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Investigating the impact of inhaled paraquat: A comprehensive evaluation protocol ☆

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ARTICLE INFO

Method name:
Evaluating the pulmonary and systemic effects of inhaled paraquat

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ABSTRACT

This paper provides a complete protocol for studying the effects of inhaled paraquat (PQ), a toxic herbicide that has negative effects systemically and on the lungs. The protocol aims to evaluate the effects of aerosolized PQ exposure on lung and systemic injury in an animal model, which will provide significant information for therapeutic interventions for PQ-induced pulmonary and systemic damage.

The protocol involves the following key components: 1. Study groups: By including control, non-treated aerosolized PQ-exposed, and treated PQ-exposed animals with various agent groups in the experiment, lung and systemic injury in each group could be evaluated, and different measured parameters could be compared among groups. 2. PQ exposure: Animals in the PQ-exposed groups are subjected to PQ aerosol inhalation, simulating occupational or accidental exposure in farmers working with this herbicide. 3. Assessment measures: To determine the degree of lung and systemic injury and its physiological effects, several assessments, such as biochemical markers, histopathological analysis, and functional tests, are used.

The protocol offers reliable and accurate results by using standardized methods and data collection. The effect of PQ exposure on lung and systemic injury could be evaluated by statistical analysis of the collected data, which also makes it easier to identify possible protective agents or interventions.

This comprehensive evaluation protocol provides an essential basis for studying the mechanisms behind PQ-induced lung and systemic injury and assessing the effectiveness of preventative or therapeutic strategies in minimizing its adverse effects.

☆ Related research article: **For a published article:** F. Amin, A. Roohbakhsh, A. Memarzia, H.R. Kazerani, M.H. Boskabady, Immediate and late systemic and lung effects of inhaled paraquat in rats. J. Hazard. Mater. 415 (2021) 125,633. 10.1016/j.jhazmat.2021.125633.

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Specifications table

Subject area:	Pharmacology, Toxicology, and Pharmaceutical Science
More specific subject area:	Respiratory Physiology, Respiratory Pharmacology, Respiratory Inflammation, Pulmonary Toxicology, Systemic Inflammation, Oxidative Stress
Name of your protocol:	Protocol for evaluating the pulmonary and systemic effects of inhaled PQ
Reagents/tools:	Paraquat dichloride (Sigma-Aldrich) Saline solution (0.9 % NaCl) Inhalation exposure system Omron CX3 nebulizer from Japan
Experimental design:	• Animal selection: Selecting suitable animals based on variables such as age, sex, and strain. • Group allocation: Assign animals to control non-treated PQ-exposed and treated PQ-exposed groups at random. • Exposure protocol: Using an inhalation exposure system, administer saline and PQ aerosols to the control, non-treated, and treatment groups. • Animal monitoring and data collection: Record parameters such as respiration rate, lung function, and clinical symptoms during and after exposure. Collect samples (such as blood, bronchoalveolar lavage fluid (BALF), tracheal smooth muscle (TSM) and lung tissue) for further analysis. • Histopathological analysis: To evaluate lung injury, sacrifice animals, isolate lung tissue, and perform a histopathological investigation. • Statistical analysis: Compare control and non-treated and treated PQ-exposed groups by analyzing data using appropriate statistical tests.
Trial registration:	N/A
Ethics:	Statement: The animal experiments conducted using this protocol complied with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. All experimental procedures involving animals were carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986, and associated guidelines. The study also adhered to the EU Directive 2010/63/EU for animal experiments. The sex of the animals used in the experiments was indicated, and where appropriate, the influence or association of sex on the results of the study was considered and reported. Ethical considerations and animal welfare were given utmost priority throughout the study. Moreover, the three R's (Replacement, Reduction, and Refinement) in animal research were observed in this protocol.
Value of the Protocol:	1. Standardized Approach: The procedure provides a standard method for assessing the effect of inhaled PQ, ensuring consistency between tests and allowing for accurate comparisons across research. This improves comprehension of the effect of the drug on the respiratory system and systemic effects and increases the reproducibility of results. 2. Ethical Considerations: The protocol ensures that animal studies follow established standards and regulations, such as the ARRIVE guidelines and appropriate authorities. By adhering to these ethical considerations, the protocol promotes the responsible and humane use of animals in research. 3. Scientific Validity: The study improves the scientific validity of the findings by following a well-designed and detailed process. The systematic strategy, which includes proper group allocation, consistent exposure protocols, and reliable data collection and analysis methodologies, increases the reliability of study.

