Electrophysiological assessment of peripheral nerves and neuro-muscular junctions in patients following self-poisoning with paraquat

Sudheera S Jayasinghe (sudheerasj@yahoo.com)
University of Ruhuna
Kithsiri D Pathirana
University of Ruhuna
Klintean Wunnapuk
Chiang Mai University
A W K Mithunika
University of Ruhuna
H M N W Chandrasiri
University of Ruhuna
J K N Hansani
South Asian Clinical Toxicology Research Collaboration
Andrew H Dawson
University of Peradeniya
Nick A Buckley
University of Peradeniya

Research Article

Keywords: Autonomic nervous system, Neuromuscular junction, Paraquat, peripheral nervous system, Poisoning, Electrophysiology

Posted Date: November 10th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1021252/v1

Abstract

Paraquat is neurotoxic. We aimed to study the electrophysiological effects of peripheral nerves and neuromuscular junction (NMJ) in the survivors of paraquat poisoning.

A cohort study was conducted on patients following paraquat poisoning. Controls were recruited. The assessments were performed around one and six weeks after the exposure. Motor nerve conduction velocity (MNCV), amplitude of compound muscle action potential (CMAP), sensory nerve conduction velocity (SNCV), F-wave studies, cardiovascular response to different stimuli, sympathetic skin response (SSR) studies, and exercise modified supramaximal slow repetitive stimulation (RNS) and electromyography (EMG) were performed.

There were 28 (21 males) patients and 56 controls. The mean (SD) age of the patients and the controls were 29 (12) and 31 (11) years.

Significant impairment at the first assessment in the SNCV of ulnar nerve, amplitude of ulnar nerve CMAP on distal stimulation, and F-wave occurrence in median, ulnar and tibial nerves; change of systolic blood pressure three minutes after standing and SSR amplitude (vs controls) was observed.

All parameters reverted to normal at six weeks after the exposure.

There was electrophysiological evidence for somatic nerve, autonomic, and NMJ dysfunction following acute paraquat poisoning which was not seen at six weeks after the exposure.

1. Introduction

Paraquat (PQ^{2+}, 1, 1'–dimethyl-4, 4' –dipyridyl) is a broad-spectrum, non-selective, contact herbicide used in deliberate self-poisoning in many countries in Asia and the Pacific, especially in agricultural communities [1, 2].

Although paraquat is classified as a Class 2 moderately toxic substance by the World Health Organization, the Pesticide Action Network Asia and Pacific considers paraquat to be a Class 1 toxic substance due to its lack of an antidote, high acute toxicity, and delayed effects. Paraquat poisoning has a very high case fatality (>50%) compared to most other pesticides, and is a leading cause for death from pesticide poisoning in many countries, including Sri Lanka before it was banned in 2008 [3, 4].

Paraquat causes toxicity by generating Reactive Oxygen Species (ROS) and other apoptosis-related molecules via three distinct pathways: 1- one electron reduction by the flavoenzyme NADPH-cytochrome P450 reductase and a subsequent redox cycle involving superoxide dismutase (SOD) and glutathione pools, 2- Inhibition of the mitochondrial electron transport chain, and 3- interaction with enzymes such as nitric oxide synthases, thioredoxin reductase, NADPH oxidase, NADH-ubiquinone oxidoreductase, and xanthine oxidase [1, 5].

Paraquat natively exists as a divalent cation (PQ^{2+}), which undergoes redox cycling to produce the monovalent cation PQ^{+} in the presence of cellular diaphorases such as NADPH oxidase and nitric oxide synthase [6].

The reduced paraquat radical is immediately re-oxidized to PQ^{2+} in the presence of molecular oxygen, forming a superoxide anion radical (O_2^{-}). Superoxide radical is then dismutated to molecular oxygen and hydrogen peroxide by SOD. Further, there is generation of the hydroxyl free radical (HO.) in the presence of iron through the Fenton reaction. The generation of such highly reactive oxygen species induces toxicity in multiple organs such as the lung, liver, kidney, and muscles [1, 5].

However, the most important target organ for paraquat toxicity is the lung, contributed to by the transport of paraquat against a concentration gradient into the lung. However, muscle is the largest reservoir and releases paraquat slowly over many days [7]. Hence, the neuromuscular junctions (NMJ) in somatic and autonomic nerves are potentially exposed for prolonged periods.

Many other organs are also affected: centrizonal hepatic necrosis, proximal renal tubular damage, myocardial damage, and skeletal muscle damage with focal necrosis are seen in post-mortem of patients with paraquat poisoning [8].

Although the case fatality following deliberate ingestion of paraquat is around 65%, there are reports of paraquat survivors even with large doses of paraquat ingestion [9–11]. Hence the morbidity of survivors is an important area to be explored.

A study conducted on patients admitted after paraquat self-poisoning showed that they had significantly low nerve conduction velocity. The parameters returned to normal after 6 weeks [12]. However, it remains unknown whether paraquat-induced oxidative stress could lead to peripheral demyelinating neuropathy

In humans, a peripheral burning sensation (perhaps indicating neuronal involvement) is a common clinical feature that indicates a poor prognosis [13]. A progressive severe toxic myopathy has also been reported after paraquat poisoning. Microstructural changes in the extrapyramidal ganglia and hippocampus have been observed in neuroimaging studies of paraquat poisoned victims [14].

Paraquat intoxication triggers an elevation of oxidative stress in the nerve, provoking lipid peroxidation and myelin protein aggregation leading to demyelination [12].

However, surprisingly, although paraquat is a potential human neurotoxicant, [15] published studies exploring neurotoxic effects of paraquat in humans are limited to XX. Therefore, we aimed to explore peripheral nerve (somatic and autonomic) and NMJ function in survivors of paraquat poisoning.

