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Two fatal intoxications by colchicine taken for saffron. Clinical, medico-legal and forensic toxicological implications

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ABSTRACT

Although fatal colchicine intoxications are rare and mostly related to suicidal intake or accidental overdose, other hypotheses should be considered when dealing with colchicine poisoning. We present a case of double, acute, and subacute, fatal colchicine intoxication in a married couple. The 70-year-old male victim suddenly died after vomiting and diarrhea. The next day his wife showed aggravating gastrointestinal symptoms and was hospitalized with a diagnosis of septic shock. A complete postmortem examination on the man was performed, together with histopathological analysis. Toxicological examination performed through liquid chromatography coupled to mass spectrometry revealed a colchicine blood peripheral concentration of 33 ng/mL. A few days after hospitalization, the woman showed a colchicine plasma concentration of 32 ng/mL. Despite veno-venous hemofiltration, she ultimately died of septic shock and multi-organ failure.

Death scene investigation revealed that, a few days before the death of the male victim, the couple had collected wild saffron and had eaten a presumed saffron risotto.

The integrated analysis of circumstantial, clinical, postmortem and toxicological data allowed to establish that the couple had died of a fatal accidental intoxication due to the ingestion of natural colchicine, mistaken for saffron. The death of the male was deemed caused by acute cardiovascular collapse induced by acute intoxication, while the female had suffered a subacute poisoning by antimitotic agent, resulting in immunosuppression and systemic infection. Toxicological analyses, promptly performed on the man for forensic purposes, directed the investigations and suggested the clinical diagnosis on the woman.

1. Introduction

Colchicine is an alkaloid extracted from Colchicum Autumnale, which inhibits microtubule polymerization, thus blocking cells in metaphase. It is used to treat a variety of diseases, including acute gout, Behcet's disease and Mediterranean fever. After oral administration, it is rapidly absorbed and distributed to tissues, with a plasma half-life of approximately 1 h [1]. The drug is metabolized by demethylation mediated by CYP3A4 [2] and mostly excreted with faeces after enterohepatic circulation. Doses of colchicine above 0.8 mg/kg are described as potentially fatal, even though a clear separation between nontoxic, toxic and lethal doses still lacks [3,4]. Although fatal colchicine intoxications are rare and mostly related to suicidal intake or accidental medication overdose [5], other hypotheses, such as homicide, should always be considered when dealing with colchicine poisoning.

2. Case history and death scene investigation

We present a case of double, acute and subacute, fatal colchicine intoxication in a couple in the Northeast part of Italy. Despite mild gastrointestinal symptoms, the couple had left for a holiday. The following day, the 70-year-old male victim suddenly died after acute vomiting and diarrhea. The next day his wife was hospitalized with a diagnosis of septic shock. A forensic autopsy was requested on the man to direct further clinical investigations on the woman; postmortem toxicology suggested a fatal colchicine intoxication of the man and prompted to perform a targeted toxicological analysis of the body fluids of the woman who, notwithstanding the supportive therapy, ultimately died 16 days after her husband.

An accurate death scene investigation (DSI) was performed by law enforcement officers, in close cooperation with the Department of Legal and Occupational Medicine and took place firstly in Folgaria, where the