Description of protocol

The herbicide PQ is often used and well-known for its harmful effects on human health and on the respiratory system [1,2]. PQ inhalation can cause serious lung injury because it accumulates in the lungs after inhalation, causing oxidative stress, inflammation, and, eventually, lung injury [3,4]. Coughing, shortness of breath, and lung fibrosis are common symptoms of PQ-induced pulmonary toxicity [5,6].

There have been continuous attempts to lessen the respiratory symptoms induced by PQ and improve the patients' health. In order to lessen the toxicity of PQ, researchers have concentrated on developing innovative treatment approaches, such as anti-inflammatory, antioxidant, and lung-repair-promoting substances [7–9]. However, it is essential to develop reliable and reproducible assessment techniques using animal models in order to evaluate the effectiveness of the possible treatments.

Animal models play an essential role in investigating the unfavorable effects of PQ and assessing the efficacy of preventative and treatment interventions. Researchers may use these models to carefully simulate human exposure events and examine the underlying pathological mechanisms as well as the possible treatment of the induced injury.

In this work, we provide an in-depth and reproducible protocol for examining the effects of PQ inhalation in an animal model. The purpose of this protocol is to offer a standardized approach to assessing the detrimental mechanisms underlying PQ-induced toxicity and the efficacy of possible treatments.

Rationale: The purpose of the study is to assess how inhaled PQ affects the systemic and respiratory systems. Determining the effects of PQ toxicity through a standardized approach would benefit the fields of pharmacology, toxicology, and pharmaceutical research. Other researchers can examine the systemic and respiratory effects of PQ and expand our understanding of its mechanism of action and potential treatment strategies by providing a clear and reproducible method.

Materials

-Paraquat dichloride (Sigma-Aldrich)
- Saline solution (0.9 % NaCl)

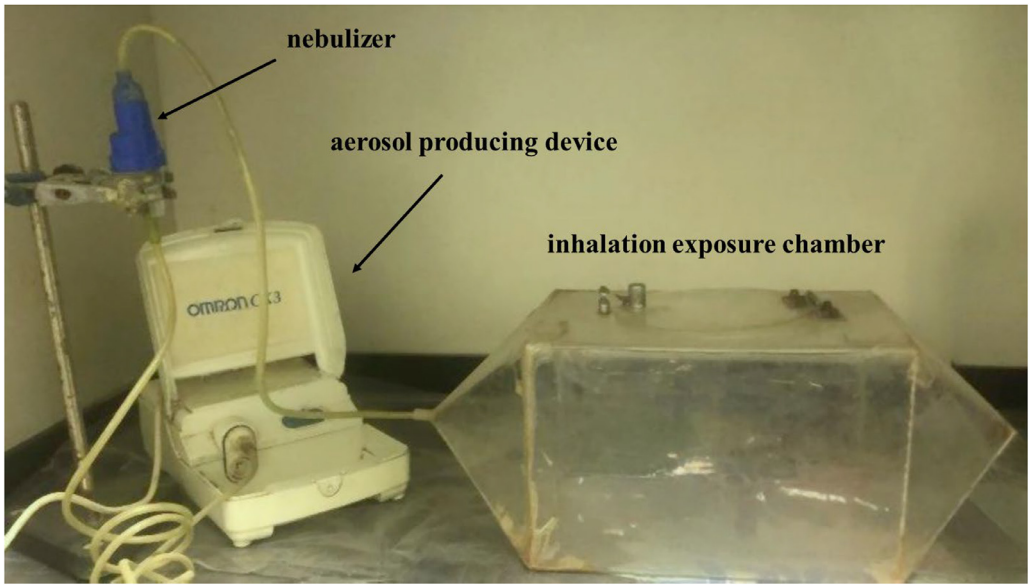

Fig. 1. Devices used for inhalation exposure.

Tools

Inhalation exposure chamber

The inhalation exposure chamber is a rectangular cube made of clear pyrex material that measures 26 × 17 × 15 cm. One side of the chamber has a passage through which air with the aerosolized material is introduced. On the other side, an entry valve was placed to allow experimental animals to enter.

