DOI: 10.2478/10004-1254-63-2012-2158

Case Report

# MONOCROTOPHOS POISONING THROUGH CONTAMINATED MILLET FLOUR

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Received in September 2011
CrossChecked in September 2011
Accepted in July 2012

Several episodes of mass poisoning by organophosphates (OPs) have been reported from the developing countries. The diagnosis of OP-poisoning is mainly based on the characteristic clinical features and history of exposure to a known OP compound. Estimation of serum and red blood cell (RBC) cholinesterase activities are helpful in confirming the diagnosis. However, there is controversy regarding a definite relationship between serum cholinesterase activity and the severity of clinical manifestations and prognosis. This report describes an episode of mass monocrotophos poisoning that occurred due to accidental ingestion of monocrotophos-contaminated millet (so called *bavta*) flour involving eight severely poisoned persons. Clinical presentation included severe abdominal pain, diarrhoea, vomiting, pupil narrowing, and difficulty breathing. On hospital admission, plasma cholinesterase (PChE) and especially RBC acetylcholinesterase (AChE) activities correlated well with clinical symptoms presented by the patients. This case study highlights the need for clinicians to be aware of OP-pesticide poisoning from food sources and the need to look for depressed PChE and AChE activities that may point to OP exposure, so that OP-poisoning can be identified immediately and patients can receive specific treatment, rather than general treatment for food poisoning.

**KEY WORDS:** *abdominal pain, acetylcholinesterase, acute OP poisoning, blood cholinesterase, diarrhoea, difficulty breathing, organophosphate pesticides, plasma cholinesterase, pupil narrowing, vomiting*

Organophosphate (OP) pesticides continue to be the most common type of pesticides involved in acute poisoning in countries like India and Sri Lanka (1, 2). A large number of them, including monocrotophos, are registered for use in India. Despite structural differences, the mechanism by which they elicit their toxicity is identical and is associated with the inhibition of the nervous tissue acetyl cholinesterase. Morbidity and mortality from OP pesticides remains especially high in rural settings where facilities for intensive care are either absent or very limited. The World Health Organization (WHO) has estimated that each year more than 200,000 people in the world die from pesticide poisoning (3). Most of the poisonings occur in Asia and at least 50 % are OP-related (1, 3). Episodes of mass poisoning due to OP pesticides have been reported from developing countries like Pakistan and India (4, 5); the first from India was reported in 1958 and occurred following the consumption of parathion-contaminated wheat, killing more than 100 people (6).

In the past five years, several episodes of mass poisoning by different pesticides have been reported to the Poison Information Centre (PIC) of the National Institute of Occupational Health (NIOH) in Ahmedabad; most notably endosulfan (7), phorate and ethion (8) poisonings. It has been observed that OP poisoning from contaminated food ingestion is all too often treated empirically for food poisoning instead of specific treatment. In this paper, we outline the

medical history of patients involved in OP poisoning with details of the investigations conducted to ascertain the involvement of monocrotophos.

## CASE HISTORY

This episode of mass poisoning occurred at Deva village about 60 km from Ahmedabad following a meal containing breads made from contaminated *bavta* (a variety of millet) flour. Estimated average consumption of the flour (based on number of breads eaten and estimated amount of flour required for preparing a bread) was 107 g in children and 200 g in adults. The affected people were initially admitted to the civil hospital in Nadiad and then to the civil hospital in Ahmedabad. The site of the incident and the hospitals were visited and the information was collected from the relatives, neighbours and the medical personnel who treated the victims. In addition, routine tests were run at the civil hospital in Ahmedabad, including haematology and urine analysis, random blood sugar, liver and renal function tests, arterial blood gases, chest X-rays, ECG, blood pressure (by sphygmomanometry), and respiratory and heart rate. Plasma cholinesterase (PChE) and red blood cell (RBC) acetylcholinesterase (AChE) activities were measured at the PIC. For the study, ethical clearance was obtained from the Ahmedabad-civil hospital. A written consent in local language was obtained from the patients, and they were informed of the importance of the study.

