

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

Manoj Kumar Pathak¹, Rohini²

¹Professor and Head, Department of Forensic Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, U.P., India

²Research Scholar, Department of Forensic Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, U.P., India

Corresponding Author: Manoj Kumar Pathak

Abstract: In the rural areas, death due to agricultural poisons is quite common especially Celphos. Death may occur immediately or get delayed for days or weeks together, where the poisoning may not be the cause of death actually. In such cases, the determination of exact cause of death may be difficult as external appearance may not give any clue or internal examination may even sometimes does not reveal anything on gross examination. 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. 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 histopathological findings have shown congestion in 100% cases and fatty changes in liver in 75% of cases of poisoning due to AIP.

Keywords: Celphos, Aluminium Phosphide(AIP), hepatotoxicity, steatosis, fatty liver, sinusoidal congestion, Scanning Electron Microscope(SEM)

Date of Submission: 04-11-2019

Date of acceptance: 20-11-2019

I. Introduction

According to the review study, a review of Aluminium Phosphide poisoning and a flowchart to treat it by Behrooz Hashemi 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. It is a well-known and highly effective outdoor and indoor insecticide and rodenticide.

As per the review article, An update on toxicology of Aluminium Phosphide by Ali Akbar Moghadamnia, it has been known as suicide poison because of its accessibility and toxicity. The fact becomes more emphasized as it has no specific antidote.^[2]

In countries like Iran, it is banned and used to protect rice. In USA, it has been registered for the indoor fumigation of agricultural compounds, animal feeds etc.^[3] It has been used as a pesticide since 1940s and the accidental cases of fatal exposure of Phosphine gas liberated from AIP have been reported in the literature in 1967 and 1980 from bulk shipment of wheat. This poisoning was not known in India before 1980, the first case being reported in 1981 from MGM medical college, Indore.

Tablets, pellets or compressed discs contain phosphide and other substances such as ammonium carbonate, which after coming in contact with acid in the stomach releases Phosphine even more rigorously.

The effect of Celphos on heart is most prominent and the most evident cause of death in first 24 hours. Its effect on liver becomes important from the fact that the undigested poison goes to the liver and is metabolised later. It is responsible for producing after effects which worsens the situation further.^[4]

According to Devan et al, the phosphine that is not expired out through the lungs may be metabolised to phosphates, hypophosphite and phosphate.^[5] According to Anger et al, the phosphine gas was absent in the blood and urine but present in the liver.^[6] As per the study, A review of Aluminium Phosphide poisoning and a flowchart to treat it by Behrooz Hashemi et al, the most common hepatotoxic findings in patients ingesting AIP tablets are elevated aspartate transaminase and alanine transaminase. Jaundice, if present may be a sign of liver impairment.^[1]

Liver

Liver is the largest mass of glandular tissue in the body and the largest internal organ, weighing approximately 1500g and accounting for nearly 2.5% of adult body weight. Many circulating plasma proteins are produced by and secreted by the liver and it plays vital role in uptake, storage and distribution of both the

nutrients and vitamins from the blood stream. It is also responsible for maintaining blood glucose levels of very low density lipoproteins. Besides, it degrades or conjugates numerous toxic substances and drugs. It is an exocrine gland which produces bile that emulsifies fat.^[7]

The pathology of liver

Liver is the site of many diseases of which some become symptomatic whereas some are diagnosed on autopsy only. The major clinical syndromes of liver disease are hepatic failure, cirrhosis, portal hypertension and cholestasis, all having their characteristic clinical manifestations. Although, there are laboratory tests for their evaluation but liver biopsy remains gold standard for diagnosis.

Drug or toxin induced liver disease

When it comes to drug or toxin induced liver disease, it may be made on the basis of a temporal association of Liver damage with drug or toxin exposure. Reactions may be classified as predictable or unpredictable.

