BJPS

Brazilian Journal of
Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902022e21600

Article

In Vitro and *In Vivo* evaluation of montmorillonite for paraquat poisoning

Xiang Guo[1], Wei Guo[2], Tiandi Li[3*], Fen Liu[4], Jinpeng Zhou[1], Meiqiong Guo[1]

[1]Emergency Management Office, Shenzhen Prevention and Treatment Center for Occupational Disease, Shenzhen, China, [2]Medical Affairs Department, The First Affiliated Hospital of Nan Chang university, Nanchang Jiangxi, China, [3]Chemical Analysis & Physical Testing Institute, Shenzhen Prevention and Treatment Center for Occupational Disease, Shenzhen, China, [4]Quality Control Department, Shenzhen Prevention and Treatment Center for Occupational Disease, Shenzhen, China

Evaluation of montmorillonite for paraquat by *in vitro* and *in vivo* test. *In vitro* test were evaluated by a batch test, taking the paraquat concentration, adsorbents, reaction environment and time as indices, the absorption rate was screened by orthogonal design. *In vivo* test was executed with rabbits. Group 1: 4 rabbits dosed with montmorillonite. Group 2: 8 rabbits dosed with 200 mg/kg paraquat. Group 3: 6 rabbits dosed with 200 mg/kg paraquat then gavage with montmorillonite 5 min later. Group 4: 6 rabbits dosed with 200 mg/kg paraquat then gavage with montmorillonite 30 min later. Blood paraquat concentration, serum cytokines, blood gas analysis and histopathology of lung were implemented. *In vitro* test found that all the four factors influence the absorption rate of paraquat ($P < 0.05$). *In vitro* test found that oral montmorillonite could change toxicokinetics parameters of paraquat ($P < 0.05$); decrease raised serum TGF-β1 and HMGB1 ($P < 0.05$) and alleviate the histopathology damage of lung. Montmorillonite might exert its protective effects on paraquat induced damage.

Key words: Paraquat. Montmorillonite. Toxicokinetics. Histopathology. *In vivo. In vitro.*

INTRODUCTION

Paraquat (N, N′-dimethyl-4, 4′-bipyridinium dichloride; PQ) is a kind of sterilizer and herbicide that is characterized by contact-kill and systemic action, and can be absorbed quickly by green plants and make them wither and die (Hawkes, 2014). It is therefore widely used in agricultural production and occupies a large market share in China. Because of its high human toxicity and mortality characteristics, PQ poisoning has become one of the most common types of pesticide poisoning in China and has thus attracted considerable attention in recent years(Yin *et al*., 2013). From 2002 to 2011, the Poison Control Center of the National Institute of Occupational Health and Poison Control of the Chinese Center for Disease Control and Prevention (National Poison Control Center, NPCC) recorded 1571 cases of PQ intoxication consultation in total, of which oral administration (81.29%) was the main type of PQ exposure and the yearly fatality rate ranged from 31% to 97%(Yin *et al*., 2013). Obviously, digestive tract exposure is the main cause for PQ poison, respiratory and intact dermic absorption in occupational setting were not usual. In most cases, death has resulted from extensive pulmonary damage, and is initially characterized by edema, hemorrhage, and, at later stages, fibrosis (Sun, He, 2017).

Without the availability of effective, specific antidotes, only moderately effective, nonspecific, life-supporting measures are taken to detoxify PQ poisoning in clinics. These measures often include the administration of oral decontaminants, such as activated charcoal (AC) or gastric lavage, for early PQ decontamination

***Correspondence:** T. Li. Chemical Analysis & Physical Testing Institute. Shenzhen Prevention and Treatment Center for Occupational Disease. Shenzhen, China. Phone: +86-13600158214. Email: flight027@126.com. ORCID: https://orcid.org/0000-0001-5427-0610. Xiang Guo - ORCID: https://orcid.org/0000-0001-9794-7871. Wei Guo - ORCID: https://orcid.org/0000-0002-5334-3221. Fen Liu - ORCID: https://orcid.org/0000-0001-6600-1055. Jinpeng Zhou - ORCID: https://orcid.org/0000-0002-1396-8665. Meiqiong Guo - ORCID: https://orcid.org/0000-0001-5191-8434

(Meredith, Vale, 1987), followed by anti-oxidant therapy (Nguyen, Malik, Howland, 2014), immunosuppression (Xu, Lu, 2019), hemoperfusion (Yen, Wang, Hsu, 2018), hemodialysis (Wang *et al*., 2017), resuscitation, and supportive care. Upon oral ingestion, PQ is usually rapidly absorbed into the small intestine and distributed to major organs, including the liver, lungs, kidneys, and muscles. Therefore, preventing the intestinal absorption of PQ after oral ingestion might be the key to successful treatment. Unfortunately, AC for gastrointestinal tract decontamination is unattainable in mainland China, and gastric lavage may cause serious side effects in emergency departments, especially in patients without respiratory protection. The development and evaluation of new gastroenteric adsorbents for PQ poisoning are therefore of great importance.

Montmorillonite is a special type of clay that is in the same family (Smectite) as bentonite (Meredith, Vale, 1987). Because of its strong adsorption capacity, montmorillonite has been widely used in the treatment of diarrhea, peptic ulcers, and gastrointestinal hemorrhage (Amdrup, Olesen, 1972). Some research has also reported the clinical application of montmorillonite for the management of chemical poisons, such as aflatoxin B1 and zearalenone (Wang *et al*., 2018). In the present study, the adsorption capacity of montmorillonite for PQ poisoning is evaluated by *in vitro* and *in vivo* experiments.

MATERIAL AND METHODS

Chemicals

Smecta® (montmorillonite powder, 3 g/bag) was obtained from Beaufour Ipsen Pharmaceutical Co., Ltd. (Tianjin, China). The native standards of paraquat dichloride and labeled standards of paraquat-ring-d8·2HCl (PQ-label) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Gramoxone® (200 g/L paraquat water solution) was obtained from Syngenta China Co., Ltd. (Shanghai, China). All solvents used were of analytical grade. Acetonitrile, formic acid, and ammonium formate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Deionized water was organically and biologically purified by a Millipore Simplicity UV water system (Shanghai, China).

