Original Paper

Quantitative Estimation of Zinc Using Trace Metal Analyzer in Zinc Phosphide Poisoning Cases

Anita Yadav, Hari Prasad, Adarsh Kumar*, AK Jaiswal, DN Bhardwaj

ABSTRACT

India is predominantly an agricultural country and therefore use of zinc phosphide (ZnP) as a rodenticide is frequent. Its misuse as a suicidal agent is consequently often reported. The present study was undertaken to quantitatively estimate the value of zinc in ZnP poisoning deaths autopsied in the mortuary of All India Institute of Medical Sciences, New Delhi, India.

Out of a total 2734 medicolegal autopsies conducted during the period from January 2010 to November 2011, 124 cases (4.5%) were reported as suspected poisoning, of which 8 cases were brought with alleged history of ZnP poisoning. Since the cause of death is usually certified on the basis of police inquest proceedings, postmortem findings and presence of phosphine in viscera during toxicological examination, a particular case may be reported as a false positive, since putrefied viscera also tests positive. Therefore, cause of death remains inconclusive in many cases.

In the present study, hospitalized deaths with alleged history of zinc phosphide poisoning were included, and estimation of zinc was done using trace metal analyzer (TMA) in different biological samples – blood, heart, kidney, liver, stomach contents and intestinal contents. By comparing the values with the quantities in control samples it was found that quantitative estimation of metal (zinc) in ZnP poisoning provides significant results.

Key Words: Zinc phosphide; ZnP; Phosphine; Chemical analysis; Trace metal analyzer; TMA

INTRODUCTION

Poisoning has always been an important cause of mortality worldwide. Poisoning can occur through various modes of exposure, including swallowing unknown, unlabeled substances. Modern drugs enter the global market in an ever increasing number via pharmaceutical companies.[1] The term 'poison' is generally used to refer to any substance which when ingested, inhaled or absorbed or when applied to, injected into, or developed within the body in relatively small amounts, by its chemical nature causes damage to the structure or functions of the body. The term 'poisoning' refers to the morbid condition produced by a poison, which may range from mild symptoms to fatal illness.[2] Poisons have always played a dominant role in literature, history, romance and crime. The uniqueness of 'poison' lies in the fact that it is a silent, subtle weapon that can be readily administered without violence or suspicion.[3] Poisoning occurs in all age groups and can be accidental, suicidal or homicidal.

The most common mode of poisoning is generally due to household agents, followed by drugs, agricultural pesticides, chemicals, poisonous plants and animal bites or stings. Certain groups of poisons are prevalent in a particular geographical area and socio-economic class depending upon the ease of availability, knowledge and intention.[4]

Dept of Forensic Medicine & Toxicology, #315, New Forensic Wing, All India Institute of Medical Sciences, New Delhi.

*(*Author for correspondence*): E-mail: dradarshk@yahoo.com

Zinc phosphide, which is a readily available rodenticide in India, is a dark grey, crystalline powder or pellet.[5] It is sold under various trade names, including Arrex, Commando, Denkarin Grains, Gopha-Rid, Phosvin, Pollux, Ridall, Ratol, Rodenticide AG, Zinc-Tox and ZP. Zinc phosphide has the chemical formula Zn_3P_2 containing 75% of zinc phosphide and 25% of antimony potassium tartrate, an emetic to cause vomiting if the material is accidentally ingested by humans or domestic animals. Accidental or intentional ingestion or inhalation of the dust gives rise to fatal poisoning in humans. Four to five grams of zinc phosphide may be fatal. Toxicity is due to liberation of phosphine gas. Zinc phosphide is insoluble in water but soluble in the hydrochloric acid of the stomach, which results in the production of highly toxic phosphine gas. The reaction is as follows[6]:

$$Zn_3P_2 + 6HCl \rightarrow \uparrow 2PH_3 + 3ZnCl_2$$

Siwach SB et al conducted a study at PGIMS, Rohtak, Haryana, and reported 559 cases of acute poisoning during March 1992 to May 1993.[7] Of these, 91.4% cases were due to self-poisoning (suicidal), 8.1% accidental and 0.5% homicidal. Aluminium phosphide was the most commonly encountered substance, followed by organophosphates and zinc phosphide in 67.8%, 13.9% and 4.3% cases, respectively.

