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Original Article

Study of Qualitative Analysis of Phosphine in Postmortem Blood

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Abstract

Aluminium phosphide is widely used as fumigant and pesticide. Its widespread use is associated with increased incidence of poisoning. Its poisoning occurs directly due to ingestion and indirectly due to inhalation. After coming into contact with gastric acid on ingestion, it produces phosphine, the main active component for poisoning. In medicolegal cases, aluminium phosphide poisoning is diagnosed by the presence of phosphine in the blood and tissue samples using silver nitrate test. An issue has been raised about the postmortem production of phosphine and hence, false positive result for phosphine with the test. In this study, postmortem blood was kept stored at room temperature for varying duration without any preservative. The blood samples were analysed for presence of phosphine using silver nitrate test. Aluminium phosphide poisoning cases gave positive results. No aluminium phosphide negative case showed positive result in this study. It was concluded that silver nitrate test doesn't give false positive reaction with stored postmortem blood.

Keywords: Aluminium Phosphide Poisoning; Ammonium Molybdate Test; Silver Nitrate Test; False Positive Test; Hydrogen Sulphide.

Introduction

Aluminium phosphide is a pesticide for indoor fumigation of agricultural commodities as well as pest control [1]. It is a solid fumigant and widely used to protect grain in storage [2, 3]. It is considered to be an ideal grain fumigant due to its toxic properties to all stages of insect, high potency, no effect on seed viability and no residue on food grain. Upon contact with the moisture in the environment, it releases phosphine gas which is the active pesticide component [1, 3].

Due to its easy availability and widespread use in the farming areas of India, there is a rise in the incidence of its poisoning and this is one of the most common cause of acute poisoning in India [2], especially in rural areas [3]. Acute poisoning occurs in two forms: direct ingestion or indirect inhalation. Its toxicity is mainly due to liberation of phosphine, when the ingested phosphide comes into contact with the gastric acid, and the gas is absorbed through GI mucosa and distributed to the tissues. In blood, it interacts irreversibly with free haemoglobin and RBC haemoglobin [3]. Phosphine acts as a strong nucleophilic reducing agents and inhibits cellular enzymes, especially cytochrome C oxidase [1, 3]. It also produces various superoxide radicals and cellular peroxides and leads to cellular injury [1].

Diagnosis of aluminium phosphide poisoning is done by positive history of ingestion and confirmation is done by Silver Nitrate Test performed with breath of the patient bedside and on blood, gastric content and viscera samples [2, 4].

In medicolegal cases, the viscera samples are

analyzed using Silver Nitrate Test for phosphine as it is highly specific and highly sensitive [5]. However, an issue has been raised about the false positive test result for aluminium phosphide using the Silver Nitrate test. The study has explained that false positive result may be due to postmortem production of phosphine in the stored samples [5].

This study has been performed to know whether there is any postmortem production of phosphine and consequently, false positive result of Silver Nitrate test with postmortem blood, stored at room temperature for various durations.

Principle of the Experiment

Aluminium phosphide reacts with hydrogen chloride gives phosphine gas which precipitates as black silver phosphide with silver nitrate.

$$AlP + 3HCl \rightarrow AlCl_3 + PH_3\uparrow$$

$$PH_3\uparrow + 3AgNO_3 \rightarrow Ag_3P\downarrow + 3HNO_3$$

Hydrogen sulphide also reacts with silver nitrate and gives black precipitates of Silver sulphide, a false positive result.

$$2AgNO_3 + H_2S\uparrow \rightarrow Ag_2S\downarrow + 2HNO_3$$

To avoid hydrogen sulphide to react with silver nitrate, seal the mouth of flask with cotton soaked with concentrated solution of lead acetate to trap hydrogen sulphide. Lead acetate reacts with H_2S and produces black lead sulphide which deposits on the soaked cotton.

$$Pb(CH_3COO)_2 + H_2S\uparrow \rightarrow PbS\downarrow + 2CH_3COOH$$

In this way, hydrogen sulphide cannot react with Silver Nitrate and false positive reaction can be avoided.