For cholinesterase estimations, two to three millilitres of blood was collected from the antecubital vein into vacutainer tubes containing EDTA, and blood samples taken to the PIC laboratory within half an hour. PChE and AChE activities were determined by spectrophotometry using a modified Ellman's method with acetyl thiocholine as substrate (9). To 1.5 mL phosphate buffer (pH 7.2, 52 mmol $L^{-1}$) containing 0.26 mmol $L^{-1}$ 5,5'-dithiobis-(2-nitrobenzoic acid), 10 µL of sample was added and the reaction was started by adding 50 µL of 156 mmol $L^{-1}$ acetyl thiocholine. The rate of change in optical density was recorded at 405 nm on RA-50 Chemistry Analyzer (Technicon) at 37 °C. Blood sugar, serum creatinine, blood urea, and serum bilirubin were analysed using standard commercial kits (Ranbaxy Diagnostics, India).

For identification of the OP pesticide, a sample of *bavta* flour was collected and stored at -20 °C in an airtight glass bottle protected from light. Information on the approximate amount of flour (bread) consumed by the individuals was also collected to estimate the amount of the pesticide ingested. The flour sample was analyzed for identification and quantification of the organophosphate pesticide using gas chromatography with a nitrogen phosphorus detector (GC-NPD, TRACE GC Ultra System, Thermo Finnigan, USA) (10). We added 50 g of flour from the sample to a 100 mL mixture of acetone and water (65:35), and shook for 3 min using a vertical blender with a homogeniser at high speed. The extract was filtered through a Buchner funnel using moderate vacuum. From the obtained filtrates, we transferred an aliquot of 40 mL, representing 20 g of the sample, to a separating funnel and partitioned liquid using a mixture of *n*-hexane and methylene chloride (1:1). The extract thus collected was evaporated to about 1 mL under a vacuum rotary evaporator. The evaporation was repeated thrice in the presence of *n*-hexane to remove the traces of methylene chloride. The final volume of the extract was made with *n*-hexane/acetone (9:1) mixture for the GC-NPD analysis. Monocrotophos (AccuStandard) was used as standard. Acetyl thiocholine and 5,5'-dithiobis-(2-nitrobenzoic acid) were from Sigma-Aldrich, USA. All other chemicals used were of analytical grade.

## CASE REPORT

### *Patients*

On 6 October 2006 around noon eight persons (five adults and three children) from two families ate a meal of *bavta* bread and a vegetable. Within two to three hours, all started complaining of severe abdominal pain, difficulty breathing, diarrhoea, and vomiting. After hospitalisation, all manifested narrowed pupils and some had frothing at the mouth. Three (all children) lost consciousness within three hours of the incident. On the same day, seven of the affected people were taken to the civil hospital in Nadiad, where they were treated for food poisoning and received intravenous (*i.v.*) fluids and antibiotics, while one adult who had mild effects got primary treatment at a nearby private hospital. Two of the children regained consciousness after five to six hours of treatment, while one child did not regain consciousness and developed respiratory distress. He was intubated and

given bag tube ventilation. The three children and the four adults were transferred to the civil hospital in Ahmedabad on the second and the third day, respectively. The PIC, NIOH was contacted for blood analysis, and it confirmed involvement of an organophosphate pesticide by establishing lower blood cholinesterase activity levels. All patients were treated for OP poisoning with atropine (*i.v.*) and 2-pralidoxime (*i.v.*); children received 9 mg to 12 mg and adults 36 mg to 50 mg of atropine over four to five days; 2-pralidoxime was administered in the dose of 2 g to 4 g to children and 10 g to 16 g to adults over three days. The child who had respiratory distress died at 5.00 am on the third day, 53 hours after poisoning.

