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Research Article

Identifying and reporting of unknown poison by qualitative methods in patients admitted with oral consumption into the emergency department KIMS hospital

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ABSTRACT

Poisoning is an important public health problem causing significant morbidity and mortality throughout the world with increasing numbers of cases worldwide including India. Though there is improvement in the therapeutic patterns, health care professionals are not able to reduce morbidity and mortality caused due to poisoning because it is difficult to identify type of poison consumed by the patient. Objectives of our study were validating the WHO standard methods to identify the different types of poisoning using standard samples by qualitative methods. And identification, reporting of unknown poison by using gastric content in patients admitted with oral consumption of unknown compound. We have followed WHO standard methods to carry out some of qualitative tests. Gastric content was collected in unsterile container during the gastric lavage to analyze unknown compound by applying different tests including tests for salicylates, ethanol, paracetamol, iron, methanol and chloral hydrate. Thin layer chromatography was carried out to analyze the pesticides.12 patients' samples were analyzed: two of samples showed presence of salicylates and one sample matched with the Rf value of malathion, two samples showed the presence of iron and seven samples did not match with any of the above tests. Our study used, WHO standard methods and thin layer chromatography to identify the type of poisons including pesticide. Study aimed in analyzing the type of poison consumed, with the help of gastric lavage possibly with a short time period, so that therapy can be started as soon as possible to reduce percentage of mortality.

Key words: *Poisoning Management; WHO; Thin Layer Chromatography; Quantitative Test*

INTRODUCTION

Poisoning is an important public health problem causing significant morbidity and mortality throughout the world. It has been estimated that some form of poison directly or indirectly is responsible for more than one million illness worldwide annually.[1] In India more than 50,000 people die every year from toxic exposure. The causes, patterns and results of poisoning depend on the various factors such as poverty, lack of education, easy availability of pesticides, the stress of the environment, insufficient knowledge of pesticide hazards, the high costs of protective equipment, poor labeling of pesticides, advanced technology in industrial and agricultural sector, rapid industrialization, introduction of newer range of drugs for treatment and massive use of pesticides in agriculture has increased the incidence of poisoning.[2] The most common agents in India appear to be pesticides (organophosphates, carbonates, chlorinated hydrocarbons, pyrethroids and aluminium/zinc phosphide), sedative drugs, chemical (corrosive acid and copper sulphate), alcohol, plant toxins (datura, croton, calotropis) and household poisons (mostly cleaning agents). In India, as agriculture is the main occupation, insecticides and other agrochemical fertilizers are used to a greater extent and the poisoning with such products are more common. According to various studies organophosphate forms the commonest poisoning agent. We have taken up 19 commonly used pesticides.[3] In India, 113,914 estimated cases of poisoning with insecticides were observed in 2005.[4] The WHO has cited a 2007

study that about 76,000 people die each year in India from pesticide poisoning.[3,5] For management of ingested poison, gastrointestinal evacuation, has been used for centuries and it is undergoing critical appraisal. The role of ipecac and gastric lavage are being questioned, while activated charcoal is gaining importance in the management of such cases. Antidotal therapy is no more the mainstay of the management and the fact that we have antidotes for only about 5% poisons, is mainly responsible for this development. Grouping the signs and symptoms produced by the poisons in to various toxidromes helps in rapid and effective management of the case. Interference with absorption of ingested poison from the gastrointestinal tract is the mainstay of poison management. Because few specific antidotes are available to treat poisonings, absorption prevention, observation, and supportive care are the clinician's greatest assets. The challenge for clinicians managing poisoned patients is to identify those who are most at risk of developing serious complications and who might potentially benefit from gastrointestinal decontamination.[6,7] Thin layer chromatography is carried out to analyze the pesticides which is a sophisticated method of separating and identifying mixtures of two or more compounds. The separation is accomplished by the distribution of the mixture between two phases: one that is stationary and other is moving or mobile phase. Chromatography works on the principle that different compounds will have different solubility and adsorption to the two phases, which will allow for their separation. Thin Layer Chromatography (TLC) is a solid-liquid technique in which the two phases are a solid, stationary phase and a liquid, mobile phase. The solid phase using in today's laboratory is a plastic plate covered with an adsorbent, in this case, silica gel. Aluminium is another common solid phase used. Because silica is a polar molecule, the components of the solution we use in lab today will be separated based on their relative polarities. The more polar molecules, the higher affinity it will have for the more polar silica plate and will therefore spend less time in the mobile phase. As a result, it will move up the plate more slowly. Conversely, a less polar molecule will spend more time in the mobile phase and will therefore move up the plate more quickly. The speed at which the molecules will move up the plate thus depends on the relative difference in polarity between the stationary and mobile phases, and will vary depending on the nature of the stationary and mobile phases used for separation.[8,9] The purpose of our study is to provide healthcare professionals with current WHO recommendations for treating patients with pesticide-related illnesses or injuries. Our study helps in the identification of the type of poison and hence helps to provide a desirable therapy to the patients.[10,11]

