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Original Article

Preventive Potential of *Crocus sativus* L. (Saffron) Hydro-Alcoholic Extract and Safranal Against Systemic Inflammatory and Oxidative Alterations Caused by Paraquat in Rats

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Abstract

Introduction: Paraquat (PQ) is a toxic herbicide that induces systemic inflammation and oxidative stress. Saffron (*Crocus sativus* L.) (CS) and its constituent safranal possess antioxidant and anti-inflammatory properties, while pioglitazone, a peroxisome proliferator-activated receptor gamma (PPARγ) agonist, may also protect against inflammatory and oxidative injury. However, their preventive effects against PQ-induced systemic toxicity, alone or in combination, remain unclear. The present study aimed to investigate whether saffron, safranal, and pioglitazone and their combination can prevent systemic inflammation and oxidative stress resulting from PQ inhalation.

Methods: Ten groups of male Wistar rats (n = 7) were included in the experiment. The control animals (Ctrl) were exposed to saline, while the other groups to inhaled PQ aerosol (54 mg/m³, eight times in alternate days). The PQ-exposed rats then received different treatments as: saline (PQ group), two doses of CS hydro-alcoholic extract (CS-L, CS-H), two doses of safranal (Saf-L, Saf-H), pioglitazone (Pio), a combination of Pio with CS-L (Pio + CS), or Saf-L (Pio + Saf), or dexamethasone (Dexa). During the 16-days of PQ exposure, pioglitazone was delivered through intraperitoneal injection, whereas the other agents were administered orally during PQ exposure for 16 days. Measurements included total and differential white blood cell (WBC) counts, serum levels of catalase (CAT), superoxide dismutase (SOD), thiol, malondialdehyde (MDA), tumor necrosis factor-alpha (TNF-α), and interleukin-10 (IL-10). Data normality was assessed using the Kolmogorov–Smirnov test; group comparisons were performed by one-way ANOVA followed by Tukey's post hoc test.

Results: PQ inhalation significantly increased differential and total WBC counts, IL-10, TNF-α, and MDA levels, while decreasing CAT, SOD, and thiol levels ($P < 0.001$). Treatment with CS, Saf, Pio + CS, Pio + Saf, Pio, and Dexa significantly mitigated these effects ($P < 0.05$–0.001). Pio + CS and Pio+ Saf demonstrated stronger improvements in most variables compared to individual treatments.

Conclusion: Briefly, CS, Saf, and Pio mitigated PQ-induced systemic oxidative stress and inflammation in a preventive manner. Additionally, a synergistic effect was observed with Pio+ CS and Pio+ Saf, potentially involving PPARγ-mediated pathways in the beneficial properties of CS and Saf.

Introduction

Paraquat (PQ) is a commonly used pesticide that efficiently controls weed growth globally. PQ has a distribution volume of around 1-2 L/kg and distributes across many tissues, with the kidney and lungs containing higher concentrations.[1] Exposure to this hazardous material can result in lung edema and pulmonary fibrosis in animals and humans, mostly associated with oxidative stress and inflammatory responses.[2,3] Furthermore, PQ exposure can cause multi-organ failure and death, the severity of which is determined by its concentration in the bloodstream.[4] This negative effect is principally caused by the production of lipid peroxidation and free radicals, which eventually lead to collagen synthesis and the accumulation of matrix proteins.[1] Furthermore, it has been found that exposure to PQ causes a rise in tumor necrosis factor-

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alpha (TNF-α), interleukin (IL)-6, and IL-1β, which in turn promote systemic and pulmonary inflammation.[5] Exposure to PQ through inhalation has been shown to increase total and differential WBC counts, which indicates activation of inflammatory processes. PQ also disturbs the oxidative balance by decreasing thiol content, increasing malondialdehyde (MDA) levels, and suppressing the functions of catalase (CAT) and superoxide dismutase (SOD).[2,6]

PQ poisoning treatment involves the use of various substances, including activated charcoal, anti-inflammatory drugs, immunosuppressive medications, and antioxidants like salicylate and acetylcysteine.[7] However, these therapies do not provide a complete treatment for PQ toxicity, emphasizing the importance of discovering novel medications to prevent and manage PQ-induced lung or systemic complications. Currently, research is increasingly focused on the potential advantages of herbal medications with antioxidant and anti-inflammatory properties as alternative therapeutic methods for several disease prevention and management.[8-10] Over the past few decades, the therapeutic potential of medicinal plants has attracted considerable attention in addressing PQ-induced toxicity. Several examples include naringenin,[11] berberine,[12] quercetin,[13] *Zataria multiflora*,[14-17] and *Ginkgo biloba*,[18] which have been recognized for their potential in managing PQ-induced toxicity.

Saffron, scientifically identified as *Crocus sativus* L. (CS), is a perennial plant from the Iridaceae family. It is primarily grown in Iran, Spain, India, Greece, and Morocco.[19] CS has traditionally been utilized for its antidepressant, anti-inflammatory, antispasmodic, and aphrodisiac properties.[20,21] The main bioactive components present in CS include crocin, safranal, and picrocrocin.[22] Safranal (Saf) contributes to the characteristic aroma of saffron and exhibits potential antioxidant, anti-inflammatory,[23] anticancer,[24] and anti-asthmatic[25] effects. Modern pharmacological studies have explored the potential of CS in various therapeutic areas, including cancer prevention,[26] cardiovascular health,[27] and anti-asthmatic[28] effects.

