Mephedrone and chemsex: a case report

Legal Medicine

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	Rossana Cecchi
Abstract:	The chemsex or slamsex phenomenon has attracted attention worldwide, with concerns also expressed by health professionals for the spread of sexually transmitted diseases.Mephedrone or 4-methylmethcathinone, a substituted cathinone homolog of ephedrine, is one of the most popular substances used as a cheaper alternative to other traditional drugs. Fatal cases of chemsex are still rare. We present here the first case-report to the best of our knowledge of a mephedrone-related acute toxicity case in Parma (Italy) detected and quantitated in biological specimens (2.0 mg/L in urine sample, 1.1 mg/L in bile and 1.0 mg/L in central blood while 0.8 mg/L in peripheral blood). None of the other most common drugs of abuse could be detected. Autopsy findings such as facies edematosa, oedema and polyvisceral congestion, interstitial petechiae are compatible elements with a death from acute cardio-respiratory failure, with peri-mortem agony of few minutes in which the cardiac hypertrophy, the moderate aortocoronary sclerosis and mephedrone injection have played a substantial role in the evaluation of the final cause due to an accidental acute intoxication with mephedrone.
Suggested Reviewers:	
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- did you test the syringe ?
The syringe could not be tested by our laboratory since it was seized by the Police.

- do you have pictures from the histological exams ?
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Based on your valuable suggestions, we reviewed the article as per journal guidelines

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Cover letter

UNIVERSITÀ
DI PARMA

DIPARTIMENTO DI MEDICINA E CHIRURGIA
SERVIZIO DI MEDICINA LEGALE
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Via Gramsci, 14 - 43126 Parma
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e-mail: medleg@unipr.it -

Parma, 27th May 2019

Dear Editor,

We would like to submit a case report entitled "Mephedrone and chemsex: a case report." authored by Dr Luca Anzillotti, Dr Luca Calò, Dr Antonio Banchini, Dr MariaLaura Schirripa, Dr Francesca Marezza and myself, which we hope can be suitable for the publication on Legal Medicine.

In this work we present the first case-report to the best of our knowledge of an analytically confirmed chemsex mephedrone-related acute toxicity case in our area and we also reviewed the literature for adverse effects of the substance.

We believe that the toxicological identification of these substances plays a fundamental role in attributing potential risks of acute harm from specific compounds. We aim to contribute to the relevant field of emergency medicine and toxicological areas by adding valuable information on life threatening effects caused by these drugs.

Hoping that the paper is suitable for its publication on Legal Medicine, please do not hesitate to contact me for any doubt/question.

Best Regards,

Dear reviewers, we thank you for your comments and suggestions. Please find below the responses/rebuttals for each comment, hoping that the paper is now suitable for its publication on Legal Medicine.

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Highlights

- A mephedrone-related acute toxicity case
- Chemsex: misuse of mephedrone in a sexual context
- Effects of mephedrone similar to those of cocaine, amphetamines, and metamphetamines
- Mephedrone cross-reacts with methamphetamine screening tests

Title:

Mephedrone and chemsex: a case report.

Authors:

Luca Anzillotti [1], Luca Calò[1], Antonio Banchini[1] , MariaLaura Schirripa [1], Francesca Marezza[1], Rossana Cecchi[1]

Affiliation:

[1]Legal Medicine, Department of Medicine and Surgery, University of Parma

Viale Gramsci 14, 43126, Parma – Italy

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Rossana Cecchi Email: rossana.cecchi@unipr.it

Telephone number: +390521903145

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ABSTRACT

The chemsex or slamsex phenomenon has attracted attention worldwide, with concerns also expressed by health professionals for the spread of sexually transmitted diseases.

Mephedrone or 4-methylmethcathinone, a substituted cathinone homolog of ephedrine, is one of the most popular substances used as a cheaper alternative to other traditional drugs.

Fatal cases of chemsex are still rare. We present here the first case-report to the best of our knowledge of a mephedrone-related acute toxicity case in ~~the Italian territory of~~ Parma (Italy) detected and quantitated in biological specimens (2.0 mg/L in urine sample, 1.1 mg/L in bile and 1.0 mg/L in central blood while 0.8 mg/L in peripheral blood). None of the other most common drugs of abuse could be detected. Autopsy findings such as facies edematosa, oedema and polyvisceral congestion, interstitial petechiae are compatible elements with a death from acute cardio-respiratory failure, with peri-mortem agony of few minutes in which the cardiac hypertrophy, the moderate aortocoronary sclerosis and mephedrone injection have played a substantial role in the evaluation of the ~~death~~final cause due to an accidental acute intoxication with mephedrone.

Keywords: Mephedrone, chemsex, cathinones, acute intoxication, forensic toxicology.

1. Introduction

In 2017, 51 new psychoactive substances (NPS) were detected in Europe for the first time, bringing to over 670 the number of substances monitored by the European Authority**[1]**.

The identification of NPS in biological samples is crucial in the forensic field to evaluate the spread of NPS among the population and to control the introduction of novel compounds. Within the NPS recently the use of synthetic cathinones is increasing in popularity, particularly amongst clubbers and for the practice of "chemsex".