2. Materials And Methods

A cohort study was conducted with matched controls over two years in a tertiary care hospital in the Southern Province of Sri Lanka with the approval of the Ethical Review Committee, Faculty of Medicine, University of Ruhuna, Sri Lanka. Consecutive patients admitted to the hospital with a history of paraquat poisoning and a positive urine paraquat test (sodium dithionite test) [16–18] were recruited to the study after obtaining informed written consent.

Acute neurotoxicity studies are generally done in few days to 14 days after exposure to the substance [19, 20]. Studies on sub-acute toxicity were carried out up to 90 days [20, 21]. We selected the midpoints of these time frames to record the data on nerve function; that is around one week and six weeks after the ingestion.

Two controls matched for age (±3 years), sex, and occupation (according to International Standard Classification of Occupation; ISCO-88 by International Labour Organization) were recruited for each patient. The controls were visitors of patients from the same hospital who volunteered to participate in the study. Controls did not have a history of acute exposure to any poison. People who had diabetes mellitus, known neurological disease, history of head injury, or who were on long-term medication were excluded from both arms of the study.

Sensory and motor nerve conduction studies, F-wave studies, and electromyography were performed to assess the function of somatic nerves. Autonomic nerve function was assessed with cardiovascular reflex-based autonomic nerve function tests (heart rate response to Valsalva maneuver, heart rate response to deep breathing, heart rate response to standing, and blood pressure response to sustained handgrip) and sympathetic skin response (SSR). Exercise modified slow repetitive supra-maximal stimulation was used to assess the function of the neuromuscular junction.

The Neuropack MEB-9400A/K EMG/EP (Nihon Koden) system was used for electrophysiological assessments, which were carried out by the trained principal investigator (SJ). A consultant neurologist (KP) was also involved in interpretation of the findings. All the tests were performed in the Clinical Neuroscience Centre, Department of Medicine, Faculty of Medicine, University of Ruhuna, Sri Lanka.

The patients were assessed at one and six weeks after the exposure. Assessments on controls were done once.

2.1 Peripheral nerve function

2.1.1 Somatic nerve function

2.1.1.1. Sensory nerve conduction studies

Orthodromic sensory nerve conduction velocities (SNCV) of median and ulnar nerves were performed on both hands with supra maximal stimulation [22].

2.1.1.2 Motor nerve conduction studies

Motor nerve conduction studies of the median, ulnar, and common peroneal nerves were carried out on both sides with supra maximal stimulation [22]. Nerve conduction velocity (NCV) and the amplitude of the compound muscle action potential (CMAP) on distal stimulation were recorded [22].

2.1.1.3 F-wave studies

Minimum reproducible F-wave latency and percentage of F-wave occurrence were measured in median, ulnar and tibial nerves on both sides. Sixteen supra maximal stimulations were given to each nerve [22, 23].

2.1.1.4 Electromyography (EMG)

EMG studies were performed on the deltoid and the first dorsal interosseous muscle on the dominant side. A concentric needle electrode was inserted into the above muscles at rest and during contraction. Attention was paid to spontaneous activity at rest, amplitude of the motor units, polyphasia, and the interference pattern during contraction of the muscle [22].

2.1.2 Muscle power

Muscle power was assessed in flexors and extensors of the wrist, biceps, triceps, dorsi flexors, and plantar flexors of the feet and toes, flexors and extensors of the knee, flexors and extensors of the hip, abductors and adductors of the thigh. The muscle power was graded according to the Medical Research Council scale for grading muscle function from 0 – 5 [24].

2.1.3 Tendon reflexes

Tendon reflexes were assessed in knee, ankle, triceps, biceps, and supinator [24].

2.1.4 Autonomic nerve function

Autonomic function tests were based on the cardiovascular reflexes to a variety of stimuli as described by D J Ewing and B F Clarke (1982) [25]. These tests are the heart rate response to Valsalva manoeuvre, heart rate response to deep breathing, heart rate response to standing, blood pressure response to standing, and blood pressure response to sustained handgrip.

According to Neuropack S1, QP948BK user guide heart rate variability (R-R interval) based autonomic function tests were performed with reference, recording, and ground surface electrodes attached to the second intercostal space at the right border of the sternum, left anterior axillary line at the lowest rib, and right anterior axillary line at the lowest rib, respectively. The R-R intervals during the test were analysed by autonomic nervous system testing software. (Neuropack S1, QP948BK)

For the blood pressure response to sustained handgrip, resting diastolic blood pressure was measured. The subject was then instructed to squeeze the hand dynamometer as hard as possible and then to maintain 30% of maximum voluntary pressure for up to 5 minutes; diastolic pressure was recorded at the

end of each minute. If the rise reached 16 mmHg within 5 minutes, it was considered normal and the procedure was discontinued. If not, the rise of diastolic blood pressure just before handgrip release at 5 minutes was recorded [25–27].

In sympathetic skin response, the median nerve was stimulated with an intensity of 25 mA as described by Neuropack S1, QP948BK user guide. Six simulations were given with at least a 30 seconds interval between consecutive stimuli. Acquired wave forms were superimposed and rectified to get the maximum amplitude. Maximum SSR amplitude and average SSR latency were recorded.

2.2 NMJ function

NMJ function was assessed with slow repetitive supra maximal stimulation of the median nerve of the dominant upper limb. Ten stimulations at a rate of 3Hz with filter setting of 20 Hz – 5 kHz were administered. Laboratory temperature was maintained at 25^0C.

The nerve was stimulated with a supra maximal stimulation at the wrist at rest (A), immediately after 30 seconds maximal isometric exercise (B), and two minutes after the exercise (C) [28]. Participants were asked to abduct the thumb against fixed resistance for the isometric exercise [28]. Post exercise facilitation and post exercise exhaustion were assessed after the isometric exercise [28, 29].