Recent findings have highlighted the involvement of the peroxisome proliferator-activated receptor gamma (PPARγ) pathway in modulating inflammatory reactions and oxidative stress.[29] Activation of PPARγ has been shown to suppress the production of pro-inflammatory cytokines and reduce oxidative damage in models of lung injury or systemic inflammation and oxidative stress.[29,30] Therefore, pharmacological activation of this receptor may represent a promising strategy to attenuate PQ-induced toxicity. Pioglitazone (Pio), a well-known PPARγ agonist, has demonstrated anti-inflammatory and antioxidant effects in several experimental models.

In addition, dexamethasone (Dexa), a potent glucocorticoid with well-established anti-inflammatory and immunosuppressive properties, is frequently used as a reference anti-inflammatory drug in experimental studies.[29,30] Including Dexa in the present study allows comparison of the effects of the tested compounds with a standard anti-inflammatory treatment.

The present study was designed to determine whether CS hydro-alcoholic extract, Saf, and pioglitazone (Pio) could offer protection against the oxidative and inflammatory disturbances caused by PQ in rats. By exploring this question, the research intends to enhance understanding of the therapeutic value of these agents in mitigating PQ-induced damage.

Materials and methods

Chemicals

The chemicals utilized in this study and their respective providers are as follows:

Sigma-Aldrich Chemical Co. St. Louis, MO, USA: PQ and dexamethasone;

Samisaz Pharmaceutical Company, Iran: pioglitazone (Pio);

Betagene laboratory equipment, Mashhad, Iran: ethanol 96%;

Novin zafran, Mashhad, Iran: CS and Saf.

Plant material and extract preparation

Samples of CS were obtained and authenticated by Mrs. Molaei. A reference specimen was deposited in the Herbarium of the Faculty of Agriculture at Ferdowsi University of Mashhad (Voucher No. 143-0319-1).

To prepare the hydro-alcoholic extract, 10 grams of the dried and chopped plant material were subjected to extraction using a Soxhlet apparatus with a mixture of 25 ml distilled water and 25 ml ethanol. After extraction, the solvent was evaporated under reduced pressure, and distilled water was added to adjust the final concentration of the extract to 10 g% of plant material.[31]

Animals

Male Wistar rats, eight to nine weeks old, were provided by the animal center of Mashhad University of Medical Sciences (MUMS), Mashhad, Iran. The average weight of the rats was 230 ± 34 g. The animals were kept in steel cages with controlled environmental conditions, such as a 12-hour light/dark cycle, 22 ± 2 °C temperature, and 54 ± 2% relative humidity. Throughout the experiment, the rats had adequate provisions, including unrestricted access to food and water. The methods used for research on rats fully followed the standards and rules provided by the MUMS Animal Experiments Ethical Committee (ID: 961810). Before the start of the experiment, the animals were randomly assigned to the experimental groups. Outcome measurements and laboratory analyses were performed by investigators who were blinded to the group allocation.

Experimental groups

Seventy healthy male Wistar rats were used in the study and distributed into ten experimental groups, each consisting of seven rats.

1. Control group: This group received saline aerosol exposure for 30 minutes per session, repeated eight times every other day throughout the 16-days experimental

period.
2. PQ groups: Animals in these groups inhaled PQ aerosol at a concentration of 54 mg/m³ for 30 minutes every other day, totaling eight exposures. Throughout the 16-days PQ exposure period, various treatments were administered to the following groups.
2.1. PQ group: received saline
2.2. CS-L group: received 20 mg/kg CS hydro-alcoholic extract
2.3. CS-H group: received 80 mg/kg CS hydro-alcoholic extract
2.4. Saf-L group: received 0.8 mg/kg Saf
2.5. Saf-H group: received 3.2 mg/kg Saf
2.6. Pio group: received 5 mg/kg Pio
2.7. Pio + CS-L group: received a combination of CS-L and Pio
2.8. Pio + Saf-L group: received a combination of Saf-L and Pio
2.9. Dexa group: received 0.03 mg/kg Dexa.

It is important to point out that PQ was dissolved using saline as the solvent. An Omron CX3 nebulizer (Japan) was used to produce PQ aerosol with particles ranging from 3 to 5 µm. The nebulizer operated at an airflow rate of 8 L/min, and the generated aerosol was delivered into an exposure chamber following the procedure described in a previous study.[14] A PQ concentration of 54 mg/m³ was maintained within the exposure box.[17] The dosages, methods of administration, and duration of treatment were determined based on similar investigation.[2,32] Pio was administered intraperitoneally (i.p.) whereas the other treatment agents were administered orally (Figure 1).

Blood sample preparation, WBC count analysis, and biochemical parameter evaluation

At the end of the treatment period (day 17), the rats were anesthetized using xylazine (5 mg/kg, i.p.) and ketamine (50 mg/kg, i.p.). Blood samples (5 mL) were obtained directly from the heart. From these samples, 4 mL was centrifuged at 2000 rpm for 10 minutes to isolate the serum, which was preserved at −70 °C for later analysis of cytokines and oxidative stress indicators.

Using previously described methods, the total and differential WBC counts were determined.[33] The assessment of thiol, CAT, SOD, and MDA was carried out using standard procedures described previously.[6]

For the determination of blood cytokine levels (TNF-α and IL-10) enzyme-linked immunosorbent assay (ELISA) kits from Karmania Pars, Kerman, Iran, were utilized. The experiments were performed in compliance with previously described procedures[34] and the manufacturer's instructions. Blood samples from six randomly selected rats in each experimental group were analyzed to determine cytokine concentrations.

