Salbutamol in acute organophosphorus insecticide poisoning - A pilot dose-response phase II study

Fazle Rabbi Chowdhury, [1*, 2, 3] Md, Mustafezur Rahman, [1, 4] Parash Ullah, [1, 5]
Abdul Mumith Ruhan, [1] Md. Shafiqul Bari, [1] M.M.Jahangir Alam, [1] Md. Moyeen Uddin, [1]
Shomik Maruf, [6] Md. Ismail Patwary, [1] Michael Eddleston [7, 8]

[1] Department of Medicine, Sylhet MAG Osmani Medical College,
Sylhet, Bangladesh

[2] Centre for Tropical Medicine and Global Health
Nuffield Department of Medicine, University of Oxford, Oxford, UK

[3] Mahidol Oxford Tropical Medicine Research Unit, Mahidol University, Bangkok, Thailand

[4] Department of Gastroenterology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh

[5] Department of Medicine, Shaheed Suhrawardy Medical College Hospital, Dhaka, Bangladesh

[6] International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh

[7] Pharmacology, Toxicology, & Therapeutics, University/BHF Centre for Cardiovascular Science,
University of Edinburgh, UK,

[8] South Asian Clinical Toxicology Research Collaboration,
University of Peradeniya, Sri Lanka

Running Title: Salbutamol for acute organophosphorus insecticide poisoning
Word Count: Main text: 2628, Abstract: 292, Table: 02, Figure: 04

*** Correspondence:** Fazle Rabbi Chowdhury**,** Peter Medawar Building for Pathogen Research, South Parks Road, University of Oxford, OX1 3SY. Tel: +44 07392757139, Email: fazle.chowdhury@ndm.ox.ac.uk

Abstract

Background: Treatment of acute organophosphorus (OP) insecticide poisoning is difficult, with many patients dying despite best care. Pre-clinical studies have shown benefit from salbutamol, possibly due speeding alveolar fluid clearance or reducing bronchoconstriction. In this small pilot dose-response study, we aimed to explore whether addition of nebulised salbutamol to standard care might improve resuscitation.

Methods: We performed a single-blind phase II study comparing the effect of two different doses of nebulized salbutamol versus saline placebo, in addition to standard treatment. Primary outcome was oxygen saturations over the first 60 min of resuscitation; secondary outcomes included heart rate, incidence of dysrhythmias, time to 'atropinisation', atropine dose required, and mortality.

Result: Seventy-five patients were randomised to receive 5 mg (Salb5, n=25) or 2.5mg (Salb2.5, n=25) of salbutamol, or saline placebo (NoSalb, n=25), by nebuliser. Oxygen saturations did not differ between groups over the first 60 min of resuscitation (median AUC NoSalb: 1376 [95% CI 1282 to 1470], Salb2.5: 1395 [1305 to 1486], Salb5: 1233 [1100 to 1367]; p=0.9898). Heart rate was also similar across the three arms. Median time to full atropinisation, and atropine dose required, were the same for all three arms (NoSalb 15.0 [10-16] min & 12.6 [8.0-13.4] mg, Salb2.5 15.0 [10-16] min & 12.6 [9.3-16.8] mg, and Salb5 15.0 [10-20] min & 12.6 [10.7-20.6] mg; p=0.4805 and p=0.1871, respectively). Three (12%) patients died in the Salb2.5 and Salb5 groups and two (8%) in the NoSalb group.

Conclusion: This pilot study, within the limitations of its small size and variation between patients, found no apparent evidence that administration of nebulised salbutamol improved resuscitation of patients with acute OP insecticide self-poisoning. The data obtained provides a basis to design further studies to ultimately test the role of salbutamol in OP insecticide poisoning.

Key words: Salbutamol, Nebulization, Organophosphorus, Insecticide, Poisoning

INTRODUCTION

Self-poisoning with pesticides is one of the three most important global means of suicide,[1] being particularly important in lower and middle-income countries (LMIC) where highly hazardous pesticides (HHPs) cause over 10,000 deaths each year.[2, 3] Organophosphorus (OP) insecticides are responsible for many of these deaths. They inhibit acetylcholinesterase (AChE), causing cholinergic overstimulation in the autonomic nervous system, central nervous system and neuromuscular junction (NMJ).[4] Patients die early from acute respiratory failure, especially if it occurs before hospital presentation, or later from cardiovascular collapse, delayed NMJ dysfunction, or complications of pre-hospital toxicity (eg. hypoxic brain injury or aspiration pneumonia) and mechanical ventilation.[5-7] Acute respiratory failure results from failure of central respiratory drive and NMJ dysfunction, as well as bronchorrhoea and bronchoconstriction that cause severe hypoxia.[6, 8]

Medical management is difficult.[9] Mainstays of treatment include immediate resuscitation and oxygen, administration of the muscarinic antagonist atropine, and assisted ventilation.[9, 10] Incremental (or double) bolus dosing of atropine is highly effective;[11] the role of oxime AChE reactivators, such as pralidoxime, is unclear.[12, 13] Case fatality for self-poisoning remains high, especially in the under-resourced district hospitals that admit the majority of patients worldwide. While regulatory action to remove HHPs from agricultural practice is likely to be the most effective public health response [14, 15], OP insecticides will continue to be used for the foreseeable future and better treatment options are urgently required.

By antagonising muscarinic receptors, atropine turns off the production of the bronchial fluid that fills the lungs. However, it does not speed fluid removal from the lung. A treatment that increases fluid removal from alveoli, to complement atropine-induced cessation of production, could speed up the return of effective oxygen exchange and improve resuscitation. Salbutamol can accelerate alveolar fluid

clearance by enhancing salt and water transfer across alveolar and distal airways.[16] It may reverse OP insecticide induced bronchoconstriction, improving respiratory mechanics by decreasing airflow resistance and peak airway pressures and increasing dynamic compliance.[17] Animal studies have shown that salbutamol reverses OP insecticide-induced bronchoconstriction.[18, 19]

A nebulised dose of salbutamol – available widely in LMIC rural hospitals - might be able to access the alveolar epithelium and increase the rate of fluid removal after OP poisoning. Therefore, a transient effect of nebulised salbutamol should not be a limitation since co-administered atropine will stop fluid production before salbutamol's effect wears off. Although intravenous (IV) salbutamol might be better able to reach the alveoli in patients with bronchorrhoea, we selected the nebulised route for this first study due to safety considerations and wide availability of nebulisers.