The decrement responses were quantified [22] by calculating the percentage change in amplitude as follows:

2.2.1 At rest - the first train of stimuli (A) [22]

Percentage change in amplitude of the action potential between the first and the n^{th} (n = the second or fourth or fifth action potential) of the first train of stimuli $(A_1\text{-}A_n)/A_1 \times 100$

2.2.2 Immediately after 30 seconds maximal isometric exercise - the second train of stimuli (B) [22]

Percentage change in amplitude between the first and n^{th} action potential of the second train of stimuli $(B_1\text{-}B_n)/B_1 \times 100$

2.2.3 Two minutes after the exercise - the third train of stimuli (C) [22]

Percentage change in amplitude between the first and n^{th} action potential of the third train of stimuli $(C_1\text{-}C_n)/C_1 \times 100$

Post exercise facilitation was assessed by calculating the ratio of the amplitude of the first action potential in the second train to the amplitude of the first action potential of the first train (B_1/A_1) [28, 30] and

post exercise exhaustion by calculating the ratio of the amplitude of the first action potential of the third train to the amplitude of the first action potential of the first train (C_1/A_1) [28].

2.3 Estimation of plasma paraquat

Blood was collected within 12 hours of exposure. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to estimate the plasma paraquat levels [31].

The severity of paraquat poisoning was quantified with the Severity Index of Paraquat poisoning (SIPP) (time since ingestion in hours multiply by plasma paraquat level in µg/ml) [32].

2.4 Statistical analysis

Data from the controls and the patients were compared with unpaired T-tests. Comparisons of the first (at one week following exposure) and the second (at six weeks following exposure) assessment of patients used paired T-tests. F-wave in the first assessment of the patients vs controls and the first assessment vs the second assessment were analysed with Mann-Whitney U test and Wilcoxon Signed Ranks test respectively. Bonferroni correction was used in multiple comparisons. Correlation between SIPP score with the neurotoxicity outcomes was analysed with Spearman's correlation coefficient.

3. Results

A total of 99 patients with paraquat poisoning were admitted to the hospital during the study period. Forty nine patients died during the hospital stay and nineteen left against medical advice. Three patients did not consent for electrophysiological assessment. Electrophysiological assessments were carried out in 28 patients at one week (mean 9 ± 4 days - first assessment) and 18 patients turned up for the second assessment at six weeks after the exposure. There were 56 controls.

Seven of the 28 patients were female. The mean (SD) age of the patients and controls were 29 (12) and 31 (11) years, respectively. The mean height (SD) of the patients and controls were 160 (5.9) cm and 158.9 (11.3) cm, respectively. The median (IQR) plasma paraquat concentration of survivors within 12 hours of exposure was 0.01 (0.001-0.15) µg/ml. The median (IQR; range) SIPP score of the survivors was 3.2 (0.08-6.46; 0.01-35.76).

None of the patients had co-ingested any other agrochemical.

Among 28 patients, six patients received activated charcoal, 14 patients received Fuller's earth, 12 patients received both and seven patients did not receive either of them.

Some of the patients were treated with immunosuppressants; two patients received cyclophosphamide (intravenous (IV) 1g stat, oral 250 mg twice daily for three days), dexamethasone (IV 8mg three times daily for three days followed by oral dexamethasone for a maximum of six days) and methylprednisolone (IV 1g daily for three days). Six patients received cyclophosphamide (IV 1g stat, oral 250 mg twice daily) and methylprednisolone (IV 1g daily for three days).

3.1 Somatic nerve function

Comparison of the results of somatic nerve function in the patients and the controls is shown in Table 1.

EMG was performed in 20/28 patients in the first assessment (eight patients had painful cannula sites on the dominant hand) and 15/18 in the second assessment. None of the patients showed spontaneous activity, fibrillation potentials, high amplitude of the motor units, polyphasia or reduced interference pattern.

3.2 Autonomic nerve function

The results of the first and the second assessment of autonomic function in the patients and the controls are shown in Table 2. Statistically significant autonomic dysfunction was found in the change of SBP three minutes after standing and SSR amplitude. All the parameters reverted to normal in the second assessment.

The test of heart rate response to Valsalva manoeuvre was performed only on 12 patients since the rest of the patients had mouth ulcers as a result of mucosal burns due to paraquat.

The blood pressure response to sustained hand grip was not able to perform in eight patients due to painful cannula sites on the hand. Only 4/20 patients were able to complete the test due to fatigue. This was significantly less than that of the second assessment of the controls ($p<0.01$), however no useful information on autonomic response was able to be derived from this test.

3.3 NMJ function

Significant decrement response was observed 30 seconds after an isometric exercise at the first assessment of the patients compared to the controls (Table 3).

None of the decrement responses was significant in the second assessment compared to the controls.

Maximum decrements observed in the patients at rest, just after isometric exercise, and two minutes after isometric exercise, were 28%, 20%, and 13% respectively.

3.4 Muscle power and tendon reflexes

All patients and the controls showed muscle power of 5 and tendon reflexes of 1 – 2.

Statistically significant correlations were observed with SIPP and electrophysiological parameters at the first assessment in heart rate response to standing, heart rate response to Valsalva manoeuvre and F-wave occurrence of tibial nerve with Spearman's correlation coefficient (Table 4).

4. Discussion

This was the first study in humans to have measured the effects of acute paraquat poisoning on peripheral nerves (somatic and autonomic) and the NMJ. Significant impairment of function was found at the first assessment in the SNCV of ulnar nerve, amplitude of ulnar nerve CMAP on distal stimulation, F-wave occurrence in median, ulnar and tibial nerves and change of systolic blood pressure three minutes after standing. The decrement response in NMJ after exercise augmentation was significantly greater in the patients at one week after the exposure. All these electrophysiological changes had become insignificant at the time of the second assessment in six weeks. However, it needs to be noted that for any damage to be evident with electrophysiology, 50% of the nerve fibres should be affected as unaffected fibres compensate for the damaged ones [22]. Therefore, less than 50% of residual nerve injury cannot be excluded.