MATERIALS AND METHODS

Study design

A prospective, observational, analytical study has been conducted for a period of 6 months on patients admitted to the emergency department, Kempegowda Institute of Medical Science and Research Center (KIMS) Hospital, Bangalore due to poisoning. The study was carried according to permission granted by ethical committee of Visveswarapura Institute of Pharmaceutical Sciences (VIPS), Bangalore.

Inclusion criteria

All poisoning patients admitted into the emergency department with oral consumption of poisoning.

Exclusion criteria

1. Patients admitted with other route of poisoning.
2. Patients came with gastric lavage done in other medical centre.

Study procedure

We have followed the WHO standard methods to carry out some of the qualitative tests. Collected and analyzed standard samples were kept as standard reference. Gastric aspiration/content was collected in unsterile container during the gastric lavage to analyze the unknown compound. Collected gastric aspiration was filtered using filter paper and the clear solution will be taken for analysis. Well-designed data collection form was designed to document all the relevant data title, demographic details, type of poison, time of consumption, first aid done, complication and other relevant details. Different tests were performed to identify the unknown compound in the gastric content of the patient and the positive results have been documented and reported to the emergency department. Quantitative tests for salicylates, ethanol, paracetamol, iron, methanol and chloral hydrate as per the WHO standard were carried out.

Prepare the developing container

Thin layer chromatography was carried out to analyze the pesticides.

A beaker with a watch glass on the top was used for the developing container for TLC. The solvent were transferred into beaker to a depth of just less than 0.5 cm.

Prepare the TLC plate

The TLC plates were washed and wiped. Silica gel was used as adsorbent and the TLC plates were prepared with the silica gel (Figure 1).

Spot the TLC plate

If the sample was not already in solution, about 1 mg in 1 mL of a volatile solvent such as hexanes, ethyl acetate, or methylene chloride were dissolved. As a rule of thumb, a concentration of 1% usually works well for TLC analysis. If the sample is too concentrated, it will run as a smear or streak (see troubleshooting section below); if it is not concentrated enough, nothing can be seen on the plate. In the organic teaching labs, we used 10μL microcaps - they are easier to handle than the smaller ones used in research labs. The microcap was dipped into the solution and then gently touched the end of it onto the proper location on the TLC plate.

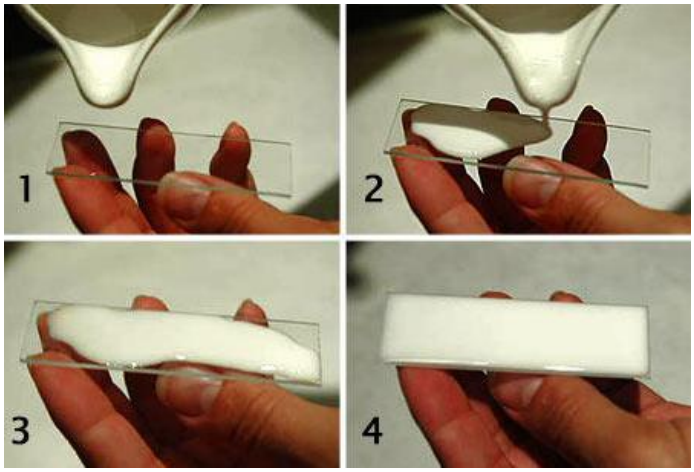

Figure 1: Prepare the TLC plate

Develop the plate

The prepared TLC plate has been placed in the developing beaker which covered with the watch glass, and left it undisturbed. The solvent was poured up the TLC plate by capillary action and allowed the plate to develop until the solvent was about half a centimeter below the top of the plate. The plate has been removed from the beaker and immediately marked the solvent front with a pencil and kept the plate to dry.