We therefore performed a pilot proof of concept dose response randomised controlled trial (RCT) using two doses of nebulised salbutamol to test its ability to speed the resuscitation of OP insecticide poisoned patients. Subsequent studies will be required to assess different dosing regimens before moving, if the data support it, into a large phase III RCT.

METHODS

This dose-response phase II RCT was a feasibility and proof of concept pilot study conducted in the Department of Medicine at the Sylhet M. A. G. Osmani Medical College Hospital (SOMCH), Sylhet, Bangladesh. Ethics approval was received from the SOMC research ethics review committee. Written informed consent was taken in their own language from each patient or their relative (for unconscious patients and those under 16 years). The trial was registered (NCT02160548) at clinicaltrials.gov.

Participants

We approached all patients with OP insecticide self-poisoning admitted to the adult wards that required standard resuscitation with atropine, oxygen and fluids. Exclusion criteria were age <12 years, patient not requiring atropine or oxygen, prior receipt of atropine and lack of informed consent. The OP insecticide was identified from the history, the empty bottle brought in by the patient or relative, and/or from the patient/relative identifying the pesticide on a chart showing all locally available OP insecticides.

Outcome, objectives and hypotheses

The primary aim was to test the efficacy of salbutamol at improving oxygenation over the first 60 min of resuscitation. Our hypothesis was that addition of the beta-adrenergic agonist salbutamol to standard care and atropine would improve oxygenation and speed resuscitation. Secondary outcomes included heart rate, incidence of dysrhythmias, time to 'atropinisation', atropine dose required for resuscitation, incidence of aspiration or pneumonia, and mortality.

Randomisation and allocation

Patients were randomised into three study arms to receive nebulised salbutamol 5 mg (Salb5) or 2.5 mg (Salb2.5), or saline 5 mL as placebo (NoSalb). Randomisation and allocation sequence generation were done independently by a clinician who was not associated with the care or assessment of the patients by means of a random number table. Allocations were concealed in sequentially numbered, opaque, sealed envelopes.

In this RCT, the nebulized salbutamol or saline was not masked to the research clinician. However, the primary outcome (oxygen saturations) was recorded using an objective automated method; the study staff was not able to influence the recording of these data. Data analysis was done masked to allocation. Randomisation occurred after baseline data had been entered, and could not be altered by study doctors. The recruiting doctor could not predict allocation before randomisation.

Intervention and study drugs

Patients were seen by study doctors within 30 min of admission and treated as per standard management guidelines with doubling doses of atropine until a clinical response.[20] All patients received standard oxygen supplement, 4 litre/min via nasal mask until full atropinisation and vital stability. The aims for full atropinisation were to produce a clear chest on auscultation with no wheeze, heart rate >80 beats/min, pupils no longer pin point, dry axillae and systolic blood pressure >80 mm of Hg. Patients did not receive any atropine or pralidoxime before enrolled to this study. The initial dose of atropine was usually three ampules (1.8 mg), which was then doubled to 6, 12, and 24 ampule doses in every five minutes interval if there was no response. A partial response would result in a similar dose, rather than a doubling dose, being administered. All patients received pralidoxime iodide 1 g IV as a single dose. Due to the dearth of intensive care beds in the hospital, intubation and ventilation were rarely available and patients were managed on open adult medical wards with a dedicated cardiac monitor (Infunix; IP-4050). Readings for peripheral oxygen saturations and heart rate were recorded automatically every minute; screen shots were taken every ten minutes to provide hard records of data for each ten min period.

Patients received a single nebulised dose of salbutamol or saline immediately after the first dose of IV atropine. Salbutamol sulphate (5 mg/mL vials, Ventolin respirator solution, GlaxoSmithkline, UK), 0.9%

sodium chloride for infusion (saline), atropine sulphate (0.6 mg/mL ampules, Chemist laboratories, BD) and pralidoxime iodide (500 mg/20 mL ampules, Hindustan medicines, India) were purchased locally.

Sample size

No similar study has been previously performed; therefore, no data were available that could be used to generate a sample size calculation. This study was designed to provide initial data on efficacy and safety that would allow the design of a larger phase III study of nebulised salbutamol or a similar scale study assessing IV salbutamol. We aimed to enrol 25 patients into each study arm (total 75 patients). We are aware that the marked variability in the clinical syndrome and severity produced by different OP insecticides would confound the results.[21] However, we believed that this size trial was both practical and useful for initiating a set of studies on salbutamol in OP insecticide poisoning.

Statistical analysis

Graphpad Prism v 7.0 was used for analysis. Demographic factors and clinical characteristics were summarised with counts (%) for categorical variables and median (interquartile range [IQR]) for continuous variables, as none was expected to be normally distributed. The main analysis was carried out on an intention-to-treat basis. For the primary outcome, we compared the mean area-under-the-curve (AUC, calculated by Prism using the trapezoid method) value of oxygen saturations between three arms using a one-way ANOVA test. The $AUC_{0\text{-}60min}$ was based on a saturation of 70%, the lowest admission saturation in a patient who survived (with sensitivity analyses revising the baseline to 60% and 80%). We also did post-hoc analysis in Prism assessing (log-rank Mantel-Cox test) time to sustained saturations > 95 %, censored at 60 minutes or death. For non-parametric secondary outcomes, all groups were compared using a Kruskal–Wallis test; if significant, we then planned to perform pairwise comparisons with a non-parametric Mann–Whitney test. For parametric outcomes, we compared the odds ratios across the pairs.

