Original Article

Protective effect of montelukast on paraquat-induced lung toxicity in rats

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Summary **In the current study, the possible protective effect of montelukast (Mont, 50 mg/kg, *p.o.* given 2, 24, and 48 h after paraquat injection) against acute paraquat toxicity (PQ, 25 mg/kg, *i.p.*) in rats was evaluated. The effects of treatment on selected oxidative stress parameters in lung tissues as well as certain inflammatory markers in serum were evaluated. The obtained results revealed that Mont can ameliorate the biochemical alterations in the lung tissues which were induced by PQ in rats. This was evidenced by the significant reduction in lung tissues lipid peroxidation, protein carbonyl content and DNA fragmentation, as well as by normalization of glutathione and myeloperoxidase activities. Moreover, the elevated levels of serum TNF-α, and LDH induced by paraquat were reversed in Mont-treated rats. Meanwhile, lung paraquat concentration was significantly reduced after Mont treatment compared with PQ alone group. Additionally, a higher survival percentage was observed in rats treated with Mont (80%) compared with the PQ alone group (30%) during 7 days observation. The results indicate that Mont protects lung tissue by balancing oxidant-antioxidant status, inhibiting neutrophil infiltration, and by regulating the generation of inflammatory mediators. In conclusion, the obtained results emphasize the beneficial effect of Mont in ameliorating the toxicity signs of paraquat in rats. The current study greatly recommends the usage of Mont in management of paraquat toxicity.**

Keywords: Paraquat, montelukast, lipid peroxidation, glutathione, protein carbonyl, DNA fragmentation, myeloperoxidase, tumor necrosis factor (TNF-α)

1. Introduction

Paraquat (PQ) is a widely used nonselective contact herbicide and a highly toxic compound for humans and animals (*1,2*). Irrespective of its route of exposure, paraquat is rapidly distributed in most tissues and most highly concentrated in the lungs, producing pneumotoxicity and lung injury (*1,3*). The strong pneumotoxicity of paraquat is mainly due to its accumulation in the lung through a polyamine uptake system and to its ability to induce redox cycling, leading to oxidative stress-related cell death and inflammation (*4*). Death is usually associated with respiratory insufficiency injury due to an oxidative insult to the alveolar epithelium with subsequent obliterating fibrosis (*1,5*). The paraquat-induced lung injury is morphologically characterized by an early destructive phase, in which the alveolar type I and type II epithelial cells are damaged and a second proliferative phase defined by alveolitis, pulmonary edema and infiltration of inflammatory cells (*6*). Thus, research for the treatment of paraquat toxicity has mainly focused on alleviating the lung injury.

The mechanism of paraquat toxicity involves the generation of the superoxide anion, with subsequent formation of more toxic reactive oxygen species (ROS), such as hydrogen peroxide and hydroxyl radical and the oxidation of the cellular NADPH, the major source of reducing equivalents for the intracellular reduction of paraquat, which results in the disruption of NADPH-requiring biochemical processes (*7-10*).

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Management of paraquat poisoning has remained mostly supportive, as there is no specific antidote or effective treatment for PQ poisoning has been identified so far. This management has been directed towards the modification of the toxicokinetics of paraquat before its cellular uptake (*11*). Additional protective measures have also been adopted recently, to prevent the generation or to scavenge ROS (*1*) and to reduce the inflammation (*4,10,12*).

Cysteinyl leukotrienes, namely leukotriene LTC4, LTD4, and LTE4, are potent proinflammatory lipid mediators derived *via* the 5-lipoxygenase pathway from arachidonic acid. They are mainly secreted by eosinophils, mast cells, monocytes, and macrophages, and play a crucial role in inflammation, bronchoconstriction, edema formation, and airway remodeling of asthmatics (*13-16*).

Anti-leukotriene agents, including leukotriene receptor antagonists and synthesis inhibitors, are known to be effective in several inflammatory models. The selective LTD4 receptor antagonist, montelukast (Mont), is used mainly to reduce eosinophilic inflammation in the airways of asthmatic patients (*17-18*), and is also effective in management of allergic rhinitis, COPD and idiopathic pulmonary fibrosis (*16*). Moreover, montelukast was reported to have beneficial effects in management of experimental gastric mucosal ulceration (*19*), colitis (*20*), oxidative renal damage (*21*), hepatopathy (*22*), burn-induced multiorgan damage (*23*), and renal ischemia/reperfusion injury (*24*). The protective effects of montelukast against vital organs injury in these experimental models were attributed, at least in part, to its ability to inhibit neutrophil infiltration, and to regulate the generation of inflammatory mediators in addition to its pronounced antioxidant potential.

Accordingly, in the current study, montelukast was chosen as a candidate to study its putative protective effect on paraquat-induced lung toxicity in rats. The effect of treatment on a selected oxidative stress markers and neutrophile migration in the lung tissues in addition to certain inflammatory markers in the serum, after three doses administration of Mont to rats exposed to acute PQ toxicity, were studied. The overall aim of this study was to find a new and a possible effective treatment that can protect against PQ-induced lung toxicity.

