Study of Renal Histopathology In Case of Celphos Poisoning by SEM

Abstract: Nearly 30,000 people die every year because of pesticide poisoning, worldwide. In rural areas, death due to agricultural poisons is quite common especially Celphos. It is one of the compounds of Phosphorus, which is naturally produced along with other disagreeable substances during the decay of the animal bodies. The death may be immediate or can be delayed for days or weeks. The symptoms present in the case of poisoning are more or less similar to the presentable symptoms of poisoning due to some other poison also and mimics those of upper respiratory tract infection. The determination of exact cause of death needs to be studied at organs and tissues level for studying the detailed effect. The histopathological findings in kidney are congestion and necrosis along with degeneration and regeneration of tubular epithelium.

Keywords: Renal toxicity, tubular necrosis, CRRT, DIC.

INTRODUCTION

According to Mehrpour et al., nearly 30,000 people die every year because of pesticide poisoning, worldwide. Out of these, most common pesticide agents are organophosphates and phosphides, Aluminium Phosphide in particular. In the rural areas, death due to agricultural poisons is quite common especially Celphos. Metal phosphides make a large proportion of the pesticides that are being used currently. It is a well-known and highly effective outdoor and indoor insecticide and rodenticide. The highest incidence of poisoning was reported in case of household agents followed by drugs and agricultural pesticides. The phosphate gas released after oral intake of AlP is mainly excreted by kidneys and lungs (Mehrpour, O. et al., 2012).

The fate of Aluminium Phosphide inside the body is slightly different depending on the contents of the stomach at the time of ingestion. After ingestion, phosphate is released due to contact between AIP and water/ acid in the Gastrointestinal tract, whereas some Phosphide may be absorbed by the Gastrointestinal tract without hydrolysis and get converted to phosphate (Hashemi-Domeh, B. et al., 2016). As per the article, Validation of qualitative test for phoshpine gas in human tissues, Phosphine is not new to science and modern chemistry. It is one of the compounds of Phosphorus, which is naturally produced long with other disagreeable substances during the decay of the animal bodies and has fishy or garlicky odour due to presence of substituted Phosphine’s (Raina, A. et al., 2003).

The preceding symptoms, as well as the biochemical and physiological changes that occur in response to phosphate exposure, can be grouped into three possible categories of phosphate action neural, metabolic, and redox related. According to Mechanism of Phosphine toxicity, Phosphine initially causes agitation followed by convulsions in humans followed by lethargy. Acetylcholine is an excitatory neurotransmitter and the role of the esterase is to attenuate acetylcholine signalling, exposure to phosphate would be expected to inhibit the attenuation. The net result would be overactive acetylcholine signalling, which would most likely be expressed as hyperactivity and in extreme cases, excitotoxicity (Nath, N. S. et al., 2011).

According to Acute Aluminium phosphide poisoning; an update, ingestion is usually suicidal in intent, uncommonly accidental and rarely homicidal due to its offensive odour (Wahab, A. et al., 2008).
The kidney

The kidneys are a pair of bean-shaped organs on either side of spine, below the ribs and behind your belly. The kidneys’ job is to filter blood, remove wastes, control the body’s fluid balance, and maintain the electrolyte balance of the body. All of the blood in the body passes through them several times a day (Hoffman, M. 2020).

The primary route in which the body eliminates substances is through the kidneys. The main function of the kidney is the excretion of body wastes and harmful chemicals into the urine. The functional unit of the kidney responsible for excretion is the nephron. Each kidney contains about one million nephrons (Urinary Excretion).

The pathology of kidney

The most important diseases of the urinary system are
• Developmental disorders
• Glomerular diseases
• Vascular kidney diseases
• Neoplasms
• Infectious diseases

The most important disorders which produce typical histopathological changes are polycystic kidney disease and multicystic renal dysplasia, and are included under developmental disorders.

Glomerular diseases arise from immunological injury, metabolic disorders, and circulatory disorders. The most common cause of renal injury and renal failure in the elderly is hypertension. It is most commonly manifested in the form of acute tubular necrosis as a result of complication of renal hypoperfusion. Neoplasm is more common in case of malignant tumours compared to benign neoplasms. Infectious diseases arise mainly due to bacterial infections, reaching the kidney hematogenously (Damjanov, I. 2012).