Role of the funding source:

The funding source had no role in study design, data collection, data analysis, and data interpretation; or writing of the report; or in the decision to submit for publication. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

RESULTS

From 1st Oct 2013 to 1st Dec 2015, 158 patients with acute symptomatic OP insecticide self-poisoning were assessed on admission to SOMCH. Eighty-three were excluded from the study (figure 1) while seventy-five were consented and randomised into the trial: 25 to saline placebo (NoSalb), 25 to salbutamol 2.5 mg (Salb2.5), and 25 to salbutamol 5 mg (Salb5), in addition to usual care.

Baseline characteristics

Patient groups were broadly similar at baseline in this pilot study (table 1), although a greater number of Salb5 patients had consumed the more toxic WHO Class Ib insecticides (Salb5 52%, Salb2.5 32%, NoSalb 24%). Clinically, more Salb5 patients had GCS <7/15 (figure 2) although NoSalb patients had lower mean oxygen saturations at baseline (table 1). According to the Peradeniya Organophosphorus Poisoning (POP) severity scale [22], a higher number ($p<0.001$) of moderately poisoned patients were recruited to the Salb5 group compared to NoSalb (64% vs 28%); although there was a similar number of severely poisoned patients across groups (figure 2). The mean heart rate in the Salb5 group was modestly higher than the other two groups (table 1). Time to presentation was similar across the three groups (figure 3) with four outliers in the Salb2.5 (one) and Salb5 (three) groups.

Primary outcome – oxygen saturations

Mean peripheral blood oxygen saturations improved more rapidly in NoSalb and Salb2.5 groups, reaching 95.0% by 16 and 18 min respectively, than in the Salb5 group, which transiently reached 95.0% at 30 min before dipping down to the low 90s for the remaining 30 min (figure 4A). The median (IQR) area-under-the-curve (AUC) for peripheral oxygen saturations over the first 60 minutes, using 70% as the baseline, was similar for all three groups: 1376 (95% CI 1282 to 1470), 1395 (1305 to 1486), and 1233 (1100 to 1367) for NoSalb, Salb2.5 and Salb5 groups, respectively (ANOVA across all three groups, p=0.9898; figure 4B). Revising the baseline to 60% or 80% in a sensitivity analysis had no effect on the results. Again, on post-hoc analysis assessing time to saturation >95%, no difference (p=0.2963) was seen between study groups (figure 5).

Secondary outcomes

The median time for atropinisation and the median dose of atropine required for full atropinisation in each arm was the same, at 15 minutes and 12.6 mg (or 21 ampules), respectively (table 2, p=0.4805 and p=0.1871 by Kruskal-Wallis, table 2). The mean heart rate increased across all three arms initially, before falling back towards 100 bpm at 10 min (figure 4C). The heart rate was consistently higher in the Salb5 group throughout resuscitation until the last 15 min when the mean Salb2.5 heart rate increased towards 115 bpm (figure 4C). The heart rates and rhythms were never hazardous.

There were no differences in the incidence of aspiration pneumonia or NMJ dysfunction (intermediate syndrome) between the groups (table 2). Five (20%) NoSalb patients developed paralytic ileus compared to one (4%) in both Salb2.5 and Salb5 groups. The number of patients discharged without complications was similar between groups: 22 (88%) Salb5 vs 21 (84%) Salb2.5 vs 20 (80%) NoSalb

patients (table 2). Three (12%) patients died in Salb2.5 and Salb5 groups while two (8%) patients died in the NoSalb group. All but one death occurred during resuscitation (figure 4D).

DISCUSSION

This RCT showed no apparent benefit from using a single 2.5 mg or 5 mg dose of nebulised salbutamol in addition to standard care in a tertiary level Bangladeshi hospital. Oxygenation over the first 60 min was non-significantly worse in the patients receiving nebulised salbutamol 5 mg compared to no salbutamol or salbutamol 2.5 mg despite similar doses of atropine and a similar time to atropinisation. As expected [23, 24], there was no indication of adverse cardiac effects of the nebulised salbutamol, although the heart rate of patients receiving salbutamol 5 mg was higher than the other groups for the first 45 min (suggesting possible systemic exposure). There was no trend for benefit for other secondary outcomes such as mortality, intermediate syndrome, or aspiration pneumonia, although the study was not powered to detect such differences.

However, as expected due to the small size of the study, there was variation in severity between the three arms of the study with more sick patients in the higher dose of salbutamol arm. Much bigger studies designed using the data collected in this study and further phase II studies, and accounting for variation in OP compound and poisoning severity, will be required to answer the question of effectiveness.

In a guinea pig study, Chavez et al showed immediate (5 minutes post intervention) benefit of using salbutamol 10 μg/kg by the intra-peritoneal (IP) route 90 minutes after parathion poisoning.[18] Administration of 2 mg/ml aerosolized salbutamol over 2 min also produced significant improvement (more than 50% reversion) of respiratory function lasting 5-10 min [19]. However, in both studies, animals did not receive atropine or any other treatment for parathion intoxication. The study was

therefore quite different to the situation with poisoned humans and therefore of questionable relevance. We hypothesised that salbutamol might offer benefit over and above atropine by increasing the rate of fluid extraction from the alveoli through activation of epithelial sodium channel (ENaC) pumps and by treating bronchoconstriction, both of which should result in a lower requirement for atropine and a faster time to atropinisation and resuscitation. That neither occurred might suggest that sufficient salbutamol did not get to the alveoli, dissolving instead in the fluid filling the lungs. We did not differentiate our recording of bronchospasm and bronchorrhoea, so it is possible that the salbutamol effectively treated the bronchospasm while atropine dosing was continued for the alveoli bronchorrhoea. Oxygen saturations did not rise because the alveoli were filled with fluid.

Intravenous salbutamol either as a bolus 250 mcg, or an infusion starting at 5 mcg/min, might be a more effective way of stimulating the alveoli channels. However, this also offers a greater likelihood of adverse effects than nebulised salbutamol. The BALTI trial used intravenous infusions of salbutamol for 7 days (15mcg/kg/hr for up to seven days) but found it to be poorly tolerated, and possibly hazardous, in patients with acute respiratory distress syndrome (ARDS). The difference between ARDS and OP insecticide poisoning is that salbutamol treatment would only be required during acute resuscitation in the latter, reducing the risk of adverse effects.