A study conducted in Japan showed that patients with paraquat self-poisoning had significantly lower nerve conduction velocity. The parameters returned to normal after 6 weeks which is compatible with the current study [12].

SNCV and amplitude of CMAP of ulnar nerve of the patients showed significant reduction compared to the controls. The same findings were not seen in the median and common peroneal nerves.

Accordingly, ulnar nerve seemed to be affected more than median and common peroneal nerves in this study. Vulnerability of nerves to toxic damage has been associated with smaller nerve diameter and increased fibre length [33]. In a study that assessed median and sural nerve conduction velocities in workers exposed to phenoxy herbicides, sural nerve was significantly affected and it was attributed to the increased length, smaller diameter, presence of few fibres and lesser degree of myelination of sural nerve compared to median nerve [33]. The ulnar nerve diameter is smaller than of median throughout its course, and significantly smaller than median nerve at several sites. Further, the number of fascicles was lower in ulnar nerve than median nerve [34]. Therefore, the ulnar nerve may be more vulnerable than the median or common peroneal nerves to paraquat induced damage.

In a study conducted by Hichor et al. (2017), it was revealed that mice exposed to paraquat displayed disorganization of myelin sheaths and deregulation of myelin genes. It was found that paraquat-induced oxidative stress in the nerve resulting in lipid peroxidation and myelin protein aggregation that leads to demyelination. Paraquat intoxication was found to disturb the steady-state level of myelin gene expression through alteration of positive and negative signals for myelination. They suggested that these findings may explain the peripheral nerve defects observed in humans exposed to paraquat. Therefore, it was suggested that demyelination could be a cause of defects in conduction velocity of peripheral nerves observed in patients with paraquat intoxication [12].

The electrophysiological findings of demyelinating type peripheral neuropathy are slowing of conduction velocity, marked prolongation of distal latency, or both [35]. In our study we demonstrated significant reduction in SNCV which suggests demyelination of nerves following paraquat poisoning.

Another major mechanism of peripheral neuropathy is axonal loss [35]. Exposure to toxins is an accepted cause for axonal loss [22]. Axonal type peripheral neuropathy primarily affects amplitude of CMAP and amplitude sensory nerve action potential (SNAP) [35]. In this study we assessed only the amplitude of CMAP which showed a significant reduction for the ulnar nerve. Thus, this supports that paraquat poisoning may also cause axonal damage to peripheral nerves. However, amplitude changes can also occur with demyelination due to secondary axonal loss.

Our results for the F-wave occurrence showed a statistically significant reduction in all three nerves tested (median, ulnar and tibial) in the patients one week after exposure to paraquat compared to the controls. F-waves are low amplitude responses produced by antidromic activation of motor neurons. F-waves are the most sensitive and reliable nerve conduction study for evaluating polyneuropathies [36]. Occurrence of F-waves reflects the excitability of motor nerves that determines the probability of a recruitment response in individual axons [36–39]. Thus the reduced occurrence of F-waves implies impairment of motor nerve excitability following acute exposure to paraquat.

The autonomic nerve assessment suggested that paraquat poisoning affects sympathetic integrity, with a significant reduction in SBP 3 min after standing and amplitude of SSR noted in patients one week after paraquat ingestion.. However, parasympathetic integrity was not affected.

Neuronal uptake of paraquat has not been studied but muscles have a very high uptake of paraquat [40]. However, we were not able to elicit evidence of myotoxicity. Reactive oxygen species generated in muscle would be expected to affect NMJ function [41]. The gold standard method to assess the function of NMJ is a single-fibre electromyogram (SFEMG) [42]. Repetitive nerve stimulation is commonly used when facilities and expertise to do SFEMG are not available. The current study used slow (rather than fast) repetitive stimulation to minimize the discomfort to the participants, but exercise modification was introduced to augment the function of NMJ [43]. We observed significant decrement response just after the exercise but neither at rest nor few minutes after the exercise.

Animal studies suggest co-formulants of paraquat may also contribute to neurotoxicity. Commercial paraquat inhibited the amplitude of miniature end-plate potentials (m.e.p.ps) and end-plate potentials (e.p.ps) of mouse diaphragm. Interestingly pure paraquat did not have these effects. By the analysis of components in commercial paraquat, they found that paraquat by-products and the added emulsifying agent were responsible for these findings. The study emphasized the clinical significance their findings stating that neuromuscular blocking may contribute to the respiratory failure in paraquat intoxicated patient [44].

Paraquat may also disrupt calcium homeostasis which is critical for both muscle and nerve function [45]. Paraquat increased the basal activity of plasma membrane Ca^{2+}- ATPase (PMCA), which plays a critical role in Ca^{2+} homeostasis, and inhibited its sensitivity to calmodulin at low doses, while inhibiting both these components at high doses. It was suggested that PMCA is a sensitive target of oxidation in primary

neurons and the inactivation of PMCA under prolonged oxidative stress could lead to altered Ca^{2+} signalling [45].

Inflammatory cytokines such as interferon-γ may also contribute to neurodegenerative and neurochemical effects of paraquat [46].

Paraquat-induced myopathy characterized by muscle necrosis and increased plasma creatine kinase has been reported in animals and humans [47, 48]. However, EMG in this study did not show any evidence to support chemically induced myositis (spontaneous fibrillation potentials activity) or denervation (fibrillation potentials, high amplitude motor units, polyphasia, and reduced interference). The reduction in amplitude of CMAP is consistent with either muscle or axonal damage [22]. The lack of any other evidence of myotoxicity may imply axonal damage.