Experimental animals

Male New Zealand white rabbits (5 months old, weighing 2.35 ± 0.01 kg) were obtained from the Animal Experiment Center of Southern Medical University, China. The rabbits were handled in accordance with the Standard Guide for the Care and Use of Laboratory Animals, the research was approved by Ethics Committee of Shenzhen Prevention and Treatment Center for Occupational Disease (No. LL-202009). All methods were carried out in compliance with the ARRIVE guidelines.

In vitro batch test

The adsorption of different adsorbents (activated charcoal, montmorillonite, fuller's earth, and marine sand) was evaluated by a batch test. A dose of PQ (5, 25, or 50 mg) was dissolved in a normal solution and mixed with 5 mL simulated gastric fluid or simulated intestinal fluid in a 15-mL acrylic resin centrifuge tube with a cover, and the final PQ concentrations were 1, 5, and 10 mg/L, respectively. Then, 50, 250, or 500 mg (ten times the PQ dose) of adsorbents were added and mixed by an IKA KS 4000 orbital shaker (Staufen, Germany) for different amounts of time. Samples for the PQ concentration analyses were taken from the centrifuge tube and then measured by LC-MS/MS. From the concentration of PQ in the simulated digestive juices before adsorption (C_0) and that in the simulated digestive juices during the adsorption time (C_x), the absorption rate of each component was calculated according to Eq. (1):

$$\text{Absorption rate (\%)} = (C_0 - C_x)/C_0 \times 100. \quad (1)$$

All *in vitro* tests were repeated three times. Taking the PQ concentration (1, 5, and 10 mg/L), different adsorbents (activated charcoal, montmorillonite, fuller's earth, and marine sand), reaction environment (pH = 2.0 and pH = 6.8), and reaction time (1, 10, 30, 60, and 120 min) as indices, the absorption rate of PQ was screened by an L_{25} ($2^1 \times 3^1 \times 5^2$) orthogonal design as Table I.

TABLE I - Orthogonal design of *in vitro* test

Level	Adsorbents	Time (min)	PQ concentration (mg/ml)	Reaction environment
1	activated charcoal	1	1	simulated gastric fluid (pH2.0)
2	montmorillonite	10	5	simulated intestinal fluid (pH6.8)
3	fuller's earth	30	10	
4	marine sand	60		
5	Control	120		

In vivo experiment of adsorbents for PQ poisoning

A group of 24 rabbits was randomly divided into four groups with random number table method.

Group 1: Montmorillonite control group (4 rabbits); dosed with 2000 mg/kg montmorillonite in saline (concentration: 500 mg/mL) by oral gavage without PQ.
Group 2: PQ poisoning group (8 rabbits); dosed with 200 mg/kg PQ in saline (concentration: 100 mg/mL) by oral gavage without montmorillonite.
Group 3: Immediate treatment group (6 rabbits); dosed with 200 mg/kg PQ in saline (concentration: 100 mg/mL) by oral gavage with 2000 mg/kg montmorillonite in saline (concentration: 500 mg/mL) 5 min later.
Group 4: Delayed treatment group (6 rabbits); dosed with 200 mg/kg PQ in saline (concentration: 100 mg/mL) by oral gavage with 2000 mg/kg montmorillonite in saline (concentration: 500 mg/mL) 30 min later.

The rabbits' general conditions were observed. In groups 2, 3, and 4, 0.5-mL blood samples were collected from an indwelling arterial needle and anticoagulated with heparin sodium before PQ exposure and 10, 20, and 40 min and 1, 1.5, 2, 3, 4, 8, 24, 72, and 168 h after PQ exposure. Another 2.0-mL blood sample was collected from an indwelling arterial needle before PQ exposure and 168 h after PQ exposure for cytokine analysis and blood gas analysis. All experimental animals were sacrificed with excessive phenobarbitone anesthetization at 168 h after exposure, general dissection was conducted, and the pathology of the lung was observed. The effectiveness of the montmorillonite was estimated by calculating the change of the toxicokinetic parameters, cytokine analysis, blood gas analysis, and histopathological examination.

Analytical methods for PQ concentration

0.5 mL of simulated digestive juices (*in vitro*) or 0.5 mL of an anticoagulated blood sample (*in vivo*) was centrifuged at 3,000 rpm for 5 min to obtain 0.2 mL of supernatant. Each sample was pipetted into a 1.5-mL vial and spiked with 40 µL of the labeled internal standard working solution (1.0 µg/mL) to create a concentration of 40.0 ng/mL. The samples were vortex-mixed with 0.8 mL of an acetonitrile-formic acid deionized water solution (acetonitrile:0.1% formic acid deionized water = 3:1) for 5 min, then centrifuged at 12,000 rpm for 5 min, and 0.5 mL of the supernatant was then transferred to auto-injection vials.

Chromatographic separation was performed by a Shimadzu LC-20ADXR HPLC system (SHIMADZU, Japan) composed of an autosampler and an HPLC pump. The column used was an XBridge®BEH-HILIC, 100 mm × 2.1 mm × 2.5 µm (Waters Corporation, Milford, MA, USA). The analytes were separated with isocratic elution by using 40% 100 mmol/L ammonium formate in a 0.5% formic acid deionized water solution and 60% acetonitrile for 10 min. The flow rate was 400 µL/min, the column temperature was 30 °C, and the injection volume was 2 µL. The surveyor HPLC system pump pressure was the maximum of 400 bar.

For the MS/MS analysis, an AB Sciex API 4000$^+$ triple quadrupole mass spectrometer (AB SCIEX,

Redwood City, CA, USA) was used. The instrument was operated with an electrospray ionization (ESI) source. The source was operated in positive-ion mode using multiple reaction monitoring (MRM). The instrument parameters were as follows: sheath gas pressure, 50 psi; auxiliary gas pressure, 55 psi; capillary temperature, 650 °C; spray voltage, 5000 V; collision gas pressure, 12 psi. The positively-charged molecular ion of PQ at m/z 171.2 was selected as a precursor ion, and the two transitions of 155.2 and 77.2 were selected as the quantitative transitions. The positively-charged molecular ion of isotope-labeled internal standard PQ-d8 at m/z 179.2 was selected as a precursor ion, and the two transitions of 162.2 and 82.2 were selected as the quantitative transitions.

Toxicokinetic analysis

To estimate the toxicokinetic parameters, either one- or two-compartment pharmacokinetic models were fit to the blood data from the various *in vivo* studies. The concentrations of PQ in the peripheral blood at different times after dosing were input into drug analysis system (DAS) 3.0 software, which calculated the kinetic curve and toxicokinetic parameters, including $t_{1/2}$, Cl, AUC, and Vd.