In this trial, the overall mortality was 10.6%, with no significant difference between the groups. A survey done in 2007 across Bangladesh reported a mortality of about 15% for OP poisoning [25]. The mortality in this study may have been lower due to chance or because of improved management, administration of atropine, and active monitoring of patients. Clinical trials can be useful to optimize and improve the management of OP poisoning. Unfortunately, ICU beds and mechanical ventilation were not available for patients in the study hospital - a problem widespread in Bangladesh and in other Asian district

hospitals that see the majority of cases. Deaths occurred from acute respiratory failure due to a lack of resources.

Limitations

This study was a dose-response RCT, aiming to collect data that might allow further studies to be designed. Due to its size, it was not powered to detect modest differences in the incidence of death or respiratory complications. Since we found no evidence of improved oxygenation with high dose, there is no mechanistic indication that a small benefit may occur with this treatment. However, there is marked variability between OP insecticides in the clinical syndromes produced and severity of poisoning [21]; again, due to the small size of this pilot study, there were differences within the three groups in terms of the OPs involved and poisoning severity. This will have complicated the analysis of the oxygenation data and may have hidden a benefit for some patients. Due to the size of the study and therefore limited data, we also did not perform more refined time-dependent analysis and modelling. Much bigger, phase III RCTs will be required to control for the variation and to look for clinical benefit from salbutamol. Patients will need to be stratified on the basis of the OP insecticide ingested and severity of clinical features and cholinesterase inhibition, as done previously [26].

During resuscitation, several patients were restless, vomiting or defecating, and it was therefore difficult to maintain patient monitor probes attached continuously. This resulted in some missing data; however, since we took readings at one min intervals and used the AUC for the whole 60 min, we could still analyse the primary and key secondary outcomes. We could not intubate our patients whose GCS was less than seven due to lack of ICU facility, increasing mortality in the study.

Conclusion:

We found no evidence that nebulised salbutamol is likely to be beneficial at speeding atropinisation and resuscitation of poisoned patients, although the small size of the study and variation in poisoning severity due to variation in OP ingested, dose, and time to admission prevented small effects being noted. Management of such patients remains a great challenge [9, 10], especially in the resource-poor

district hospitals in rural Asia that admit most patients. More evidence is required with which to guide therapy. Further studies of nebulised salbutamol or a pilot study of intravenous salbutamol until atropinisation is obtained may determine whether attempting to increase fluid removal from lungs is a viable therapeutic approach. Larger studies will then be required to determine whether salbutamol is beneficial to patients.

Contributors:

ME and FRC designed and set up the RCT, and wrote the first draft of the paper. MMR, PU and AMR enrolled the patients and collected all the data. FRC, MSB, MMJA, MMU and MIP ran the trial centre and monitored the management of the patients. FRC, ME and SM did the statistical analysis. All authors had a role in improving the study design and in reviewing and editing the final version of the report.

Funding Information:

Funding came from the award of a Lister Research Prize Fellowship awarded to ME

Disclosure Statement:

We declare that we have no conflict of interest.

Acknowledgements:

We especially thank Dr. Shamim Ara Keya and Dr. Nazia Hassan for database management. We also thank the doctors and staff of the Medicine Department, Sylhet M.A.G. Osmani Medical College Hospital, especially Dr. Ratan Bhomik, Dr. Kaiser Khokon, Dr. Nobendu Chowdhury, and Dr. Mohammed Mirazur Rahman for patient care, and to the hospital administration authority for their full support and cooperation.

REFERENCES

1. World Health Organization. Preventing suicide. A global imperative. ed. Geneva: WHO; 2014.

2. Eddleston M. Patterns and problems of deliberate self-poisoning in the developing world. Q J Med. 2000;93:715-731.

3. Gunnell D, Eddleston M, Phillips MR, et al. The global distribution of fatal pesticide self-poisoning: systematic review. BMC Public Health. 2007;7:357.

4. Lotti M. Clinical toxicology of anticholinesterase agents in humans. In: Krieger RI, Doull J, editors. Handbook of pesticide toxicology Volume 2 Agents. San Diego: Academic Press; 2001. p. 1043-1085.

5. Eddleston M, Eyer P, Worek F, et al. Differences between organophosphorus insecticides in human self-poisoning: a prospective cohort study. Lancet. 2005;366:1452-1459.

6. Eddleston M, Mohamed F, Davies JOJ, et al. Respiratory failure in acute organophosphorus pesticide self-poisoning. Q J Med. 2006;99:513-522.

7. Chowdhury FR, Bari MS, Alam MM, et al. Organophosphate poisoning presenting with muscular weakness and abdominal pain--a case report. BMC Res Notes. 2014;7:140.

8. de Candole CA, Douglas WW, Lovatt Evans C, et al. The failure of respiration in death by anticholinesterase poisoning. Brit J Pharmacol. 1953;8:466-475.

9. Eddleston M, Chowdhury FR. Pharmacological treatment of organophosphorus insecticide poisoning: the old and the (possible) new. Br J Clin Pharmacol. 2016;81:462-470.

10. Eyer P, Eddleston M, Thiermann H, et al. Are we using the right dose? - a tale of mole and gram. Br J Clin Pharmacol. 2008;66:451-452.

11. Abedin MJ, Sayeed AA, Basher A, et al. Open-label randomized clinical trial of atropine bolus injection versus incremental boluses plus infusion for organophosphate poisoning in Bangladesh. J Med Toxicol. 2012;8:108-117.

12. Buckley NA, Eddleston M, Li Y, et al. Oximes for acute organophosphate pesticide poisoning. Cochrane Database Syst Rev. 2011;2:CD005085.

13. Worek F, Thiermann H, Wille T. Oximes in organophosphate poisoning: 60 years of hope and despair. Chem Biol Interact. 2016;259:93-98.