*Analysis*

The data on the clinical status, outcome, and blood cholinesterase activities of seven patients are shown in Table 1. All had significantly lower activities of PChE and AChE compared to reference intervals of 2900 U $L^{-1}$ to 5800 U $L^{-1}$ and 1700 U $L^{-1}$ to 2300 U $L^{-1}$, respectively (8). The children (Patient 1, 2, and 3) were drowsy at the time hospitalisation and had very low activities of AChE, ranging from 0 U $L^{-1}$ to 61 U $L^{-1}$. One child (patient 1), who had narrowed, pinpoint pupils and the lowest PChE and AChE activities, died while the rest survived. Patients 4, 5, 6, and 7 showed comparatively higher activities of PChE and AChE. They were conscious at the time of

**Table 1** *Patient data at the time of hospital admission*

| Parameter | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 | Patient 6 | Patient 7 |
|---|---|---|---|---|---|---|---|
| Sex | Male | Male | Male | Male | Female | Female | Female |
| Age / years | 7 | 5 | 8 | 48 | 45 | 30 | 26 |
| Body weight / kg | 17 | 15 | 22 | 42 | 50 | 47 | 62 |
| Level of conscioness | Drowsy | Drowsy | Drowsy | Conscious | Conscious | Conscious | Conscious |
| *PChE / $UL^{-1}$ | 81 | 126 | 401 | 874 | 945 | 675 | 520 |
| **AChE / $UL^{-1}$ | 0 | 61 | 0 | 640 | 493 | 652 | 468 |
| AChE / PChE | 0 | 0.48 | 0 | 0.73 | 0.52 | 0.97 | 0.90 |
| Routine investigations *** | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| Pupils | Constricted, pinpoint | Semi-constricted, not pinpoint | Constricted, not pinpoint | Semi-constricted, not pinpoint | Semi-constricted, not pinpoint | Semi-constricted, not pinpoint | Semi-constricted, not pinpoint |
| Other symptoms | Tachycardia, stupor, no fever | Fever | Fever | No fever | Bradycardia, no fever | No fever | No fever |
| Blood pressure syst/diast / mm Hg | 110/75 | 100/70 | 108/74 | 114/70 | 110/70 | 110/70 | 116/78 |
| Heart rate / bpm | 148 | 90 | 90 | 64 | 55 | 80 | 98 |
| Respiratory rate / bpm | 36 | 32 | 52 | 20 | 22 | 22 | 24 |

** Normal range (2900 to 5800) U $L^{-1}$*
*** Normal range (1700 to 2300) U $L^{-1}$*
**** Includes routine urine analysis, arterial blood gases, chest X-ray, and ECG*
*PChE – plasma cholinesterase*
*AChE - acetylcholinesterase*

**Table 2** *Patient data - other routine haematological and biochemical findings*

| Parameter | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 | Patient 6 | Patient 7 |
|---|---|---|---|---|---|---|---|
| Blood haemoglobin / g $dL^{-1}$ | 11.6 | 11.1 | 11.7 | 11.0 | 11.5 | 10.9 | 11.9 |
| Total WBC count | 12,100 | 19,200 | 10,800 | 10,200 | 9,100 | 10,600 | 9,100 |
| Differential WBC count (N/L/M/E/B) | 55/38/04/03/00 | 50/46/02/02/00 | 56/40/02/02/00 | 59/35/03/02/01 | 53/31/04/02/00 | 51/33/04/02/00 | 60/34/04/01/01 |
| $SpO_2$ / % | 96 | 98 | 98 | - | - | - | - |
| Random blood sugar * / mg $dL^{-1}$ | 128 | 118 | 110 | 132 | 110 | 115 | 120 |
| Blood urea ** / mg $dL^{-1}$ | 26.1 | 22.2 | 24.4 | 30.1 | 28.0 | 24.2 | 22.0 |
| Serum creatinine *** / mg $dL^{-1}$ | 0.62 | 0.44 | 0.72 | 0.87 | 0.84 | 0.72 | 0.83 |
| Serum total bilirubin **** / mg $dL^{-1}$ | 0.67 | 0.70 | 0.82 | 1.22 | 1.10 | 0.78 | 0.52 |
| Complications developed | Respiratory failure | No | No | No | No | No | No |
| Outcome | Died after 53 h | Complete recovery | Complete recovery | Complete recovery | Complete recovery | Complete recovery | Complete recovery |