2. Materials and Methods

2.1. *Chemicals and drugs*

Paraquat dichloride was purchased from Sigma (St Louis, MO, USA). Montelukast was from Merck Sharp & Dohme Ltd. (Hoddesdon, Hertfordshire, UK). All other chemicals and reagents used were of highest analytical grade and were obtained from Sigma.

2.2. *Animals*

This study was performed using adult male Wistar rats (225 ± 25 g) obtained from the animal facilities of King Saud University. Animals were kept under standard laboratory conditions (12/12 h light/darkness, 22 ± 2ºC room temperature, 50-60% humidity) for at least 1 week before starting the experiments. Animals were allowed free access to tap water and rat chow ad libitum during the entire experiment. All experiments were carried out in accordance with the recommendations of King Saud University Committee Acts for using experimental animals which are complied with the international acts.

2.3. *Experimental protocol*

Animals were divided into three groups of rats (n = 10). One group of animals, served as control, was treated with saline (0.5 mL, *p.o.*) for three days. Paraquat group was treated with a single dose of paraquat (PQ, 25 mg/kg, *i.p.*) followed by oral saline administration after 2, 24, and 48 h. The third group was given montelukast (Mont, 50 mg/kg, *p.o.*) 2, 24, and 48 h after single injection of PQ (25 mg/kg, *i.p.*). All animals were sacrificed under light ether anesthesia, in the third day, after 3 h from the last treatment. Blood samples were collected, from 16 h fasted animals, and serum was separated immediately and stored at -80ºC for biochemical analysis. Lungs were removed immediately after killing, separated from the other organs, washed in ice-cold saline solution, blotted and weighed before subjecting to homogenization. Relative lung weight of each animal was calculated as a percentage of the absolute body weight on the sacrifice day.

In another set of experiments, animals were kept under the same conditions and treated as described above. Abnormal findings, including weakness and dyspnea, were noted and recorded if present. The lethality was registered every day until day 7 from PQ injection.

Paraquat dose (25 mg/kg, *i.p.*) was chosen according to the dose used in previous studies and after preliminary studies carried out by the author of the current study. This dose is known to produce severe lung toxicity and death in rats within few days (*4,25,26*).

2.4. *Determination of lung PQ concentration*

Measurement of PQ contents in lung tissues gives an indication about the severity of toxicity. Determination of lung PQ concentration was carried out according to the method of Fuke *et al.* (*27*) with minor modifications. Briefly, lung samples were homogenized in ice-cold 50 mM phosphate buffer containing 0.1% (v/v) Triton X-100 (pH 7.4). The homogenate was kept on ice, centrifuged at 3,000 × g, 4ºC, for 10 min. Aliquots of the lung supernatants were treated with

5-sulfosalicylic acid (5% in final volume) and then centrifuged (13,000 × g, 4ºC for 10 min). The resulting supernatant fractions were alkalinized with NaOH 10 N (pH > 9) and then gently mixed with the reductant sodium dithionite to give the blue color, characteristic of the PQ cation radical. The absorbance was read at 490 nm and the results were expressed as percentage of PQ group value.

2.5. *Estimation of lung malondialdehyde (MDA) and glutathione (GSH) activities*

Tissue samples were homogenized with ice-cold 150 mM KCl for the determination of MDA and glutathione levels. The MDA levels were assayed for products of lipid peroxidation by monitoring thiobarbituric acid reactive substance formation by the spectrophotometric method, as described previously by Ohakawa *et al*. (*28*). Lipid peroxidation was expressed as nmol MDA/g tissue. Glutathione was determined by the spectrophotometric method, based on the use of Ellman's reagent as described by Ellman (*29*), and the results were expressed as μmol GSH/g tissue.

2.6. *Estimation of lung protein carbonyl contents*

Protein carbonyl contents (PCO, ketones and aldehydes) were estimated according to the method of Levine *et al*. (*30*) using 2,4-dinitrophenylhydrazine (DNPH). Lung samples (0.2 g) were rinsed in 10 mM HEPES buffer (pH 7.4) and homogenized in phosphate buffer (pH 7.4). After centrifugation at 10,000 × g for 10 min, 0.5 mL of the supernatant was taken into tubes. Then equal volume of DNPH in 2 M HCl was added to each tube. The blank contained 2 M HCl only. All tubes were vortexed every 10 min for 1 h in dark and then mixed with 30% trichloroacetic acid. After centrifugation, the pellet was washed three times with 1 mL of ethanol: ethylacetate (1:1, v/v). The final pellets were dissolved in 1 mL of 6 M guanidine HCl in 20 mM potassium dihydrogen phosphate (pH 2.3). The difference in absorbance between the DNPH-treated and HCl-treated samples was determined at 370 nm. Lung tissue PCO contents were expressed as percentage of control normal group value.