14. Chowdhury FR, Dewan G, Verma VR, et al. Bans of WHO Class I Pesticides in Bangladesh-Suicide Prevention without Hampering Agricultural Output. Int J Epidemiol. 2017;

15. Gunnell D, Fernando R, Hewagama M, et al. The impact of pesticide regulations on suicide in Sri Lanka. Int J Epidemiol. 2007;36:1235-1242.

16. Mutlu GM, Factor P. Alveolar epithelial beta2-adrenergic receptors. Am J Respir Cell Mol Biol. 2008;38:127-134.

17. Morina P, Herrera M, Venegas J, et al. Effects of nebulized salbutamol on respiratory mechanics in adult respiratory distress syndrome. Intensive Care Med. 1997;23:58-64.

18. Segura P, Chavez J, Montano LM, et al. Identification of mechanisms involved in the acute airway toxicity induced by parathion. Naunyn Schmiedebergs Arch Pharmacol. 1999;360:699-710.

19. Chavez J, Segura P, Vargas MH, et al. Paradoxical effect of salbutamol in a model of acute organophosphates intoxication in guinea pigs: role of substance P release. Am J Physiol Lung Cell Mol Physiol. 2007;292:L915-923.

20. Eddleston M, Dawson A, Karalliedde L, et al. Early management after self-poisoning with an organophosphorus or carbamate pesticide - a treatment protocol for junior doctors. Crit Care. 2004;8:R391-R397.

21. Eddleston M, Eyer P, Worek F, et al. Differences between organophosphorus insecticides in human self-poisoning: a prospective cohort study. Lancet. 2005;366:1452-1459.

22. Senanayake N, de Silva HJ, Karalliedde L. A scale to assess severity in organophosphorus intoxication: POP scale. Hum Exp Toxicol. 1993;12:297-299.

23. Lewis LD, McLaren M, Essex E, et al. Plasma concentrations of salbutamol in acute severe asthmatics. Aust N Z J Med. 1990;20:204-207.

24. Webb J RJ, Clark TJ. Comparison of different methods of administration of beta2-sympathomimetics in patients with asthma. Br J Dis Chest. 1982;76:351-357.

25. Faiz MA. Baseline survey on cases of poisoning and its outcome. Poison Information Centre DMC, Dhaka, Bangladesh. Dhaka, Bangladesh, Poison Information Centre, Dhaka Medical College, Dhaka, Bangladesh. 2007 1-31.

26. Eddleston M, Eyer P, Worek F, et al. Pralidoxime in acute organophosphorus insecticide poisoning--a randomised controlled trial. PLoS Med. 2009;6:e1000104.

FIGURE LEGENDS

Figure 1: Trial Profile

Flow diagram as per the CONSORT guidelines. Reasons for trial exclusion were not required atropine (n=29), not required oxygen (n=47), and did not give consent (n=7)

Figure 2. Glasgow Coma Scale (GCS) & Peradeniya Organophosphorus Poisoning (POP) severity scores on admission

Median and IQR (black dots represent fatal cases).

Figure 3 Time to presentation for patients across the three arms of the study

Figure 4. Peripheral oxygen saturations and heart rate after administration of saline placebo or salbutamol

A) Mean (SEM) and **B)** area-under-the-curve (AUC; using base of 70%), showing mean [IQR]) for peripheral oxygen saturation over the first 60 min for the three groups. Patients who died are marked with black dot. **C)** Mean (SEM) for heart rate over the first 60 min for the three groups. **D)** Time to death for patients

Figure 5. Comparison of Time to event (Oxygen saturation >95%) analysis between the three arms of the study

Table 1. Baseline demographic and clinical characteristics

	A NoSalb (n=25)	**B Salb2.5 (n=25)**	**C Salb5 (n= 25)**
Age (years; median, IQR)	30 (20 to 44)	30 (19 to 44)	25 (21 to 36)
Men (number; %)	13 (52%)	15 (60%)	16 (64%)
Poisons ingested (number; %)			
WHO Class Ib toxicity (dichlorvos/ dicrotophos)	6 (24%)	8 (32%)	13 (52%)
WHO Class II toxicity (chlorpyrifos)	11 (44%)	10 (40%)	7 (28%)
WHO Class III toxicity (malathion)	8 (32%)	7 (28%)	5 (20%)
Time between poisoning and start of treatment (min; median, IQR) *	263 (213 to 376)	205 (175 to 254)	246 (205 to 364)
< 120 mins (number; %)	0 (0)	2 (8%)	0 (0%)
120-240 mins (number; %)	11 (44%)	14 (56%)	11 (44%)
> 240 mins (number; %)	14 (56%)	8 (32%)	14 (56%)
Clinical status on admission			
Oxygen saturations (%; mean, SD)	82.4 (15.2)	84.0 (12.3)	85.1 (12.3)
Heart rate (bpm; mean, SD)	104.3 (21.3)	97.4 (19.6)	110.0 (22.5)
Systolic BP (mmHg; mean, SD)	113.6 (15.2)	103.4 (13.9)	113.6 (24.9)
Respiratory rate (RR; mean, SD)	20.3 (2.6)	20.8 (2.9)	22.2 (3.7)
GCS (median, IQR)	10 (8 to 15)	13 (8 to 15)	12 (7 to 13)
GCS 14 or 15 (number; %)	9 (36%)	11 (44%)	5 (20%)
GCS 11 to 13 (number; %)	3 (12%)	2 (8%)	9 (36%)
GCS 7 to 10 (number; %)	12 (48%)	9 (36%)	5 (20%)
GCS 3 to 6 (number; %)	1 (4%)	3 (12%)	6 (24%)
Peradeniya Organophosphorus Scale (POP) [22]			
Mild (0-3)	17 (68%)	13 (52%)	8 (32%)
Moderate (4-7)	7 (28%)	11 (44%)	16 (64%)
Severe (8-11)	1 (4%)	1 (4%)	1 (4%)
Clinical features on admission			
Miosis (number; %)	25 (100%)	25 (100%)	25 (100%)
Sweating (number; %)	25 (100%)	25 (100%)	25 (100%)
Salivation (number; %)	25 (100%)	25 (100%)	25 (100%)
Vomiting (number; %)	25 (100%)	23 (92%)	24 (96%)
Bronchospasm (number; %)	24 (96%)	23 (92%)	22 (88%)
Confusion (number; %)	14 (56%)	13 (52%)	14 (56%)
Muscle weakness (number; %)	14 (56%)	12 (48%)	14 (56%)
Lacrimation (number; %)	5 (20%)	7 (28%)	6 (24%)
Diarrhoea (number; %)	1 (4%)	3 (12%)	4 (16%)

