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Histopathological study of Lungs in case of Aluminium Phosphide poisoning by SEM

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Abstract: *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 phosphine gas released after oral intake of AlP is mainly excreted by kidneys and lungs. So, the effect on lungs is quite evident and needs to be explored.*

Keywords: *Celphos, Aluminium phosphide, Scanning Electron Microscope(SEM), ARDS-Acute Respiratory, Distress Syndrome, Atelactasis.*

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I. Introduction

According to review article, A review of Aluminium phosphide poisoning and a flowchart to treat it, by Behrooz Hashemi-Domeneh et al, the use of pesticides has been increasing in recent years and has increased the quality and quantity of agricultural products. Metal phosphides make a large proportion of the pesticides that are being used currently. The compounds of phosphides among pesticides are mainly those of Aluminium, magnesium and calcium. The reason behind the popularity lies in the fact that they are potent, cheap and have no undesirable effects on the agricultural products.[1]AlP has been used as pesticide since 1940s and the incidence of poisoning. According to review article, Aluminium Phosphide Poisoning : A challenge for the Physician, phosphine is rapidly absorbed from stomach or lungs by simple diffusion, oxidized slowly and excreted in urine as hypophosphie and also exchanged through lungs unchanged. The toxic effects appear 1-60 min after ingestion.[2] According An update on toxicology of Aluminium phosphide by Ali Akbar Moghadamnia, to the phosphine gas released after oral intake of AlP is mainly excreted by kidneys and lungs. [3]So, the effect on lungs is quite evident and needs to be explored.

Lungs

According to Human Anatomy by Matthew Hoffman,The lungs are a pair of spongy, air-filled organs located on either side of the chest (thorax). The inhaled air enters into the lungs through trachea its tubular branches, called bronchi. The bronchi then divide into smaller and smaller branches (bronchioles) and finally end in clusters of microscopic air sacs called alveoli. In the alveoli, gaseous exchange takes place, i.e. Oxygen from the air is absorbed into the blood and Carbon dioxide, travels from the blood to the alveoli, where it can be exhaled. Between the alveoli is a thin layer of cells called the interstitium, which contains blood vessels and cells that help support the alveoli.

The lungs are covered by a thin tissue layer called the pleura. The same kind of thin tissue lines the inside of the chest cavity -- also called pleura. A thin layer of fluid acts as a lubricant allowing the lungs to slip smoothly as they expand and contract with each breath.[4]

The lung toxicity

Irritant gases interfere with the normal respiratory process and obstruct the respiratory pathway, leading to suffocation and difficulty in breathing. Further, hypoxia leads to various microscopic changes. Irritant gases including chlorine, hydrochloric acid, ammonia, nitrogen oxides, sulfur oxides, sulfur or nitrogen mustards, and phosgene follow a similar pathologic process and time course of disease onset in humans. Where the inhaled irritant gases indicate the time course between causal chemical exposures and development of clinically significant symptoms upto a few months, the action of phophine released from Celphos is instantaneous. The

mechanism of toxic action exerted by irritant gases generally involves widespread and severe injury of the epithelial lining of the bronchioles that leads to acute respiratory symptoms which can include lung edema within days. Risk of BO from irritant gases is driven substantially by toxicokinetics affecting concentrations occurring at the bronchiolar epithelium. Highly soluble irritant gases that cause BO generally follow a threshold-dependent cytotoxic mechanism of action that at sufficiently high doses results in severe inflammation of the upper respiratory tract and the bronchiolar epithelium concurrently. This is followed by acute respiratory distress, pulmonary edema, and post inflammatory concentric fibrosis that become clinically obvious within a few months. Phosphine is heavier than air and less soluble in water. But after taking Aluminium Phosphide pellets, the phosphine liberated interferes with the respiratory pathway leading to severe effects at macroscopic and microscopic level. As a respiratory poison, it affects the transport of Oxygen or interferes with the utilization of oxygen by various cells in the body. Exposure results in pulmonary edema.(Wikipedia)

According to review article, A review of Aluminium phosphide poisoning and a flowchart to treat it, by Behrooz Hashemi-Domeneh et al, the most common respiratory signs and symptoms are tachypnoea, dyspnoea, crepitation and rhonchi.In adult patients, Respiratory Distress Syndrome and pulmonary edema are common. Accumulation of bloody or full-protein liquids in the pleural space is also evident.