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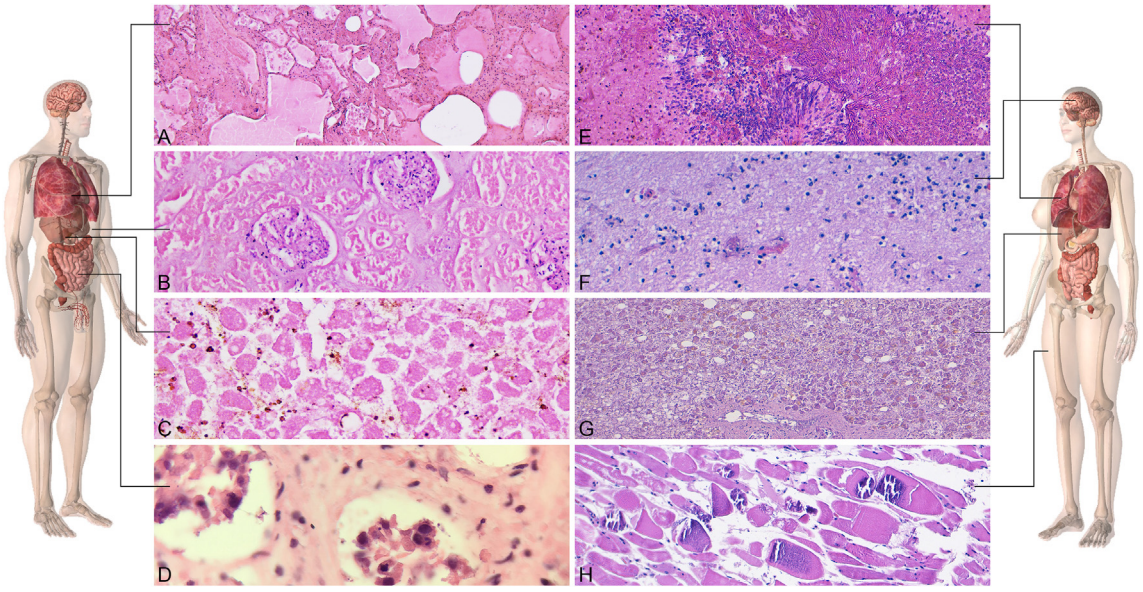

Fig. 1. Histopathology results of the male victim, to the left side, and female victim, to the right side of the Figure. Male victim: A) lung, massive alveolar edema (50×, H&E). B) kidney, tubule cells with acute tubular necrosis (100×, H&E). C) liver, hepatocellular vacuolization (400×, H&E). D) stomach, gastrointestinal mucosa with enlarged, hyperchromatic nuclei and ring mitosis (630×, H&E). Female victim: E) lung, pulmonary infarction and colonies of Aspergillus (50×, H&E). F) brain, cerebral emboli of Aspergillus (200×, H&E). G) liver, hepatic microvescicular steatosis (50×, H&E). H) dissolution of striated muscle with coagulation necrosis and rhabdomyolysis (400×, H&E).

couple had been staying in holiday.

Meanwhile the neighbors reported they had seen, a few days before the death of the man, the couple coming back from a walk with a basket full of lilac-colored flowers, collected to prepare what they thought was a saffron risotto. On the basis of the toxicological results pertaining to the male victim, as suggested by forensic pathologists, who recommended to look for both medications and natural colchicine sources, the DSI was extended from the secondary to the primary site of presumed intoxication, at the house of the victims. No colchicine-containing medications nor plant/flowers residues were discovered. However, in the kitchen garbage were retrieved an empty box of rice and onion skin residues, elements necessary to prepare an Italian risotto.

3. Methods of investigation

3.1. Clinical data

Clinical data regarding the home therapy of the couple were obtained through the family physician. Clinical records of the woman were requested to the Hospital where she was hospitalized.

3.2. Autopsy and histopathology

Complete post-mortem examinations, with accurate external examination, careful dissection and extensive sampling of biological fluids and organs for further investigations were performed. Hematoxylin and eosin staining was used for histopathology.

3.3. Toxicological analysis

In the male victim, a comprehensive toxicological screening was performed by means of multiple-analytical techniques such as immunoenzymatic, gas-chromatography, and liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS) or high resolution and high accuracy mass spectrometry (HRMS) after liquid–liquid sample extraction for acid, neutral and basic fractions.