Fasciculation (number; %)	0 (0%)	5 (20%)	3 (12%)
Arrhythmia (number; %)	0 (0%)	2 (8 %)	4 (16%)
Convulsion (number; %)	0 (0%)	0 (0%)	0 (0%)

* Time of ingestion was not known for one patient in the Salb2.5 arm

Table 2. Summary of outcomes plus comparative statistics

	NoSalb (n=25)	**Salb2.5 (n=25)**	**Salb5 (n= 25)**	
Atropinisation				
Time required for full atropinisation (min; median, IQR)	15.0 (10 to 16)	15.0 (10 to 16)	15.0 (10 to 20)	p=0.4805 (by Kruskal-Wallis)
Dose of atropine required for atropinisation (mg; median, IQR)	12.6 (8.0 to 13.4)	12.6 (9.3 to 16.8)	12.6 (10.7 to 20.6)	p=0.1871 (by Kruskal-Wallis)
Complications				
Aspiration pneumonia (number, %)	1 (4%)	1 (4%)	1 (4%)	
Intermediate syndrome (number, %)	0 (0%)	1 (4%)	1 (4%)	
Paralytic Ileus (number, %)	5 (20%)	1 (4%)	1 (4%)	
Tachyarrhythmia (number, %)	0 (0%)	0 (0%)	0 (0%)	
Outcome				
Death	2 (8%)	3 (12%)	3 (12%)	
Discharged without complication	22 (88%)	21 (84%)	20 (80%)	

Figure 1

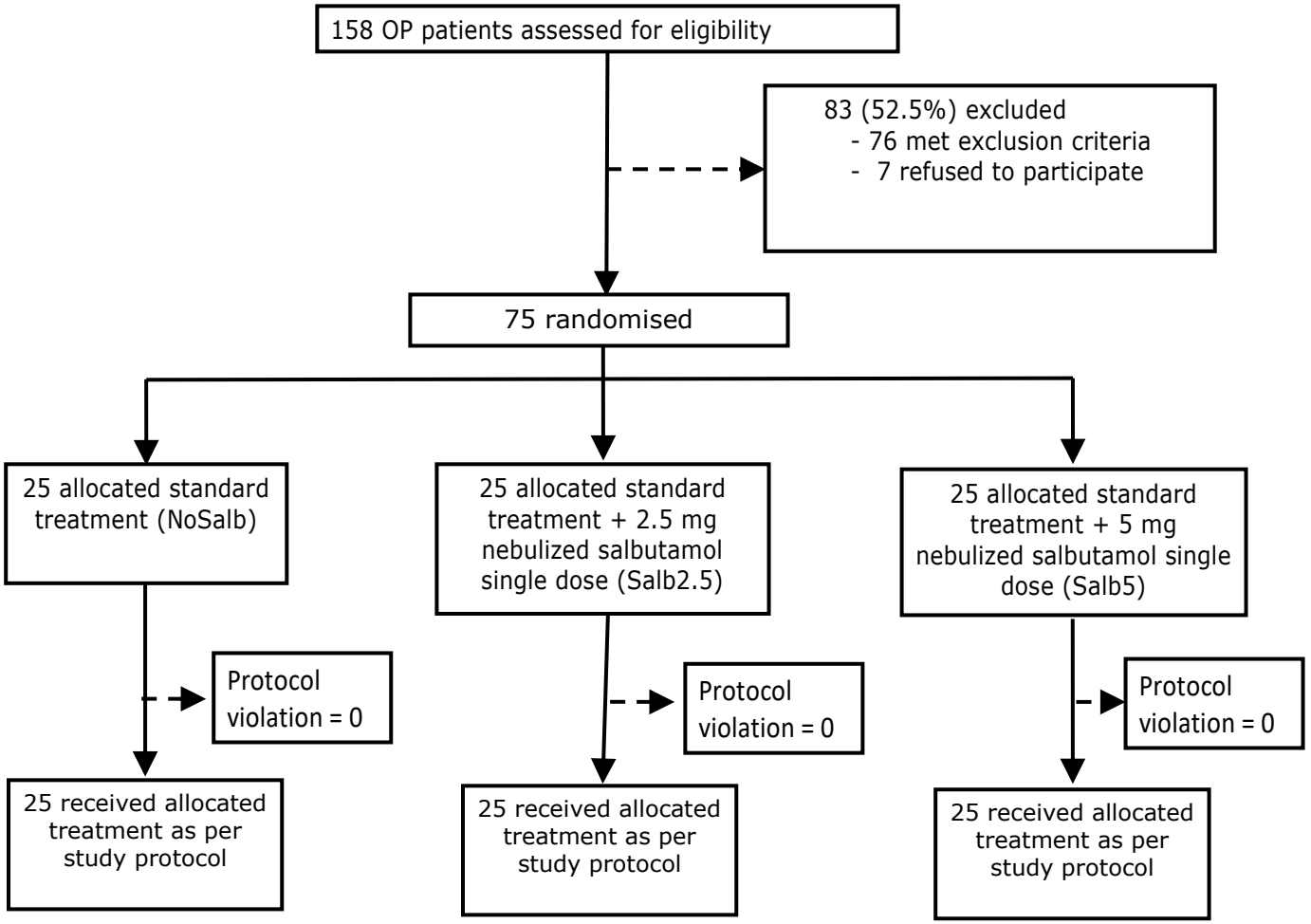

158 OP patients assessed for eligibility
83 (52.5%) excluded
- 76 met exclusion criteria
- 7 refused to participate
75 randomised
25 allocated standard treatment (NoSalb)
25 allocated standard treatment + 2.5 mg nebulized salbutamol single dose (Salb2.5)
25 allocated standard treatment + 5 mg nebulized salbutamol single dose (Salb5)
Protocol violation = 0
Protocol violation = 0
Protocol violation = 0
25 received allocated treatment as per study protocol
25 received allocated treatment as per study protocol
25 received allocated treatment as per study protocol