To confirm the blackening is due to presence of phosphine, Ammonium Molybdate test is done with blackened Whattman's paper, producing canary yellow colour of Ammonium phosphomolybdate $(NH_4)_3PO_4.12MoO_3$.

Material and Methods

In the present study, femoral blood samples from autopsy cases were analysed for presence of phosphine. For the study, 10 cases with history of Aluminium phosphide poisoning and 15control cases (without anypoisoning) were taken. Aliquots were prepared and stored at room temperature without any preservative for various durations i.e. 2 days, 1 week, 1 month, 3 month, 6 month, 12 month and 18 month as shown in the table 1.Samples were analysedon the day of collection to confirm / negate Aluminum Phosphide poisoning. To avoid loss of phosphine present / formed in the samples, the samples were open only once during the study and then discarded.

Chemicals/reagents

Silver nitrate from Qualigens, India; suprapure water from Rions, India; ammonium heptamolybdate tetrahydrate, lead acetate, hydrochloric acid and nitric acid from Merck, India were used.

Glasswares

Conical flask from Borosil, India was used.

Miscellaneous

Whattman's filter paper andcotton.

Preparation of reagents

- Silver Nitrate solution–1g of silver nitrate salt is dissolved in 10 ml of distilled water.
- Lead Acetate solution–saturated solution of lead acetate is made up in distilled water.
- Ammonium Molybdate solution–10 N sulphuric acid solution is dissolved in 100ml distilled water.2.5 gm ammonium molybdate is dissolved in 30 ml of distilled water. 20 ml of the prepared sulphuric acid solution is added to the ammonium molybdate solution. Total solution is made up to the 100 ml by adding distilled water.

Steps of the experiment

1. Lead acetate impregnated cotton was prepared by dipping pieces of cotton in freshly prepared saturated solution of lead acetate and air dried.
2. Silver nitrate impregnated paper was prepared by putting few drops of freshly prepared silver nitrate solution on the Whattman's filter paper and spread it to over enough areas to cover the mouth of flask. Paper was air dried but not in direct sunlight.
3. In a conical flask 5ml blood was taken. 1ml of distilled water and few drops of hydrochloric acid were added to it. Neck of flask was packed with lead acetate impregnated cotton pieces. Mouth of flask was covered with silver nitrate impregnated Whattman's filter paper (figure 1) and the arrangement was heated for 5-10 min at 50^0C. Colour deposition over the cotton and paper were examined. Positive reaction was shown by blackening of the filter paper (figure 2).
4. Blackened filter paper was cut in small pieces

and heated for few minutes with diluted nitric acid. The extract was evaporated to the dryness for 2-3 times. Concentrated nitric acid was added to the residue followed by freshly prepared ammonium molybdate reagent Formation of canary yellow colour confirmed presence of phosphide in blood (figure 3).

Fig. 1: Conical flask containing blood and $AgNO_3$ impregnated paper on its mouth

Fig. 2: Blackening of $AgNO_3$ impregnated paper showing presence of Phosphine

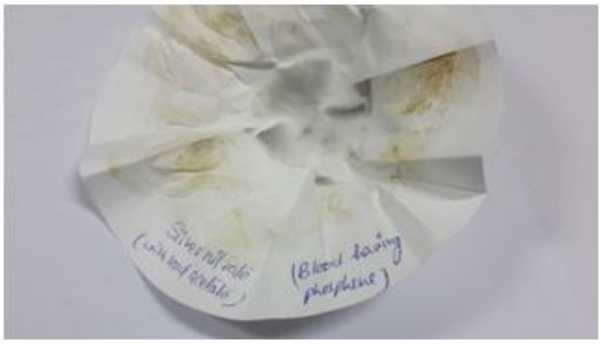

Fig. 3: Canary yellow colour with ammonium Molybdate due to presence of phosphine

Result and Discussion

The tests were done with standard procedure for phosphine with lead acetate impregnated cotton trapping of hydrogen sulphide. Interpretation of the result was done as follows:

(i) Blackening of silver nitrate impregnated Whattman's paper only is considered positive reaction for presence of phosphine,

(ii) Blackening of both silver nitrate impregnated Whattman's paper and lead Acetate cotton are considered positive reaction for presence of both phosphine and hydrogen sulphide,

(iii) Blackening of lead acetate cotton only is considered positive reaction for presence of hydrogen sulphide, and

(iv) Positive result is confirmed by ammonium molybdate test showing canary yellow colour with presence of phosphine.