Statistics

The Kolmogorov-Smirnov test was used to determine the normality of the data distribution. Tukey's test was employed for multiple comparisons following the one-way ANOVA test. The findings were presented as means ± SEM in accordance with previous similar studies.[1,2] All statistical analyses were performed using GraphPad Prism 8 (GraphPad Software, Inc., San Diego, CA, USA). A p-value of less than 0.05 was considered indicative of statistical significance.

Results

Total and differential WBC

The results indicated that exposure to PQ significantly elevated the total WBC count as well as the differential counts, including eosinophils, lymphocytes, monocytes,

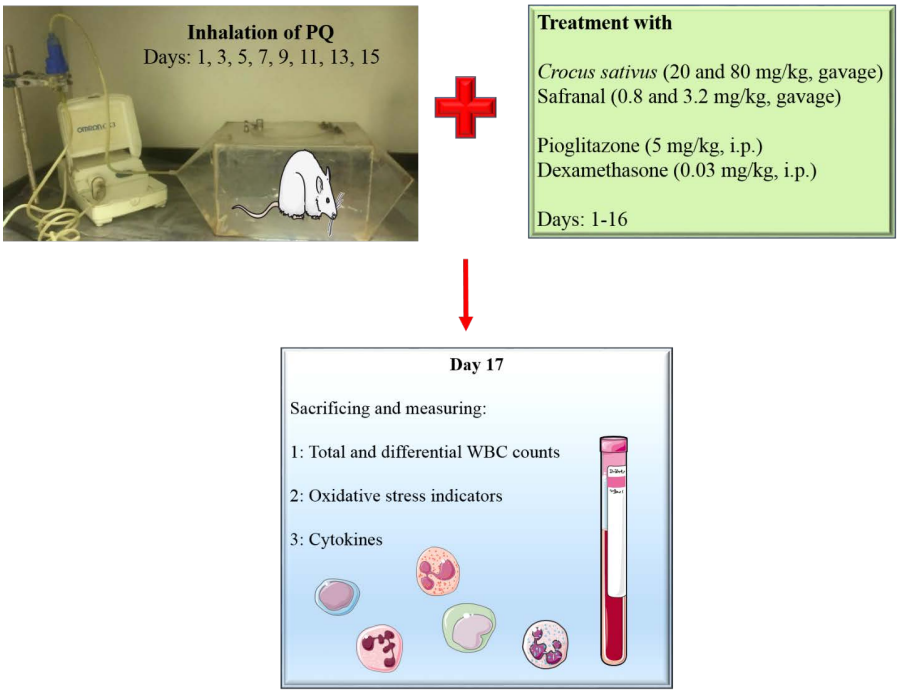


Figure 1 Experimental procedure of exposing rats to PQ and treatment groups (Images from https://smart.servier.com)