Visualize the spots

If there were any colored spots, we circled them lightly with a pencil. Most samples were not colored and needed to be visualized with a UV lamp. (Figure No. 2)

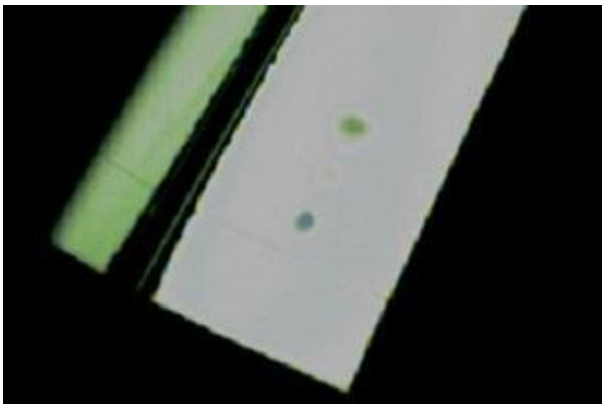

Figure 2: Spots are visualized under UV.

Tests for salicylates, ethanol, paracetamol, iron, methanol and chloral hydrate were carried out. These are color tests where the color of the samples were compared with the color of the standards. Thin layer chromatography was used to determine the Rf values of the pesticides which were kept as standards.

RESULT

The study was conducted in the department of ED, KIMS Hospital, Bangalore. We have followed the WHO standard methods to carry out some of the qualitative tests. Tests for salicylates, Phosphides and Phosphorous, Ethanol, Paracetamol, iron, methanol and chloral hydrate are carried out which showed the positive results. Standard pesticides are run using TLC. The commonly used pesticides in the rural areas are collected and the TLC was carried out. Out of 19 pesticides, we have got the Rf values of 7 pesticides. The R values of these 7 pesticides were found out using different ratios of solvent systems (Table 1), (Figures 3 & 4).

Some are UV sensitive and some are not sensitive to UV lamp. Rf value is calculated using the formula:

$$Rf = \frac{\textit{Distance travelled by the compound from the origin}}{\textit{Distance travelled by the solvent from the origin}}$$

Table 1: Rf Value of 7 Pesticides with Solvents and Their Ratio

Pesticide	Rf value	Solvents	Spot Color	UV Sensitive
Isopropyl amine	0.56	Acetone and hexane (50: 50)	Dark Purple	NO
Mixin-B	0.929	Acetone and hexane (50: 50)	Light Brown	NO
Planofix	0.216	Hexane and chloroform (60:40)	Light Brown	NO
Confidor	0.55	Hexane and acetone (50:50)	Dark Purple	Yes
Kilin -2	0.95	Hexane and acetone (50:50)	Yellow	Yes
Ribuimidacloprid	0.56	Cyclohexane, acetone and chloroform (75:25:5)	White	NO
Malathion	0.75	Hexane and chloroform (60:40)	Yellow	NO

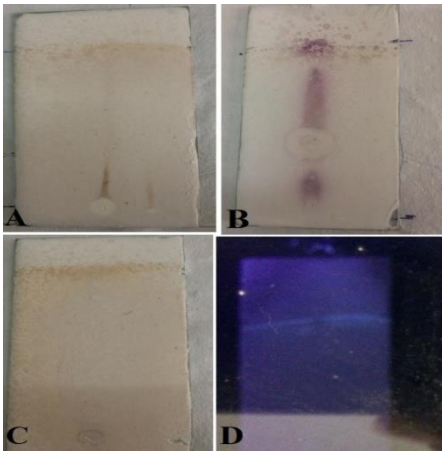

Figure 3: A: (Round up) isopropyl amine, B: Mixin-B, C: Planofix, D: Confidor UV

12 patients' samples were analyzed and compared with Rf values of 7 pesticides: two of the samples showed the presence of salicylates and one sample matched with the Rf value of malathion, two samples showed the presence of iron and seven samples did not match with the any of the Rf value of pesticides in Table 1.