2.7. *Determination of lung DNA fragmentation*

Estimation of DNA fragmentation was determined by colorimetric diphenylamine assay as described by Burton (*31*). Lung samples from different groups were homogenized in chilled lysis buffer (10 mM Tris-HCl, 20 mM EDTA, 0.5% Triton X-100, pH 8.0). Homogenates were then centrifuged at 27,000 × g for 20 min to separate intact chromatin in the pellet from fragmented/damaged DNA in the supernatant fractions. Perchloric acid was added to the pellets and supernatant samples to reach a final concentration of 0.5 N. Samples were heated at 90ºC for 15 min and centrifuged at 1,500 × g for 10 min to remove protein. The supernatants were left to react with diphenylamine for 18 h at room temperature and the absorbance was measured at 600 nm. DNA fragmentation was expressed as a percentage of total DNA appearing in the supernatant fractions. Treatment effects were reported as percentage of control fragmentation.

2.8. *Determination of lung myeloperoxidase activity*

Myeloperoxidase (MPO) activity in tissues was measured according to the modified method of Bradley *et al.* (*32*). Samples of lung tissues were homogenized in 50 mM potassium phosphate buffer pH 6.0 (PB), and centrifuged at 40,000 × g for 10 min. The pellets were then suspended in 50 mM PB containing 0.5% hexadec yltrimethylammonium bromide. After three freeze-and-thaw cycles, the samples were centrifuged at 40,000 × g for 10 min. Aliquots (0.3 mL) were added to 2.3 mL of reaction mixture containing 50 mM PB, *O*-dianisidine, and 20 mM H_2O_2 solution. One unit of enzyme activity was defined as the amount of MPO present that caused a change in absorbance, measured at 460 nm, for 3 min. MPO activity was expressed as U/g tissue.

2.9. *Estimation of serum tumor necrosis factor (TNF)-α and lactate dehydrogenase (LDH) activities*

Rat serum tumor necrosis factor (TNF)-α was estimated using enzyme-linked immunosorbent assay (ELISA) kit specific for the rat TNF-α according to the instruction manual of R&D kit with absorbance at 450 nm. The concentration of TNF-α was expressed as pg/mL. The activity of serum lactate dehydrogenase (LDH) was assayed using the commercial kit of bioMerieux, SA, France. The absorbance was measured at 340 nm and the enzyme activity was expressed as U/L.

2.10. *Statistical analysis*

Statistical analysis was done using a GraphPad Prism 3.0 (Graph-Pad Software, San Diego, CA). All data were expressed as means ± SEM. Groups of data were compared with analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Values of $p < 0.05$ were considered significant.

3. Results

Data of the current study showed that, there was no behavioral changes were noticed on day 1 after PQ injection, as compared to normal rats. However, starting from day 2 symptoms including rapid shallow respiration, dyspnea, loss of appetite, piloerection, and cyanosis were observed in paraquat-treated rats. These

symptoms were less apparent in the group treated with PQ + Mont. By the end of the 7 days post treatment, the survival was 100% in control normal group, while three of ten animals (30%) of PQ group and eight of ten animals (80%) of PQ + Mont group were survived.

Relative lung weight (RLW) was assessed to give an indication about the edema degree (Figure 1). No significant differences were obtained in RLW values among control and PQ + Mont group. However, in comparison to these groups, animals from the PQ group showed a significant RLW increase at 72 h after PQ exposure ($p < 0.001$, $p < 0.01$, respectively).

Measurement of PQ in lung tissues gives an indication about the severity of toxicity. Administration of 3 doses of Mont (50 mg/kg, *p.o.*) after PQ injection, produced a 60% reduction in lung concentration of PQ compared with PQ alone group (Figure 2).

Lung malondialdehyde levels, as an index of lipid peroxidation, were significantly increased in PQ-treated group (to 2 fold, $p < 0.001$, as compared to the control normal group). However, in montelukast treated rats, malondialdehyde levels were significantly reduced (to 31%, $p < 0.001$ compared with PQ-only group) (Figure 3).

Contents of lung GSH, a key antioxidant, were decreased significantly ($p < 0.001$) in PQ group compared with normal rats. Meanwhile, the lung GSH content was significantly restored back to near control normal levels upon montelukast treatment (to 102%, $p < 0.01$, compared with PQ group) (Figure 4).

Data in figure 5 showed that, administration of PQ produced a 2.3 fold increase in protein carbonyl content in the lung tissues compared with control normal rats ($p < 0.001$). On the contrary, post treatment with Mont prevented the increase in protein carbonyl content induced by PQ in lung tissues (to 40%, $p < 0.001$, compared with PQ group) (Figure 5).