** Normal range <130 mg $dL^{-1}$*
*** Normal range (15 to 40) mg $dL^{-1}$*
**** Normal range (0.5 to 1.40) mg $dL^{-1}$*
***** Normal range (0.50 to 1.10) mg $dL^{-1}$*

hospitalisation and did not develop any complications. The ratios of AChE to PChE activity varied from 0 to 0.97 (Table 1) and did not correlate with the clinical picture at the time of admission or with patient outcome. Cholinesterase activities, especially AChE, correlated well with the clinical picture of the patients on the day of admission. The activities of blood urea, serum creatinine and total bilirubin were within normal range (Table 2). The analysis of the *bavta* flour revealed very high amounts of monocrotophos (938 mg $kg^{-1}$). Estimated ingestion of the pesticide by the subjects ranged from 75 mg to 225 mg. The child who died after respiratory distress was estimated to have ingested about 113 mg of the pesticide.

## DISCUSSION

Monocrotophos is a highly toxic OP pesticide belonging to the WHO class 1b. Approximately 6000 tons of this pesticide are used annually in India. It is a small (molar mass: 223.2 g $mol^{-1}$), water-soluble molecule that can be absorbed rapidly by the oral route. The time course of the described events leading to severe toxicity within a few hours points toward quick absorption of this pesticide. Quick absorption of and delayed treatment seem to be the main culprits for very high morbidity observed in this episode.

Symptoms and signs of OP poisoning vary with the age of the affected person. Young children present with altered levels of consciousness, rather than with the classic DUMBELS (diarrhoea/diaphoresis, urination, miosis, bradycardia/bronchospasm, emesis, lacrimation, salivation) signs, which are common in adults. After retrospective examination of 36 children aged two to eight years, Lifshitz et al. (11) observed a decreased level of consciousness including coma, stupor and hypotonicity upon exposure to organophosphates or carbamates in Israel. Similarly, all the three children (aged five to eight years) in our report were drowsy at the time of hospital admission. The child who presented with narrowed pupils and the lowest PChE and AChE activities died after the development of respiratory distress. It is unclear whether this was due to a larger exposure or greater sensitivity. Four adults showed comparatively higher

activities of PChE and AChE and were all conscious at the time of admission and did not develop any complications. This suggests that cholinesterase activities, especially AChE activities, correlate well with the clinical picture of the patient, which is in line with our earlier observations about acute ethion (OP pesticide) poisoning (8). Even though we know that the poisoning was due to ingestion of contaminated food, we did not establish the exact reason, but it was most probably unintentional.

Pesticides are known to affect lipid, protein, and carbohydrate metabolism. OPs increase Ach, which contributes to the secretion of insulin and glucagon by activating protein kinase C and by increasing the efficiency of free cytosolic calcium on exocytosis of insulin granules. Oral administration of 1/10 $LD_{50}$ of monocrotophos caused reversible hyperglycaemia in rats, peaking 2 h following administration, accompanied by significant inhibition of acetylcholinesterase (AChE) activity. At 4 h following administration, glucose was back to normal (12). Similarly, feeding broiler chicks with poultry mash containing 2 mg $kg^{-1}$ of monocrotophos for eight weeks significantly decreased blood glucose concentration and serum AChE activity compared to control, without significant changes in blood urea nitrogen, total red blood cell (RBC) count, packed cell volume, haemoglobin, eosinophil and monocyte count (13). We, however, found no changes in blood glucose levels after acute monocrotophos poisoning. This may be due to the reversible nature of hyperglycaemia, as has already been observed in experimental rats (12).