Table 1 shows the result of the study. With all the cases with aluminium phosphide poisoning, silver nitrate test gave positive results which were later confirmed by ammonium molybdate test. The cases without aluminium phosphide poisoning gave negative results with silver nitrate.

We have also repeated the same test with decomposed blood but without lead acetate impregnated cotton trapping forhydrogen sulphidein 2 cases and they gave false positive result (figure 4). However, it gave negative result with ammonium molybdate test which showed that it was due to hydrogen sulphide production in the stored blood and not due to phosphine (figure 5).

This study was performed to know if there is any postmortem production of phosphine, and subsequently, false positive result with blood using standard Silver Nitrate Test. In this study, we had stored

Table 1: Result of the Silver Nitrate Test for Phosphine

Duration of preservation	Aluminium Phosphide Positive Cases(n= 10)		Aluminium Phosphide Negative Cases(n= 15)	
	Spot test	Confirmatory test	Spot test	Confirmatory test
0 days	+ve	+ve	-ve	-ve
2 days	+ve	+ve	-ve	-ve
1 week	+ve	+ve	-ve	-ve
1 month	+ve	+ve	-ve	-ve
3 month	+ve	+ve	-ve	-ve
6 month	+ve	+ve	-ve	-ve
12 month	+ve	+ve	-ve	-ve
18 month	+ve	+ve	-ve	-ve

Fig. 4: spot test with decomposed blood witouth and with lead acetate trapping

Fig. 5: negative Molybdate teat with decomposed blood due to H_2S

the femoral blood samples from both the aluminium phosphide poisoning cases and from cases without any poisoning. The samples were stored at various duration, upto 18 months at room temperature without any preservative. All the samples were opened only for once and then discarded to avoid any loss of phosphine.

During this study there was no false positive result for phosphine with standard silver nitrate test with lead acetate impregnated cotton trapping of hydrogen sulphide. False positive result is given by hydrogen sulphide produced during the storage but can be confirmed by negative ammonium molybdate test. To avoid this false positive phenomenon, lead acetate impregnated cotton trapping of hydrogen sulphide method has been used.

Conclusion

This study concludes that silver nitrate test give positive result with blood only if there is antemortem presence of phosphine. Stored and decomposed blood samples don't give false positive result for phosphine. The false positive result is only due to produced hydrogen sulphide during decomposition.

Limitation

This study was done on small number of samples; a large scale study is needed to confirm the findings.

References

1. National Pesticide Information Centre. Medical Case profile. Inhalational Risks from Phosphide Fumigants [Internet]. [cited 2015 Jun 6]. Available from: http://npic.orst.edu/mcapro/Phosphine.pdf.
2. Wahab A, Zaheer MS, Wahab S, Khan RA. Acute aluminium phosphide poisoning: an update. Hong Kong J Emerg Med. 2008; 15(3): 152–5.
3. Proudfoot AT. Aluminium and zinc phosphide poisoning. Clin Toxicol. 2009 Feb; 47[2]: 89-100.
4. Mehrpour O, Jafarzadeh M, Abdollahi M. A Systematic Review of Aluminium Phosphide Poisoning. Arch Ind Hyg Toxicol [Internet]. 2012 Jan 1 [cited 2015 Jun 6];63[1]. Available from: http://www.degruyter.com/view/j/aiht.2012.63.issue-1/10004-1254-63-2012-2182/10004-1254-63-2012-2182.xml.
5. Raina A, Shrivastava HC, Mathur N, Dogra TD. Validation of qualitative test for phosphine gas in human tissues. Indian Journal of Experimental Biology.2003; 41: 909-11.
6. DFS Manual of Toxicology.1st ed. Directorate of Forensic Sciences, MHA, Govt of India.Selectiveand Scientific Publisher: New Delhi; 2005: 218.