Enzyme-linked immunosorbent assay (ELISA) assay

Blood samples were collected from an indwelling arterial needle before PQ exposure and 168 h after PQ exposure, and the plasma was prepared by centrifugation and then frozen at -80 °C for subsequent analysis. The levels of rabbit transforming growth factor-β1 (TGF-β1), high-mobility group protein 1 (HMGB1), and monocyte chemotactic protein 1 (MCP-1) were assayed using ELISA kits (Shanghai J&L Biological Co., Ltd., Shanghai, China). The procedure was performed according to the manufacturer's instructions.

Histopathological examination

Lung tissue dissected from the right lower lobe was fixed with 10% paraformaldehyde solution, and the specimen was then sectioned to tissue blocks with a thickness of about 0.5 cm. Conventional gradient alcohol dehydration was then performed by a VT1000A automatic dehydration machine, paraffin embedding was performed by an EG1150H/C tissue-embedding machine, and serial sectioning was conducted. The paraffin-embedded sections were stained with hematoxylin and eosin (HE) following standard procedures by an ST5010 automatic tissue staining apparatus (LEICA, Germany). Histopathological changes of the lung tissue were observed under an Eclipse Ni optical microscope system (Nikon, Japan).

Blood gas analysis

The arterial blood was drawn before PQ exposure and 168 h after PQ exposure for blood gas analysis by an i-STAT 1 blood gas analyzer (Abbott Laboratories, USA). The recorded parameters of pH, PaO_2, $PaCO_2$, and PaO_2/FiO_2 were subsequently calculated.

Statistical analysis

The data are represented as the mean ± standard deviation, and analysis was performed with SPSS 16.0 software for Windows (SPSS Inc., Chicago, IL, USA). Parameters were compared among multiple groups with one-way analysis of variance (ANOVA). When $P < 0.05$, the LSD t-test was applied.

RESULTS

In vitro batch test

The orthogonal test results for the absorption rate of PQ are presented in Table II. All the factors including the adsorbent ($F = 122.70$, $P = 0.00$), time ($F = 8.14$, $P = 0.00$), PQ concentration ($F = 14.97$, $P = 0.00$), and reaction environment ($F = 49.25$, $P = 0.00$) were found to significantly influence the absorption rate. From greatest to least, the factors affected the absorption rate in the following order: adsorbents > time > PQ concentration > reaction environment. The orthogonal experiments also indicated the optimal prescription to be as follows: $A_2T_2C_2E_1$.

TABLE II - Results of Orthogonal Test

No.	A	T	C	E	Absorption Rate (%)			
					1	2	3	Mean
1	4	4	2	1	25.91%	26.43%	35.92%	29.42%
2	2	1	3	1	99.41%	99.39%	99.40%	99.40%
3	4	5	2	2	24.30%	5.77%	10.77%	13.61%
4	4	3	3	2	-1.61%	3.05%	7.45%	2.96%
5	4	1	1	1	19.04%	26.73%	19.86%	21.88%
6	5	5	3	1	24.95%	21.25%	11.24%	19.15%
7	5	3	1	1	12.30%	12.86%	21.59%	15.58%
8	2	2	3	2	97.38%	97.53%	97.31%	97.41%
9	3	5	2	1	99.51%	99.51%	96.35%	98.46%
10	4	2	3	1	69.34%	84.64%	67.67%	73.88%
11	2	3	2	1	99.38%	99.28%	99.46%	99.37%
12	5	1	2	2	0.70%	1.14%	-7.09%	-1.75%
13	3	3	3	2	51.44%	49.64%	50.46%	50.51%
14	1	3	2	1	92.32%	92.39%	92.25%	92.32%
15	1	1	3	1	96.19%	96.53%	96.61%	96.44%
16	2	5	1	2	79.29%	78.48%	78.18%	78.65%
17	1	2	2	2	89.07%	88.87%	89.94%	89.29%
18	5	4	3	2	3.51%	0.38%	1.69%	1.86%
19	3	4	3	1	54.62%	60.09%	62.04%	58.92%
20	1	5	3	1	98.82%	98.74%	98.96%	98.84%
21	5	2	2	1	50.83%	56.42%	57.83%	55.03%
22	1	4	1	2	57.09%	59.65%	63.26%	60.00%
23	3	2	1	1	47.53%	46.32%	44.89%	46.25%
24	2	4	2	1	99.32%	99.40%	99.28%	99.33%
25	3	1	2	2	78.30%	83.50%	70.20%	77.34%
$\overline{K_1}$	87.38%	58.66%	44.47%	66.95%				
$\overline{K_2}$	94.83%	72.37%	65.24%	46.99%				
$\overline{K_3}$	66.29%	52.15%	59.94%					
$\overline{K_4}$	28.35%	49.91%						
$\overline{K_5}$	17.97%	61.74%						
R	76.89%	22.46%	20.77%	19.96%				

General observation of experimental animals

Poisoning symptoms and death were not observed in the animals in the montmorillonite control group (Group 1) or treatment groups (Groups 3 and 4) during the experimental periods. Two animals in the PQ poisoning group (Group 2) moved slowly and refused food and water at 24 h after PQ exposure. These two animals died at 30 h and 75 h after PQ exposure, respectively, and the subsequently-presented results exclude these two animals.

Concentration-time curve of PQ poisoning rabbit model for gastrointestinal adsorbents

Figure 1 presents the changes in the plasma PQ concentrations within different groups, and the concentration-time curve of PQ was fitted with a two-compartment kinetic model. The plasma PQ concentrations of the treatment groups (Groups 3 and 4) were significantly lower than that of the PQ poisoning group (Group 2) ($F = 5.80$, $P = 0.01$), but there was no significant difference between Groups 3 and 4 ($P = 0.51$). No interaction between the group and time was observed ($F = 1.53$, $P = 0.07$).