Predictable drug or toxins affect all people in a dose-dependent fashion whereas unpredictable reactions depend upon individual host variations, particularly the propensity to mount an immune response to drug-related antigen.^[8]

According to Aluminium Phosphide Poisoning: A Challenge for the physician by Vijay Kumar Verma et al, in case of AIP poisoning, in blood the phosphine changes the circular dichroic spectra of haemoglobin. The change in Haem-iron leads to conformational changes of the prosthetic group. The level of phosphine in blood is positively co-related to the clinical grades of AIP toxicity and to the dose of the pesticide consumed. The occurrence of intravascular haemolysis with AIP poisoning in a patient with normal G6PD levels is of significance as jaundice in patients with this poisoning is often attributed to the hepatic damage alone.^[9] According to the study, Management of Celphos poisoning with a novel intervention: A ray of hope in the darkest of clouds by Sukhminder et al, death due to acute hepatocellular toxicity and fulminant hepatic failure has also been reported in acute poisoning. On analysing blood gases, combined respiratory and metabolic acidosis are revealed.^[10]

According to Forensic Science Scanning Electron Microscopy and News in this Field by Kotrly M et al. (2009) Scanning electron microscope is an analytical method in the field of forensic science, employed for primary information about unknown samples and materials.^[11] According to the study done by Masaki et al, recent Scanning Electron Microscopic studies made on the liver demonstrate that SEM has advantage over the conventional TEM (Transmission Electron Microscope) in that a wide en face view of the tortuous liver cell plates and sinusoidal walls can easily be obtained and thus has contributed much to the elucidation of the tri-dimensional architecture of Hepatocytes. In human the Kupffer's cell can be clearly discriminated from the endothelial cells under SEM while studying the histology of the liver.^[12]

II. Material And Methods

The sample of heart 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^o 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 single liver 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 liver taken after autopsy. Figs 3-7 are SEM micrographs of liver in case of Aluminium phosphide poisoning.



Figure1.Gross image of the liver in case of Celphos poisoning showing overall congestion along with haemorrhagic spots.

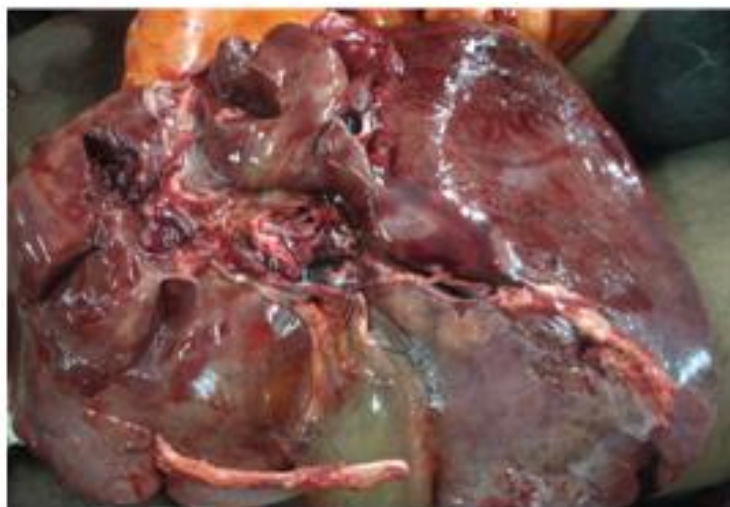


Figure3.Gross image of the under surface of the liver along with gall-bladder showing edema and congestion along with haemorrhagic spots even in porta-hepatis area.

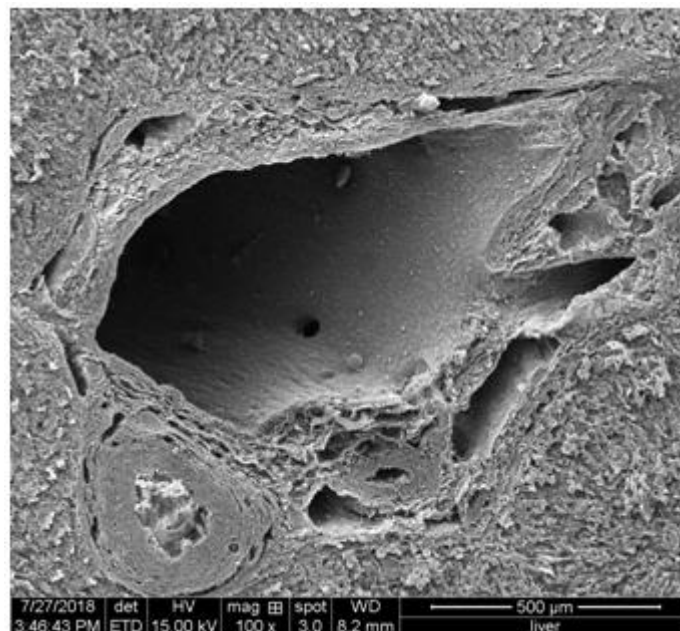


Figure 2 showing cross section of liver lobules, central vein, sinusoid, hepatocytes and opening of sinusoid into central vein at 100X