Effect of Mont on PQ-induced DNA fragmentation in rat lung tissues is illustrated in Figure 6. The results showed that oral administration of PQ induced an increase of 2.9 fold in DNA fragmentation in the rat lung tissues compared with normal control, demonstrating tissue degeneration. Post administration

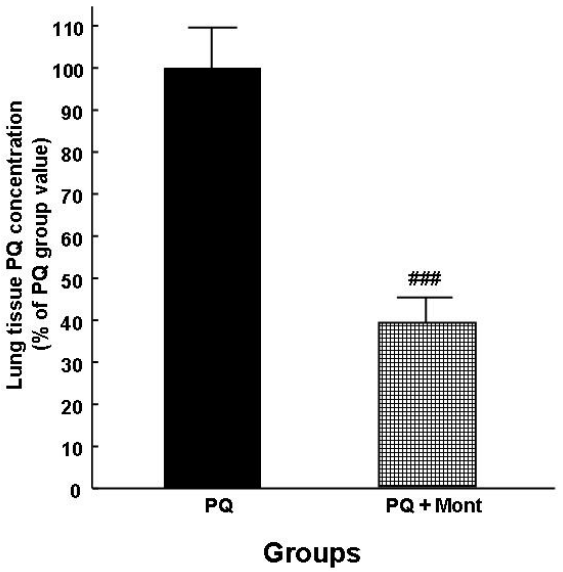

Figure 2. The lung paraquat concentration in paraquat (PQ), and paraquat + montelukast (PQ + Mont) treated rats. Values are expressed as percentage of paraquat treated rats ($n = 10$ rats). ### Significantly different from PQ treated group at $p < 0.001$.

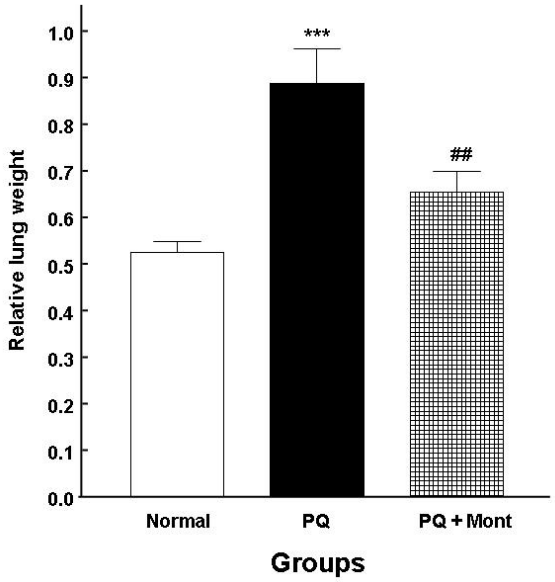

Figure 1. The relative lung weight in normal, paraquat (PQ), and paraquat + montelukast (PQ + Mont) treated rats. Values are expressed as mean ± SEM of ($n = 10$ rats). *** Significantly different from normal group (saline) at $p < 0.001$. ## Significantly different from PQ treated group at $p < 0.01$.

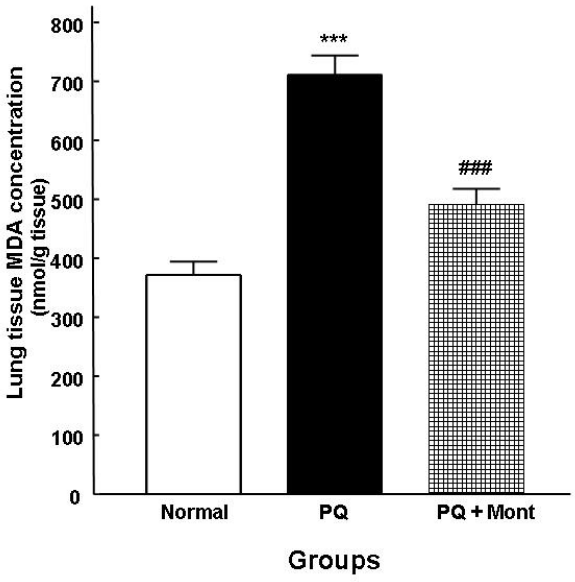

Figure 3. The lung malondialdehyde (MDA) concentration in normal, paraquat (PQ), and paraquat + montelukast (PQ + Mont) treated rats. Values are expressed as mean ± SEM of ($n = 10$ rats). *** Significantly different from normal group (saline) at $p < 0.001$. ### Significantly different from PQ treated group at $p < 0.001$.

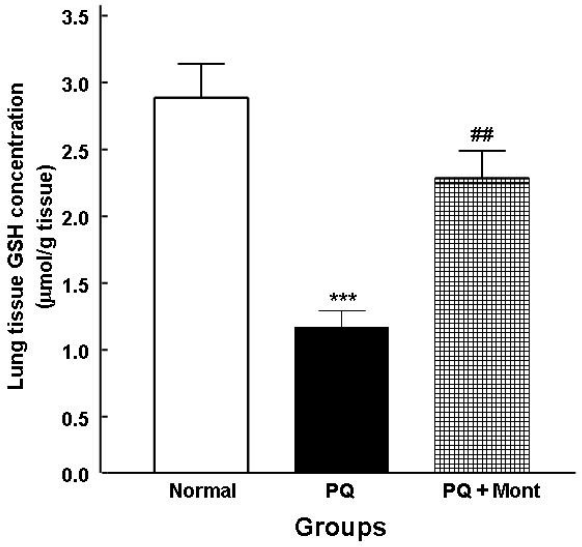

Figure 4. The lung glutathione (GSH) concentration in normal, paraquat (PQ), and paraquat + montelukast (PQ + Mont) treated rats. Values are expressed as mean ± SEM of (n = 10 rats). *** Significantly different from normal group (saline) at $p < 0.001$. ## Significantly different from PQ treated group at $p < 0.01$.