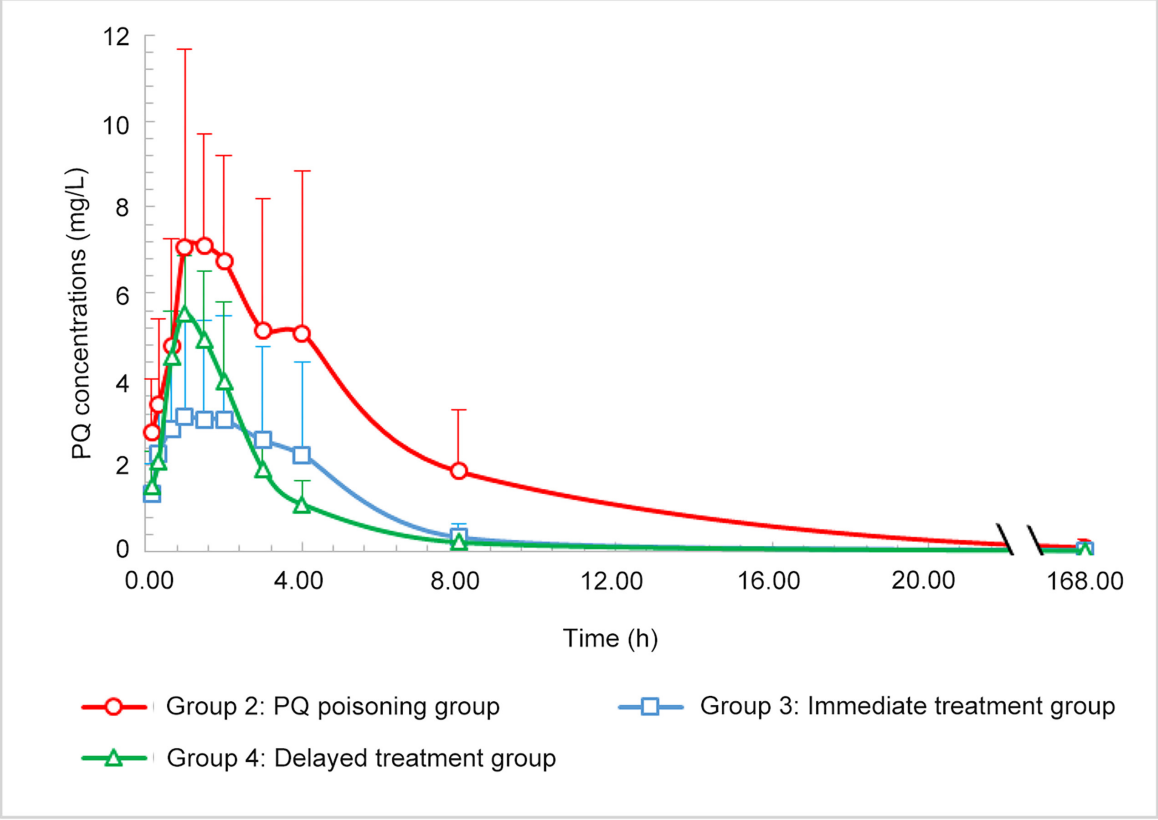

FIGURE 1 - Concentration-time curve of PQ for different groups.

Toxicokinetic parameter changes of a poisoning rabbit model for adsorbent treatment

The toxicokinetic parameter changes of the poisoning rabbit model for the gastrointestinal adsorbent treatment groups and the control group are summarized in Table III.

TABLE III - Kinetics parameters changes of groups ($\bar{x} \pm s$)

Parameters	Unit	Group			*F*	*P*
		2	3	4		
$AUC_{(0-t)}$	mg/L*h	72.89±35.15	19.78±14.79	17.92±5.42	26.75	0.00*
$AUC_{(0-\infty)}$	mg/L*h	76.05±34.30	19.95±14.75	17.94±5.43	30.92	0.00*
$AUMC_{(0-t)}$	h*h*mg/L	993.68±325.32	106.66±86.21	82.01±28.92	107.44	0.00*
$AUMC_{(0-\infty)}$	h*h*mg/L	1356.91±585.59	127.26±80.14	83.40±29.11	72.44	0.00*
$MRT_{(0-t)}$	h	14.43±3.17	5.48±0.88	4.54±0.46	121.44	0.00*
$MRT_{(0-\infty)}$	h	19.03±8.42	8.70±4.67	4.61±0.40	25.9	0.00*
$VRT_{(0-t)}$	h^2	340.65±145.82	99.12±57.87	76.78±14.35	36.96	0.00*
$VRT_{(0-\infty)}$	h^2	813.79±771.92	567.20±702.32	82.70±7.42	6.15	0.00*
λ_z	1/h	0.07±0.04	0.08±0.08	0.14±0.05	6.57	0.00*
$t_{1/2z}$	h	15.47±12.11	20.75±17.48	5.95±3.75	6.25	0.00*
T_{max}	h	2.16±1.47	1.61±1.26	1.14±0.39	2.74	0.08
Vz	L/kg	67.36±55.40	105.14±138.46	103.68±60.18	5.71	0.00*
CLz	L/h/kg	2.98±0.99	21.76±19.93	12.41±4.64	5.22	0.01*
C_{max}	mg/L	9.21±4.43	4.23±2.27	6.03±1.03	9.5	0.00*

Note: * Statistically significant

Enzyme-linked immunosorbent assay (ELISA) assay

Serum levels of TGF-β1, HMGB1 and MCP-1 were measured by ELISA before and 168 h after PQ exposure were shown in Table IV. In PQ poisoning group (Group 2), serum TGF-β1and HMGB1 levels significantly elevated on 168 h compared to montmorillonite control group (Group 1). Rabbits treated with montmorillonite whether 5 min (Group 3) or 30 min (Group 4) following PQ exposure had significantly reduced serum TGF-β1 and HMGB1 levels compared to PQ group (Group 2), however, there were no different between 2 treatment groups (Group 3 and Group 4). Serum MCP-1 levels have no significantly changes between different groups.

TABLE IV - Cytokines changes of groups ($\bar{x} \pm s$)

Groups	TGF-β1 (pg/ml)		HMGB1 (ng/ml)		MCP-1 (pg/ml)	
	0 h	168 h	0 h	168 h	0 h	168 h
1	151.50±19.36	173.53±20.00	77.70±11.46	76.23±1.73	63.79±8.31	59.30±8.14
2	171.89±19.28	230.21±30.26	88.04±12.61	96.92±9.17	69.59±13.13	76.61±6.91
3	162.71±22.55	171.06±14.91	80.14±10.01	81.14±5.98	69.94±6.89	66.83±9.53
4	159.60±19.17	166.94±16.91	82.29±6.94	81.12±7.27	70.84±10.41	68.14±8.01
F	8.05		3.99		1.64	
P	0.00		0.02		0.22	

Histopathological examination

The results of the general observation are displayed in Figure 2. The lungs from the animals in the montmorillonite control group (Group 1) exhibited smooth surfaces, a normal pink color, and good textural elasticity. In the PQ poisoning group (Group 2), all the animals (6/6) had irregular dark-red or dark-gray plaques on the surfaces of the lungs that were characterized by clear boundaries with the normal lung tissue and a relatively tough texture, especially in the lower lobes of both lungs. Otherwise, a small portion (1/6) of the animals in the montmorillonite treatment groups (Groups 3 and 4) had similar lesions as the animals in the PQ poisoning group, but there were fewer lesioned areas and the degrees of the lesions were lesser.