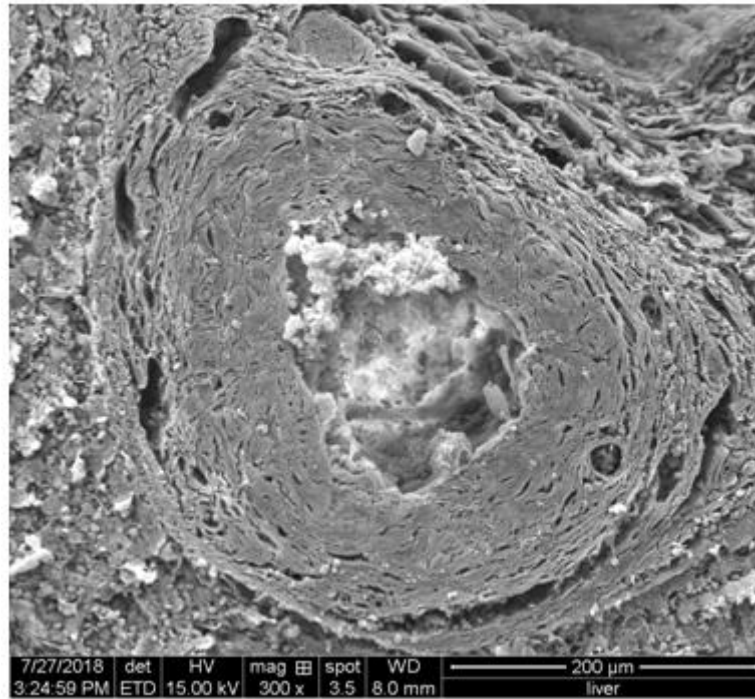


Figure 3 showing micrograph of centrilobular necrosis at 300x

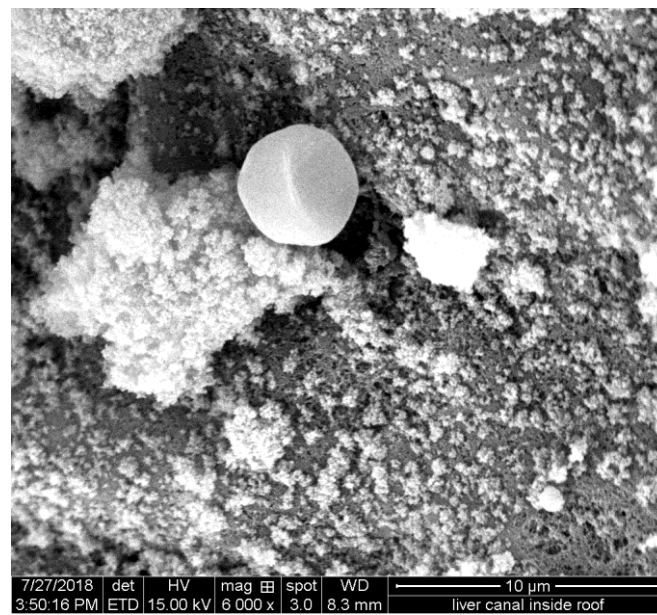


Figure 4 showing micrograph at 6000x showing lymphocytic infiltration along with broader areas of necrosis.

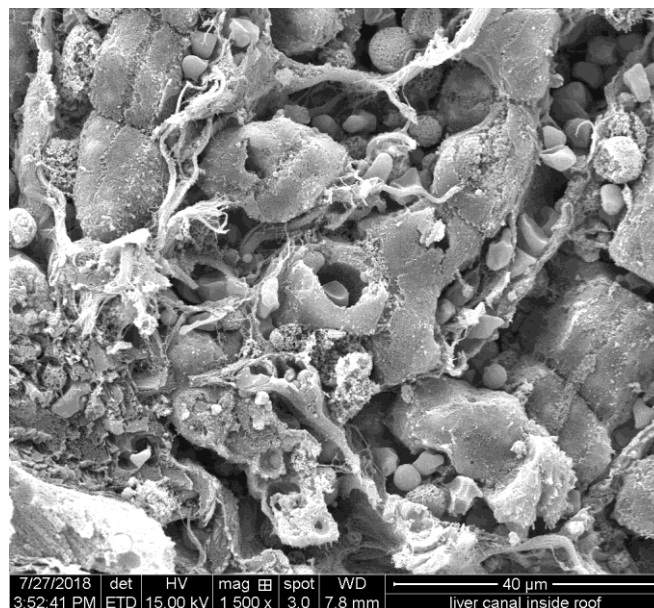


Figure 5 showing closer view of hepatic lobules showing hepatic plates and sinusoids at 1500X.