Screening analysis was performed on an LTQ-Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) with an electrospray source (ESI) working in positive mode. HRMS spectra were recorded in the mass range m/z 80–600 using a target mass resolution of 30000. Chromatography was performed on an Atlantis T3 (150 × 2.1 mm, 3 µm) column (Waters Corporation, MA, USA) with a flow of 300 µL/min following a gradient elution program based on water (0.1% formic acid) and acetonitrile (0.1% formic acid). Detection of colchicine was achieved on the basis of accurate mass measurements of MH^+ ion (400.17546 m/z), the correspondence between the observed isotopic pattern, and the calculated one, along with the relative retention time (RRT) with respect to the internal standard (IS: d3-methadone).

The confirmation and quantitation of colchicine in ante-mortem samples (plasma collected from the female victim) and post-mortem samples (blood collected from the male victim) were achieved following the procedure proposed by Jiang et al. with only slight modifications, using a LC-MS/MS system equipped with a XEVO TQ-S MICRO (Waters Corporation, MA, USA) tandem mass-spectrometer set in electro-spray ionization (ESI) positive mode [6]. Detection of the target analyte was performed by multiple reaction monitoring (MRM) observing two transitions for colchicine (m/z 400.2 → 310.2 and m/z 400.2 → 358.3; RT 3.21 min). More than 10 data-points were collected over the chromatographic peak. The MRM transition 400.2 → 358.3 of colchicine was filtered as qualifier and d3-methadone was used as IS. The estimated lower limit of detection (LLOD) and lower limit of quantitation (LLOQ) were 0.1 ng/mL and 0.3 ng/mL, respectively. The assay was estimated to be linear in the range 0.3–50 ng/mL.

4. Results

4.1. Case 1. The male victim

4.1.1. Clinical data

The man had been heart transplanted in 2011 for a post-infarction severe dilated cardiomyopathy and was assuming the immunosuppressive drugs Cyclosporine and Everolimus, as well as antihypertensive and diuretic therapy.

4.1.2. Autopsy and histopathology

The autopsy revealed brain swelling (1460 g), together with severe pulmonary edema and congestion (right lung 700 g, left lung 620 g). The transplanted heart was markedly enlarged (580 g), despite the absence of hypertrophy (interventricular sept and free ventricular wall thickness: 15 mm) and showed a left descending coronary narrowing of 65%. Gallstones with complete obstruction of cystic duct were observed. The stomach was empty and within the small bowel a modest amount of semiliquid stool was identified.

As shown in Fig. 1, massive alveolar edema was microscopically confirmed; the kidney, severely affected by postmortem changes, showed detachment of the cells from the basal membranes as well as large hyperchromatic nuclei and infiltration of lymphomonocytic cells. Hepatic lobules had sustained autolysis, therefore only hepatocellular vacuolization could be identified. The mucosa of the stomach, the colon and the small intestine showed cells with enlarged, hyperchromatic nuclei and ring mitosis, in the context of moderately preserved villi and tubules. Histopathology of the heart showed a focal subendocardial substitutive fibrosis and wavy myofibers.

4.1.3. Toxicological analysis

Toxicological analysis revealed a colchicine blood peripheral concentration of 33 ng/mL (Fig. 2), within the potentially fatal concentrations reported in the Literature (9–250 ng/mL) [7].

4.2. Case 2. The female victim

4.2.1. Clinical data

During hospitalization, as the couple had experienced similar gastrointestinal symptoms, an infective disease/food poisoning was suspected. The woman showed atrial fibrillation, acute kidney failure, metabolic acidosis and massive D-dimer elevation. On day 2, clinical conditions worsened with confusion, cardiac arrest and liver failure (Fig. 3). White blood cells drop to 0.40×10^9/L and remained extremely low till the day before death, while procalcitonin rose to values indicative of systemic infection (17.49 μg/L).

Septic shock, confirmed by chest X-ray, bronchoalveolar lavage and aspiration, multi-organ failure and persistent levels of colchicine ultimately led the woman to death.

4.2.2. Toxicological analysis

Plasma samples demonstrated a toxic colchicine plasma concentration, as shown in Fig. 3, with only minimum response to veno-venous hemofiltration.