Nebulizer

A nebulizer is a device utilized to disperse a solution containing allergenic or toxic substances (such as ovalbumin or PQ) as an aerosol. This device consists of a small cylindrical container made of plastic and comprises two primary components. The lower part functions as a reservoir for the inhalation solution, while the upper part acts as a lid, securely placed atop the lower portion.

The nebulizer operates by drawing in air, generated by an aerosol device, through a plastic tube connected to the lower section. As the air passes through, it transforms the solution within the nebulizer into fine particles. Subsequently, these particles blend with the incoming air and are directed into the inhalation chamber via an interface located on the upper part of the nebulizer (Fig. 1).

Aerosol-producing device

The aerosol-producing device plays an essential role in generating the required airflow to transform the PQ solution into an inhalable aerosol for laboratory animals. This device is connected to the nebulizer using a plastic tubing, allowing for a seamless transfer of the aerosolized solution. The airflow from the device enters the nebulizer at a rate of 8 ml/min, providing proper aerosolization of the solution.

PQ and its solution preparation method

PQ, a solid powder with a molecular weight of 16.275 g/mol, was used in the study. To prepare the PQ solution, 400 mg of PQ was dissolved in 300 ml of normal saline. This resulted in a PQ concentration of 1.33 mg/ml in the solution. A volume of 4.5 ml of the PQ solution was poured into the nebulizer chamber.

Considering that the nebulizer has an air output of 3.7 l and a liquid output of 0.15 ml/min, the calculated dose of PQ in the aerosol was 54 mg/m^3.

Additionally, a low-dose PQ solution was prepared by dissolving 200 mg of PQ in 300 ml of normal saline. This resulted in a PQ concentration of 0.66 mg/ml in the solution. The aerosol generated from this low-dose solution contained 27 mg/m^3 of PQ.

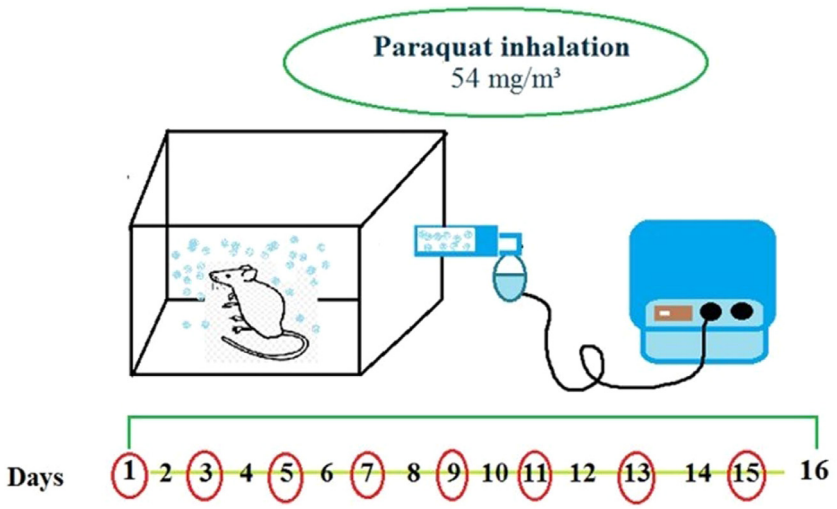

Fig. 2. Overview of the study process in rats exposed to inhaled PQ.

$$400\ mg\ \ PQ \div 300\ ml\ \ NS = 1.33\ mg/ml$$

1 ml	*1.33 mg/ml*
0.15ml (Air Neb. OutPut)	*X (mg/ml PQ)*

$$X = \frac{1.33\ mg/ml \times\ 0.15\ ml}{1\ ml} = 0.2\ mg/ml$$

$$0.2\ mg/ml \div 3.7\ \left(Liquid\ Neb.\ OutPut\right) = 0.054\ mg/ml$$

$$0.054\ mg/ml \times\ 1000\ ml/m^3 = 54\ \boldsymbol{mg/m^3}\ >>>>>\ \boldsymbol{High\ Dose}$$

1 ml	*0.66 mg/ml*
0.15 ml (Air Neb. OutPut)	*X (mg/ml PQ)*

$$X = \frac{0.66\ mg/ml \times\ 0.15\ ml}{1\ ml} = 0.1\ mg/ml$$

$$0.1\ mg/ml \div 3.7\ \left(Liquid\ Neb.\ OutPut\right) = 0.027\ mg/ml$$

$$0.027\ mg/ml \times\ 1000\ ml/m^3 = 27\ \boldsymbol{mg/m^3}\ >>>>>\ \boldsymbol{Low\ Dose}$$

Exposure of rats to inhaled PQ

During the experiment, male Wistar rats were exposed to PQ aerosol for 30 min every other day over a period of 15 days to induce poisoning. To ensure safety during the exposure, the researcher wore a mask and special clothing while adding the aerosol solution to the nebulizer chamber.

