CASE-SERIES

Histochemistry based confirmation of the diagnosis of Copper Sulphate Poisoning: Case-Series

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ABSTRACT

The suspected suicidal death cases from copper sulphate poisoning is not uncommon in West Bengal as well as in some other parts of India. But due to extreme delay in laboratory confirmation report from Forensic Science Laboratory (FSL) regarding presence of copper in toxic doses in the preserved samples the final opinion regarding cause of death in medico legal autopsy cases is kept pending. The histochemical method of rubeonic (rubeanic) acid staining of hepatocytes to determine the presence of copper in toxic level there, can be used as an alternative method to confirm copper sulphate poisoning. In this study, slides of liver biopsy from twelve autopsy cases of suspected copper sulphate poisoning are stained with rubeonic acid. All of them showed positive results indicating confirmation of copper sulphate poisoning. Hence, this method can be adopted as an alternative to toxicological analysis of copper sulphate poisoning.

INTRODUCTION

Copper as a metal is essential for our body and it does not do any harm in small quantities. But its compounds like copper sulphate, which is also known as blue vitriol, cause toxicity by inhibiting many enzymes of human body like glucose 6 phosphate dehydrogenase and glutathione reductase as mentioned by Reddy KSN.[1] It causes intravascular haemolysis and reduction in free radical scavenging activity respectively as observed by Aggrawal A.[2] The minimum lethal dose of copper sulphate is 10 gm. as mentioned by Pillay VV.[3] At one time, copper sulphate was widely used as a suicidal poison. Although suicidal cases from copper sulphate ingestion has decreased over the decades but in some parts of India like West Bengal many suicidal poisoning cases by ingestion of copper sulphate are often found as it is cheap and easily available which is consistent with the observation by

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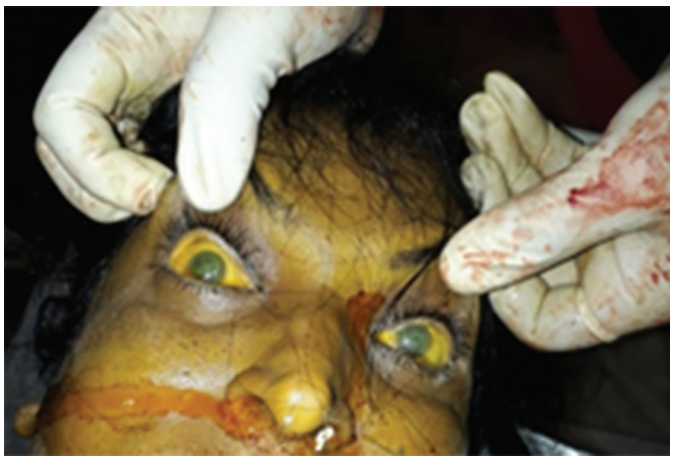
Saravu K. et al.[4] Analysing the phone call data collected at Poison Information and Research Centre(PIRC), R.G.Kar Medical College and Hospital (RGKMCH), Kolkata, which is the only PIRC in West Bengal, between November, 2018 to October,2019, it was seen about 16 percent of all metallic poisoning cases is due to ingestion of copper sulphate and it was presented in a podium presentation at TOXOCON-13 by Chakrovarty A.[5]But in medico legal autopsy cases, confirmation of copper sulphate poisoning by toxicological analysis of the preserved samples can hardly be done as there is extreme delay in getting the chemical examiner's report from FSL where preserved viscera are routinely sent for that purpose. Moreover, the blood samples collected from dead bodies with such type of poisoning at mortuary is haemolysed either as a direct effect of copper on erythrocytes or due to inhibition of different enzymes by toxic dose of copper. Thus biochemical marker like ceruloplasmin estimation is not possible from there which is consistent with findings of Badiye A. et al.[6]There is also a possibility of complete elimination of circulating metals from blood. So there is a demand for a faster, dependable and scientifically valid alternative method for qualitative assessment of copper sulphate poisoning.

Figure 1: jaundice detected on upper bulbar conjunctiva (case no.1)



In another incident, a middle aged male after losing his job consumed copper sulphate with suicidal intent, was admitted at RGKMCH after being referred from a rural hospital and expired there within approximately 10 hours from ingestion of copper sulphate which is the shortest time period with fatal outcome among all cases included in this study. Here greenish-blue discolouration of mucosa of stomach was found that was consistent with retention of a portion of poison there.

Although there were variations in presentation of different cases macroscopically, but microscopically with rubeonic acid stain the findings were almost similar as shown in fig-4 and fig-5 in contrast with the negative control (fig-6).

CASE SERIES

A brief account of some selected cases is detailed below. More details including personal identification, clinical features and time interval between ingestion of poison and death are given in table-1.