The chemistry of Phosphine

Phosphine is a reducing agent that can complex with metal ion cofactors at the active site of enzymes. It forms the basis of phosphine-mediated inhibition of enzymes such as cytochrome c oxidase and catalase. As a reducing agent, phosphine may also reduce disulfides. Its capacity to reduce a disulfide was reported in 1970, but the reaction was found to be extremely slow in a neutral solution . Recently, direct redox interaction between phosphine and cysteine at the reactive disulfide of glutathione reductase was proposed to be the mechanism by which phosphine inhibited the enzyme.

Phosphine is strongly thermodynamically favoured to act as a reducing agent [9, 59]. So, Phosphine itself (or a proposed but highly unstable hydroxy phosphine derivative) [10] is the toxic form of the element. The more oxidised oxyacids, hypophosphite, phosphite, and phosphate are not toxic. Thus, while phosphine may act on cysteine residues *in vivo*.

It has been noted that fumigation moderately induces superoxide dismutase, but inhibits the activity of peroxidase and catalase [29, 46, 48]. The net effect is the enhanced conversion of superoxide to hydrogen peroxide by the enzyme superoxide dismutase, but lack of detoxification of the resulting hydrogen peroxide by conversion to water via catalase or peroxidase. Phosphine has also been shown to react chemically with hydrogen peroxide to generate an even more reactive oxygen species, the hydroxyl radical [49

Effect of ingestion of Aluminium Phosphide

According to the article Acute Alyminium phosphide poisoning: an update, by A Wahab et al, AlP causes widespread organ damage due to cellular hypoxia. Involvement of the respiratory system may lead to dyspnoea, which may further progress to type I and II respiratory failure. According to An update on toxicology of Aluminium phosphide by Ali Akbar Moghadamnia, Phosphine inhibits mitochondrial Cytochrome oxidase and cellular oxygen utilization. According to the article, Management of Celphos poisoning with a novel intervention: A ray of hope in the darkest of clouds, toxicity is charecterised by chest tightness, cough and shortness of breath. Severe exposure can lead to accumulation of fluid in the lungs. A number of patients present the symptom of respiratory distress, sometimes alone or in combination with other organ dysfunction.

According to review article, A review of Aluminium phosphide poisoning and a flowchart to treat it, by Behrooz Hashemi-Domeneh et al,after inhalation of small amounts of phosphine, patients experience respiratory tract irritation,and dyspnoea. Tightness in chest, headache, nausea and vomitingare other manifestations. In some cases numbness accompanying ataxia,paraesthesia, tremor, muscle weakness and diplopia has also been seen. Inhalation of higher amount of gas can lead to ARDS.

II. Material And Methods

The sample of lungs was collected from the dead body of a person who had committed suicide by consuming Celphos. The mentioned case was 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 4^0 C for 30 days. 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 lungs sample from different viewing angles focusing a particular area at different magnifications.

III. Result

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

Fig 1 and 2 are gross images of the lungs taken after autopsy. Figs 3-7 are SEM micrographs of lungs in case of Aluminium phosphide poisoning.

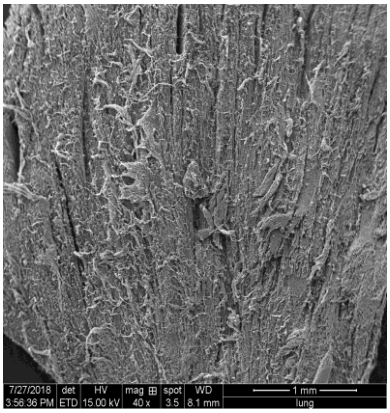

Figure 1. The micrograph showing epithelial lining of wall of bronchiole of human lung.