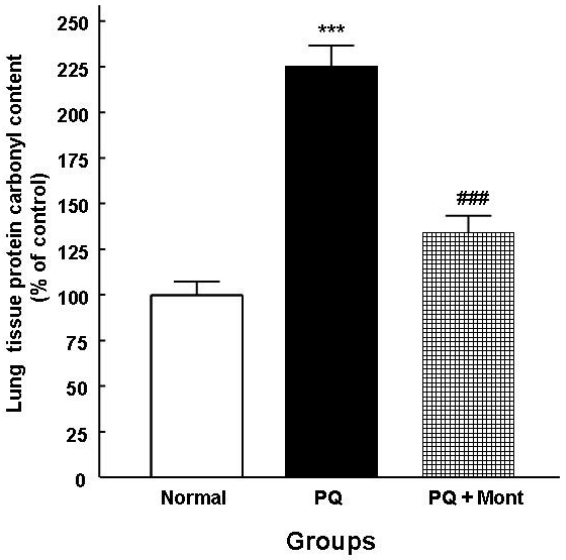

Figure 5. The lung protein carbonyl content in normal, paraquat (PQ), and paraquat + montelukast (PQ + Mont) treated rats. Values are expressed as percentage of control normal rats (n = 10 rats). *** Significantly different from normal group (saline) at $p < 0.001$. ### Significantly different from PQ treated group at $p < 0.001$.

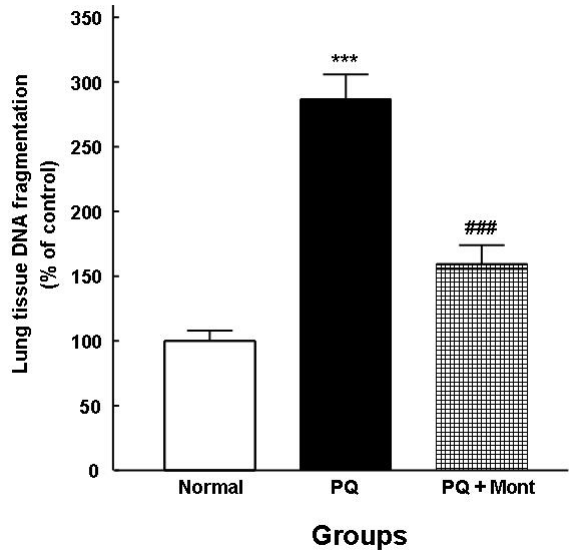

Figure 6. The lung DNA fragmentation in normal, paraquat (PQ), and paraquat + montelukast (PQ + Mont) treated rats. Values are expressed as percentage of control normal (n = 10 rats). *** Significantly different from normal group (saline) at $p < 0.001$. ### Significantly different from PQ treated group at $p < 0.001$.

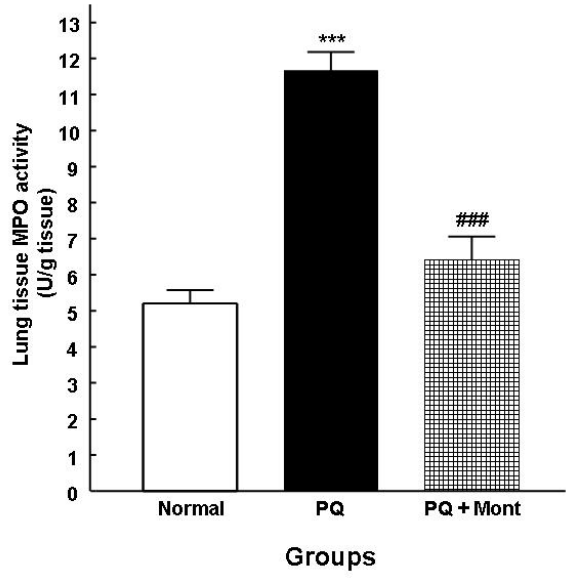

Figure 7. The lung myeloperoxidase (MPO) activity in normal, paraquat (PQ), and paraquat + montelukast (PQ + Mont) treated rats. Values are expressed as mean ± SEM of (n = 10 rats). *** Significantly different from normal group (saline) at $p < 0.001$. ### Significantly different from PQ treated group at $p < 0.001$.

of Mont significantly decreased PQ-induced lung DNA fragmentation (to 44%, $p < 0.001$, compared with PQ group). The results indicate the protective ability of Mont against PQ-induced DNA fragmentation in rat lung tissues.