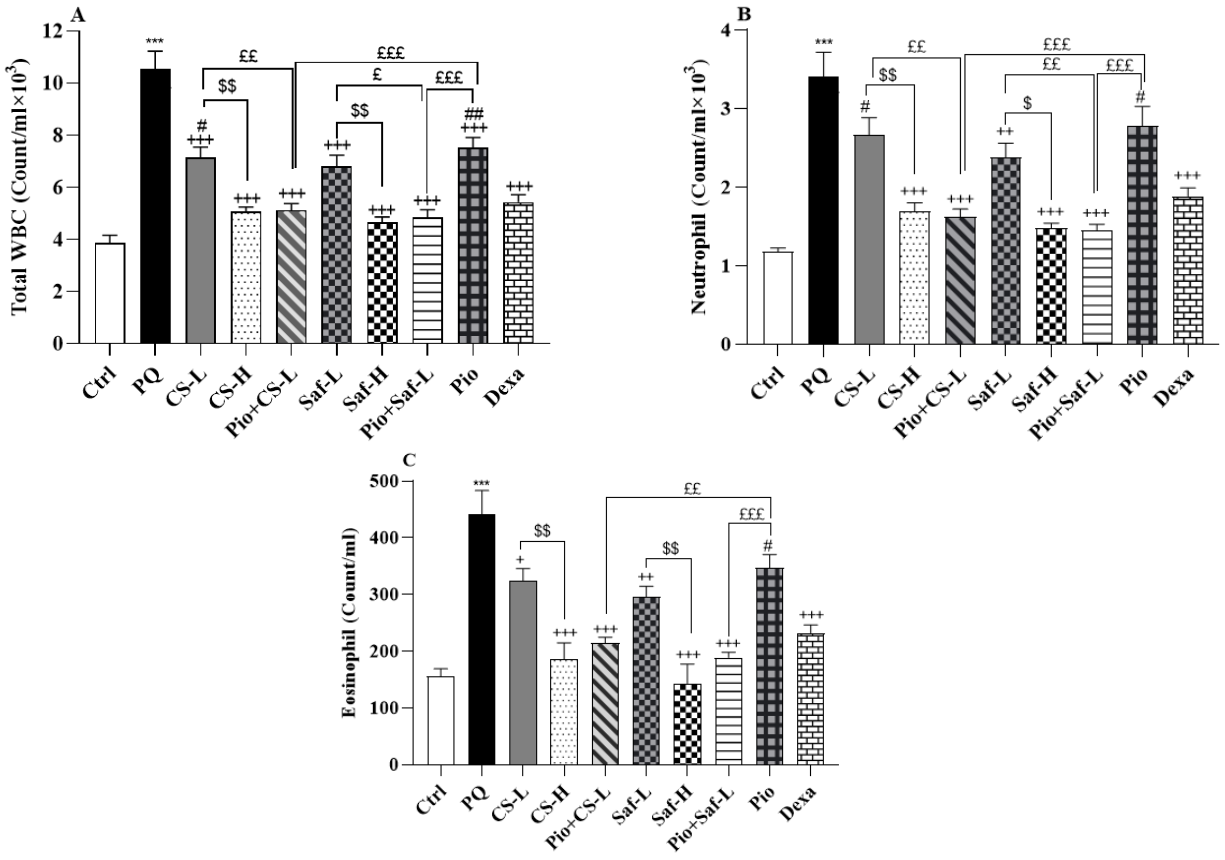


Figure 2 A: Total WBC, B: neutrophil, and C: eosinophil counts in the blood (Counts/ml) of the Ctrl group, PQ (54 mg/m^3), exposing groups to PQ and treated with CS-L (20 mg/kg, 16 days, gavage), CS-H (80 mg/kg, 16 days, gavage), Saf-L (0.8 mg/kg, 16 days, gavage), Saf-H (3.2 mg/kg, 16 days, gavage), Pio (5 mg/kg, 16 days, i.p.), Pio + CS-L, Pio + Saf-L and Dexa (0.03 mg/kg, 16 days, gavage). Dexa: dexamethasone, Pio: pioglitazone, PQ: paraquat, CS: *Crocus sativus*, Ctrl: control, Saf: safranal, L: low dose, H: high dose.
*** $P < 0.001$ statistical differences versus Ctrl group; + $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.001$ statistical differences versus PQ group; $ $P < 0.05$, $$ $P < 0.01$ statistical differences versus CS-H or Saf-H groups; # $P < 0.05$, ## $P < 0.01$ versus Dexa group; £ $P < 0.05$, ££ $P < 0.01$, and £££ $P < 0.001$ versus Pio + CS-L and Pio + Saf-L. The results are expressed as mean ± SEM (n= 7 in each group). One-way ANOVA followed by Tukey's multiple comparison test was applied for comparisons among different groups.

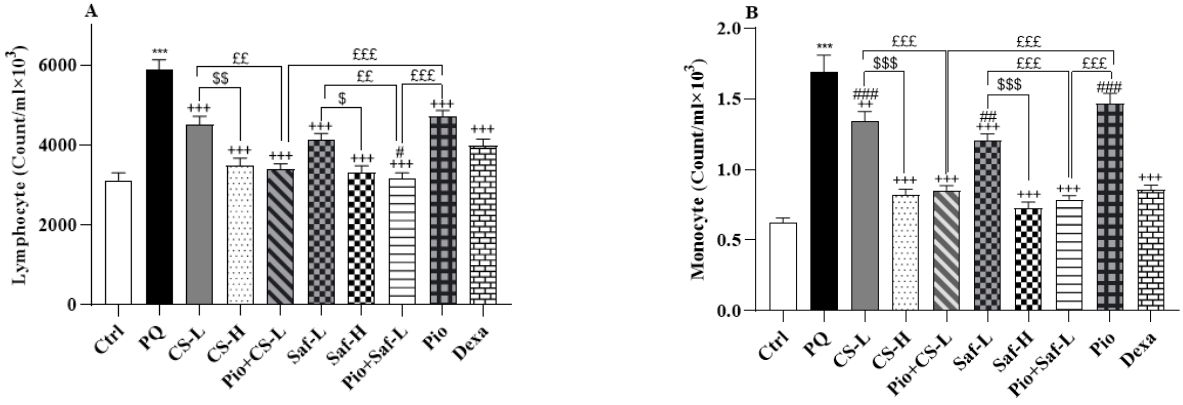


Figure 3 A: Lymphocyte and B: Monocyte counts in the blood (Counts/ml) of the Ctrl group, PQ (54 mg/m^3), exposing groups to PQ and treated with CS-L (20 mg/kg, 16 days, gavage), CS-H (80 mg/kg, 16 days, gavage), Saf-L (0.8 mg/kg, 16 days, gavage), Saf-H (3.2 mg/kg, 16 days, gavage), Pio (5 mg/kg, 16 days, i.p.), Pio + CS-L, Pio + Saf-L and Dexa (0.03 mg/kg, 16 days, gavage). Dexa: dexamethasone, Pio: pioglitazone, PQ: paraquat, CS: *Crocus sativus*, Ctrl: control, Saf: safranal, L: low dose, H: high dose.
*** $P < 0.001$ statistical differences versus Ctrl group; ++ $P < 0.01$, +++ $P < 0.001$ statistical differences versus PQ group; $ $P < 0.05$, $$ $P < 0.01$, $$$ $P < 0.001$ statistical differences versus CS-H or Saf-H groups; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.01$ versus Dexa group; ££ $P < 0.01$, and £££ $P < 0.001$ versus Pio + CS-L and Pio + Saf-L. The results are expressed as mean ± SEM (n= 7 in each group). One-way ANOVA followed by Tukey's multiple comparison test was applied for comparisons among different groups.

and neutrophils, compared to the control group; all differences were statistically significant ($P<0.001$) as shown in Figures 2 and 3.

Relative to the PQ-treated group, co-treatment with CS-L, CS-H, Pio + CS-L, Saf-L, Saf-H, Pio + Saf-L, Pio, or Dexa led to a marked decrease in both total WBC and lymphocyte counts ($P<0.001$). Moreover, neutrophil counts were reduced in the Saf-L group ($P<0.05$) as well as in the CS-H, Pio + CS-L, Saf-H, Pio + Saf-L, and Dexa groups. Eosinophil numbers also declined following

treatment with CS-L ($P<0.05$), Saf-L ($P<0.01$), and CS-H, Pio + CS-L, Saf-H, Pio + Saf-L, and Dexa ($P<0.001$). Additionally, compared to the PQ group, the number of monocytes was significantly lowered in the CS-L ($P<0.01$), CS-H, Pio + CS-L, Saf-L, Saf-H, Pio + Saf-L, and Dexa groups ($P<0.001$), as illustrated in Figure 3B.