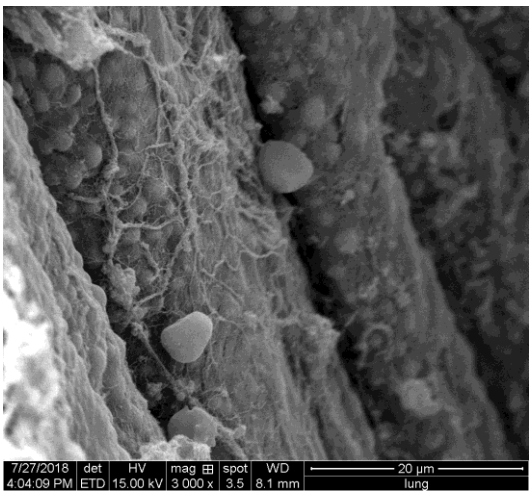

Figure 2magnified view of alveolus showing subtle ridges on the surface of the alveolar lining with round shaped lymphocytic and macrophages(infiltration).Edema along with necrosis is also visible.

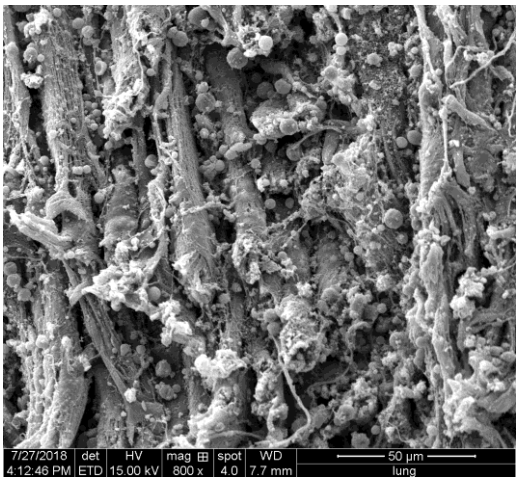

Figure 3 Micrograph showing diffused vessel injury with edema and atelectasis. The areas of necrosis and edema are evident.

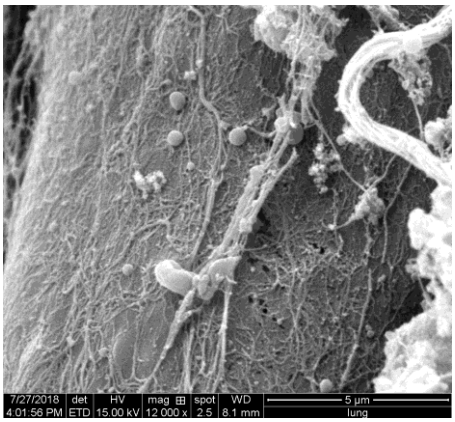

Figure 4. Highly magnified micrograph of epithelial lining of an alveolus.Necrosis is marked at various sites with desquamated respiratory epithelium

IV. Discussion

According to Aluminium Phosphide Poisoning Autopsy Findings, by Ashok Kumar Jain et al, congestion and edema are the foremost features observed in lungs on gross examination.[11]

Fig 1 is the micrograph of lung at a magnification of which showed general edema. The clinical presentation resembled to that of partially collapsed lung. In case of collapsed lung, the space between the wall of chest cavity and the lung itself fills with the air, causing all or a portion of the lung to collapse.It is called pneumothorax. Air usually enters this space called the pleural space, through an injury to the chest wall or a hole in the lung.(emedicinehealth.com).[12]According to Atlas of histopathology, by Ivan Damjanov, the thickening of alveolar walls is also evident which results in reduction in volume of alveolar air space.[13]

Fig 2 is the micrograph of lung showing interior of an alveolus with the subtle ridges on the surface of the alveolar lining. These indicate underlying capillaries and are covered by type I peumocytes. Disease which either reduce the number of alveoli or lead to fibrosis and thickening of the alveolar walls and the blood-air barrier greatly reduce the efficiency of gas exchange, which is also evident in fibrotic lung or emphysemic lung. .[14]. According to " wheat pill(Aluminium Phosphide) Poisoning" , Lungs show alveolar thickening and dilated capillaries in case of Aluminium Phosphide poisoning.[15]