4.2.3. Autopsy and histopathology

Postmortem examination demonstrated a pulmonary aspergillosis, complicated by fungal systemic spread, involving the brain, trachea, lung, where pulmonary infarction was also noted, esophagus, stomach, small bowel and heart. Hepatic steatosis, rhabdomyolysis and acute kidney failure were also observed (Fig. 1).

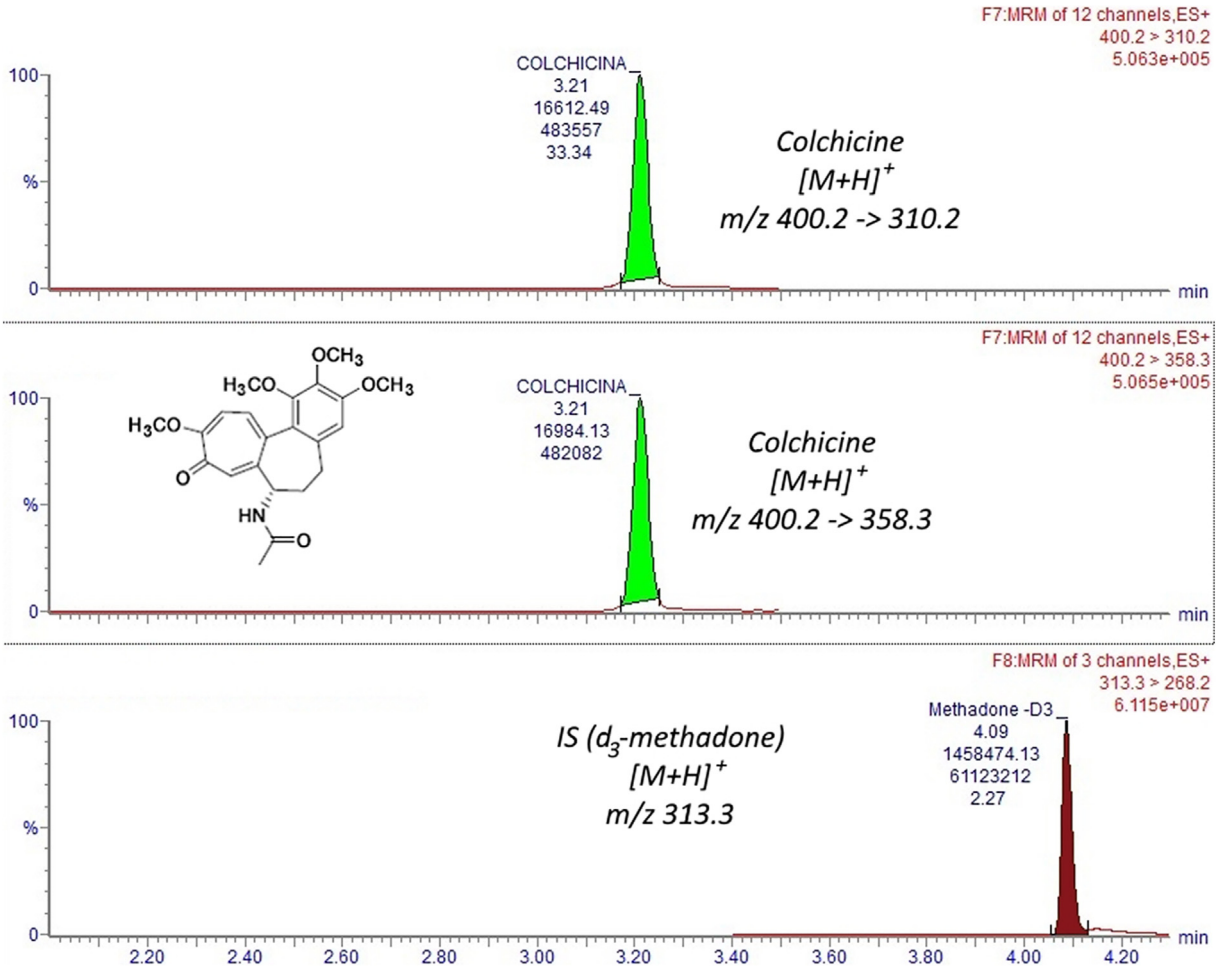

Fig. 2. Multiple reaction monitoring (MRM) chromatogram of the post-mortem blood sample collected from the male victim. A) colchicine transition *m/z* 400.2 → 310.2 at 3.21 min; B) colchicine transition *m/z* 400.2 → 358.3 (qualifier) at 3.21 min; C) Extracted ion chromatogram (XIC) of the d_3-methadone (IS) *m/z* 313.3.

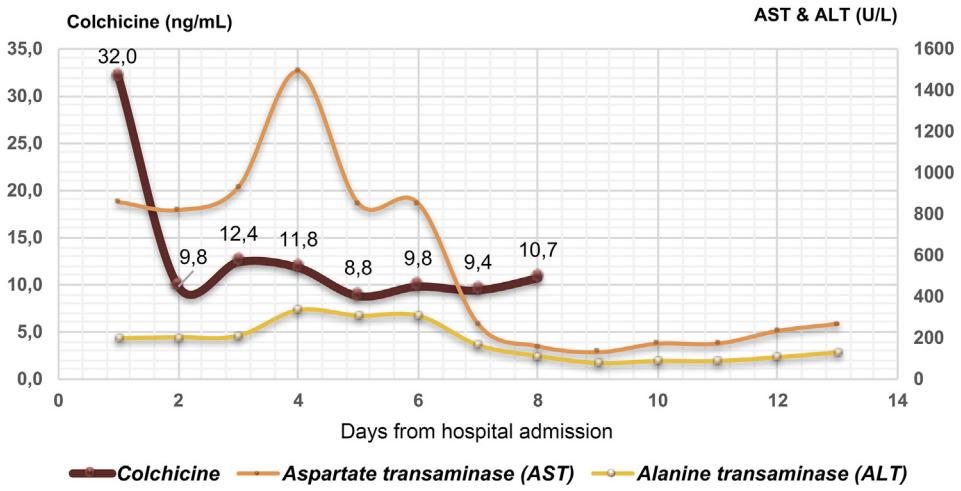

Fig. 3. Plasma levels of colchicine and aspartate/alanine transaminase of the female victim since Hospital admission. An initial sharp reduction of colchicine plasmatic levels was followed by a plateau of concentrations.

5. Discussion

In the presented cases, the death scene investigation (DSI), performed through an intense collaboration between forensic pathologists and law enforcement officers, allowed to exclude that a colchicine-based drug assumption had occurred, according to the prescribed home therapy, which was confirmed by the family physician.

Highly poisonous colchicine is found in flowers of Colchicum Autumnale, which grows spontaneously in the Northeastern part of Italy. The plant is commonly mistaken for other wild herbs, including Crocus Sativus, from which the real saffron