar, I. Aydin, M. Agilli, F.N. Aydin, M. Oztosun, H. Gul, et al., The relation between oxidative stress, inflammation, and neopterin in the paraquat-induced lung toxicity, Hum. Exp. Toxicol. 34 (2) (2015) 198–204.
[17] Y. Roohbakhsh, V. Baradaran Rahimi, S. Silakhori, H. Rajabi, P. Rahmanian-Devin, A. Samzadeh-Kermani, et al., Evaluation of the effects of peritoneal lavage with rosmarinus officinalis extract against the prevention of postsurgical-induced peritoneal adhesion, Planta Med. 86 (6) (2020) 405–414.
[18] O. Ernst, T. Zor, Linearization of the bradford protein assay, J. Vis. Exp. 38 (2010) 1918.
[19] M. Ghadiri, V. Baradaran Rahimi, E. Moradi, M. Hasanpour, C.C.T. Clark, M. Iranshahi, et al., Standardised pomegranate peel extract lavage prevents postoperative peritoneal adhesion by regulating TGF-β and VEGF levels, Inflammopharmacology 29 (3) (2021) 855–868.
[20] V.R. Askari, V. Baradaran Rahimi, A. Assaran, M. Iranshahi, M.H. Boskabady, Evaluation of the anti-oxidant and anti-inflammatory effects of the methanolic extract of Ferula szowitsiana root on PHA-induced inflammation in human lymphocytes, Drug Chem. Toxicol. 43 (4) (2020) 353–360.
[21] X. Shi, W. Zhu, T. Chen, W. Cui, X. Li, S. Xu, Paraquat induces apoptosis, programmed necrosis, and immune dysfunction in CIK cells via the PTEN/PI3K/AKT axis, Fish Shellfish Immunol. 130 (2022) 309–316.
[22] X. Liu, H. Yang, Z. Liu, Signaling pathways involved in paraquat-induced pulmonary toxicity: molecular mechanisms and potential therapeutic drugs, Int. Immunopharm. 113 (Pt A) (2022) 109301.
[23] Z.D. Zhang, Y.J. Yang, X.W. Liu, Z. Qin, S.H. Li, J.Y. Li, Aspirin eugenol ester ameliorates paraquat-induced oxidative damage through ROS/p38-MAPK-mediated mitochondrial apoptosis pathway, Toxicology 453 (2021) 152721.
[24] Z. Zhang, Q. Nian, G. Chen, S. Cui, Y. Han, J. Zhang, Klotho alleviates lung injury caused by paraquat via suppressing ROS/P38 MAPK-regulated inflammatory responses and apoptosis, Oxid. Med. Cell. Longev. 2020 (2020) 1854206.
[25] W. Li, M. Li, K. Chen, Y. Tang, R. Yin, L. Lan, et al., Oxaloacetate acid ameliorates paraquat-induced acute lung injury by alleviating oxidative stress and mitochondrial dysfunction, Front. Pharmacol. 13 (2022) 1029775.
[26] P. Saha, S. Durugkar, S. Jain, P.A. Shantanu, S.R. Panda, A. Jala, et al., Piperine attenuates cigarette smoke-induced oxidative stress, lung inflammation, and epithelial-mesenchymal transition by modulating the SIRT1/nrf2 Axis, Int. J. Mol. Sci. 23 (23) (2022) 14722.
[27] M. Mohammadi, H. Najafi, Z. Mohamadi Yarijani, G. Vaezi, V. Hojati, Protective effect of piperine in ischemia-reperfusion induced acute kidney injury through inhibition of inflammation and oxidative stress, J Tradit Complement Med 10 (6) (2020) 570–576.
[28] J. Yan, S.C. Xu, C.Y. Kong, X.Y. Zhou, Z.Y. Bian, L. Yan, et al., Piperine alleviates doxorubicin-induced cardiotoxicity via activating PPAR-γ in mice, PPAR Res. 2019 (2019) 2601408.
[29] G. Fredrickson, F. Barrow, K. Dietsche, P. Parthiban, S. Khan, S. Robert, et al., Exercise of high intensity ameliorates hepatic inflammation and the progression of NASH, Mol. Metabol. 53 (2021) 101270.
[30] P. Malczynska-Sims, M. Chalimoniuk, Z. Wronski, J. Marusiak, A. Sulek, High-intensity interval training modulates inflammatory response in Parkinson's disease, Aging Clin. Exp. Res. 34 (9) (2022) 2165–2176.
[31] L. Wang, J. Lavier, W. Hua, Y. Wang, L. Gong, H. Wei, et al., High-Intensity interval training and moderate-intensity continuous training attenuate oxidative damage and promote myokine response in the skeletal muscle of ApoE KO mice on high-fat diet, Antioxidants 10 (7) (2021) 992.
[32] Q. Zhang, F. Shen, W. Shen, J. Xia, J. Wang, Y. Zhao, et al., High-Intensity interval training attenuates ketogenic diet-induced liver fibrosis in type 2 diabetic mice by ameliorating TGF-β1/smad signaling, Diabetes Metab Syndr Obes 13 (2020) 4209–4219.
[33] G. Aykutoglu, M. Tartik, E. Darendelioglu, A. Ayna, G. Baydas, Melatonin and vitamin E alleviate homocysteine-induced oxidative injury and apoptosis in endothelial cells, Mol. Biol. Rep. 47 (7) (2020) 5285–5293.
[34] J. Jiang, E. Mehrabi Nasab, S.M. Athari, S.S. Athari, Effects of vitamin E and selenium on allergic rhinitis and asthma pathophysiology, Respir. Physiol. Neurobiol. 286 (2021) 103614.
[35] E. Abdelrazik, H.M. Hassan, Z. Abdallah, A. Magdy, E.A. Farrag, Renoprotective effect of N-acetylcystein and vitamin E in bisphenol A-induced rat nephrotoxicity; Modulators of Nrf2/NF-κB and ROS signaling pathway, Acta Biomed. 93 (6) (2022) e2022301.
[36] D. Xie, J. Hu, Z. Yang, T. Wu, W. Xu, Q. Meng, et al., Vitamin supplementation protects against nanomaterial-induced oxidative stress and inflammation damages: a meta-analysis of in vitro and in vivo studies, Nutrients 14 (11) (2022) 2214.
[37] C. Groussard, C. Plissonneau, L. Josset, F. Capel, M. Mura, E. Gouraud, et al., Beneficial effects of high intensity interval training and/or linseed oil supplementation to limit obesity-induced oxidative stress in high fat diet-fed rats, Nutrients 13 (10) (2021) 3531.
[38] Z. Kavyani, P. Dehghan, M. Khani, M. Khalafi, S.K. Rosenkranz, The effects of camelina sativa oil and high-intensity interval training on liver function and metabolic outcomes in male type 2 diabetic rats, Front. Nutr. 10 (2023) 1102862.