Original Article

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Effects of Sildenafil and Vitamin E on Paraquat-Induced Pulmonary Fibrosis in Rats

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Abstract

Paraquat, specifically concentrated in lung tissue, leads to lung damage through oxidative and inflammatory processes. Pulmonary fibrosis occurs due to inflammation and an imbalance in the antioxidant system. Drugs like sildenafil, which inhibits phosphodiesterase enzymes, are used to treat pulmonary arterial hypertension. Antioxidants such as vitamin E can restore the oxidant-antioxidant balance. This study aimed to evaluate the effects of sildenafil and vitamin E on paraquat-induced pulmonary fibrosis. Male Wistar rats were used in animal studies. Paraquat was administered orally (R1-1) at a single dose of 40 mg/kg to induce lung fibrosis. Three groups were studied: Sildenafil administered orally (10 mg/kg) for 14 days. Vitamin E administered orally (500 mg/kg) for 14 days. Simultaneous administration of vitamin E (500 mg/kg) and sildenafil (10 mg/kg) for 14 days. Biochemical and histopathological tests were performed and compared with a control group. Paraquat administration caused fibrosis and an oxidant-antioxidant imbalance in lung tissue. Treatment with sildenafil and vitamin E increased glutathione (total GSH) and superoxide dismutase (SOD) levels while decreasing malondialdehyde (MDA) and hydroxyproline (HYP) levels compared to the paraquat group. Tumor Necrosis Factor-α (TNF-α) showed no significant changes in the study group compared to the control group. Treatment with sildenafil and vitamin E improved the oxidant-antioxidant balance in lung tissue exposed to paraquat. Additionally, this study demonstrated significant antioxidant effects of sildenafil and vitamin E against paraquat-induced oxidative damage.

Keywords: Paraquat, Sildenafil, Vitamin E, Pulmonary Fibrosis.

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1. Introduction

Poisoning with herbicides and agricultural chemicals remains a significant global public health concern (1). Paraquat (11'-Dimethyl-44'-bipyridinium dichloride) is a widely used herbicide associated with various health issues, including Parkinson's disease (2), pulmonary fibrosis (3), and infertility problems (4). Paraquat enters the body through ingestion, inhalation, and skin contact, with rapid absorption and lung storage. Its toxicity primarily involves superoxide anion production via the redox cycle, leading to the generation of reactive oxygen species (ROS) such as hydrogen peroxide and hydroxyl anion (5). ROS contribute to lipid peroxidation, mitochondrial membrane disruption, DNA and protein damage, and depletion of antioxidant

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reserves, including glutathione. Additionally, paraquat disrupts the mitochondrial respiratory chain complex, separating oxidation from phosphorylation (6). Sildenafil, a phosphodiesterase type 5 inhibitor (7), is rapidly absorbed after oral administration, reaching peak plasma concentration within an hour (8). It is FDA-approved for treating pulmonary hypertension and has potential benefits in conditions like stroke, dementia, and neurodegenerative disorders due to increased angiogenesis and neurogenesis (9). Sildenafil's relaxing effect on arterial smooth muscle cells relies on nitric oxide (NO) signaling (10). Pulmonary fibrosis, a chronic respiratory disease, results from an imbalance in the antioxidant system and inflammation. Combining antioxidant compounds, such as vitamin E supplements, with standard treatments can reduce oxidative stress, inhibit fibroblast expansion, and mitigate lung damage (11). Vitamin E, known for its high antioxidant properties, has attracted attention in improving pulmonary fibrosis symptoms in animal models (12, 13). Studies have identified anti-inflammatory and antioxidant effects of sildenafil (14). Recent research also suggests that sildenafil significantly improves lung function in pulmonary fibrosis patients (15, 16). Given paraquat's oxidative stress mechanism, antioxidants may play a crucial role in managing its toxic effects (17).

This study aims to evaluate the separate and combined effects of sildenafil (a NO producer) and vitamin E (an antioxidant) in preventing or reducing paraquat-induced toxicity.