MATERIALS AND METHODS

In this study undertaken from January 2010 to November 2011, all autopsies conducted in the mortuary of AIIMS, New Delhi with alleged history of zinc phosphide poisoning were included, along with 50 control cases without any history of poisoning. The biological samples collected comprised gastric and intestinal contents, blood, liver, kidney and heart. Blood was preserved in sodium fluoride vials, while the viscera were preserved in saturated solution of common salt. The samples were then analyzed using silver nitrate test method to screen for phosphide poisoning.

Reagents and Chemicals: Suprapure acetic acid, nitric acid, liquor ammonia, and sulphuric acid were obtained from Merck. Nitrogen gas of 99.99% purity was used. Micropipettes (Eppendorf) of volume 10–100 microlitre and 100–1000 microlitre were used.

Reagents used for the Preparation of Zinc

1. Preparation of Zn standard: 1000 ppm soln of zinc was prepared by dissolving suitable amount of zinc chloride in 100 ml of ultrapure water, followed by 2–3 drops of nitric acid. 1 ppm of Zn soln was prepared by diluting 1000 ppm soln using N1V1=N2V2 formula.
2. Preparation of buffer solution: Ammonium acetate buffer was prepared by dissolving 5.55 ml of suprapure acetic acid in 10 ml of ultrapure water. Subsequently, 3.7 ml of suprapure ammonia was added slowly and pH was adjusted to 4.6 by adding a few drops of suprapure acetic acid. Finally the volume was made up to 50 ml.

Silver Nitrate Test: All the biological samples were qualitatively analyzed by silver nitrate test for phosphine gas as per standard procedure[8]: 5 ml of biological material was taken in a conical flask filled with a guard tube containing lead acetate soaked cotton. Few ml of cadmium sulphate solution was added. It was acidified with dilute sulphuric acid. The mixture was heated gently on water bath at 40–60°C. The gas evolved was allowed to come in contact with silver nitrate ($AgNO_3$) paper. It would turn grey or yellowish brown or black due to reaction of phosphine with silver nitrate solution. The sample which did not qualify the test was excluded from the study other than the control cases.

Digestion Procedure: Samples were digested in a microwave digestion system (680 Microwave Digestion System from Aurora Instruments) (**Fig 1**). The vessels of microwave digester were cleaned thoroughly by nitric acid and water mixture (1:1) and dried. One ml or 1 gm of the biological sample was transferred into the liner vessel and 15 ml of 34.5% was added to each vessel and left for a few minutes to outgas. Vessels were loaded in the microwave digester oven and the machine was run on the temperature program as given in **Table 1**. The

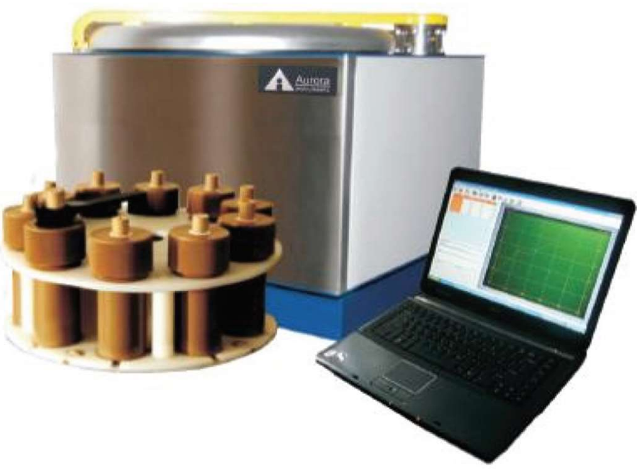

Fig 1: Microwave digestor
Aurora Instruments Ltd, Canada Model - MW 680

Table 1 Program for digestion

Steps	Time (in sec)	Starting Temp (ºC)	Ending Temp (ºC)
1.	210	28	100
2.	600	100	160
3.	600	160	170

samples were then cooled down and opened in a fumehood and transferred to 50 ml volumetric flask, and with the help of ultrapure water made up to the mark.