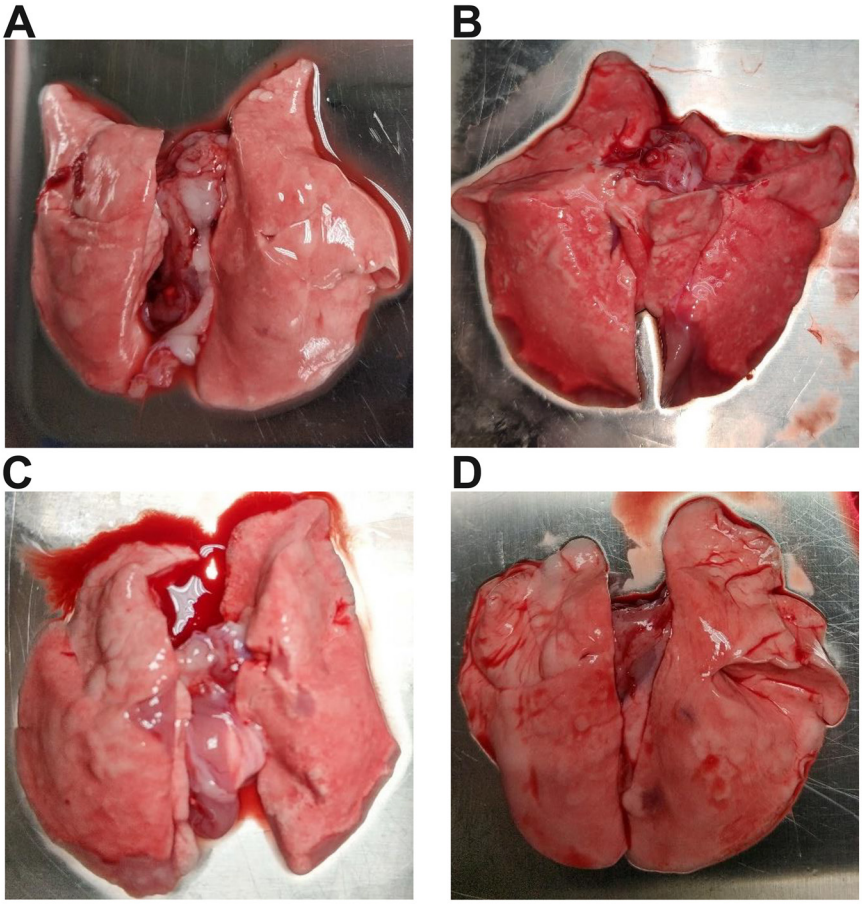

FIGURE 2 - General observation for lungs in different groups (A: Montmorillonitec control group; B: PQ poisoning group; C: Immediate treatment group; D: Delayed treatment group).

The results of HE staining are displayed in Figure 3. Lung tissues from the animals in the montmorillonite control group (Group 1) exhibited normal structures and no histopathological changes; the alveolar walls in the lung tissues were thin and integral, and there were no abnormal exudates in the pulmonary interstitia. In comparison, in the PQ poisoning group (Group 2), the alveolar tissues of all six experimental animals showed obvious congestion and edema, the swelling of capillary vessels, widened alveolar septa, the formation of partially-visible membranes, and the extravasation of red blood cells in the pulmonary interstitia. A small portion (1/6) of the animals in the montmorillonite treatment groups (Groups 3 and 4) had only minimal damage, and the histopathological changes in their tissues were minor as compared with those in the tissues from the animals in the PQ poisoning group.

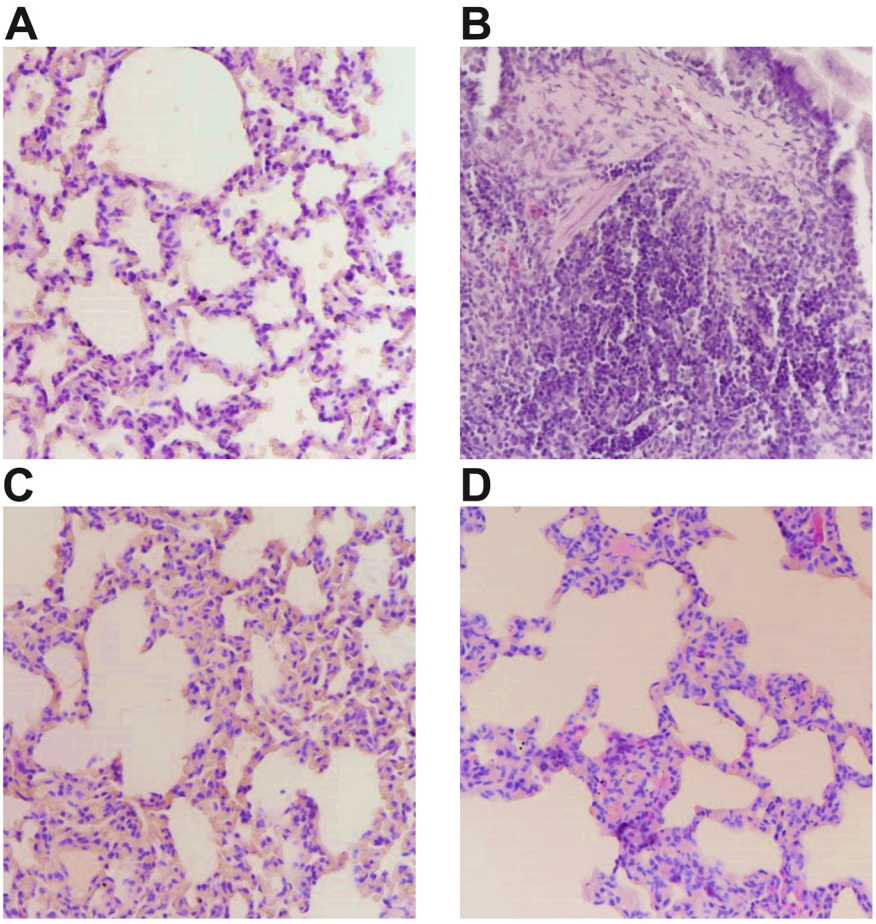

FIGURE 3 - Histopathological observation for lungs in different groups (HE, 200×. A: Montmorillonitec control group; B: PQ poisoning group; C: Immediate treatment group; D: Delayed treatment group).