2. Materials and Methods

2.1. Experimental Animals and Paraquat-Induced Pulmonary Fibrosis

Fifty adult male Wistar rats (200–220 g) were obtained from the Razi Institute in Shiraz, Iran. The rats were housed in groups of five under a 12-hour light-dark cycle at a temperature of 22 °C and humidity of 50-60%. After 3 days of acclimatization, the rats were randomly divided into five groups, each containing 10 rats: Group I: Received normal saline (1 mL/d, p.o.) for 14 days. Group II: Received orally paraquat (40 mg/kg, single dose) on day 1, followed by normal saline (1 mL/d, p.o.) for 14 consecutive days. Group III: Received orally paraquat (40 mg/kg, single dose) on day 1, followed by sildenafil (10 mg/kg/d, p.o.) for 14 consecutive days. Group IV: Received orally paraquat (40 mg/kg, single dose) on day 1, followed by vitamin E (500 mg/kg/d, p.o.) for 14 consecutive days. Group V: Received orally paraquat (40 mg/kg, single dose) on day 1, followed by both sildenafil (10 mg/kg/d, p.o.) and vitamin E (500 mg/kg/d, p.o.) for 14 consecutive days. On the fourteenth day, the rats in all five groups were anesthetized with sodium pentobarbital (3%), and their lung tissue was completely isolated. Lung tissues were washed in normal saline and stored in a freezer at -80 °C for subsequent biochemical and pathological tests

2.2. Preparation of Lung Tissue Samples

Lung tissues isolated from rats were homogenized in a specific volume of HCl-Trizma buffer (200 mg of tissue in 1 ml of buffer) using a homogenizer. The samples were then centrifuged and their supernatant was separated. The supernatant was used for determining biochemical factors.

2.3. Determination of Malondialdehyde (MDA) by Thiobarbituric Acid Colorimetry in Lung Tissue (R1-2)

In a microtube, 100 microliters of the sample were mixed with 100 microliters of trichloroacetic acid (20%) and 100 microliters of thiobarbituric acid (TBA) solution (50 mmol). The resulting mixture was heated at 95°C for one hour. Gradually, the MDA present in the sample reacted with TBA, resulting in a pink or red solution. The reaction product was centrifuged for 5 minutes, and the supernatant was separated and poured into a microplate. The intensity of the resulting color was read at a wavelength of 532 nm, and the MDA concentration in the serum was expressed in

µmol/mL.

2.4. Determination of Glutathione Concentration (total GSH) in Lung Tissue(R1-6)

Fifty microliters of the sample were mixed with 50 microliters of trichloroacetic acid (10%) and centrifuged to separate excess protein. The sample was then mixed with 150 microliters of 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB, 1 mmol). Glutathione in the sample reacted with DTNB, resulting in a color change. The intensity of the resulting color was read at a wavelength of 412 nm using an ELISA Reader. total GSH concentration was expressed in µM/mg tissue (18).

2.5. Superoxide Dismutase (SOD) Activity in Lung Tissue

Five micrograms of lung homogenate were mixed with sodium pyrophosphate buffer, phenazine methosulfate (PMT), and nitro-blue tetrazolium (NBT). The reaction was initiated by adding nicotinamide-adenine dinucleotide (NADH). The reaction mixture was incubated at 30 °C for 90 seconds and stopped by adding 1 ml of glacial acetic acid. The absorption intensity of the mixture was measured at 560 nm. Each unit of superoxide dismutase activity was determined as the enzyme concentration required to inhibit color production by 50% in 1 minute under the study conditions (19).

2.6. Determination of Tumor Necrosis Factor-α

The blood sample was centrifuged, and the amount of serum TNF-α protein was measured using the Bradford technique and the Rat TNF alpha ELISA kit produced by Abcam, Germany (20).

2.7. Determination of Hydroxyproline in Lung Tissue

The total collagen content in the left lung was measured using a colorimetric assay (21). The left lung was dried at 80°C until it reached a constant weight. Overnight hydrolysis of the dried lung tissue in 12 N HCl at 120 °C was performed under vacuum conditions in a glass vial. NaOH was used to adjust the pH to 7, and distilled water was added to achieve a sample volume of 30 mL. After mixing the sample solution (1.0 mL) with 1.0 mL of chloramine T solution (0.05 mol/L), a 20-minute incubation occurred at room temperature. Next, a 20% dimethyl benzaldehyde solution was added to the mixture and incubated at 60 °C for 20 minutes. Analyses of each sample were conducted by measuring absorbance at 557 nm. The results are expressed in micrograms of hydroxyproline per gram of wet lung weight using the standard hydroxyproline curve.