5. Limitations

The major limitation was the limited number of paraquat survivors we studied. However, this is still the largest neurotoxicity study of paraquat to date as paraquat poisoning is highly lethal. Many patients refused further medical care and study involvement when they were informed about the dire prognosis and lack of any effective antidote. Electrophysiological investigations are time-consuming, cause discomfort, and are not required for the medical management of these potentially fatally poisoned patients. These difficulties may explain the lack of any previous published longitudinal studies of neurotoxicity after paraquat poisoning.

The patients with clinical features of paraquat poisoning were recruited to the study irrespective of the severity of poisoning. However, most severely poisoned patients either died or discharged themselves against medical advice when it was clear there was no antidote. Therefore, neurotoxicity in severely poisoned patients was not able to be studied.

The first assessment was not able to be performed exactly one week after exposure as patients needed to be transported to the Clinical Neuroscience Centre from the hospital and it was only possible to do this when they were clinically stable. As the effect of paraquat poisoning on the nerve and neuromuscular function is likely to be transient, it is unclear whether such a variation in the timing of conducting nerve and neuromuscular function assessment may have had impacts on the observed results.

Approximately 35% of the enrolled patients did not attend for follow-up testing despite reminders. Thus, our study can exclude clinically apparent long-term neurotoxicity but lacks the power to eliminate the possibility that there is a minor degree of persistent sub-clinical neurological damage.

6. Conclusions

The function of peripheral nerves and NMJ was impaired after single acute exposure to paraquat. There was evidence for both axonal and myelin sheath lesions. While long-term impairment was not

demonstrated in this study with electrophysiological means, it may be worthwhile assessing the structural lesion with direct methods such as visualization of nerve fibres with electron microscopy.

Declarations

Acknowledgments

The authors would like to acknowledge the participants of the study, the administrative staff, the healthcare professionals including the Consultants who supported the study at Teaching Hospital, Karapitiya, Sri Lanka, the Heads and staff members of the Department of Pharmacology and Department of Medicine, University of Ruhuna, Sri Lanka. We are grateful to the members and the Clinical Research Assistances of the South Asian Clinical Toxicology Research Collaboration. A special word of gratitude is extended to Professor A. Fernando and Professor P. L. Ariyananda.

Author contributions

Conceptualization and study design: SSJ, NAB, AHD, KDP

Formal analysis and investigation: SSJ, NAB, AHD, KDPKW

Writing - original draft preparation: SSJ, HMNWC, AWKM, JKNH

Writing - review and editing: NAB, AHD, KDP

Funding acquisition: NAB

Resources: KDP

Supervision: NAB, AHD

Additional information

Funding

This study was supported by the South Asian Clinical Toxicology Research Collaboration, and was funded by the Wellcome Trust/ National Health and Medical Research Council International Collaborative Research Grant GR071669MA and NHMRC project grant 1030069.

Conflicts of interest/Competing interests

The authors have no conflicts of interest to declare that are relevant to the content of this article

Availability of data and material - Not applicable

Code availability - Not applicable

Ethics approval

Ethics approval for the study was granted by the Ethical Review Committee, Faculty of Medicine, University of Ruhuna, Sri Lanka. This study was performed in line with the principles of the Declaration of Helsinki.

Consent to participate

Informed written consent was obtained from all individual participants included in the study.

Consent for publication - Not applicable

References

1. Gawarammana, I. B., Buckley, N. A. Medical management of paraquat ingestion. *Br J Clin Pharmacol*. 2011;**72(5)**:745-57.

2. Blanco-Ayala, T., Anderica-Romero, A. C., Pedraza-Chaverri, J. New insights into antioxidant strategies against paraquat toxicity.*Free Radic Res*. 2014;**48(6)**:623-40.

3. Eddleston, M. Patterns and problems of deliberate self-poisoning in the developing world. *QJM*. 2000;**93(11)**:715-31.

4. Dawson, A. H., Eddleston, M., Senarathna, L., Mohamed, F., Gawarammana, I., Bowe, S. J., et al. Acute human lethal toxicity of agricultural pesticides: a prospective cohort study. *PLoS Med*. 2010;**7(10)**:e1000357.

5. Ranjbar, A. Evidence of oxidative damage in paraquat toxicity. *Zahedan Journal of Research in Medical Sciences* 2014.

6. Rappold, P. M., Cui, M., Chesser, A. S., Tibbett, J., Grima, J. C., Duan, L., et al. Paraquat neurotoxicity is mediated by the dopamine transporter and organic cation transporter-3. *Proc Natl Acad Sci U S A*. 2011;**108(51)**:20766-71.

7. Watts, M. Paraquat: Pesticide action network Asia & the Pacific. 2011.

8. Soontornniyomkij, V., Bunyaratvej, S. Fatal paraquat poisoning: a light microscopic study in eight autopsy cases. *J Med Assoc Thai*. 1992;**75 Suppl 1**:98-105.

9. Wilks, M. F., Fernando, R., Ariyananda, P. L., Eddleston, M., Berry, D. J., Tomenson, J. A., et al. Improvement in survival after paraquat ingestion following introduction of a new formulation in *Sri Lanka. PLoS Med*. 2008;**5(2)**:e49.

10. Mettananda, K. C., de Silva, A. P., de Silva, H. J. Acute systemic paraquat intoxication: survival without long-term complications. *Ceylon Med J*. 2008;**53(4)**:136-7.

11. Lheureux, P., Leduc, D., Vanbinst, R., Askenasi, R. Survival in a case of massive paraquat ingestio,n.*Chest*. 1995;**107(1)**:285-9.

12. Hichor, M., Sampathkumar, N. K., Montanaro, J., Borderie, D., Petit, P. X., Gorgievski, V., et al. Paraquat Induces Peripheral Myelin Disruption and Locomotor Defects: Crosstalk with LXR and Wnt Pathways. *Antioxid Redox Signal.* 2017;**27(3)**:168-83.