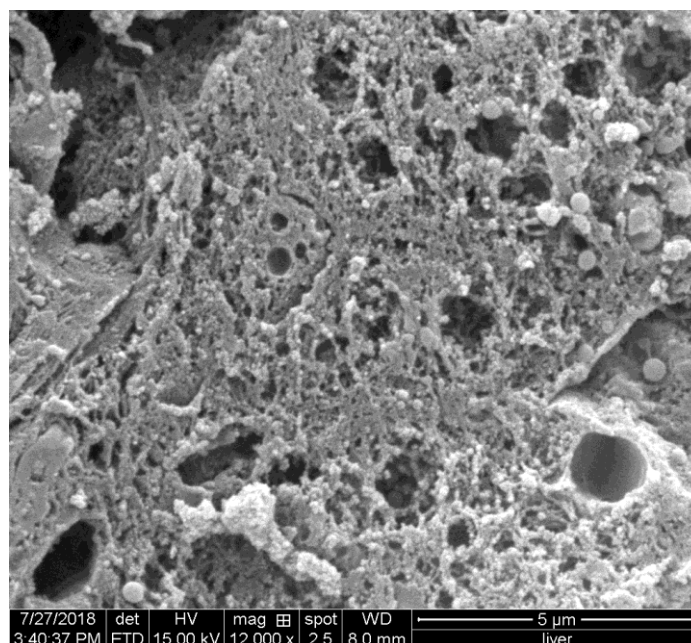


Figure 6 shows micrograph at 12000X depicting fatty changes in liver.

IV. Discussion

Fig 1 is the gross image of liver taken at the time of autopsy in case of Celphos poisoning. It showed overall congestion along with haemorrhagic spots. According to the article, Aluminium Phosphine Poisoning Autopsy Findings by Ashok Kumar Jain et al, congestion has been seen in liver in 88% cases.^[3]

Fig 2 is also the gross image of the liver with gall bladder intact. It showed edema and congestion along with haemorrhagic spots.

In fig 3, the micrograph shows cross section of liver in case of death due to Aluminium Phosphide poisoning. According to Curran's atlas of histopathology, hepatic lobule is a roughly hexagonal mass of tissue, which consists of anastomosing plates of hepatocytes. Terminal hepatic venule is the relatively larger venule at the centre, into which the sinusoids drains. General congestion of sinusoids and central vein is evident in the micrograph.^[7] According to Survey the Histopathological Findings in Autopsy of Poisoned Patients with Rice Tablet(Aluminium Phosphide), in a study on 80 poisoned patient with phosphine sinusoidal congestion was observed in 45% cases and congestion of central vein in 15% cases.^[7] According to the review article, An update on toxicology of Aluminium Phosphide by Ali Akbar Moghadamnia, central vein congestion along with congestion of sinusoids has been observed.^[2] In the study, A review of Aluminium Phosphide poisoning and a

flowchart to treat it by Behrooz Hashemi et al, the most common finding was sinusoidal congestion along with vacuolation of Hepatocytes.^[1]

In fig 4, we have observed centrilobular necrosis with areas of necrosis at several spots. Along with this, general oedema and congestion is also visible in the micrograph. According to Survey the Histopathological Findings in Autopsy of Poisoned Patients with Rice Tablet(Aluminium Phosphide),centrilobular necrosis was evident in 1% case only but the result has varied in different studies.^[13] According to Curran's atlas of histopathology, the pathologic changes referred to as ischemic necrosis is are most severe in hepatocytes in zone 3, which surrounds the terminal hepatic venule. It is referred as centrilobular necrosis.^[7] Similar result has also been obtained in the study, An update on toxicology of Aluminium Phosphide by Ali Akbar Moghadamnia.^[2]

Zone 3 of the liver acinus is the first to be affected in the condition of hypoperfusion and hypoxia in the case of congestive heart failure also. Hepatocytes in zone 3,undergo ischemic necrosis, while no noticeable changes are seen in zone 1 and 2. Necrosis of such type is called centrilobular necrosis.^[7]

According to Atlas of histopathology by Ivan Damjanov, massive hepatic necrosis caused by toxins is characterised by coagulative necrosis which is predominantly located in perivenular zone.^[14]