2.8. Histopathology

After sacrificing the animals, the lung samples were dissected. The lungs were fixed with a 10% buffered formalin solution. Subsequently, the lung specimens were dehydrated and embedded in paraffin. Histological examination of the tissues involved cutting 5 µm sections using a rotary microtome, deparaffinization, and staining with hematoxylin and eosin. Light microscopy was used to examine all sections (×200).

2.9. Statistical Analysis

The data were analyzed using GraphPad Prism 8 (GraphPad Software, Inc.). Results were expressed as means ± SD and analyzed using one-way ANOVA followed by Tukey–Kramer multiple comparison tests. Before analysis, the normal distribution of data was evaluated. Statistically significant differences were considered at $P<0.05$.

2.10. Ethics Approval

The animals were provided free access to food and water, and all experiments were conducted under the "Guide for the Care and Use of Laboratory Animals" (NIH publication No. 85-23, revised 2011; Albus, 2012). The Committee approved the animal experiment protocol at Shiraz University of

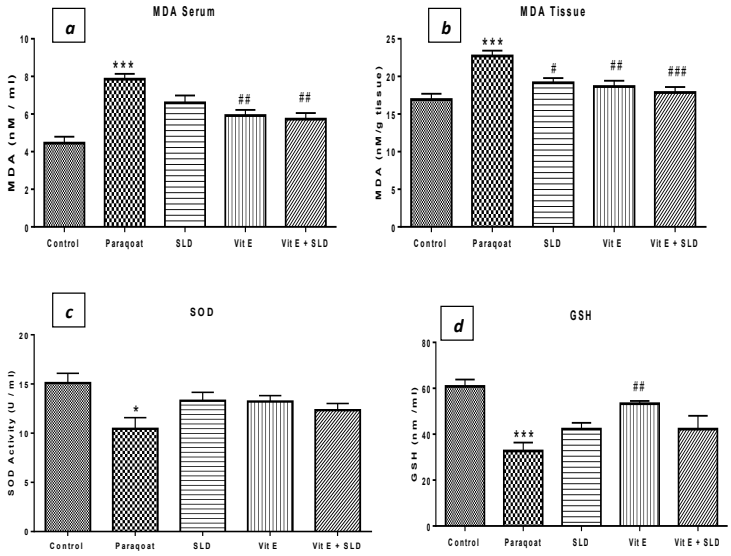

Figure 1. Effects of sildenafil (SLD) and vitamin E (VitE) on serum malondialdehyde (MDA) a, tissue malondialdehyde (MDA) b, superoxide dismutase (SOD) c, and tissue glutathione peroxidase (GSH-Px) d. *: compare to Control (p<0.05), ***: compare to Control (p<0.001).
#: compare to Paraquate (p<0.05), ##: compare to Paraquate (p<0.01), ###: compare to Paraquate (p<0.001).

Medical Sciences (Approval ID: IR.SUMS. AEC.1401.113).

3. Results

3.1. Analysis of MDA,Total GSH, and SOD Levels

As depicted in Figure 1, paraquat intoxication led to a significant increase in MDA (malondialdehyde) content in both blood and lung tissue (Figure 1a, 1b) when compared to the control group (P<0.05). Treatment with vitamin E resulted in decreased MDA levels in blood and lung tissue over 14 days (P<0.05). Additionally, the combined use of sildenafil and vitamin E reduced malondialdehyde levels in the serum and lung tissue of rats. Conversely, SOD (superoxide dismutase) activity significantly decreased in lung tissue after paraquat intoxication compared to the control group (P<0.05) (Figure 1c). However, treatment with sildenafil and vitamin E increased SOD activities in lung tissue (P<0.05). Furthermore, total GSH (glutathione) concentration levels were significantly reduced in lung tissue following paraquat intoxication (P<0.05). After treatment with sildenafil and vitamin E, total GSH concentration showed a significant increase (P<0.05) (Figure 1d).

3.2. Analysis of the Effect of Sildenafil on Tissue TNF-α Levels

In this study, we investigated tissue

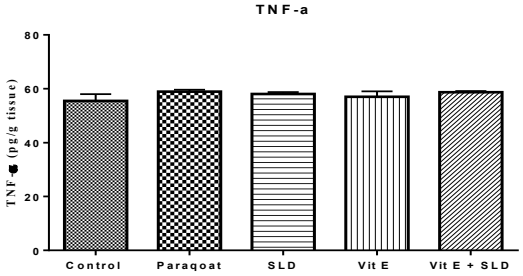

Figure 2. Effects of sildenafil(SLD) and vitamin E on pulmonary fibrosis induced by paraquat. tumor necrosis factor-α levels (pg/ml) were compared.