13. Gawarammana, I. B., Dawson, A. H. Peripheral burning sensation: a novel clinical marker of poor prognosis and higher plasma-paraquat concentrations in paraquat poisoning. *Clin Toxicol (Phila)*. 2010;**48(4)**:347-9.

14. Wu, B., Song, B., Tian, S., Huo, S., Cui, C., Guo, Y., et al. Central nervous system damage due to acute paraquat poisoning: a neuroimaging study with 3.0 T MRI. *Neurotoxicology*. 2012;**33(5)**:1330-7.

15. Prasad, K., Tarasewicz, E., Mathew, J., Strickland, P. A., Buckley, B., Richardson, J. R., et al. Toxicokinetics and toxicodynamics of paraquat accumulation in mouse brain. *Exp Neurol*. 2009;**215(2)**:358-67.

16. Gil, H. W., Hong, J. R., Jang, S. H., Hong, S. Y. Diagnostic and therapeutic approach for acute paraquat intoxication. *J Korean Med Sci*. 2014;**29(11)**:1441-9.

17. Yamashita, J. Clinical studies on paraquat poisoning; prognosis and severity index of paraquat poisoning using the urine levels. *Nippon Hinyokika Gakkai Zasshi*. 1989;**80(6)**:875-83.

18. Scherrmann, J. M., Houze, P., Bismuth, C., Bourdon, R. Prognostic value of plasma and urine paraquat concentration. *Hum Toxicol*. 1987;**6(1)**:91-3.

19. Ali, R., Ali, R., Jaimini, A., Nishad, D.K., Mittal, G., Chaurasia, O. P., et al. Acute and sub acute toxicity and efficacy studies of Hippophae rhamnoides based herbal antioxidant supplement. *Indian journal of pharmacology*. 2012;**44(4)**:504-8.

20. Guidance for industry: Single dose acute toxicity testing for pharmaceuticals. Center for Drug Evaluation and Research (CDER) 1996. Aug, Available from: http://www.fda.gov/downloads/Drugs/…/Guidances/ucm079270.pdf

21. Muralidhara, S., Ramanathan, R., Mehta, S. M., Lash, L. H., Acosta, D., Bruckner, J.V. Acute, subacute, and subchronic oral toxicity studies of 1,1-dichloroethane in rats: application to risk evaluation. *Toxicological sciences : an official journal of the Society of Toxicology*. 2001;**64(1)**:135-45.

22. Aminoff, M. J. Electrodiagnosis in clinical neurology. 5 ed. B SD, editor. USA: Elsevier; 2005. 335-55 p.

23. Thompson, L. L. The F- wave. Lowery lee Thompson MD, editor. Boston: Little Brown and company; 1981. 87-9 p.

24. Tsai, Y. A., Chuang, T. Y., Yen, Y. S., Huang, M. C., Lin, P. H., Cheng, H. Electrophysiologic findings and muscle strength grading in brachioplexopathies.*Microsurgery*. 2002;**22(1)**:11-5.

25. Ewing, D. J., Clarke, B. F. Diagnosis and management of diabetic autonomic neuropathy. *Br Med J (Clin Res Ed)*. 1982;**285(6346)**:916-8.

26. Hilz, M. J., Dutsch, M. Quantitative studies of autonomic function. *Muscle & nerve*. 2006;**3**3(1):6-20.

27. Andrew, A. L., Myers, S. Serial grip strengh testing- its role in assesment of wrist and hand disability.*internet journal of surgery.* 2004.

28. Engel, L. S., Keifer, M. C., Checkoway, H., Robinson, L. R., Vaughan, T. L. Neurophysiological function in farm workers exposed to organophosphate pesticides.*Arch Environ Health*. 1998;**53(1)**:7-14.

29. Misra, U. K, Nag, D., Khan, W. A., Ray, P. K. A study of nerve conduction velocity, late responses and neuromuscular synapse functions in organophosphate workers in India. *Arch Toxicol.* 1988;**61(6)**:496-500.

30. Misra, U. K., Nag, D., Khan, W. A., Ray, P. K. A study of nerve conduction velocity, late responses and neuromuscular synapse functions in organophosphate workers in India. *Arch Toxicol*. 1988;**61(6)**:496-500.

31. Wunnapuk, K., Medley, G. A., Liu, X., Grice, J. E., Jayasinghe, S., Gawarammana, I., et al. Simple and sensitive liquid chromatography-tandem mass spectrometry methods for quantification of paraquat in plasma and urine: application to experimental and clinical toxicological studies. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2011;**879(28)**:3047-52.

32. Min, Y. G., Ahn, J. H., Chan, Y. C., Ng, S. H., Tse, M. L., Lau, F. L., et al. Prediction of prognosis in acute paraquat poisoning using severity scoring system in emergency department. *Clin Toxicol (Phila)*. 2011;**49(9)**:840-5.

33. Singer, R., Moses, M., Valciukas, J., Lilis, R., Selikoff, I. J. Nerve conduction velocity studies of workers employed in the manufacture of phenoxy herbicides.*Environ Res.* 1982;**29(2)**:297-311.

34. Brill, N. A., Tyler, D. J. Quantification of human upper extremity nerves and fascicular anatomy. *Muscle & nerve*. 2017;**56(3)**:463-71.

35. Chung, T., Prasad, K., Lloyd, T. E. Peripheral neuropathy: clinical and electrophysiological considerations. *Neuroimaging Clin N Am*. 2014;**24(1)**:49-65.

36. Fisher, M. A. F-waves–physiology and clinical uses.*ScientificWorldJournal*. 2007;**7**:144-60.

37. Kimura, J. Electrodiagnosis in deseases of nerve and muscle. USA: F A Davis company; 1983. 353-77 p.

38. A practical-guide. A practical guide to diagnosis,first aid and hospital treatment, paraquat poisoning. London, Uk: Health assessment and envionmental safety department of Syngenta and the Medical Toxicology Unit, Guy's & St Thomas'Hospital NHS Trust, London, UK; 2003.