Blood gas analysis

Arterial blood gas parameters were measured before PQ exposure and 168 h after PQ exposure are summarized in Table V, and no significant differences between groups were found.

TABLE V - Blood gas parameters changes of groups ($\bar{x} \pm s$)

Groups	PO_2 (mmHg)		Sat O_2 (%)		PCO_2 (mmHg)		TCO_2 (mmHg)		pH		BE (mmol/L)		HCO_3 (mmol/L)	
	0 h	168 h	0 h	168 h	0 h	168 h	0 h	168 h	0 h	168 h	0 h	168 h	0 h	168 h
1	83.75±3.30	87.25±4.92	95.25±0.50	96.25±0.50	34.63±1.36	34.53±3.44	21.00±5.10	20.33±3.40	7.29±0.10	7.35±0.06	-5.33±5.43	-6.00±4.24	19.97±4.43	19.43±3.29
2	93.00±10.60	90.50±13.84	96.50±1.22	97.17±1.33	32.00±3.61	36.48±5.34	18.17±4.12	25.33±7.61	7.33±0.07	7.41±0.11	-8.67±5.20	0.00±9.14	17.28±4.15	24.28±7.47
3	91.67±6.65	83.67±11.98	96.00±1.10	95.50±1.64	31.72±3.79	33.63±2.50	16.00±3.58	25.50±3.21	7.28±0.13	7.46±0.04	-8.67±4.55	0.67±3.50	15.03±3.46	24.67±3.09
4	88.17±8.70	89.50±6.53	96.17±0.41	97.50±0.55	32.12±5.69	33.65±3.78	19.17±6.18	24.50±4.55	7.35±0.08	7.45±0.04	-7.33±7.39	-0.50±5.17	18.27±6.02	23.47±4.36
F	0.57		2.69		0.59		0.16		1.15		0.18		0.17	
P	0.64		0.08		0.63		0.92		0.36		0.91		0.92	

DISCUSSION

In the years 1965-1967, bipyridylium herbicides such as PQ were found to bind strongly to soil and clay minerals, a common feature shared by many other organic cations (Knight, Tomlinson, 2010). Investigations of the adsorption capacities and chemical compositions of a variety of soils revealed that montmorillonite was a particularly strong binding agent *in vitro* (Faust, Zarins, 1969; Knight, Tomlinson, 2010). Present study not only researched the adsorption capacity of different kinds of adsorbents, but also explored the influencing factors of the adsorption capacity via orthogonal *in vitro* experiments. The results also support the findings of former reports(Faust, Zarins, 1969; Knight, Tomlinson, 2010); montmorillonite was found to have a stronger adsorption capacity than activated charcoal or fuller's earth, its adsorption reaction was fast and complete, and it is also appropriate for use in the stomach acid environment. Therefore, montmorillonite powder was used as the adsorbent for follow-up *in vivo* experiments.

The LD_{50} values of PQ for rats, guinea pigs, and monkeys are 126, 22, and 50 mg/kg, respectively (Dinis-Oliveira *et al.*, 2008). PQ is primarily absorbed through the digestive tract, and the toxic effects of PQ poisoning rapidly progress after absorption (Chui, Poon, Law, 1988). In a previous report (Kan *et al.*, 2010) the toxicokinetic parameters of PQ in rabbits after oral administration were found to be as follows: C_{max}, 14.46 ± 2.35 mg/L; T_{max}, 1.63 ± 0.31 h; $AUC_{(0-t)}$, 177.61 ± 14.62 mg × h/L. In the present study, the toxicokinetic parameters were found to be similar, but the C_{max} and AUC values were lower than those found in the previous study; this may have been caused by the low absorptivity and bioavailability of the PQ solution used in the present study. At the time when Clark undertook his experiments with adsorbent substances in rodents, the assumption was made that fuller's earth or bentonite could combine with PQ and decrease the mortality of experimental rats administered with 200 mg/kg PQ (Clark, 1971). It was also found in the present study that montmorillonite powder has a similar adsorption capacity, and that either immediate or delayed treatment with montmorillonite will impact the digestive tract absorption of PQ, result in lower toxicokinetic parameters related to the internal loading of PQ, such as AUC, C_{max}, and MRT, and ultimately reduce the fatality rate in treatment groups.

Previous studies showed that several cytokines, chemokines, and fibrogenic mediators play important roles in the molecular mechanisms of pulmonary inflammation and fibrosis induced by PQ (He *et al.*, 2020; Khalighi *et al.*, 2016; Wang *et al.*, 2020). Inflammatory cells secrete a variety of pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6, TGF-β1, HMGB1, and chemokine MCP-1, which intensify inflammatory reactions and lead to lung injury (Amirshahrokhi, Khalili, 2016; Chen *et al.*, 2019; Chen, Fu, 2018; Huang *et al.*, 2020; Wang, Luo, Zhao, 2014).

TGF-β1 is secreted by numerous cell types, including fibroblasts and macrophages. It is an important regulator of cell growth and differentiation and has been identified as the most important pro-fibrotic cytokine. TGF-β1 has been implicated as a mediator of chronic inflammation and fibrosis in many tissues, including lung tissue. It stimulates the proliferation and differentiation of alveolar epithelial cells and fibroblasts, as well as the production of matrix metalloproteinases and collagen. Several studies have reported the increase of TGF-β1 after PQ exposure (Chen, Fu, 2018; Hua *et al.*, 2017; Zhang *et al.*, 2014). In the present research, the serum TGF-β1 was found to be significantly elevated 168 h after PQ exposure; as montmorillonite reduced the PQ concentration in blood, the TGF-β1 levels in the animals in the treatment group were lower than those in the animals in the PQ poisoning group.