is derived [8,9]. Despite the impossibility of retrieving information from the couple, on the basis of the circumstantial data, and particularly of the absence of medications and of the retrieval of elements pointing towards the ingestion of natural elements, the accidental ingestion of Colchicum Autumnale flowers, mistaken for saffron, appeared as the most probable hypothesis. Neither flowers left nor plant residues were found, despite extensive research in both the primary and secondary death scenes. However, the presence in the kitchen garbage (DSI at the primary scene) of an empty box of rice and onion skin residues, elements necessary to prepare an Italian risotto, confirmed the witness statements pointing towards the colchicine-risotto (mistaken for saffron risotto).

Postmortem blood analysis performed on the male victim demonstrated a concentration of colchicine in the fatal range, thus, according to the short half-life of the drug, pointed towards an acute death. The assumption of colchicine was confirmed by histopathology, and particularly by the appearance of the intestinal mucosa, which was consistent with a toxic effect of anti-mitotic drug [11]. Furthermore, the hepatocellular vacuolization could be explained by the liver concentration of colchicine, which undergoes hepatic metabolism and enterohepatic circulation [1]. Postmortem examination of the man matched the circumstantial data of gastrointestinal symptoms and allowed to speculate that a severe dehydration with fluid loss and electrolytic imbalance had occurred.