In fig 5, fenestrated area of endothelial cell attenuation. According to Scanning Electron Microscopy of Human Liver Sinusoids, the non-fenestrated areas of endothelial cell radiate from the nuclear swelling and separate fenestrate areas. Small fenestrations are typically distributed in uniform spacing to form sieve plates. Adjacent endothelial cells showed overlapping partly.Also, a portion of sinusoidal wall showing larger fenestrations is visible through which granular or fibrous materials are seen in the space of Disse.^[12] According to Survey the Histopathological Findings in Autopsy of Poisoned Patients with Rice Tablet(Aluminium Phosphide), nuclear fragmentation is seen in case of Aluminium Phosphide poisoning.^[13] According to the review article, An update on toxicology of Aluminium Phosphide by Ali Akbar Moghadamnia, destruction of nucleolus of Hepatocytes has also been observed.^[2]

In fig 6, sinusoidal wall showing Kupffer's cell is visible which is provided with numerous cytoplasmic processes. According to Survey the Histopathological Findings in Autopsy of Poisoned Patients with Rice Tablet(Aluminium Phosphide), clusters of Polymorpho-nuclear leukocytes have been seen in case of Aluminium Phosphide poisoning. Along with this fine cytoplasmic vacuoles are also visible.^[13] According to Aluminium Phosphine Poisoning Autopsy Findings by Ashok Kumar Jain et al, small granuloma have been seen in case of Aluminium Phosphide poisoning.^[4] According to Abnormal Electrocardiogram in patients with Acute Aluminium Phosphide poisoning by Amine Ali et al leukocytic infiltration has been seen along with congestion and edema.^[15]

In fig 7, we have observed fatty changes in liver in case of Celphos poisoning. According to Atlas of Histopathology by Ivan Damjanov, steatosis is also known as fatty liver changes. It is characterized by replacement of the hepatocyte cytoplasm with fat droplets. Variable degrees of micro steatosis and macro steatosis have been seen. It is accompanied by chronic inflammation and ballooning degeneration of Hepatocytes. Sometimes, "chicken wire" fibrosis is prominent. Sometimes it is also characterized by distinct pericellular and perisinusoidal collagen deposition.^[14] According to Vijay Kumar Verma et al, hepatobiliary system showed acute hepatic failure, jaundice, hepatitis and soft tender hepatomegaly.^[9] According to Aluminium Phosphide Poisoning Autopsy Findings by Ashok Kumar Jain et al, mild fatty infiltration has been seen in 38% case while in similar study by Siwach et al, it has been reported in 100% cases.^[4] According to the review article An update on toxicology of Aluminium Phosphide by Ali Akbar Moghadamnia, fatty liver changes have been seen in such case. As per the study, hepatotoxicity manifestations develop 72 hours after AIP intoxication. Death due to acute hepato-cellular toxicity and fulminant hepatic failure has also been reported in acute intoxication.^[2]

The incidence of poisoning has been increasing steadily. It is now the commonest poisoning in Northern and Central regions of the country. It is marketed in India as tablets of Celphos, Quickphos and available in small and large packs containing greyish white tablets weighing about 3gm each containing 56% AIP and 44% Aluminium Carbonate. Fatal dose of AIP has been stated to be in the range of 150-500mg/70 Kg whereas the mortality rate in clinical reports has varied between 37-100% in different research.

The highest incidence of poisoning was reported in case of household agents followed by drugs and agricultural pesticides. The histopathological findings have shown congestion in 100% cases and fatty changes in 75% of cases of poisoning due to AIP.

As per the review article, A Systematic Review Of Aluminium Phosphide Poisoning, in an autopsy study of unnatural death in North-Western India, AIP was found to be the most common suicidal poison. It was reported that 68.4% of deaths were caused by AIP out of the deaths due to poisoning.^[2]

According to Histopathological Findings in Liver in Poisoning Cases, on 50 medico-legal autopsy cases of AIP poisoning, congestion of liver was found in 44 cases, mild fatty changes in 19 cases while only

10% cases showed haemorrhagic necrosis. It is evident from the above depiction that congestion is foremost in case of liver followed by fatty changes and haemorrhagic necrosis while it has varied in different studies.^[16]

Besides, the liver is main site of injury in case of Alcohol abuse and causes cirrhosis. Other drugs are also hepatotoxic and produce peculiar effects which are life threatening in some cases. A classic example of predictable hepatotoxin is acetaminophen, which has now become the commonest cause of acute liver failure. In idiosyncratic group, chlorpromazine, halothane sulphonamides and Allopurinol are included and affect rare persons involving a combination of direct cytotoxicity and immune mediated injury.^[8]