The CS-H treatment produced greater reductions in total WBCs, neutrophils, eosinophils, lymphocytes ($P<0.01$), and monocytes ($P<0.001$) compared to the CS-L group. Likewise, the Saf-H group showed a more pronounced decrease in total WBCs, eosinophils ($P<0.01$), neutrophils, lymphocytes ($P<0.05$), and monocytes ($P<0.001$) than the Saf-L group.

Compared to the CS-L group, the Pio + CS-L treatment produced greater reductions in total WBC count, neutrophils, lymphocytes ($P<0.01$), and monocytes ($P<0.001$). In addition, this combination lowered total WBCs, neutrophils, lymphocytes, and monocytes ($P<0.001$), as well as eosinophils ($P<0.01$), more markedly than the Pio group.

Similarly, the Pio + Saf-L group showed greater decreases in total WBCs ($P<0.05$), neutrophils, lymphocytes ($P<0.01$), and monocytes ($P<0.001$) compared to the Saf-L group. Furthermore, relative to the Pio group, the Pio + Saf-L treatment significantly reduced the counts of total WBCs, neutrophils, eosinophils, lymphocytes, and monocytes ($P<0.001$).

Compared to the CS-L group, the Dexa treatment resulted in greater reductions in total WBC count, neutrophils ($P<0.05$), and monocytes ($P<0.001$). In addition, relative to the Pio group, Dexa more markedly decreased total WBCs ($P<0.01$), neutrophils, eosinophils ($P<0.05$), and monocytes ($P<0.001$).

Oxidative stress markers

PQ inhalation significantly elevated MDA level while markedly decreasing the serum concentrations of CAT, SOD, and thiol compared to the control group ($P<0.001$) (Figure 4).

CAT and SOD levels showed a marked elevation ($P<0.001$) in the CS-H, Pio + CS-L, Saf-H, Pio + Saf-L, and Dexa groups when compared to the PQ group. In contrast, MDA concentrations were lower in the CS-L, Pio, CS-H, Pio + CS-L, Saf-L, Saf-H, Pio + Saf-L, and Dexa treatment groups. Moreover, thiol levels rose significantly in the CS-H, Saf-H, and Pio + Saf-L ($P<0.001$), and in the Pio + CS-L group ($P<0.01$).