HMGB1, a highly-conserved nonhistone DNA-binding protein, could be actively released to the cytosol and the extracellular environment and function as a cytokine to initiate inflammatory responses. In rat models of PQ poisoning, it has been shown that multiple inflammatory factors, including HMGB1, were elevated in the lung tissue or serum following PQ-induced cell death (Chen *et al.*, 2019; Huang *et al.*, 2020; Nishiyama *et al.*, 2013). Yan suggested that the development of an acute immune response presents experimental evidence that targeting any component in the HMGB1-TLR4-IL-23-IL-17A axis is sufficient for the alleviation of lung injuries following PQ exposure (Yan *et al.*, 2017). In the present research, the serum HMGB1 was found to be significantly

elevated 168 h after PQ exposure; as montmorillonite reduced the PQ concentration in the blood, the HMGB1 levels in the animals in the treatment group were lower than those in the animals in the PQ poisoning group.

MCP-1 is a member of the C-C chemokine family and regulates the migration and infiltration of monocytes/macrophages. MCP-1 is produced by several cells, such as endothelial cells, alveolar epithelial cells, and macrophages. This pro-inflammatory protein has been implicated in the pathogeneses of various inflammatory diseases(Amirshahrokhi, Khalili, 2016). It has been reported that MCP-1 plays an important role in the development of lung inflammation and fibrosis (Gao *et al.*, 2016; Prasse, Müller-Quernheim, 2009). However, it was not found in the present study that the administration of PQ caused a significant increase in the levels of serum chemokine MCP-1; as MCP-1 regulates the migration and infiltration of monocytes/macrophages, its concentration in serum was low.

The most common causes of death due to acute PQ poisoning are pulmonary fibrosis and progressive respiratory failure. As mentioned previously, pulmonary edema, hemorrhage, the destruction of alveolar epithelial cells, and ultimately fibrosis caused by inflammatory cytokines and chemokines are the main histopathological characteristics of PQ poisoning (Javad-Mousavi *et al.*, 2016; Khalighi *et al.*, 2016). In this study, lung injury in the animals in the PQ group exhibited similar features to those of acute lung injury, including interstitial pneumonia, increased lung permeability, and interstitial inflammatory cell infiltration. The characteristic PQ-induced pathological alterations, including alveolar edema, hemorrhage, inflammatory cell infiltration, diffuse alveolar collapse with an increased thickness of the alveolar walls, swollen alveolar epithelial cells, and the development of an extensive fibrosis, were observed in the lungs of the experimental animals after PQ exposure. In this study, treatment with montmorillonite was found to be very effective in decreasing the levels of PQ and preventing oxidative damage induced by PQ, which are characterized by the alleviation of PQ-induced tissue damage.

Arterial blood gas tests are used to evaluate gas exchange in humans by determining the partial pressures of carbon dioxide and oxygen, the blood pH, and the bicarbonate level to assess oxygen delivery ability, ventilator function integrity, and acid-base equilibrium systems. In theory, arterial blood gas tests have significance in prognosing PQ poisoning. Previous studies have investigated the predictive value of arterial blood gas tests in prognosing patients with PQ poisoning (Hu *et al.*, 2017; Huang, Zhang, 2011; Javad-Mousavi *et al.*, 2016) and have identified the most significant indexes of the arterial blood gas test. A previous research analysis of PQ poisoning revealed that there were significant differences between the deceased and survival groups in the PCO_2 base excess, HCO_3^-, TCO_2, and pH. They also found that the most important correlated indexes were associated with the partial pressure of carbon dioxide (PCO_2) (Hu *et al.,* 2017). According to the results of the present study, there were no obvious differences between groups, even between the PQ poisoning group and the control group. This may be due to the PQ exposure dose and sacrifice time used in the present study, the focus of damage on the lower margin of the lung, or the sampling of only a portion of the whole lung; contrarily, it may be explained by the strong compensatory ability of lung organs, as part of the healthy lung tissue could still maintain normal breathing ability.

In this study, only two time points (5 and 30 min) of montmorillonite treatment after PQ exposure were used, and the observation period only lasted 168 h. It would be more valuable if a delayed treatment time or a prolonged observation period were investigated. However, the results of the present study clearly demonstrate that montmorillonite significantly decreased the blood PQ concentration and reduced the accumulation of TGF-β1 and HMGB1 in PQ-treated rabbits. These findings suggest that montmorillonite might exert its protective effects on PQ-induced damage by reducing the load of PQ in the body and alleviating the earlier inflammation damage due to PQ-induced oxidative stress in the lungs of rabbits. Future studies are warranted to further clinically investigate the effectiveness of montmorillonite powder for PQ poisoning.

Ethics approval and consent to participate

The rabbits were handled in accordance with the Standard Guide for the Care and Use of Laboratory

Animals, the research was approved by Ethics Committee of Shenzhen Prevention and Treatment Center for Occupational Disease (No.LL-2020009).

Competing interests

The authors declare that they have no conflict of interest.

FUNDING

This study supported by the Medical Research Fund of Guangdong Province (A2021417) and Research And Development Programme of Shenzhen Prevention and Treatment Center for Occupational Disease (KPII-202001). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

AUTHORS' CONTRIBUTIONS

GX conceived and coordinated the study, designed, performed and analyzed the experiments, wrote the paper. GW carried out cytokines, blood gas and histopathology analysis. LTD and LF carried out the PQ concentration analysis. ZJP and GMQ carried out the data collection, data analysis, and revised the paper. All authors reviewed the results and approved the final version of the manuscript.

ACKNOWLEDGEMENTS

Not applicable.

REFERENCES

Amdrup E, Olesen ES. Inhibition of gastric and duodenal proteases in the treatment of haemorrhage from peptic ulcer. II. Clinical observations on the effect of bentonite. Scand J Gastroenterol. 1972;7(3):273-7.

Amirshahrokhi K, Khalili AR. Carvedilol attenuates paraquat-induced lung injury by inhibition of proinflammatory cytokines, chemokine MCP-1, NF-κB activation and oxidative stress mediators. Cytokine. 2016;88:144-53.

Chen F, Liu Z, Li W, Li D, Yan B. The significance of serum HMGB1 level in humans with acute paraquat poisoning. Sci Rep. 2019;9(1):7448.

Chen H, Fu X. Dynamics study on the role of curcumin on TGF-β1 expression and pathological changes in acute paraquat poisoned rats. Exp Ther Med. 2018;16(5):3841-46.