Trace Metal Analyzer: Voltametric determination of zinc was performed by Trace Metal Analyzer (Model 797 VA Computrace from Metrohm) (**Fig 2**). This is a three-electrode system consisting of mercury as working electrode, platinum as auxillary electrode, and potassium chloride as reference electrode.

Fig 2: Trace metal analyzer
Make - Metrohm, Switzerland. Model – 797VA

Procedure for Zinc Analysis: 10 ml of ultrapure water and 1 ml of buffer of pH 4.6 was taken in a polarographic vessel and measurements were started. The voltamogramme of the blank was recorded. 0.1 ml of the prepared sample solution was added to the polarographic vessel and then voltamogramme of the sample solution was recorded under the same conditions. Subsequently, 0.1 ml of 1 ppm zinc standard was added twice, and then voltamogramme of the standard was recorded. Finally the concentration of the metal was calculated by linear regression method using the following formula:

$$\text{Final Results} = \text{Concentration} \times \frac{\text{Cell volume}}{\text{Sample amount}} \times \frac{\text{Multiplier}}{\text{Divisor}}$$

Where, Multiplier = dilution and Divisor = sample amount taken for preparation[9]

RESULTS AND DISCUSSION

Out of a total of 2734 medicolegal autopsies conducted during January 2010 to December 2011, 124 cases were of alleged poisoning, constituting about 4.5% of the total autopsies conducted during this period. Of the 124 fatal poisoning cases, 8 were due to zinc phosphide poisoning, which constituted about 6.4% of all poisoning deaths. The maximum quantity of zinc was found in the stomach contents, i.e., 1619.85 mcg/dl in control and 6380.05 mcg/dl in cases of zinc phosphide poisoning, followed by intestinal contents (1492.66 mcg/dl in control and 6006.14 mcg/dl in cases) and blood (1067.2 mcg/dl in control and 6018.36 mcg/dl in cases). In the liver, it was 884.79 mcg/dl in control and 4299.67 mcg/dl in cases, while in the heart it was 678.05 mcg/dl in control and 3714.15 mcg/dl in cases, and in the kidneys it was 820.52 mcg/dl in control and 2898.20 mcg/dl in cases (**Table 2**).

Bhadkambekar et al in 2008 studied zinc as a marker element in the viscera of suspected metal phosphide poisoning.[10] Neutron Activation Analysis (NAA) was employed to detect and quantify the concentration of zinc in the viscera/stomach. The methodology was developed on simulated and real life viscera samples to quantify the amount of zinc using NAA. The results obtained by NAA for real-life were validated using a complementary analytical technique, viz differential pulse anodic stripping voltammetry, and the values obtained were in good agreement (within +/- 5-8%). They opined that this method could be useful in the medicolegal field for framing a definitive opinion about zinc phosphide poisoning.

Table 2: Mean values of zinc in various biological samples of controls and cases analyzed using trace metal analyzer

S.No	Type of biological sample	Quantity obtained in control cases (mcg/dl)	Quantity obtained in zinc phosphide poisoning cases (mcg/dl)
1.	Stomach contents	1619.85	6380.05
2.	Intestinal contents	1492.66	6006.14
3.	Blood	1067.2	6018.36
4.	Liver	884.79	4299.67
5.	Heart	678.05	3714.15
6.	Kidney	820.52	2898.20

CONCLUSION

It is well known that metal phosphides are cheap, highly lethal, and easily available in our country. These are widely used as grain preservatives by farmers and less-educated people. Now its misuse as an agent of suicide is also increasing in the rural areas of mostly less-educated people. Quantitative estimation of metal in phosphide poisoning needs to be done to opine the speculated poisoning conclusively. This study provides a reference value about quantitative level of zinc in normal population as well as in deaths due to zinc phosphide poisoning. We found almost 5–6 times increase in zinc level of various viscera in fatal cases. Further, our study also provides information about the biological sample in which maximum increase in the level of zinc is expected, which is blood. This indicates that blood is the best sample for quantitative analysis of zinc when opining about cause of death or any medicolegal query relating to zinc phosphide poisoning.

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