Figure 2

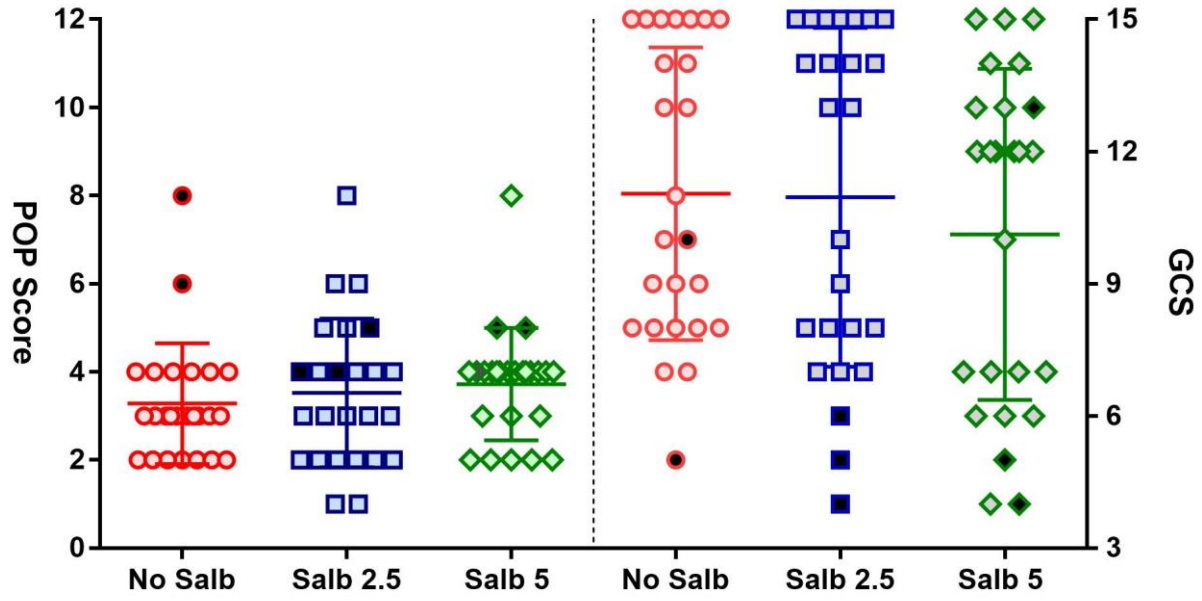

POP Score
GCS
12
10
8
6
4
2
0
15
12
9
6
3
No Salb
Salb 2.5
Salb 5
No Salb
Salb 2.5
Salb 5