Case presentation

In the present, study a woman aged 25 years had consumed Aluminium Phosphide kept in her house for preservation of wheat. It came to notice of family members after she started vomiting and complained of pain in throat which has been reported in case of acute Aluminium phosphide poisoning by A wahab et al.,(2008) She was rushed to the hospital. Along with classical presentations of poisoning, the patient presented with garlic like odour from mouth and nostrils. On further examination, it was noted that patient had bradycardia, hypotension and breathlessness. She was in gasping state at the time of admission to hospital. Gastric lavage was done with 1:5000 Potassium Permanganate. The patient was kept on respiratory support along with symptomatic management i.e. positive pressure ventilation as it inhibits cytochrome oxidase which is a respiratory enzyme, parenteral proton pump inhibitors and Intra-Venous Magnesium Sulphate to control cardiac Brady arrhythmias. It proved to be a respite for her. Toxic effects of phosphine and the metal phosphides on the kidneys are not rare and may be delayed. Patient survived for 18hrs. and then died.

According to study by Wilson et al., renal symptoms were not prominent. Urinalyses of 30 patients revealed abnormalities in 8, usually as microscopic hematuria and bile in urine, but all survived due to cardiovascular and pulmonary toxic effects (Wilson, R. et al., 1980).

Misra et al., found acute renal failure (Misra, U. K. et al., 1988; & Wahab, A. et al., 2008) along with anuria developed with blood urea. The serum creatinine level marked a rise on the second day of admission. The persistent anuria and uraemia resulted in peritoneal dialysis and the patient died after 72 hours of admission due to hepato-renal failure and ventricular tachycardia (Misra, U. K. et al., 1988).

Similar studies by Chopra et al., reported proteinuria which gradually disappeared over 10 days along with renal failure in the end (Chopra, J. S. et al., 1986).

As per Chugbet et al., Plasma Renin Activity is increased in shock due to AIP poisoning. An initially high PRA continued to rise, probably due to slow release of toxic phosphine gas, which was detected by a positive silver nitrate paper test. This rise was directly proportional to the dose of AIP consumed and there was a direct relationship between mortality and an increased PRA. The authors concluded that angiotensin converting enzyme inhibitors may have a role in combating shock in AIP poisoning (Chugh, S. N. et l 1989).

Metabolic and Electrolyte Disturbances:

According to A Review of aluminium phosphide poisoning and a flowchart to treat it, the electrolyte abnormalities in the patients of Celphos poisoning cases is manifested in the form of high or low sodium, potassium and magnesium (Hashemi-Domeneh, B. et al., 2016).

The above features are not uncommon in AIP poisoning. Hypokalaemia is quite common soon after ingestion of metal phosphides and is secondary to vomiting. It can be contributed to by catecholamine’s release. Other prominent features are metabolic acidosis or mixed metabolic acidosis and respiratory alkalosis and acute renal failure are also frequent. This can be attributed to acute adreno-cortical insufficiency resulting in impaired production of aldosterone which
regulates sodium conservation and water retention (Mehrpour, O. et al., 2012).

It was evident that AIP can also alter glucose homeostasis. Also, blood glucose was found to be higher in people who died of AIP poisoning than in those who survived (Mehrpour, O. et al., 2012).

The cases of severe and persistent hypoglycaemia have been reported by (Chugh, S. N; & Chugh, S. N. et al., 1989). It has been thought to be possibly secondary to adrenal gland damage and low circulating cortisol concentrations and low circulating cortisol concentrations (Chugh, S. N. et al., 1989) and impaired gluconeogenesis and glycogenolysis is and glycogenolysis is by Kalra G S et al., (1991), whereas hyperglycaemia has been reported by Rahman, H. A. (1999) and Shadnia, S. et al., (2005).

In a case reported by R G Bogle et al., a woman remained previously well and then hypotensive after ingesting AIP mixed with water. Further the clinical presentation marked worsening hypoxia and metabolic acidosis resulting into death (Bogle, R. G. et al., 2006).

**MATERIAL AND METHOD**

The sample of kidney was collected from the mentioned case, brought to Autopsy room of IMS, BHU. The brief history of previous medications and substance abuse was noted which could interfere the study. The collected sample was preserved in 10% formaldehyde and refrigerated at 4°C. Fixed samples were taken for SEM analysis to CDRI Lucknow. They were treated with 1% osmium tetra oxide for 1 hour. Traces of Osmium tetra oxide were removed by washing the samples with PBS buffer. The samples were dehydrated through graded series of Alcohol (30%, 50%, 70%, 90% and finally absolute) and later subjected to Critical Point Dehydration. Samples were mounted over Aluminium stubs and coated with film of Gold-Palladium using sputter coat unit. Processed samples were examined under SEM for imaging.