Lung MPO activity is an indirect evidence of neutrophil infiltration into the tissue. As illustrated in Figure 7, Lung MPO activity of the PQ-exposed animals was significantly higher (about 2.2 fold, $p < 0.001$) compared with control normal group. Post-treatment of rats with Mont, completely prevented the increase of MPO activity and significantly reduced this parameter (to 45% compared with PQ group) (Figure 7).

Results of the present investigation showed that serum TNF-α level measured after 3 days from PQ injection was significantly higher than the control normal group ($p < 0.001$, Figure 8). Moreover, serum LDH level was significantly increased after PQ injection ($p < 0.001$, Figure 9) compared with normal rats. These findings verify generalized tissue damage.

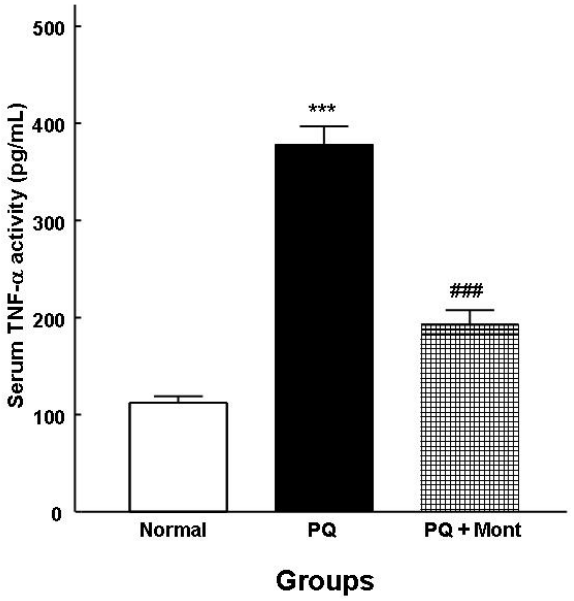

Figure 8. The serum TNF-α activity in normal, paraquat (PQ), and paraquat + montelukast (PQ + Mont) treated rats. Values are expressed as mean ± SEM of (n = 10 rats). *** Significantly different from normal group (saline) at $p < 0.001$. ### Significantly different from PQ treated group at $p < 0.001$.

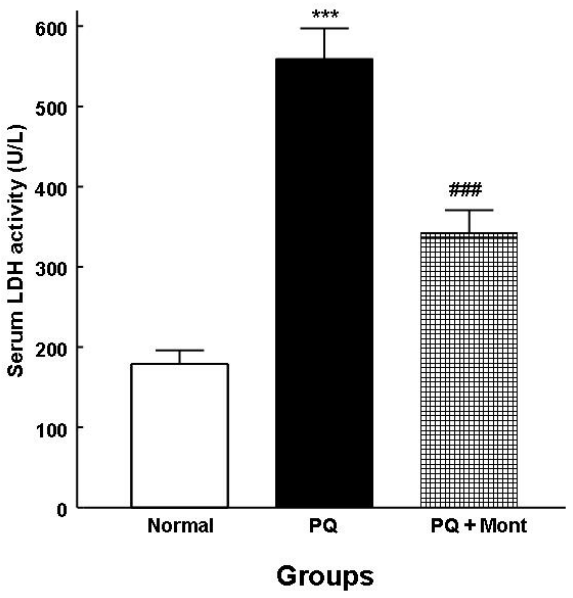

Figure 9. The serum lactate dehydrogenase (LDH) activity in normal, paraquat (PQ), and paraquat + montelukast (PQ + Mont) treated rats. Values are expressed as mean ± SEM of (n = 10 rats). *** Significantly different from normal group (saline) at $p < 0.001$. ### Significantly different from PQ treated group at $p < 0.001$.

On the contrary, PQ-induced rises in serum TNF-α and LDH levels were abolished and significantly reduced by montelukast treatment (to 49% and 39%, $p < 0.001$ compared with PQ group, respectively) (Figures 8 and 9).

4. Discussion

The current study revealed that montelukast produces a potent protection against paraquat-induced lung toxicity in rats. It was shown that administration of three doses of Mont (50 mg/kg, *p.o.*) 2, 24, and 48 h after PQ exposure (25 mg/kg, *i.p.*) resulted in a marked decrease in accumulation of PQ in the lung (about 60% compared to the PQ alone exposed rats). Consequently, the reduction in lung PQ contents resulted in prevention of PQ-induced lung toxicity. This was evidenced by the decrease in relative lung weight and the increase in the number of survived animals, to 80% compared with the 30% in PQ alone group. Additionally, the significant amelioration in the altered biochemical parameters induced by paraquat revealed the protection from toxicity.

The current findings also suggest that the PQ-induced death is mainly caused by rapid progress of respiratory failure. This assumption is supported by the observed symptoms including rapid shallow respiration, cyanosis, pulmonary edema, and the increase in relative lung weight. These findings are also concordant with the results of previous investigators (*2,33*).