A housewife attempted suicide by consuming copper sulphate, was rushed to local hospital and then to RGKMCH on the same day and expired there after 5 days. Jaundice was evident in this case which was not found in other cases as the deceased survived for longer period than other cases.(Fig-1, 2)

Figure 2: liver is soft, greasy, and yellowish due to cholestasis as seen macroscopically in cross-section (case no.1)

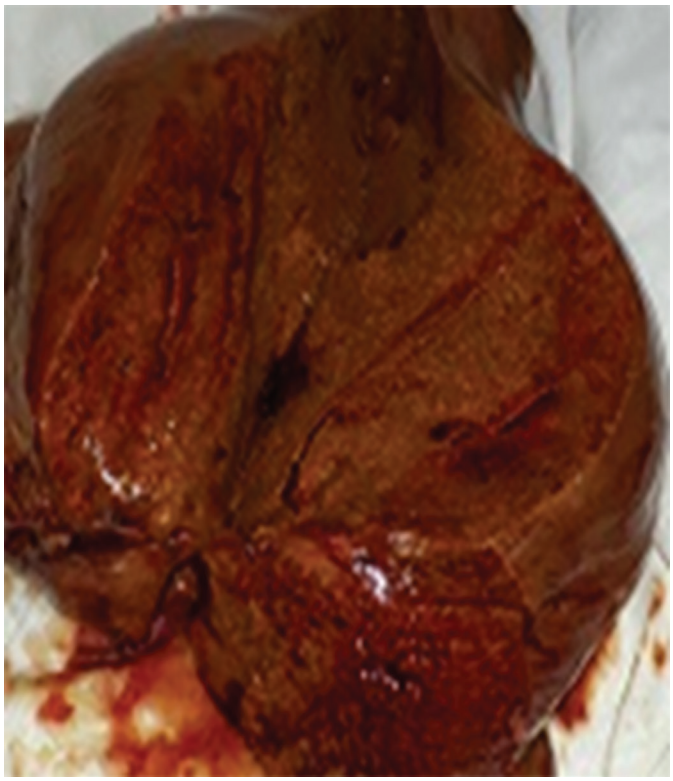


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Figure 3: mucosal congestion and submucosal haemorrhage in stomach (case no.4)

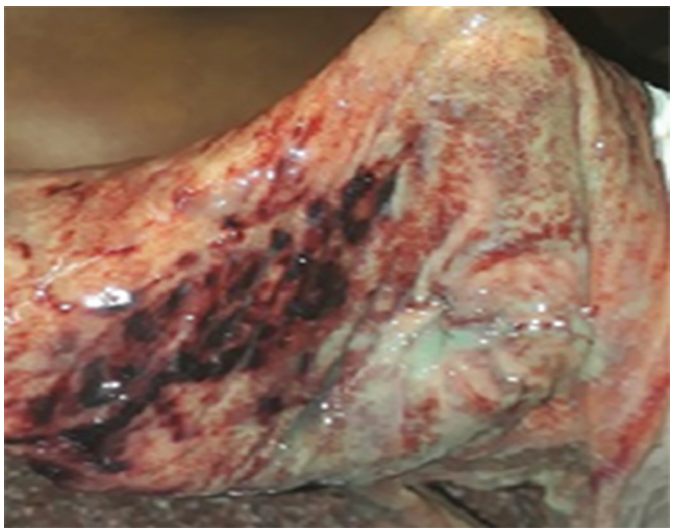


Figure 4: copper appears as greenish-black dots under microscope with rubeonic acid stain (40x)

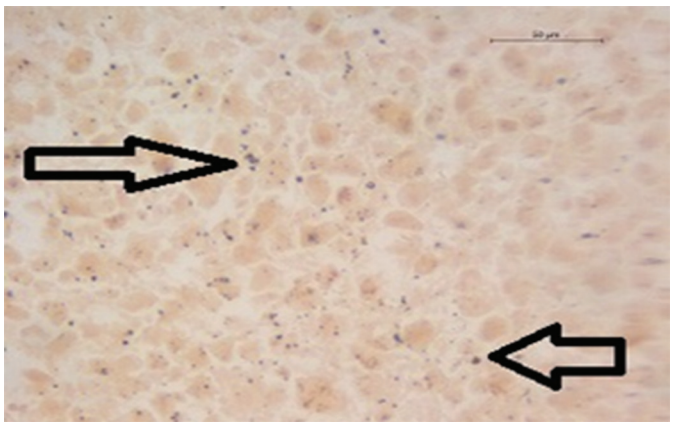
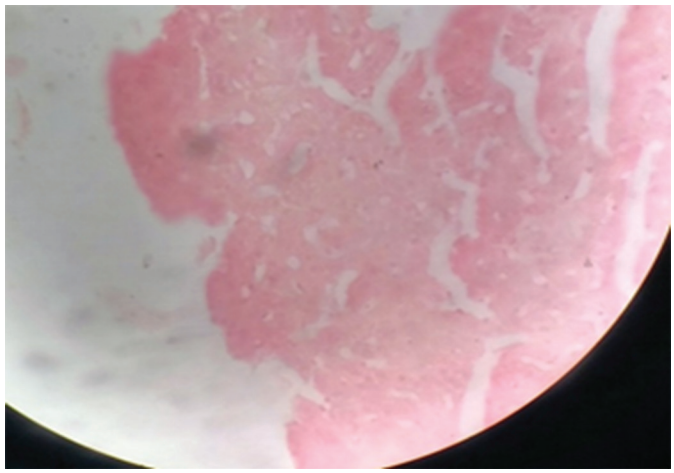