A number of micrographs were taken of single kidney sample from different viewing angles focusing a particular area at different magnifications.

**RESULT**

During autopsy, on gross examination, all organs are congested. However, the histopathological examination reveals distinct pathology in major organs.

Fig 1 and 2 are gross images of the kidney taken at the time of autopsy. Figs 3-8 are SEM micrographs of kidney in case of Aluminium phosphide poisoning.

![Figure 1](image1.jpg)

**Figure 1.** The human kidney in case of Aluminium phosphide poisoning at the time of autopsy showed overall congestion along with areas of necrosis. Petechial haemorrhages present on the surface can be noted.

![Figure 2](image2.jpg)

**Figure 2.** The dissected kidney showing internal structures at the time of autopsy. Intravascular coagulation due to internal haemorrhage and bleeding diathesis can be observed.
Figure 3. Cross-section of renal tubular epithelium. The section shows inner region of cortex near the medulla, along with glomerulla.

Figure 4. Portion of a cortex of human kidney. This image shows urinary space, glomerulus and necrotic renal tubular epithelium.

Figure 5. The details of a renal corpuscle in the cortex of the human kidney. Oocytes covering the capillary loops of the glomerulus.
**Figure 6.** Photomicrograph showing cloudy swelling of renal tubular epithelium with areas of tubular degeneration in the cross-section of cortex of human kidney.

**Figure 7.** Details of renal corpuscles in the cortex and medulla of glomeruli with necrosis of medullary part of inner tubular epithelium with process of podocytes at spaces. Endothelial cells of glomerular capillaries and urinary spaces of renal corpuscles.

**Figure 8.** Details of a portion of cortex of human kidney showing areas of acute tubular necrosis and tubular degeneration along with convoluted tubules.

**DISCUSSION**

Fig 1 is the gross photograph of human kidney in case of Celphos poisoning taken at the time of autopsy. Overall congestion. Similar finding was reported by Jain A K et al., (2005).

Fig 2 is the photograph of dissected kidney in case of AIP poisoning. Congestion was well marked. Similar findings have been reported by A k Kapoor *et al.*, (2005).

According to Verma V K *et al.*, wide spread capillary damage led to bleeding diathesis, Disseminated Intravascular Coagulation (DIC) and acute tubular necrosis (2001) as had also been reported by Mehrpour *et al.*, (2012). Shock and DIC led to terminal renal
failure. In study conducted by Jain A K et al., necrosis along with degeneration of tubular epithelium had been observed. On dissecting adrenals haemorrhagic necrosis have been found in which fat depletion in patches had been found in 33% cases (2005).

Fig 3 is the micrograph at 40X. It shows cross section of renal tubular epithelium. In study conducted by Mehrpour et al., acute tubular necrosis had been reported in case of AlP poisoning (Mehrpour et al., 2012).

Fig 4 is micrograph at 800X. It shows Portion of a cortex of human kidney showing urinary space, glomerulus and necrotic renal tubular epithelium. The kidney displays two distinct structural arrangements. The parts convolute which contains glomeruli in renal corpuscles and many cross sections of convoluted tubules and the pars radiata which contains longitudinal sections of straight tubules. In the image, a cross section of an arcuate artery is seen in the upper left and radial cortical artery is seen in the centre of the image (Curran, R. C., & Crocker, J. 2005).

Fig 5. It is micrograph at resolution 800 X. The details of a renal corpuscle in the cortex of the human kidney are evident in the micrograph. The image also shows the Podocytes covering the capillary loops of the glomerulus (Curran, R. C., & Crocker, J. 2005). Areas of necrosis were evident which was consistent with the studies made by A K Kapoor et al., (2005). According to Mehrpour et al., eccentric nucleus in the cortex and congestion within glomerulus and intra parenchymal part of the kidney had been reported (Mehrpour et al., 2012).

Fig 6. Micrograph at 1500 X showing the cross section of human kidney in case of Celphos poisoning. Details of the surface capillaries in glomeruli in renal corpuscles is evident in the image. Along with it, the primary, secondary and tertiary processes of podocytes covering the capillary surfaces can also be seen. Tubular degeneration had been reported by Mehrpour, O. et al., (2012).