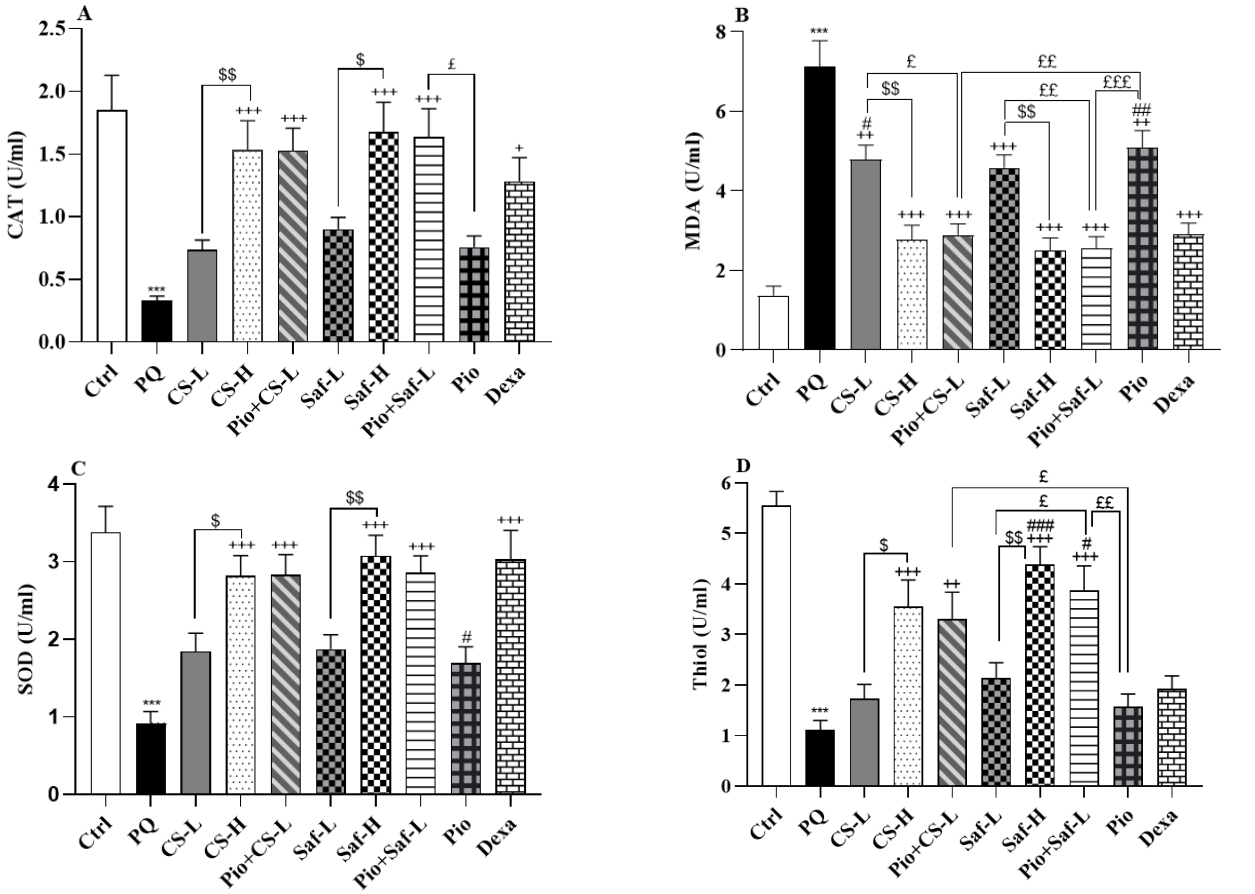


Figure 4 Oxidant and anti-oxidant biomarkers A: CAT, B: MDA, C: SOD, and D: thiol in the serum of the Ctrl group, PQ (54 mg/m^3), exposing groups to PQ and treated with CS-L (20 mg/kg, 16 days, gavage), CS-H (80 mg/kg, 16 days, gavage), Saf-L (0.8 mg/kg, 16 days, gavage), Saf-H (3.2 mg/kg, 16 days, gavage), Pio (5 mg/kg, 16 days, i.p.), Pio + CS-L, Pio + Saf-L and Dexa (0.03 mg/kg, 16 days, gavage). Dexa: dexamethasone, Pio: pioglitazone, PQ: paraquat, CS: *Crocus sativus*, Ctrl: control, Saf: safranal, L: low dose, H: high dose.
*** $P<0.001$ statistical differences versus Ctrl group; + $P<0.05$, ++ $P<0.01$, +++ $P<0.001$ statistical differences versus PQ group; \$ $P<0.05$, \$\$ $P<0.01$ statistical differences versus CS-H or Saf-H groups; # $P<0.05$, ## $P<0.01$, ### $P<0.01$ versus Dexa group; £ $P<0.05$, ££ $P<0.01$, and £££ $P<0.001$ versus Pio + CS-L and Pio + Saf-L. The results are expressed as mean ± SEM (n= 7 in each group). One-way ANOVA followed by Tukey's multiple comparison test was applied for comparisons among different groups.

Compared to the CS-L group, treatment with CS-H produced greater improvements in oxidative stress markers, including higher levels of CAT ($P<0.01$), SOD, and thiol ($P<0.05$), along with a more pronounced reduction in MDA level ($P<0.01$). Likewise, the Saf-H group showed stronger effects than the Saf-L group, reflected by increased CAT ($P<0.05$), SOD, and thiol ($P<0.01$) levels and a greater decline in MDA concentration ($P<0.01$).

The Pio + CS-L combination produced a more pronounced decline in MDA level than either CS-L ($P<0.05$) or Pio alone ($P<0.01$). This combination also caused a greater rise in thiol level compared to the Pio group ($P<0.05$). In addition, CAT activity was higher in the Pio + Saf-L group than in the Pio treatment alone ($P<0.05$). The Pio + CS-L treatment produced a greater reduction in MDA level than either the Saf-L ($P<0.01$) or the Pio groups ($P<0.001$). In addition, thiol concentration was significantly higher in the Pio + Saf-L group compared to both the Saf-L ($P<0.05$) and Pio ($P<0.01$) groups.

Dexa administration lowered MDA levels more effectively than treatment with CS-L ($P<0.05$) or Pio ($P<0.01$). Moreover, SOD activity in the Dexa group was significantly greater than that observed in the Pio group ($P<0.05$).

Cytokine levels of IL-10 and TNF-α

Exposure to PQ significantly increased the concentrations of TNF-α and IL-10 compared to the control group ($P<0.001$).

However, IL-10 level was reduced in the CS-H, Pio + CS-L, Saf-H, and Pio + Saf-L groups ($P<0.001$), as well as in the Dexa ($P<0.01$) and Saf-L ($P<0.05$) groups when compared to the PQ group. TNF-α level was also decreased by the CS-H, Pio + CS-L, Saf-H, Pio + Saf-L, Dexa ($P<0.001$), and Saf-L ($P<0.05$) groups.

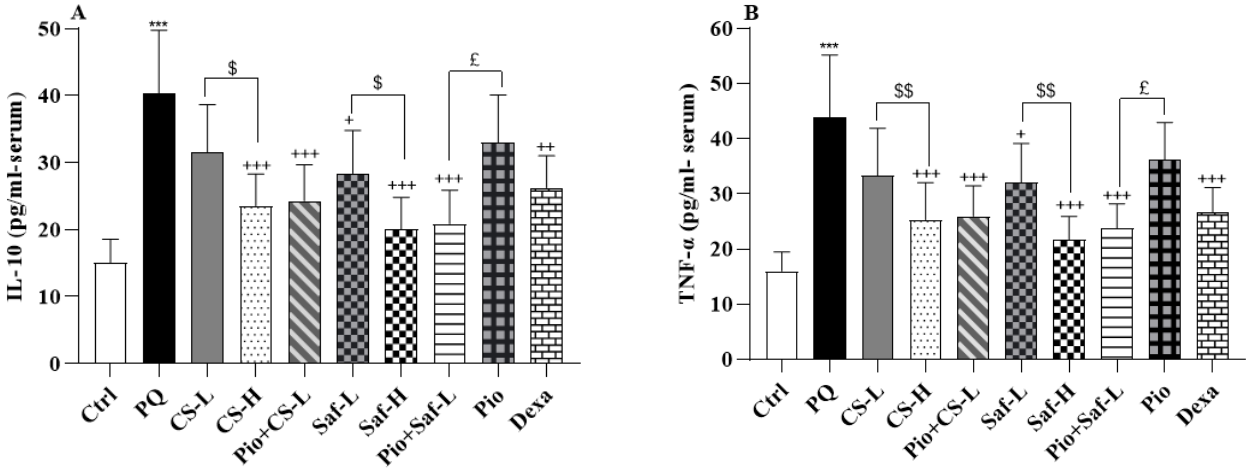
The CS-H group reduced the level of TNF-α ($P<0.01$) and IL-10 ($P<0.05$) more than the CS-L group. Similarly, the Saf-H group showed a greater reduction in TNF-α ($P<0.01$) and IL-10 ($P<0.05$) than the Saf-L group.