It is well documented that the lung is a primary target organ of PQ toxicity because it has active polyamine uptake transport systems which concentrate paraquat rapidly into type II epithelial cells of the alveoli (*34*). The established mechanism of paraquat toxicity involves the cyclic reduction and re-oxidation of paraquat which results in oxidation of NADPH to $NADP^+$ with the production of oxygen free radicals. The free radicals generated by paraquat oxidation may interact with membrane lipids leading to cell damage (*35,36*). In the same time, depletion of NADPH results in the disruption of the pathways that are dependent on this nucleotide (*37,38*) specially, those involved in the production of pulmonary surfactants (*39,40*). Rats given a high dose of paraquat develop damage to the type I and II alveolar epithelial cells and within days large areas of the alveolar epithelium are completely lost (*41*). The lungs in these animals exhibit extensive alveolar fibrosis, which in association with residual edema, and reduced gas exchange may lead to death by anoxia (*8*). In association with the above mechanism, the oxygen free radicals generated by PQ also increase neutrophil adhesion and infiltration (*42*), and generate powerful hemotactic mediators including leukotrienes (*43*) leading to endothelial damage, increased vessel permeability and produced pulmonary edema (*2,44*).

Treatment with montelukast, a CysLT1 receptor antagonist, was reported to abolish significantly the increase in vascular permeability and to reduce edema formation in lung tissues (*24*), which are concordant with the findings of the current investigation.

According to the present results, Mont proved to protect the lungs, through antagonizing the biochemical parameters that were changed upon PQ-challenging. As a direct consequence of amelioration of the paraquat-induced toxicity, 80% survival was achieved in PQ + Mont exposed rats.

The current data demonstrate that paraquat causes

oxidative inflammatory response as evidenced by alterations in serum TNF-α, LDH, and in lung MPO, MDA and GSH levels. On the other hand, the results also demonstrate that montelukast, prevents the paraquat- induced inflammatory responses. This protection seems to occur through scavenging of ROS and inhibition of MPO and inflammatory mediators induced by PQ toxicity.

In accordance with the current results, other studies showed that, antagonizing the leukotriene CysLT1 receptors with montelukast ameliorates oxidative tissue injury and improves organs functions through the mechanisms that involve an inhibitory action on tissue neutrophil infiltration (*19,24*), reduction in the release of reactive oxygen species (*19,24*), and inhibition of the inflammatory cytokines (*21,22*).

The pulmonary toxicity caused by PQ is assumed to have a connection with the activation of neutrophils (*45*). Furthermore, various inflammatory mediators including TNF-α have been expected to be increased in the lung during PQ toxicity (*11*). It is known that, TNF-α triggers the synthesis of leukotrienes and prostaglandin E2 which then stimulate the infiltration of polymorphonuclear leukocytes into the lungs and produce lung injury (*9)*.

Concerning the effect of montelukast on TNF-α, a previous study (*21*) showed that TNF-α was increased significantly in the pyelonephritic rats exposed to *E. coli* and that montelukast treatment displayed an inhibitory effect on TNF-α release along with the alleviation of neutrophil mediated parenchymal destruction, The authors suggested that the anti-inflammatory effect of montelukast may be ascribed to an inhibition of TNF-α-mediated cytotoxicity.

Tissue myeloperoxidase (MPO) activity is a sensitive and specific marker of acute inflammation and reflects polymorphonuclear cell infiltration into the parenchyma. In the present study, MPO levels were significantly increased following paraquat administration, suggesting that oxidant-generated tissue injury involves the extracellular release of MPO by activated polymorphonuclear leukocytes. Previous studies recorded that neutrophils can recruit in the lungs during the inflammatory reaction generated by PQ leading to marked elevation in lung MPO activities (*9*). On the other hand, montelukast may attenuate neutrophil recruitment and promote the resolution of inflammation by antagonizing the effects of leukotrienes, which are potent stimuli for leukocyte infiltration. Similar explanation was given by other researchers (*21,24*) upon describing the protective effect of Mont in *E. coli* induced pyelonephritis and renal ischemia/reperfusion injury in rats, respectively. These findings greatly supported the assumption that the protective effects of Mont against PQ-induced lung toxicity are also related to less infiltration of inflammatory cells with the subsequent decrease in MPO activity.

In the present study, malondialdehyde, a good indicator of the degree of lipid peroxidation, was increased in the lung of PQ-intoxicated rats, indicating the presence of oxidative damage in lung tissues. This observation is in agreement with other reports showing that lipid peroxidation is a biomarker of PQ toxicity *in vitro* (*36*) and *in vivo* (*9,46,47*). Lipid peroxidation has been implicated in a number of deleterious effects such as increased membrane rigidity, osmotic fragility, decreased cellular and subcellular components, reduced mitochondrial survival, and lipid fluidity (*48*). The current results suggest that Mont may exert its protective effect against PQ toxicity through inhibition of lipid peroxidation in lung tissues. Similarly, recent studies indicate that Mont can protect against experimental organ damage through its marked antioxidant (reduce MDA, increase GSH) in addition to its anti-inflammatory effects (reduce TNF-α, LDH) (*22,24*). Additionally, another investigator (*19*) suggested that the gastroprotective effect of montelukast on indomethacin-induced ulcerations in rats was attributed to its ameliorating effect on oxidative damage.