39. Uludag, B. K. A., Atac, C., Karatepe, A., Turman, B. F wave parameters and F -jitter. *Journal of neurological sciences (Turkish)* 2006. 2006;**23(1)**:8-13.

40. Houze, P., Baud, F. J., Mouy, R., Bismuth, C., Bourdon, R., Scherrmann, J. M. Toxicokinetics of paraquat in humans. *Hum Exp Toxicol.* 1990;**9(1)**:5-12.

41. Pesah, Y., Pham, T., Burgess, H., Middlebrooks, B., Verstreken, P., Zhou, Y., et al. Drosophila parkin mutants have decreased mass and cell size and increased sensitivity to oxygen radical stress. *Development*. 2004;**131(9)**:2183-94.

42. Stalberg, E. Neuromuscular transmission studied with single fibre electromyography. *Acta anaesthesiologica Scandinavica Supplementum*. 1978;**70**:112-7.

43. Dongren, Y., Tao, L., Fengsheng, H. Electroneurophysiological studies in rats of acute dimethoate poisoning.*Toxicology letters.*1999;**107(1-3)**:249-54.

44. Li, S., Crooks, P. A., Wei, X., de Leon, J. Toxicity of dipyridyl compounds and related compounds. *Crit Rev Toxicol*. 2004;**34(5)**:447-60.

45. Zaidi, A., Fernandes, D., Bean, J. L., Michaelis, M. L. Effects of paraquat-induced oxidative stress on the neuronal plasma membrane Ca(2+)-ATPase. *Free Radic Biol Med.* 2009;**47(10)**:1507-14.

46. Litteljohn, D., Mangano, E., Shukla, N., Hayley, S. Interferon-gamma deficiency modifies the motor and co-morbid behavioral pathology and neurochemical changes provoked by the pesticide paraquat. *Neuroscience*. 2009;**164(4)**:1894-906.

47. Fahim, M. A., Shehab, S., Nemmar, A., Adem, A., Dhanasekaran, S., Hasan, M. Y. Daily subacute paraquat exposure decreases muscle function and substantia nigra dopamine level.*Physiol Res*. 2013;**62(3)**:313-21.

48. Tabata, N., Morita, M., Mimasaka, S., Funayama, M., Hagiwara, T., Abe, M. Paraquat myopathy: report on two suicide cases. *Forensic Sci Int.* 1999;**100(1-2)**:117-26.

Tables

Table1. Somatic nerve conduction studies of the patients and the controls

Somatic nerve conduction study	Controls n=56	Patients		1st assessment of the patients vs controls		2nd assessment of the patients vs controls	
		1st assessment n=28	2nd assessment n=18	Mean difference (patients - controls)	95% CI/ P value	Mean difference (patients-controls)	95% CI/ P value
Sensory nerve conduction velocity (SNCV) (m/s)							
Median	56·8 (5·9)	55·8 (10·6)	57·0 (6·6)	-0·9	-4·7 to 2·8	0·2	-3·2 to 3·6
Ulnar	60·1 (5·4)	46.8 (23.4)	58·5 (6·7)	**-13.3**	**-19·8 to -6.8**	-1·6	-4·7 to 1·5
Motor nerve conduction velocity (MNCV) (m/s)							
Median	56·3 (4·5)	57·2 (5·5)	56·2 (4·9)	0·9	-1·3 to 3·2	-0·1	-2·6 to 2·4
Ulnar	56·9 (4·2)	56·3 (5·8)	55.8 (3·9)	-0·6	-2·8 to 1·6	-1·1	-3·3 to 1·2
Common peroneal	48·1 (5·4)	48·6 (4·5)	48·6 (5·4)	0·4	-4·7 to 5·7	0·5	-3·7 to 4·7
Amplitude of CMAP on distal stimulation (mV)							
Median	14·3 (4·3)	12·6 (3·5)	13·6 (3·7)	-1·7	-3·6 to 0·1	-0·8	-3 to 1·5
Ulnar	10·6 (2·6)	9·5 (2·6)	11·1 (1·9)	**-1·1**	**-0·06 to -2·2**	0·5	-0·7 to 1·6
Common peroneal	8·8 (3·7)	8·3 (3·9)	8·2 (4·1)	-0·5	-2·2 to 1·2	-0·6	-2·7 to 1·4
F-wave latency (ms)							
Median	26·7 (2·1)	26·8 (2·3)	26·5 (2·9)	0·1	-0·9 to 1·1	-0·2	-1·4 to 1·1
Ulnar	25·9 (4·0)	26·9 (2·3)	26·9 (2·3)	1·0	-0·6 to 2·4	1·0	-0·9 to 3
Tibial	49·5 (5·1)	50·8 (4·9)	49·9 (3·7)	1·3	-1·1 to 3·8	0·01	-2·6 to

							2·6
F-wave occurrence (%)							
Median	90 (11)	77 (20)	93 (10)	**-13**	**0·002†**	3	0·5†
Ulnar	91(11)	79 (23)	88(10)	**-12**	**0·004†**	-3	0·8†
Tibial	93 (13)	76 (26)	95 (8)	**-17**	**0·002†**	2	0·8†

Values are mean (SD), † analyzed with Mann-Whitney U test, $ analyzed with Wilcoxon Signed Ranks test, bold values are significant at p<0.05