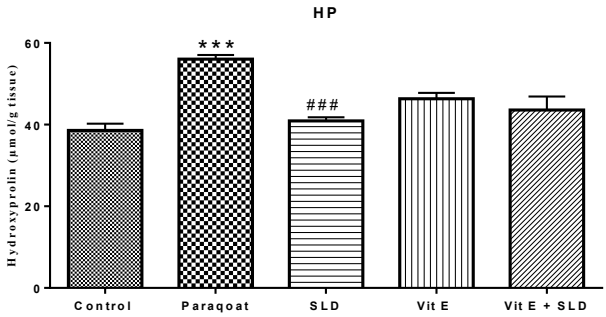

Figure 3. Effects of sildenafil (SLD) and vitamin E on pulmonary fibrosis induced by paraquat. Hydroxyproline content in lungs. Elevated levels of lung hydroxyproline content have been reduced in sildenafil and vitamin E-treated lungs.

levels of TNF-α, an inflammatory cytokine, in rat sera (Figure 2). The Paraquat intoxication group exhibited significantly increased TNF-α levels compared to the control animals ($P<0.01$). However, treatment with sildenafil and vitamin E (vitamin E + sildenafil group) did not have a significant change (Figure 2)
.

3.3. Hydroxyproline Content in Lung Tissue

The Paraquat-intoxication group exhibited a significant increase in hydroxyproline content in the lung compared to the control group ($P<0.05$). Treatment with sildenafil and vitamin E led to a significant reduction in hydroxyproline content in lung tissues. Notably, the therapeutic effect was more pronounced with sildenafil treatment, aligning with the consistent histopathological results (Figure 3).

3.4. Histopathological Results for Lung Tissues

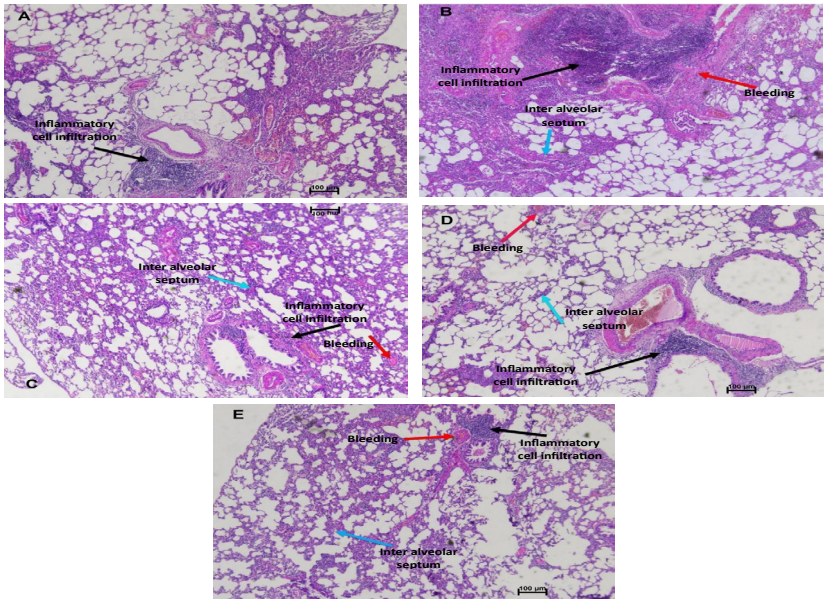

Figure 4. Representative images of H&E staining of lung tissue For control and experimental groups (magnifcation, 200×), histopathological photographs of lung tissue stained with hematoxylin and eosin are shown in Figure 4. Lungs of control rats showed normal lung morphology with normal alveolar spaces and normal thickening of alveolar septa (Figure 4(A)). The paraquat treatment led to abnormal morphologies including significant interstitial infiltration by inflammatory cells, alveolar septal thickening, and collapsed alveolar spaces (Figure 4(B)). Sildenafil and vitamin E treatments after the paraquat showed protection against paraquat-induced lung damage (Figures 4(C) and 4(D)).

Table 1. Semi-quantitative changes in lung tissue histopathology.