Figure 5 shows that the Pio + Saf-L group reduced TNF-α and IL-10 levels more than the Pio group ($P<0.05$).

Discussion

The main objective of this study was to assess the potential preventive effects of the hydroalcoholic extract of CS, Saf, and Pio on systemic inflammation and oxidative stress induced by PQ inhalation in rats. Inhalation of PQ resulted in increased total and differential WBC counts, elevated levels of MDA, IL-10, and TNF-α, as well as reduced levels of CAT, SOD, and thiol in the blood. However, treatment with CS-L, CS-H, Saf-L, Saf-H, Pio + CS-L, Pio + Saf-L, Pio, and Dexa demonstrated improvements in the PQ-induced alterations. Notably, the combination groups (Pio + CS-L and Pio + Saf-L) exhibited greater improvements in most parameters compared to the individual treatments, suggesting additive (or potentially synergistic) effects.

PQ toxicity is well-established to cause degenerative and inflammatory lesions via oxidative stress mechanisms.[6,35] PQ produces the superoxide anion, which increases the oxidative stress-related production of reactive oxygen species and toxic biomarkers.[35] These results are consistent with the current investigation, which found decreased thiol, SOD, and CAT levels and increased MDA levels in blood. Antioxidants have therefore been identified as a beneficial strategy to lessen and avoid the harmful effects induced by PQ exposure. In our study, the administration of CS-L, CS-H, Saf-L, and Saf-H could significantly decrease oxidative stress by reducing MDA, along with increasing CAT, SOD, and thiol levels. Consistent with our results another study found that CS aqueous extract (10, 20, and 40 mg/kg) reduced oxidative stress in blood by enhancing CAT, SOD, and glutathione levels in the CS-treated diabetic groups versus the untreated groups.[36] Another study reported that the administration of Saf (0.2 and 0.8 mg/kg) to rats with



ure 5 Inflammatory cytokines A: IL-10 and B: TNF-α in the serum of the Ctrl group, PQ (54 mg/m³), exposing groups to PQ and treated with CS-L (20 mg/kg, 16 days, gavage), CS-H (80 mg/kg, 16 days, gavage), Saf-L (0.8 mg/kg, 16 days, gavage), Saf-H (3.2 mg/kg, 16 days, gavage), Pio (5 mg/kg, 16 days, i.p.), Pio + CS-L, Pio + Saf-L and Dexa (0.03 mg/kg, 16 days, gavage). Dexa: dexamethasone, Pio: pioglitazone, PQ: paraquat, CS: *Crocus sativus*, Ctrl: control, Saf: safranal, L: low dose, H: high dose.

*** $P < 0.001$ statistical differences versus Ctrl group; + $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.001$ statistical differences versus PQ group; $ $P < 0.05$, $$ $P < 0.01$ statistical differences versus CS-H or Saf-H groups; £ $P < 0.05$ versus Pio + CS-L and Pio + Saf-L. The results are expressed as mean ± SEM (n= 7 in each group). One-way ANOVA followed by Tukey's multiple comparison test was applied for comparisons among different groups.

sciatic nerve injury lowered the blood MDA levels.[37]

Our data showed that inhalation of PQ significantly increased circulating cytokines, including TNF-α and IL-10. TNF-α is a well-known pro-inflammatory cytokine that plays a key role in the development of inflammatory responses associated with PQ toxicity.[32] Consistent with our findings, Ghasemi et al, showed that PQ inhalation increased the levels of TNF-α in comparison to the control group.[2] Although IL-10 is generally considered an anti-inflammatory cytokine,[38] its elevation following PQ exposure may represent a compensatory regulatory response aimed at limiting excessive inflammation and tissue injury. Another study also indicated that PQ inhalation increased IL-10 in rats versus the controls.[1] In contrast, administration of CS and Saf in the present study attenuated PQ-induced inflammatory responses. These findings are consistent with previous reports demonstrating the anti-inflammatory properties of CS and its constituents. For example, it has been reported that CS (0.1, 0.2, and 0.4 mg/ml) and Saf (4, 8, and 16 μg/ml) reduced serum inflammatory markers in sensitized guinea pigs.[39] Moreover, Boskabady et al, demonstrated that Saf (4, 8, and 16 μg/mL) lowered serum levels of cytokines in in ovalbumin-sensitized guinea pigs in comparison to the untreated group.[40]

The observed alterations in inflammatory cytokines in the present study may be closely related to modulation of the PPAR-γ signaling pathway. PPAR-γ is a nuclear receptor that plays a central role in regulating inflammatory responses and oxidative stress[41] by suppressing pro-inflammatory cytokine production, including TNF-α, and modulating immune cell activation through inhibition of NF-κB signaling.[42] Activation of PPAR-γ has also been associated with regulation of anti-inflammatory mediators such as IL-10, contributing to the resolution of inflammation.[43] Therefore, the reduction in inflammatory markers observed particularly in the pioglitazone-treated groups may be attributed, at least in part, to activation of the PPAR-γ pathway. Although direct assessment of PPAR-γ expression or activity was not performed in the present study, the cytokine profile observed is consistent with previously reported PPAR-γ–mediated anti-inflammatory mechanisms.