Table2. Autonomic nerve function of the patients and the controls

Autonomic nerve function tests	Controls (n=56)	Patients		1st assessment of the patients vs controls		2nd assessment of the patients vs controls	
		1st assessment (n = 28)	2nd assessment (n=18)	Mean difference (patients - controls)	95% CI/ P value	Mean difference (patients - controls)	95% CI/ P value
The tests of cardiac parasympathetic integrity							
Heart rate response to Valsalva manoeuvre	1·7 (0·3)	1·7 (0·6)	1·7 (0·3)	0·04	-0·2 to 0·3	-0·04	-0·2 to 0·1
Heart rate response to deep breathing	28 (10)	25 (12)	26 (11)	-2·9	-8 to 2	-2·0	-7 to 4
Heart rate response to standing	1·4 (0·3)	1·2 (0·3)	1·4 (0·3)	-0·2	-0·3 to 0·02	0·01	-0·2 to 0·2
The tests of sympathetic integrity							
SBP change 3min after standing (mmHg)	1·5 (9·3)	8·7 (11·2)	1·6 (7·3)	**7·2**	**0·006**†	-0·1	0·6†
Sympathetic skin response							
SSR Amplitude (mV)	1·3 (1·0)	0·58 (0·4)	1·3 (1·0)	**-0·7**	**<0·001**†	-0·03	0·9†
SSR average latency (ms)	1542 (159)	1550 (187)	1543 (176)	8	-73 to 90	1	-89 to 92

Values are mean (SD), † Analyzed with Mann-Whitney U test, $ Analyzed with Wilcoxon Signed Ranks test, Bold values are significant at p<0.05

Table3. Comparison of NMJ function with supramaximal slow repetitive stimulation of median nerve of the patients and the controls

Parameters	Controls (n = 56)	Patients		P value	
		1st Assessment (n = 28)	2nd Assessment (n = 18)	1st assessment vs controls †	2nd assessment vs control †
Slow repetitive stimulation at rest - the first train of stimuli (A)					
Decrement at the 2nd stimulation (%)	0·5 (-0·5 to 1·2)	-0·08 (-1·1 to 0·5)	0·07 (-0·6 to 0·8)	0·09	0·4
Decrement at the 4th stimulation (%)	0·1 (-1·2 to 1·0)	0·3 (-1·7 to 2·1)	0 (-1·2 to 0·8)	0·6	0·8
Decrement at the 5th stimulation (%)	0·4 (-1·1 to 1·6)	0 (-2·1 to 2·6)	0 (-0·9 to 1·4)	0·8	0·8
Slow repetitive stimulation just after an isometric exercise - the second train of stimuli (B)					
Decrement at the 2nd stimulation (%)	0·3 (-0·7 to 1·4)	1·7 (0·2 to 2·8)	0·3 (-0·8 to 1·0)	**0·02**	0·8
Decrement at the 4th stimulation (%)	0·7 (-2·7 to 1·5)	2·1 (1·2 to 4·7)	1·1 (-0·9 to 2·0)	**0·002**	0·2
Decrement at the 5th stimulation (%)	0·7 (-1·5 to 1·2)	1·7 (0·6 to 4·6)	0·94(-0·2 to 2·0)	**0·008**	0·5
Slow repetitive stimulation two minutes after an isometric exercise - the third train of stimuli (C)					
Decrement at the 2nd stimulation (%)	0·8 (0 to 2·9)	0·4 (-0·3 to 1·4)	0·3 (-0·8 to 1·0)	0·2	0·2
Decrement at the 4th stimulation (%)	0·8 (-0·8 to 2·4)	1·2 (-0·2 to 3·4)	1·1 (-0·9 to 2·0)	0·5	0·9
Decrement at the 5th stimulation (%)	0·7 (-1·0 to 2·3)	1·2 (-0·5 to 3·8)	0·9 (-0·2 to 2·0)	0·5	0·9
Post exercise facilitation	1·0 (0·8 to 1·2)	0·9 (0·9 to 1·1)	1·0 (0·9 to 1·1)	0·7	0·8
Post exercise exhaustion	0·9 (0·8 to 1·2)	0·9 (0·7 to 1·1)	0·9 (0·8 to 1·1)	0·3	0·8

Values are median (inter quartile range), † Analyzed with Mann-Whitney U test and $ Wilcoxon Signed Ranks test, Bold values are significant at p<0.05

Table4. Correlation of neurological outcomes with severity of poisoning

Neurological outcomes	Spearman r	P value
Somatic nerve function		
Sensory nerve conduction velocity		
Median	-0.09	0.7
Ulnar	0.2	0.5
Motor nerve conduction velocity		
Median	0.2	0.4
Ulnar	0.01	0.9
Common peroneal	-0.1	0.6
Amplitude of CMAP on distal stimulation		
Median	-0.2	0.4
Ulnar	-0.2	0.3
Common peroneal	-0.3	0.3
F-wave latency		
Median	-0.1	0.7
Ulnar	0.09	0.7
Tibial	-0.3	0.3
F-wave occurrence		
Median	0.02	0.9
Ulnar	-0.2	0.5
Tibial	**-0.5**	**0.03**
Autonomic nerve function		
Heart rate response to Valsalva manoeuvre	**0.9**	**0.04**
Heart rate response to deep breathing	0.3	0.2
Heart rate response to standing	**-0.7**	**0.03**
SBP change 3min after standing	0.4	0.1
SSR Amplitude	0.2	0.4
SSR average latency	0.05	0.9
NMJ function		

Slow repetitive stimulation at rest		
Decrement at the 2nd stimulation	0.2	0.5
Decrement at the 4th stimulation	-0.2	0.4
Decrement at the 5th stimulation	-0.1	0.6
Slow repetitive stimulation just after an isometric exercise		
Decrement at the 2nd stimulation	-0.2	0.4
Decrement at the 4th stimulation	-0.04	0.9
Decrement at the 5th stimulation	-0.04	0.8
Slow repetitive stimulation two minutes after an isometric exercise		
Decrement at the 2nd stimulation	-0.07	0.7
Decrement at the 4th stimulation	-0.08	0.8
Decrement at the 5th stimulation	0.02	0.9

Bold values are significant at $p<0.05$