Groups	Type of injury	Degree of injury
A	inter alveolar septum	-
	inflammatory cell accumulation	-
	alveolar area	-
	bleeding	-
B	inter alveolar septum	+++
	inflammatory cell accumulation	+++
	alveolar area	+++
	bleeding	+++
C	inter alveolar septum	+
	inflammatory cell accumulation	+
	alveolar area	+
	bleeding	+
D	inter alveolar septum	+
	inflammatory cell accumulation	+
	alveolar area	+
	bleeding	+
E	inter alveolar septum	++
	inflammatory cell accumulation	+
	alveolar area	+
	bleeding	+

Similar to the biochemical experiments, there were significant changes in microscopic morphology between the control and experimental groups. In the control group, the alveolar structure appeared normal, with no edema in the alveolar wall and no inflammatory cell infiltration in the lung parenchyma. However, in the Paraquat group, abundant inflammatory cell infiltration was observed, along with evident bleeding and clear membrane formation in the alveolar cavity (Figure 4 and Table 1).

4. Discussion

Lung damage represents the most serious complication of paraquat intoxication, often resulting in patient mortality. Paraquat exerts its toxic effects through oxidation reactions and subsequent inflammatory responses. Within cells, paraquat reacts with oxygen molecules, producing free radicals such as superoxide anion radical(R1-8), hydrogen peroxide, hydroxyl radicals, and peroxynitrite (22). These highly reactive radicals play a central role in lipid peroxidation within cell membranes. A critical biochemical factor in lipid peroxidation is malondialdehyde (MDA). Previous studies have demonstrated that paraquat increases MDA levels in various tissues, including the lung (21, 23). In our study, paraquat exposure elevated MDA levels in lung tissue. However, treatment with sildenafil and vitamin E significantly reduced MDA levels caused by paraquat. This finding underscores the antioxidant effects of sildenafil and vitamin E, suggesting their ability to protect lung cell membranes from lipid peroxidation damage. Possible mechanisms include membrane stabilization of polyunsaturated fatty acids and scavenging of reactive oxygen species (ROS). These conclusions align with previous research showing MDA reduction due to sildenafil and vitamin E use (24, 25). In the context of paraquat-induced lung damage, inflammation plays a pivotal role. Paraquat predominantly enhances the inflammatory response

by upregulating pro-inflammatory markers such as TNF-α (26). Consistent with other studies, we observed increased serum TNF-α levels following paraquat exposure (27, 28). The body's primary defense against ROS-induced damage involves reduced glutathione (total GSH) and superoxide dismutase (SOD) enzymes. total GSH and SOD activity serve as indicators of tissue antioxidant-oxidant balance. In paraquat poisoning, total GSH and SOD levels have been reported to decrease (18, 29-32). Similarly, our study found decreased total GSH and SOD levels in the paraquat poisoning group. This decline likely results from the utilization of these antioxidants to neutralize free radicals generated by paraquat toxicity. Furthermore, our study revealed a significant increase in hydroxyproline levels in rat lungs after paraquat treatment. Elevated hydroxyproline indicates the induction of pulmonary fibrosis by paraquat (33-35). Remarkably, treatment with sildenafil led to a statistically significant reduction in hydroxyproline content compared to the paraquat group. We propose that sildenafil and vitamin E may prevent pulmonary fibrosis by inhibiting collagen synthesis. Histopathological evidence and decreased hydroxyproline levels support the efficacy of sildenafil and vitamin E in attenuating paraquat-induced lung injury and fibrosis. These findings suggest a novel mechanism for mitigating paraquat-related complications using sildenafil and vitamin E. To elucidate the exact mode of action, further molecular investigations into inflammatory and antioxidant pathways are warranted.

5. Conclusion

The findings demonstrate that sildenafil and vitamin E effectively mitigate paraquat-induced complications in rats by modulating inflammatory pathways and antioxidant parameters. However, further investigation is needed to fully understand the precise mechanisms underlying the protective effects of sildenafil and vitamin E against paraquat-induced pulmonary fibrosis.

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Authors contributions

F. Siahpour: Literature Search, In vivo investigation, Data acquisition; H. Sadeghi: Validation, Resources, writing Reviewing and Editing; E. Panahi kokhdan: In vivo investigation, Data acquisition and Editing; M.J. Khoshnoud Conceptualization, Supervision, Data acquisition, Validation.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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