V. Conclusion

This is an original and pioneer work in the field of Forensic Nano-technology, as the electron microscopic studies at the tissue and cellular level can be a road map to the field of research and clinical investigations as the antidote for this lethal poison is not available till date. The purpose of selecting my study on this topic is that till now no reference data is available for histopathological studies by SEM. Also, there are ominous indications of gradual rise in the number of cases being reported. The effect of celphos on heart is most prominent and the most evident cause of death in first 24 hours but, the fatality rate increases as the undigested poison goes to the liver and causes after effects which worsens the condition of the patient further. Most of the patients who come into contact, even accidentally, succumb to its toxicity because of the gap between ingestion and initiation of treatment, as its fatal period is as low as 1-3 hours and ranges up to 3-4 days. The cases of survival in Celphos poisoning are rare. Also, the signs and symptoms of mild poisoning are similar to those of upper respiratory tract infections. A study on histopathological findings can reveal the consequences of consuming the poison at tissue and cellular level. Such study can be utilized for the purpose of finding its antidote which is not available till date. This can provide a guideline for the line of action for clinical purposes where the treatment is based on prognosis in such cases.

References

- [1]. Domenech B H, Zamani N, Moghaddam H H, Rahimi M, Shadnia S, Erfantalab P et al. A review of Aluminium Phosphide poisoning and a flow chart to treat it. *Arh Hig Rada Toksikol.* 2016;67:183-193.
- [2]. Moghadamnia A A. An update on toxicology of aluminium phosphide. *2012;20:25*
- [3]. Mehrpour O, Jafarzadeh M, Abdollahi M. A systematic review of aluminium phosphide poisoning. *2012;63:61-73.*
- [4]. Jain A K, Nigam M, Garg S D et al. Aluminium Phosphide Poisoning Autopsy Findings. *2005;27(1).*
- [5]. Devai I, Felfoldy L, Wittner I et al. Detection of phosphine: new aspects of the phosphorus cycle in the hydrosphere. *Nature.* 333, 1988:343-345.
- [6]. Anger F, Paysant F, Brousse F et al. Fatal Aluminium Phosphide poisoning. *Journal of analytical Toxicology(24).* 2000:90-92
- [7]. Curran R C, Crocker J. Curran's atlas of histopathology. Harvey Miller Publishers. 4th edition. 2005. 628-640.
- [8]. Kumar V, Abbas A K, Aster J C. Robbins Basic Pathology. Reed Elsevier India Private Limited. 2015;603-637.
- [9]. Verma V K, Gupta SK, Parihar A. Aluminium Phosphide Poisoning: A challenge for the physician. *2001,3(1);13-19*
- [10]. Bajwa S J S, Bajwa S K, Kaur J et al. Management of Celphos Poisoning with a novel intervention: Array of hope in the darkest clouds. *Anaesthesia Essays and Researches.* 2010 Jan-Jun;4(1):20-24
- [11]. Kotrly M et al. Forensic Science Scanning Electron Microscopy and News in this Field, *Microscope Microanal* 15,(2009) :798
- [12]. Muto M, Nishi M, Fujita T. Scanning Electron Microscopy of Human Liver Sinusoids. *1977,40(2);137-151*
- [13]. Rahbar T M, Teymourou P, Jahanbakhsh R. Survey the Histopathological Findings in Autopsy of Poisoned Patients with Rice Tablet (Aluminium Phosphide). *Journal of Guilan University of Medical Sciences.* 2010(76);56-73.
- [14]. Damjanov I. Atlas of Histopathology. Jaypee Brothers Medical Publishers (P) LTD. 2012.
- [15]. Zeggwagh A A, Louriz M. Abnormal Electrocardiogram in Patients with Acute Aluminium Phosphide Poisoning. *Advances in Electrocardiograms – Clinical Applications.* 319-327.
- [16]. Kundal R K, Kaur A, Oberoi S Set al. Histopathological Findings in Liver in Poisoning cases- A post-mortem study. *Journal of Punjab Academy of Forensic Medicine and Toxicology.* 2014;14(1),11-14.

Manoj Kumar Pathak" Histopathological study of Liver in case of Aluminium Phosphide poisoning by SEM" *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)* 13.11 (2019): 39-45.