Another study on rats (14) showed a significant decrease in haemoglobin concentrations, total RBC and white blood cell (WBC) counts and in haematocrit after chronic sublethal oral exposure (150 mg $kg^{-1}$ body weight per day) to dimethoate, another OP insecticide. The levels of blood glucose, cholesterol, urea, and total bilirubin soared, but the activities of acid phosphatase and cholinesterase significantly dropped. We, however, found no changes in blood urea, serum creatinine and total bilirubin concentrations in the poisoning episode reported here.

The oral lethal dose of monocrotophos to humans has been estimated to (5 to 50) mg $kg^{-1}$ of body weight (15), while the WHO reports that ingestion of 120 mg of monocrotophos can be fatal to humans (16). The consumption of the pesticide by the child who died was estimated to 113 mg or about 6.7 mg $kg^{-1}$ of body weight. Quite expectedly, this points to far greater susceptibility of children than of adults.

Organophosphate poisoning continues to be a serious problem in developing countries that calls for urgent revision of marketing policies, availability, safe use, and treatment facilities. This report highlights the problem of accidental pesticide poisoning in developing countries resulting in high morbidity and mortality, as facilities for immediate treatment of acutely poisoned persons are seldom at hand. It also highlights the need for clinicians to be aware of OP-pesticide poisoning from food sources and of the need to check cholinesterase activities to establish OP exposure rather than introduce food poisoning treatment alone.

*Acknowledgement*

Authors are grateful to Dr M. M. Prabhakar, medical superintendent of the civil hospital in Ahmedabad for permitting us to carry out the study. Thanks are also due to Dr P. G. Shah, residue analyst of the Centre for Organic Farming, ICAR, Anand Agriculture University, Anand, Gujarat for technical help.

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**Sažetak**

TROVANJE MONOKROTOFOSOM IZ ONEČIŠĆENOGA BRAŠNA OD PROSA

Zemlje u razvoju nerijetko se susreću sa slučajevima masovnih trovanja organofosfatima (OP). Dijagnoza trovanja organofosfatima uglavnom se zasniva na karakterističnim kliničkim simptomima koji se pojavljuju nakon izloženosti nekome od poznatih organofosfornih spojeva. U potvrđivanju dijagnoze važnu ulogu ima mjerenje aktivnosti enzima kolinesteraza u serumu i crvenim krvnim stanicama. Međutim, povezanost između aktivnosti kolinesteraze u serumu i ozbiljnosti kliničkih manifestacija i prognoze još uvijek je predmet mnogih dvojba. Ovo istraživanje donosi prikaz slučaja masovnog trovanja monokrotofosom koje je nastupilo uslijed akcidentalne konzumacije prosenog brašna (tzv. *bavta*) onečišćenog monokrotofosom. U osmero otrovanih osoba uočeni su sljedeći klinički simptomi: snažni bolovi u trbuhu, proljev, povraćanje, sužavanje zjenica i poteškoće u disanju. Razine kolinesteraza: kolinesteraze u plazmi (PChE) te posebice razine acetilkolinesteraze u crvenim krvnim stanicama (AChE), izmjerene na dan prijema u bolnicu, visoko su korelirale s kliničkim simptomima zamijećenim u bolesnika. Ovaj prikaz slučaja naglašava potrebu za boljim informiranjem liječnika o brzom prepoznavanju simptoma trovanja hranom onečišćenom OP-pesticidima i potrebom provjere jesu li u otrovanih osoba prisutne snižene razine PChE i AChE koje mogu upućivati na izloženost organofosfatima. Sve to moglo bi značajno pridonijeti ranom postavljanju dijagnoze trovanja organofosfatima, čime bi bolesnici na vrijeme mogli primiti specifičnu, a ne općenitu terapiju kao u slučajevima „običnog“ trovanja hranom.

**KLJUČNE RIJEČI:** *acetilkolinesteraza, akutno trovanje organofosfatima, bolovi u trbuhu, dijareja, kolinesteraza u plazmi, pesticidi, poteškoće u disanju, povraćanje, sužavanje zjenica*

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