Chui YC, Poon G, Law F. Toxicokinetics and bioavailability of paraquat in rats following different routes of administration. Toxicol Ind Health. 1988;4(2):203-19.

Clark DG. Inhibition of the absorption of paraquat from the gastrointestinal tract by adsorbents. Br J Ind Med. 1971;28(2):186-8.

Dinis-Oliveira RJ, Duarte JA, Sánchez-Navarro A, Remião F, Bastos ML, Carvalho F. Paraquat poisonings: mechanisms of lung toxicity, clinical features, and treatment. Crit Rev Toxicol. 2008;38(1):13-71.

Faust SD, Zarins A. Interaction of dequat and paraquat with clay minerals and carbon in aqueous solutions. Residue Rev. 1969;29:151-70.

Gao Q, Li Y, Pan X, Yuan X, Peng X, Li M. Lentivirus expressing soluble ST2 alleviates bleomycin-induced pulmonary fibrosis in mice. Int Immunopharmacol. 2016;30:188-93.

Hawkes TR. Mechanisms of resistance to paraquat in plants. Pest Manag Sci. 2014;70(9):1316-23.

He F, Wang Y, Li Y, Yu L. Human amniotic mesenchymal stem cells alleviate paraquat-induced pulmonary fibrosis in rats by inhibiting the inflammatory response. Life Sci. 2020;243:117290.

Hu L, Lin F, Li H, Tong C, Pan Z, Li J, et al. An intelligent prognostic system for analyzing patients with paraquat poisoning using arterial blood gas indexes. J Pharmacol Toxicol Methods. 2017;84:78-85.

Hua XF, Li XH, Li MM, Zhang CY, Liu HJ, Sun T, et al. Doxycycline attenuates paraquat-induced pulmonary fibrosis by downregulating the TGF-β signaling pathway. J Thorac Dis. 2017;9(11):4376-86.

Huang C, Zhang X. Prognostic significance of arterial blood gas analysis in the early evaluation of paraquat poisoning patients. Clin Toxicol (Phila). 2011;49(8):734-8.

Huang M, Guo M, Wang K, Wu K, Li Y, Tian T, et al. HMGB1 mediates paraquat-induced neuroinflammatory responses via activating RAGE signaling pathway. Neurotox Res. 2020;37(4):913-25.

Javad-Mousavi SA, Hemmati AA, Mehrzadi S, Hosseinzadeh A, Houshmand G, Rashidi Nooshabadi MR, et al. Protective effect of Berberis vulgaris fruit extract against Paraquat-

induced pulmonary fibrosis in rats. Biomed Pharmacother. 2016;81:329-36.

Kan X, Zhang XY, Dong J, Li WS, Lu ZQ. [Toxicokinetics of paraquat in rabbits]. Zhonghua Lao Dong W Sheng Zhi Ye Bing Za Zhi. 2010;28(10):756-59.

Khalighi Z, Rahmani A, Cheraghi J, Ahmadi MR, Soleimannejad K, Asadollahi R, et al. Perfluorocarbon attenuates inflammatory cytokines, oxidative stress and histopathologic changes in paraquat-induced acute lung injury in rats. Environ Toxicol Pharmacol. 2016;42:9-15.

Knight B, Tomlinson TE. The interaction of paraquat (1: 1′-dimethyl 4:4′-dipyridylium dichloride) with mineral soils. J Soil Sci. 2010;18(2):233-43.

Meredith TJ, Vale JA. Treatment of paraquat poisoning in man: methods to prevent absorption. Hum Toxicol. 1987;6(1):49-55.

Nguyen V, Malik DS, Howland MA. Methylene blue protects against paraquat-induced acute lung injury in rats. Int Immunopharmacol. 2014;20(2):358.

Nishiyama R, Shinoda M, Tanabe M, Oshima G, Takano K, Miyasho T, et al. Hemoadsorption of high-mobility group box chromosomal protein 1 using a column for large animals. Eur Surg Res. 2013;51(3-4):181-90.

Prasse A, Müller-Quernheim J. Non-invasive biomarkers in pulmonary fibrosis. Respirology. 2009;14(6):788-95.

Sun B, He Y. [Paraquat poisoning mechanism and its clinical treatment progress]. Zhonghua Wei Zhong Bing Ji Jiu Yi Xue. 2017;29(11):1043-46.

Wang G, Lian C, Xi Y, Sun Z, Zheng S. Evaluation of nonionic surfactant modified montmorillonite as mycotoxins adsorbent for aflatoxin B(1) and zearalenone. J Colloid Interface Sci. 2018;518:48-56.

Wang X, Luo F, Zhao H. Paraquat-induced reactive oxygen species inhibit neutrophil apoptosis via a p38 MAPK/ NF-κB-IL-6/TNF-α positive-feedback circuit. PLoS One. 2014;9(4):e93837.

Wang Y, Chen Y, Mao L, Zhao G, Hong G, Li M, et al. Effects of hemoperfusion and continuous renal replacement therapy on patient survival following paraquat poisoning. PLoS One. 2017;12(7):e0181207.

Wang YL, Zheng J, Zhang XF, Zhang Y. Attenuation of paraquat-induced inflammation by inhibitors of phosphorylation of mitogen-activated protein kinases in BV(2) microglial cells. J Neurol Sci. 2020;410:116679.

Xu YG, Lu YQ. Systematic review and meta-analysis of the efficacy and safety of immunosuppressive pulse therapy in the treatment of paraquat poisoning. J Zhejiang Univ Sci B. 2019;20(7):588-97.

Yan B, Chen F, Xu L, Xing J, Wang X. HMGB1-TLR4-IL23-IL17A axis promotes paraquat-induced acute lung injury by mediating neutrophil infiltration in mice. Sci Rep. 2017;7(1):597.

Yen TH, Wang IK, Hsu CW. Hemoperfusion for paraquat poisoning. Kidney Int. 2018;94(6):1239.

Yin Y, Guo X, Zhang SL, Sun CY. Analysis of paraquat intoxication epidemic (2002-2011) within China. Biomed Environ Sci. 2013;26(6):509-12.

Zhang Z, Ding L, Wu L, Xu L, Zheng L, Huang X. Salidroside alleviates paraquat-induced rat acute lung injury by repressing TGF-β1 expression. Int J Clin Exp Pathol. 2014;7(12):8841-7.

Received for publication on 12th September 2021
Accepted for publication on 07th December 2021