In colchicine fatalities, gastrointestinal symptoms usually occur in the first 4–12 h from colchicine assumption [10] and can lead to hypovolemic shock and cardiovascular collapse [12]. Moreover, the patient was assuming anti-hypertensive and diuretic drugs, which could have worsened the colchicine-induced hypotension.

Though the exact cause and mechanism of death are often uncertain [12], it has been shown that colchicine can lead to acute cardiogenic shock through both a direct and indirect mechanism [12–15]. Indeed, colchicine-induced hypovolemia and compensatory mechanisms, which involve an increased myocardial oxygen demand, can impair cardiac performance [14].

Autopsy evidence of pulmonary edema and congestion, in a victim with enlarged heart and with a long-time ischemic disease history, suggested that a deficit in the heart pump function had occurred, as shown in hemodynamic studies of colchicine poisoning [12]. The colchicine direct toxicity, which was reported to affect cardiac conduction and contractility [16,17], probably contributed to the myocardial damage and to the depression of the myocardial function, induced by hypovolemia and by ineffective compensative mechanisms, leading to acute pulmonary edema.

CYP3A4 inhibitors, as Cyclosporine, which the man was assuming as anti-rejection drug, may slow down the metabolism of colchicine, especially in elderly patients, thus precipitating its harmful effects [18].

The clinical data of the woman were consistent with the systemic effects of the second-phase of colchicine intoxication (24–72 h) [10]. As colchicine is mainly metabolized through hepatic clearance, the acute liver failure, developed during the second day of hospitalization and confirmed by histopathology, which demonstrated a microvescicular steatosis, was probably responsible for the persistence of high colchicine levels and for the inability to remove the poison from the body [13]. The high affinity binding to tissues can also be responsible for the plateau of colchicine concentration, as well as for the limited efficacy of veno-venous hemofiltration [19]. Colchicine shows high volume of distribution and low plasma protein binding (30–50%) [1], so the concentration of the xenobiotic may be higher in blood than in plasma [20]. Although it was not possible to quantify the blood concentrations in the female victim, the plasma levels seemed to be consistent with those identified in the male victim.

The white cells trend observed in the woman, with severe leukopenia followed by rebound leukocytosis, is typical of colchicine intoxications [10]. Clinical and histopathological data, and particularly the identification of cerebral emboli of Aspergillus in the brain (Fig. 1), coupled to pulmonary aspergillosis with infarction, confirmed the systemic infection by Aspergillus Flavus. Therefore, colchicine led the female victim to death firstly through multi-organ failure, proved by transaminase concentrations and histopathology of liver, kidneys and striated muscle, and secondary through systemic fungal infection and severe drug-induced immunosuppression.

6. Conclusions

The integrated analysis of circumstantial, clinical, postmortem and toxicology data allowed to establish that the couple died of a fatal accidental intoxication due to the ingestion of natural colchicine, mistaken for saffron. Close collaboration between judicial authority and forensic pathologists is essential to identify the manner of death.

Toxicological analyses, promptly performed on the man for forensic purposes, suggested the clinical diagnosis on the woman. This epitomizes the potential preventive role of forensic medicine [21]. The fatality of the male victim was deemed caused by cardiovascular collapse due to hypovolemic losses induced by acute colchicine intoxication, while the female victim suffered a subacute poisoning by an antimitotic agent, resulting in immunosuppression and secondary systemic infection. As shown in the presented case report, in order to identify a drug-related death with a high degree of probability, the forensic pathologist has to reconstruct the exact mechanism of death of the victim/s, as well as the physio-pathological chain of events from intoxication to death, integrating all circumstantial, DSI, autopsy, histopathology and toxicology data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.legalmed.2019.04.003.

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