Fig 3 is the photomicrograph of alveolus showing Pneumocyte type II cell are are evident. The increase is number of macrophages is evident. The ridge indicates an underlying capillary. The intercellular junction between two alveolar cells shows edematous appearance. According to Atlas of Histopathology, dilatation of the terminal air spaces is also associated with chronicinflammation of the bronchi. Macrophage, pneumocyte type II cells are also evident.[14]

Fig 4 is magnified image showing epithelial lining of alveolus. Necrosis is marked at various sites with desquamated respiratory epithelium.The flabbiness of the lining depicts cell death and edema which is also marked in case of partially collapsed lung. The intra-alveolar pore is noticed which shows broadening to an extent. Chronic irritation as a result of obstruction to the respiratoty passage leads to an increase in alveolar macrophages, which release various toxic factors, resulting in induction of fibroblast recruitment in the alveolar wall. Fibroblasts increase collagen deposition leading to fibrosis and thickening of the alveolar walls.[14]According to review article, A Systematic review of Aluminium Phosphide poisoning, alveolar thickening has been observed along with dilated capillaries in lungs in case of AlP poisoning.[16]

The risk of mortality associated with phosphide poisoning in humans ranges between 30% and 100%. According to current figures Aluminium Phosphide is the most common cause of poisoning deaths in India, Sri Lanka, Iran, Oman and Morocco.[1]

According to Aluminium Phosphide Poisoning Autopsy Findings, by Ashok Kumar Jain et al, thickening of alveoli by haemolysed RBC and dilated capillaries has been observed in case of Aluminium Phosphide poisoning.A long with this red hepatisation and round cell infiltration has also been observed.[11]

According to the article, Management of Celphos poisoning with a novel intervention: A ray of hope in the darkest of clouds, the accumulation of fluid in the lungs after exposure can have a delayed onset of 72 hours or more after exposure which is in relevance with the maximum fatal period of AlP. ARDS and exudative pleural effusions can also develop in cases of survival. In initial course of the treatment, patients developed severe pulmonary edema. Afterwards, the oxygen scarcity could not be made up even with the help of ventilator because of very high pressures in the pulmonary alveoli. Increased levels of inflammatory markers i.e. cytokines and interleukins have also been marked in ARDS, which leads to increased capillary permeability. This can be attributed to combined effect of increase in capillary permeability due to global

hypoxia and ARDS.[10] According to review article, Aluminium Phosphide Poisoning : A challenge for the Physician, injury to alveolar capillary membrane by phosphine after inhalation leads to ARDS, which is rare if more than 2-3 tablets have been ingested.[2]

According to the article Aluminium Phosphide poisoning:Effect of correction of severe metabolic acidosis on patient outcome, the patents had shown an increase in respiratory rate. Some of the patients were brought to the hospital in gasping condition. The increased metabolic rate could be attributed to metabolic acidosis. After its correction, no significant change in respiratory rate was observed immediately. Although significant difference in respiratory rate of survivors and non-survivors had been noted between 24-48 hours.[17]

V. Conclusion

The mechanism of phosphine inactivation of acetylcholine esterase is still unknown.Even now the antidote for this fatal poison is not known and it is killing mankind. The initial treatment is based on prognosis if the cause of symptoms following toxicity remains unknown because the initial presentation resembles those of upper respiratory tract infection. Although few lives have been saved in some cases, there still remains a big question mark to the nature of poison, the amount of poison, the time interval between consumption and resuscitation and so on. It can be concluded that more structured trials are needed in this poisoning, because mortality rates are still higher instead of improved studies and efforts in this arena. Also, strict legislations are needed to control sale of Aluminium Phosphide, better it should be sold in designed packs which can liberate phosphine slowly while it should be difficult to ingest it.

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