Figure 5: copper shown as greenish black dots under microscope with rubeonic acid stain (10x)

Figure 6: Showing the negative control for copper in hepatocytes.



DISCUSSION

Liver, the organ commonly involved in copper sulphate poisoning shows features of fatty infiltration, centrilobular necrosis and biliary stasis as mentioned by Karmakar RN. [7] The hepatic lesion will be both cellular as well as obstructive as opined by Singh ML et al.[8] Rubeonic acid or dithiooxamide is an organic compound which acts as a chelating agent according to Wikipedia.[9] It can also be used for toxicological analysis of copper sulphate poisoning as described by Kannan K.[10][11] But only the histochemical test for detection of copper within hepatocytes by staining with rubeonic acid should be considered as the confirmatory test for copper sulphate toxicity. As described by Bancroft JD, at first, 0.1% rubeonic Acid in absolute ethyl alcohol is prepared which is later mixed with 10 percent aqueous solution of sodium acetate to prepare the working solution. Then the test slides are placed in a coplin jar filled with rubeonic-acetate solution for at least 16 hours at 37°C. It is then washed with 70% ethyl alcohol followed by rinsing with distilled water,dried and lightly counterstained with 0.5% aqueous neutral red for 1 minute. At the end it is washed with distilled water, dried, mounted in synthetic resin and examined under microscope.[12][13] Then copper appears as green-black granules in liver cells (Fig-4 & 5). Being a micronutrient copper is present in food and its Recommended Dietary Allowance (RDA) is 2mg. per day as mentioned by Park K.[14] Dietary source of copper include shellfish, liver, nuts, legumes, bran and organ meats according to Jameson J et al. [15] Copper from dietary source is not visible prominently with rubeonic acid stain at normal concentration within hepatocytes. Copper may also be detected prominently with rubeonic acid stain in some diseased condition like Wilson's disease with high sensitivity and specificity as mentioned in a study

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Table-1: Summary of case description of the deceased persons under study. (n=12)

Case no.	Sex	Age (years)	*Approximate interval between ingestion of copper sulphate and		Autopsy findings
			First Admission	Death declaration	
1	female	38	2 hours	120 hours	Features of jaundice (Fig-1), liver is soft, fatty with yellowish colour due to cholestasis (fig-2) and other findings as mentioned below
2	male	32	2 hours	48 hours	Congested mucous membrane of stomach, haemorrhagic areas in submucosa (fig 3), kidney is congested and haemorrhage seen on cut section.
3	male	26	3 hours	48 hours	
4	male	50	6 hours	10 hours	
5	female	55	5 hours	12 hours	
6	male	30	4 hours	25 hours	
7	male	27	6 hours	12 hours	
8	female	36	4 hours	22 hours	
9	male	45	5 hours	25 hours	
10	female	40	6 hours	11 hours	
11	female	37	6 hours	15 hours	
12	male	34	4 hours	21 hours	

*as mentioned in attendance/admission report at casualty and death certificate issued at the place of study.

by Jain S et al.[16] This was also supported by a study by Lesna M. et al.[17]But supporting history and other biochemical investigations would differentiate between the diseased condition and poisoning. In another study by Irons RD et al., it was found that there was a linear relationship between microscopical evaluation of the stain and actual tissue copper level.[18] In this study, we have found the presence of greenish black granules of copper in the collected specimen of liver in all those twelve cases with suspected copper sulphate poisoning even when the death has occurred within 10 hours of copper sulphate ingestion (Fig-4 & 5). So from these findings, we can confirm that death has occurred in those suspected cases due to ingestion of copper sulphate in toxic doses.

CONCLUSION

Histochemical confirmation of copper sulphate poisoning by rubeonic Acid stain can be an established method to prove that copper sulphate has exerted its toxic effects reaching at hepatocytes and thus has caused death even when the chemical examiner's report is awaited. This method can be adopted by the department of Forensic Medicine and Toxicology (FMT) of any medical college where autopsy examination is routinely done.

Limitations of the study: This study is only based on qualitative assessment of copper sulphate poisoning. Quantitative assessment cannot be done by this method. Other copper compounds may also show similar histochemical findings.

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