Figure 3

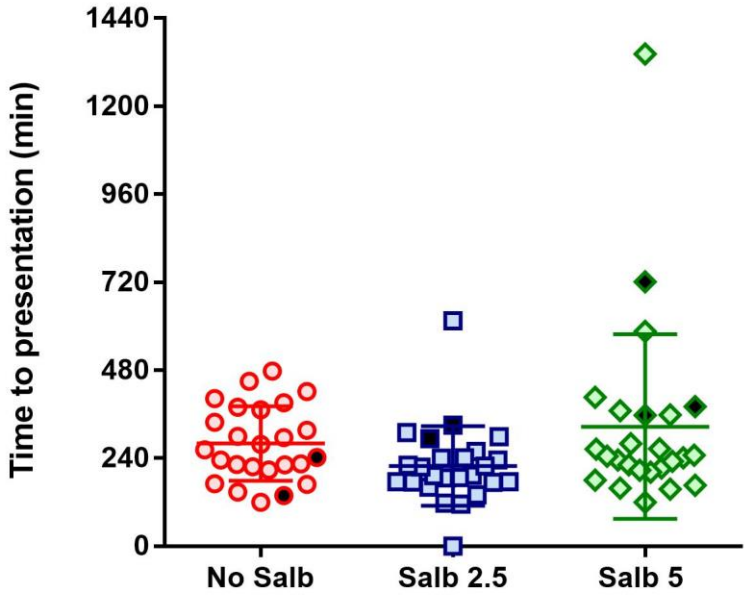

Time to presentation (min)
1440
1200
960
720
480
240
0
No Salb
Salb 2.5
Salb 5

Figure 4

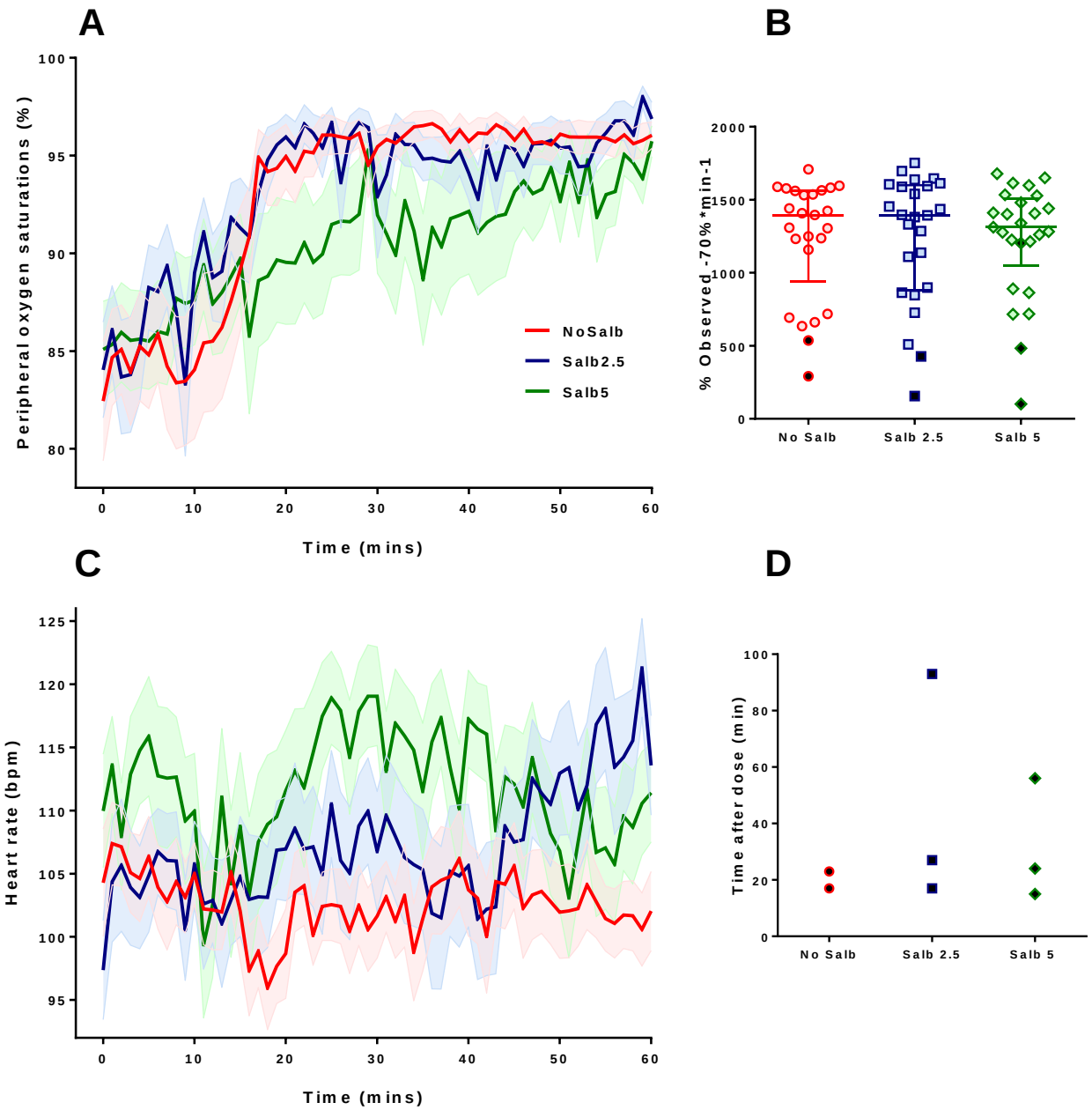
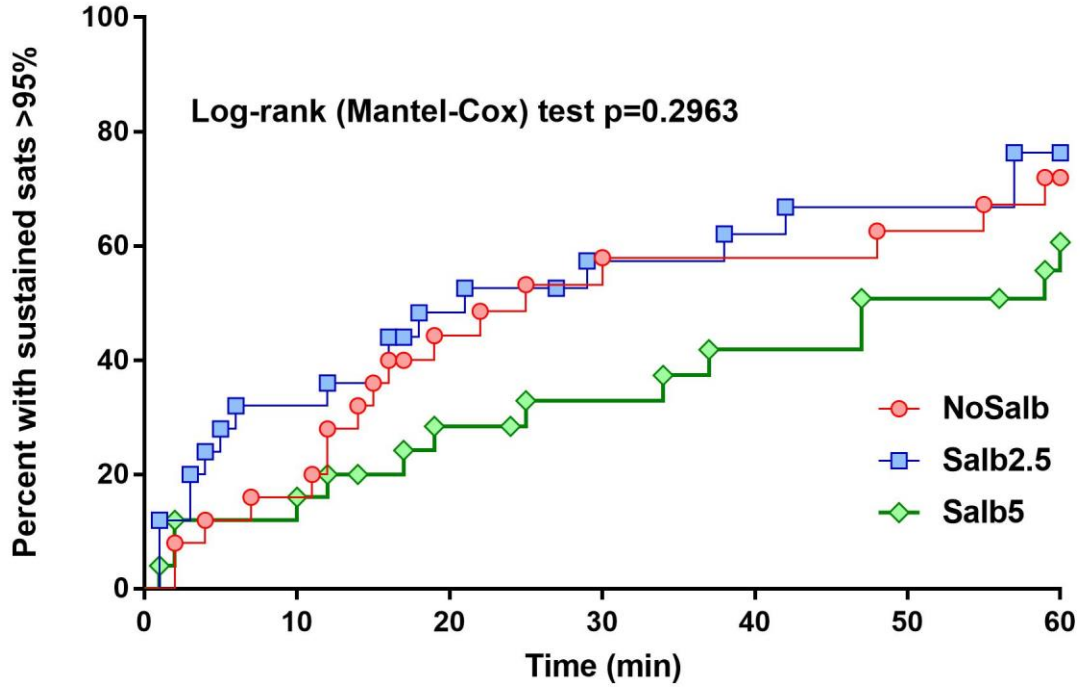

A
Peripheral oxygen saturations (%)
100
95
90
85
80
0
10
20
30
40
50
60
Time (mins)
NoSalb
Salb2.5
Salb5
B
% Observed -70%*min-1
2000
1500
1000
500
0
No Salb
Salb 2.5
Salb 5
C
Heart rate (bpm)
125
120
115
110
105
100
95
0
10
20
30
40
50
60
Time (mins)
D
Time after dose (min)
100
80
60
40
20
0
No Salb
Salb 2.5
Salb 5

Figure 5


Log-rank (Mantel-Cox) test p=0.2963
Percent with sustained sats >95%
Time (min)
NoSalb
Salb2.5
Salb5
0
20
40
60
80
100
0
10
20
30
40
50
60