Fig 7. The micrograph at a resolution of 1500 X shows the overall cloudy swelling of renal tubular epithelium with areas of tubular degeneration in the cross section of cortex of human kidney. In the image a portion of the cortex is seen. The renal corpuscle surrounded by a number of convoluted tubules are evident (Curran, R. C., & Crocker, J. 2005). Degeneration, infiltration and tubular dilation were marked which had also been reported by Sinha U S et al., (2002).

Fig 8. It is SEM image at a resolution on 3000X of human kidney in case of death due to Celphos poisoning. It shows the cortex of human kidney. The micrograph shows pars convolute of the cortex containing renal corpuscles with glomeruli and cross section of proximal and distal convoluted tubules. The portions of pars radiate regions of the cortex are seen running along both sides of the pars convolute (Curran, R. C., & Crocker, J. 2005). Tubular degeneration and renal damage is evident as reported by Sinha U S et al., (2002).

In the victims of all survival groups, tubular necrosis in kidney was a common finding, which was consistent in the victims for longer survival period. The glomeruli generally appeared normal and showed mild congestion in few cases. Another important finding in kidney was intratubular haemorrhage with RBC within tubules. The same finding has been reported by Mishra UK et al., in occupational phosphine exposure in Indian workers (Misra, U. K. et al., 1988). Wilson et al., reported microscopic hematuria in Acute Aluminium Phosphide poisoning abroad a grains freighter—epidemiologic clinic and pathologic findings (Wilson, R. et al., 1980). The cause of tubular haemorrhages can be attributed to be the result of toxic nephritis in AlP poisoning; the periodic Schiff staining of kidney has demonstrated both ischemic as well as toxic acute tubular necrosis showing tubular necrosis with both disrupted as well as intact basement membrane. The ischemic tubular necrosis can be explained on the basis of cardiovascular collapse/failure added with respiratory depression, which is evident in case of AlP poisoning cases.

Irrespective of survival period, congestion was present in all organs, including kidneys. Petechial haemorrhages present on the surface were reported during autopsy in case of AlP poisoning. Surface haemorrhagic spots can be explained on the basis of free radical mediated micro vascular damage, as reported by Chugh et al., (1989).

Tripathi et al., has reported haemorrhage as a result of peripheral capillary leak and increased hydrostatic pressure within vessel due to congestion due to cardiovascular failure which is root cause of death in AlP poisoning (Tripathi, S. K. et al., 1992).

According to VK Verma et al., AlP affects glucose metabolism leading to hypoglycaemia in addition to the effect on the renal cortex. The above changes along with hyperglycaemia can be attributed to the wide variety of changes in Mg$^{2+}$, Ca$^{2+}$, phosphate, citrate or cortisol levels which act as active stimulatory or inhibitory modulators to enzymes and hormones that catalyse and regulate glucose metabolism (Verma, V. K. et al., 2001).

In 1989, Singh and his co-workers demonstrated that serum Magnesium concentrations were increased, possibly secondary to release from damaged cardiac myocytes and hepatocytes and these findings were confirmed in subsequent studies (Singh,
Although no antidote is available for Celphos till date, the study conducted by Prashant Nasa et al., in the case report Use of continuous renal replacement therapy in acute aluminium phosphide poisoning: a novel therapy, there has been presentation of two cases of acute Aluminium Phosphide poisoning in shock after approximately 3hr. of ingestion. Along with other resuscitative measures, Continuous Renal Replacement Therapy (CRRT) started earlier, proved to be life-saving. CRRT helped in maintaining metabolic milieu and resolution of shock state till AP got excreted (Nasa, P. et al., 2013).

Conclusion
As there are ominous indications of gradual increase in the number of cases being reported for celphos poisoning, it is the demand of the time to explore more for at least having a check to the deaths caused by it. The death is not always suicidal but accidental also when the person is unaware of the toxicity of Celphos or the Phosphine released by it. It being used as a preservative for grains makes the situation more alarming for country like India, where agriculture is one of the occupations. This work in the field of forensic Nano-technology can be a road map to the field of research for making antidotes which is not available till date. The recent approach of using alternative and resuscitative measures along with CRRT which uses haemodialysis has proved to be fruitful but needs further research.

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Author’s contribution
Both authors performed or assisted the autopsy. Both authors were involved in writing of this manuscript. Both authors read and approved the final manuscript. Ethics approval and consent to participate. The present study was approved by “Institutional Ethical Committee” of Institute of Medical Sciences, Banaras Hindu University, Varanasi. All the information has been taken under consideration of medical Ethical committee.

Reference
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