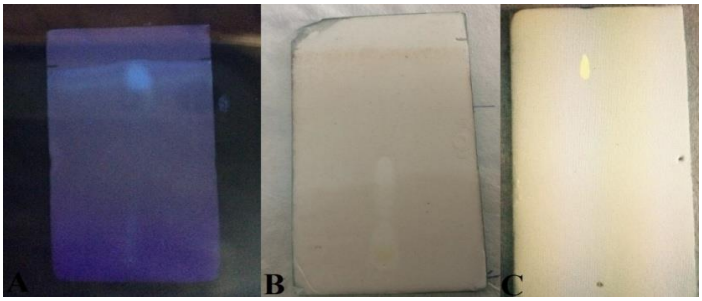

Figure 4: A: Kilin-2 under UV, B: Ribu Imidacloprid, C: Malathion

DISCUSSION

Tests for the salicylates, phosphides, phosphorous, ethanol and paracetamol, iron, methanol and chloral hydrate are carried out using the WHO standard methods. All these tests are colored tests. The color of the standards is compared with the color of the samples. Whereas in TLC, the Rf value of the standards are compared with Rf value of the samples. The solvent systems used to run one standard, differs from one another. TLC for Planofix was carried using hexane: acetone in the ratio of 50:50. But we were not able to get the results. The experiment was repeated using hexane: chloroform in the ratio of 60:40. The spot developed was dark brown and this is not UV sensitive. TLC for kilin- 2 was carried out using hexane: chloroform in the ratio of 60:40. There were no results using that. The experiment was carried out using the hexane: acetone in the ratio of 50:50. The results were obtained and this is UV sensitive. The same way the experiment was carried out for Triclonex-L. The experiment was carried out using the solvent systems; acetone: benzene in the ratio of 60:40, hexane: acetone in the ratio of 50:50 and benzene: acetone in the ratio of 9:1.

The Ribuimidacloprid was carried out using the solvent system acetone: benzene in the ratio of 60:40 and hexane: acetone in the ratio of 50:50 there were no results with these solvent systems.

The results were obtained using cyclohexane: acetone: chloroform in the ratio of 75:25:5.The negative results were obtained for Bavistin fungicide when we used solvent systems of acetone: benzene in the ratio of 60:40, hexane: acetone in the ratio of 50:50 and hexane: acetone in the ratio of 8:2.Negative results were obtained for Cuman- L when we used the solvent system, acetone: benzene in the ratio of 60:40 .the results were obtained using hexane: acetone 50:50.Malathion gave negative results when we used hexane: chloroform in the ratio of 60:40.The results were obtained when we used hexane: acetone in the ratio of50:50. Mancozeb 75% showed the negative results when we used the solvent system hexane: acetone 50:50 and acetone: benzene in the ratio of 60:40.Trimethoxan 25% showed the negative results with the hexane: acetone in the 50:50 and hexane: chloroform in the ratio of 60:40 and acetone: benzene in the ratio of 60:40.

CONCLUSION

Though there is an improvement in the therapeutic patterns, health care professionals are not able to reduce morbidity and mortality caused due to poisoning, because it is difficult to identify the type of poison consumed by the patient. Hence, there is a greater need to find the ways to identify the type of poison. Our study used, WHO standard methods and thin layer chromatography to identify the type of poisons including pesticide. Study aimed in analyzing the type of poison consumed, with the help of gastric lavage possibly with a short time period, so that the therapy can be started as soon as possible to reduce the percentage of mortality.

Limitations

Limitation of study must be stated for better interpretation. Less number of samples were analyzed as the samples were unable to obtain. Samples obtained from the study site were very less during our study period. The prepared solvent system was not appropriate to run few standards with TLC. Standard methods for the preparation of solvent systems were not available in the literatures. Hence we were unable to perform the standard run on the TLC.

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