The interplay between inflammation and oxidative stress noted here suggests a potential bidirectional relationship, whereby increased oxidative stress can drive systemic inflammation (mobilizing eosinophils, neutrophils, monocytes, and macrophages), which in turn further amplifies oxidative stress.[35,44] PQ exposure is well known to induce oxidative stress and inflammatory responses, which are often reflected by alterations in both total and differential WBC counts, as reported in previous PQ studies.[1,15] In the current work, treatment with CS and Saf could considerably ameliorate the alterations induced by PQ in total and differential WBC counts. Supporting our findings, it has been shown that CS (0.1, 0.2 and 0.4 mg/ml) and Saf (4, 8 and 16 μg/ml) reduced total and differential WBC counts in ovalbumin-sensitized guinea pigs.[45]

A dose–response relationship was observed in the present study, as the higher doses of CS and Saf (CS-H and Saf-H) were more effective in attenuating PQ-induced alterations than the lower doses (CS-L and Saf-L). This finding suggests that the protective effects of these compounds may be dose dependent. Moreover, no significant differences were found between the effects of Saf and CS across the measured parameters, indicating that both treatments exerted comparable protective effects against PQ-induced oxidative stress and inflammation. This observation may suggest that Saf, as a major bioactive constituent of CS, contributes substantially to the overall protective effects of the plant extract. Nevertheless, further investigations are required to clarify the specific role of Saf and to determine the potential contributions of other active constituents present in CS.

Prior studies have consistently documented the anti-inflammatory and antioxidant properties of PPAR-γ agonists such as pioglitazone. For instance, Pio has been shown to inhibit the increase in myeloperoxidase activity as well as the production of TNF-α protein and messenger RNA (mRNA) at dosages of 5 and 10 mg/kg.[15] Additionally, studies have shown that Pio treatment can lead to a decrease in WBC counts in individuals with metabolic syndrome and advanced diabetic nephropathy.[46] Moreover, there is evidence supporting the efficacy of Pio in alleviating systemic inflammation and oxidative stress induced by inhaled PQ.[1] These findings validate the results of the present study regarding the effect of Pio on PQ-induced systemic oxidative stress and inflammation.

A notable aspect of our study is the pronounced, possibly additive or synergistic, benefit seen in groups receiving combined Pio with CS-L or Saf-L. These combinations achieved greater improvement across multiple outcomes than their individual components, suggesting that CS and Saf might themselves modulate PPAR-γ activity. To confirm a direct interaction, future mechanistic studies employing PPAR-γ receptor antagonists are recommended.

This investigation provides important and novel insights into the effects of CS and Saf on oxidative stress and systemic inflammation induced by inhaled PQ. Our findings suggest that PPAR-γ modulation may underlie the protective effects of both CS and Saf. Future studies should explore their anti-apoptotic potential *in vitro* and *in vivo*, investigate interactions with nuclear factor erythroid 2-related factor 2 (Nrf2) signaling, and assess potential benefits in cellular models. Such research will enhance our mechanistic understanding and guide therapeutic development.

In this investigation, Dexa was used as the positive control due to its well-established antioxidant and anti-inflammatory efficacy in the context of PQ toxicity.[2] Our results indicate no significant differences between the effects of Dexa and those of CS and Saf on most parameters, suggesting that the protective effects conferred by CS and Saf are comparable to those of Dexa in the measures studied.

This study has several strengths. It used a well-established

PQ inhalation model to evaluate systemic oxidative stress and inflammation and included comprehensive assessments of inflammatory cells, cytokines, and oxidative stress markers. The study also examined both the hydroalcoholic extract of CS and its major constituent Saf at two different doses, allowing evaluation of dose-dependent effects. In addition, the inclusion of Pio as a PPAR-γ agonist, both alone and in combination with CS and Saf, provided insight into a possible mechanistic pathway involved in their protective effects, while Dexa was used as a positive control to compare the efficacy of the tested compounds with a known anti-inflammatory drug. However, some limitations should also be considered. The exact bioactive compounds in the CS extract responsible for the observed effects were not fully characterized, and the study did not employ a specific PPAR-γ antagonist to directly confirm the involvement of this signaling pathway. Furthermore, the investigation was limited to systemic biochemical and hematological parameters, and additional molecular, cellular, and histopathological analyses would help to further clarify the underlying mechanisms of action.

Conclusion

Administration of CS, Saf, Pio, or Dexa effectively mitigates PQ-induced systemic inflammation and oxidative stress when applied preventively. Remarkably, the combination of Pio with either CS or Saf consistently produced superior protective outcomes compared with individual treatments, suggesting greater protective effects (and a possible synergistic interaction). These findings provide compelling evidence that the protective effects of CS and Saf may be mediated, at least in part, through the modulation of the PPARγ pathway.

Ultimately, these results underscore the significant therapeutic potential of CS, Saf, and Pio as strategies for alleviating PQ-induced toxicity. By elucidating these protective mechanisms, our findings provide a foundation for future translational research aimed at developing novel adjuvant therapies for managing inflammation-related disorders characterized by severe oxidative stress.

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Availability of Data And Materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work the author(s) used ChatGPT and Quillbot in order to rephrase to reduce plagiarism, improve the language and grammar. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Ethical Approval

Rats were treated according to Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press) and institutional guidelines for animal care and use (Department of Physiology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran). All experimental procedures were approved and conducted in accordance with the guidelines and regulations provided by the ethical committee of MUMS for Animal Experiments (ID: 961810).

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