In the current investigation, a severe depletion in lung GSH in PQ-exposed rats was observed. GSH, presents in high concentrations in lung epithelial cells (*49*). Depletion of GSH was shown to intensify lipid peroxidation and predispose alveolar cells to oxidative damage (*9,50*). Moreover, GSH, as a non-enzymatic radical scavenger, has the capability to interact with free radicals to yield more stable elements (*51*). As reported by Ross (*52*), cell injury and enhanced cell susceptibility to toxic chemicals are related to the diminished GSH biosynthesis. Accordingly, GSH plays a critical role in limiting the propagation of free radical reactions, which would otherwise result in extensive lipid peroxidation (*53,54*). On the other hand, montelukast, as an inhibitor of neutrophil infiltration, can reduce the oxidative injury of the lung and restore the GSH levels significantly. Similar explanation was previously discussed (*21,22*).

Moreover, GSH is also involved in numerous processes that are essential for normal biological function, such as DNA and protein synthesis (*55*). It is also considered to protect cells from toxic substances through conjugation resulting in a less toxic intermediate and thus, reducing the injury level of cells (*56*). However, its importance becomes particularly evident when the redox balance is disrupted due to excessive consumption of GSH this will greatly facilitate the development of the toxicity caused by pro-oxidant xenobiotics such as PQ (*54,57,58*) which is concordant with the obtained results in the current study.

In the present study, PQ administration increased both the carbonyl group content and DNA fragmentation in the lung. On the contrary, Mont treatment, by

antagonizing PQ effects, significantly reduced both parameters. The significant increase in carbonyl group content in the lung of rats exposed to PQ is in agreement with the previous studies (*4,9,11*). As previously reported, the cellular damage mediated by PQ is essentially due to its redox-cycling leading to continuous superoxide radical production (*35*) with simultaneous generation of the hydroxyl radical (*59*), which has been implicated in the initiation of membrane injury by lipid peroxidation (*60*) and cytotoxicity *via* mitochondrial dysfunction (*11,61*). The produced reactive oxygen species are also known to produce oxidative modification in DNA and proteins leading to fragmentation of polypeptide chains, increased sensitivity to denaturation and formation of protein–protein cross linkages as well as modification of amino acid side chains to hydroxyl or carbonyl derivatives (*62*).

In the current study, Mont was shown to protect against PQ-induced elevation of carbonyl groups contents. A plausible explanation for this protection can be conferred by the potent scavenging effect of Mont on hydroxyl radical. Among ROS, hydroxyl radical is thought to be the most damaging species and mainly responsible for protein oxidation and lipid peroxidation (*63*).

Similarly, the PQ-induced lung DNA fragmentation in rats was observed previously with other investigators (*4,64*). The mechanism by which paraquat damages DNA may involve the generation of oxygen free radicals (*35*) with subsequent alteration of the oxidative balance within the cell followed by increase in hydroxyl radicals leading to direct damage of DNA (*65,66*). On the other hand, Mont can protect against PQ-induced elevation of DNA fragmentation through antagonizing the previous cascade through inhibiting the reactive oxygen species and hydroxyl radicals generation and balancing oxidant-antioxidant status. Similar explanation was previously discussed (*24*).

The protective mechanism of Mont against PQ toxicity can also be explained through the ability of Mont to stabilize the mast cells followed by inhibition of the release of inflammatory mediators and cytokines. In this context, Mont was previously reported to protect against smoking-induced lung injury in rats through inhibition of the release of inflammatory mediators and cytokines from lung mast cells (*67*).

Although similar protecting effects against PQ toxicity can be attained with other anti-inflammatory drugs like dexamethasone (*11*), sodium salicylate (*4*), or cromolyn (*44*), the additional benefit which is demonstrated by Mont in the present work is the potent antioxidant potential.

5. Conclusion

Paraquat is a strong pnumotoxicant and the toxicity is chiefly due to free radical generation with subsequent increase in lipid peroxidation, protein carbonyl, DNA fragmentation, and depletion of glutathione in lung tissues. This effect is accompanied by neutrophile migration and increase in the myeloperoxidase activities in the lung tissues. Release of inflammatory mediators such as TNF-α is also increased during PQ toxicity.

Treatment with montelukast directly after paraquat intoxication increases the incidence of survival of the rats. This occur through antagonizing the effect of free radical generation, neutrophile migration, and reduction of the release of inflammatory mediators induced by paraquat. The overall effect of montelukast is a marked protection of the lung from injury. It is highly probable that the antioxidant in addition to the anti-inflammatory effects of montelukast could contribute to its protective effect against paraquat-induced lung toxicity.

This is the first experimental study, after searching in literature to investigate the use of montelukast for treatment of paraquat toxicity, the study greatly recommends the administration of montelukast directly after paraquat toxicity and concluded that montelukast may constitute an effective and promising treatment for management of paraquat poisonings.

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