The rats were then placed inside the inhalation box, as depicted in Fig. 2. The connections between the nebulizer and the inhalation box were established, and the nebulizer was turned on under a hood. Following this, the researcher promptly left the room. Each time, only 4.5 ml of PQ solution was added to the nebulizer chamber.

Considering that the output volume of nebulizer is 0.15 ml/min and the output air volume is 3.7 l/min, the solution transformed into an aerosol within the 30-min exposure duration. To ensure safety, one and a half hours after activating the nebulizer (allowing sufficient time to confirm the absence of PQ aerosol under the hood), the researcher re-entered the room wearing a mask and special clothing. The nebulizer was then turned off, and the animal was transferred to the animal room.

It is advisable to equip the inhalation room with a robust fan to facilitate proper ventilation and maintain a safe environment during the experiment.

Experimental design

1. Animal selection: Choose appropriate animal models (e.g., rats or mice) depending on important parameters such as age, sex, and strain.
2. Group allocation: Assign animals at random to control, non-treated aerosolized PQ-exposed, and treated PQ-exposed animals with various agent groups, providing sufficient sample sizes for statistical significance.
3. Exposure protocol: Using an inhalation exposure system, provide inhaled PQ to the non-treated and treated groups, while control animals receive a sham exposure or an inert substance.
4. Monitoring and data collection: Record data such as respiratory rate, lung function, clinical symptoms, and body weight during and after exposure. Collect samples for further examination (including blood, BALF, and lung tissue).
5. Histopathological analysis: Sacrifice animals, collect lung tissue, and process samples for histopathological analysis. In order to evaluate lung injury, inflammation, or fibrosis, use staining procedures such as hematoxylin and eosin.
6. Statistical analysis: Compare control and treatment groups using proper statistical tests, determining the significance of observed differences.

According to the results of our previous studies, exposing rats to PQ in this method led to increased levels of total and differential white blood cell counts, nitrite, malondialdehyde, interleukin (IL)−10, and interferon-gamma levels, increased tracheal smooth muscle responsiveness to methacholine, and inducing lug pathological changes, but a decrease in amounts of thiol, superoxide dismutase, catalase, IL-17, and tumor necrosis factor alpha (TNF-α) in the blood and the BALF due to both doses of PQ in comparison to the control group [1,6].

These findings strongly support the validity and effectiveness of the protocol in replicating PQ-induced pulmonary and systemic deficits in humans. The observed alterations in various parameters indicate a complex immunological and oxidative stress response, mirroring the pathological changes seen in human cases of PQ toxicity. The consistency between our results and the characteristics of PQ-induced lung and systemic injury in humans [10,11] further underscores the utility of this method as a reliable model for studying the detrimental effects of PQ exposure on the respiratory system.

Study guidelines

- The protocol follows ethical guidelines and regulations, including compliance with the ARRIVE guidelines for animal research and relevant legislation such as the U.K. Animals (Scientific Procedures) Act, 1986, and EU Directive 2010/63/EU for animal experiments.
- Guidelines for human subjects do not apply to this study because it is primarily concerned with animal experiments.

Protocol validation

We used the provided methods to conduct pilot tests to validate the protocol. Significant changes in respiratory and systemic parameters, lung histology, and clinical signs were found between the control and PQ groups, validating the ability of the protocol to identify the respiratory and systemic effects of inhaled PQ.

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.

CRediT authorship contribution statement

Mahboobeh Ghasemzadeh Rahbardar: Writing – original draft. **Sima Beigoli:** Writing – original draft. **Mohammad Hossein Boskabady:** Conceptualization, Methodology, Supervision.

Data availability

Data will be made available on request.

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Supplementary material *and/or* additional information [OPTIONAL]

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- **Additional information** can include anything that is not directly related to your protocol, e.g., more general background information, useful links etc. As the protocol article format doesn't feature an introduction, you might want to use this section to highlight information you would typically include in your introduction.

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