The "chemsex phenomenon", also referred to as Sex Under the Influence of Drugs, has attracted media and political attention worldwide **[2],** also known as "slamsex" when intravenous administration occurs for a more intense high. The use of drugs in a sexual context is not new and has been already documented over the decades with many substances, usually taken to prolong sexual pleasure and activity, increase sexual self-confidence as well as enhance the perceived quality of sex **[2,3].** The phenomenon initially emerged in the United Kingdom and Western Europe among lesbian, gay, bisexual, transgender (LGBT) population and it refers to the use of crystallized methamphetamine, mephedrone, γ-hydroxybutyrate (GHB) and, to a lesser extent, drugs of abuse like cocaine and ketamine to facilitate sex, frequently in a polydrug-use combinations **[4-5,11]**.

Synthetic cathinones such as mephedrone are an emerging class of drugs with psychostimulant and hallucinogenic effects similar to those of cocaine, amphetamines, and metamphetamines. Mephedrone, in particular, a substituted cathinone homolog of ephedrine, is one of the most popular NPS recreationally used in countries such as Italy, France, USA and the UK as a cheaper alternative to other drugs of abuse, related to the class of cathinones, firstly identified in 2007.

Moreover, intoxications related to the use of such substances have been reported, in Italy as well, whereas fatal cases are less common **[6-15].**

Mephedrone is a semi-synthetic compound included in the class of synthetic cathinones and sold as a white powder defined "meow meow" ,"4-MMC", "bubbles", "crab" or "M-Cat **[37]**.

Mephedrone powder can be insufflated (snorted effects within minutes), swallowed (effects in 45-120 minutes), often after wrapping in tissue paper or, more rarely, injected **[10,16]**, however, the most common routes are inhalation and oral ingestion, but due to its high solubility in water, it is also taken by injection **[10,17-18]**. Mephedrone users may develop tolerance quickly, and as a consequence, tend to consume higher doses more frequently **[18]**, posing the consumer at high risk for overdose. Most of the mephedrone effects seem similar to those documented for amphetamine, methamphetamine and 3,4-methylenedioxy-N-methylamphetamine (MDMA) **[36],** implicitly supporting a sympathomimetic activity. The effects of ring-substituted cathinones include feelings of empathy (openness, love, closeness) and awareness of senses **[18]** however, in-depth studies on the pharmacokinetics or pharmacodynamics, human toxicity, addiction or acute overdose potential and long-term effects of the cathinones are still ongoing **[20-22]**. Since their discovery, many analytical methods were developed and validated for its detection in biological specimens, as well as in seizures [**41-49**].

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We present here the first case-report to the best of our knowledge of a confirmed chemsex mephedrone-related acute toxicity case in our region detected and quantitated in biological specimens by means of gas chromatography/mass spectrometry (GC-MS) and liquid chromatography tandem mass spectrometry analyses (HPLC/MS-MS). Papers on full investigation of modes of analyses on the detection of isomers and various mephedrone derivatives, including GC-MS, NMR and IR spectra of possible metabolites have also been published recently **[13, 24].**

2. Case Report

A 56 years old man was found dead in his flat, only wearing a sex belt with a harness around the waist; no evidence of violence was observed.

A decedent's friend which was at the death scene, declared to the police that the man purchased a dose of mephedrone powder for practicing chemsex together. Then, the man decided to dilute the chemical substance in physiological water while performing sexual activities, to make it injectable intramuscularly in his shoulder through an insulin-syringe type found at the death scene, at about 11.00 pm. After few minutes from the injection, the man suddenly collapsed and head-butted to the table leg. He was reviewed by the medical staff that declared his death at 00.51 am. On the death scene, police found different insulin-syringes types, a dish with 4 small envelops, three of which with white powder inside, two 10 mL glass vials and a flask of ~~physiological water~~saline solution with a syringe on top of the cap.

2.1 Autopsy findings

The deceased was an Italian male, 177 cm in height and 85 kg in weight; the corpse was in good condition, the body appeared well-nourished. No external injuries or other remarkable evidences were found, except for a needle puncture mark and a contused lacerated wound on the left side of his upper face area and an abrasion in the zygomatic region, both most likely due to the fall. These findings were both superficial and were deemed not meaningful for the cause of the death. The internal examination of the body did not reveal any organ abnormalities that could explain the exitus. From autopsy examination performed after 38 hours, oedematous facies, widespread polyvisceral oedema and congestion, interfissural pulmonary petechiae, a moderate aortocoronary sclerosis and cardiac hypertrophy were detected; heart was 560 g. During autopsy examination, urine, bile and blood samples (central and femoral venous blood) were collected and stored at -20°C temperature until toxicological analysis. Due to the sudden death and the availability of many other biological samples, hair collection was not deemed necessary by the coroner.

2.2 Histological findings

Samples of organs were collected and formalin fixed, paraffin embedded and stained with haemotoxilyn-eosin (HE), figures 2-4. Heart: intense hyperaemia and congestion, interstitial oedema, signs of sub-endocardial contraction bands necrosis, fibro-fatty myocardial replacement, signs of neutrophil leukocytosis, micro-haemorrhages in the myocardial tissue. Lungs: intense hyperaemia and congestion, plenty of alveolar macrophages that fill in the alveoli, sub-segmental atelectasis, intra-alveolar and interstitial oedema, sparse leukocyte infiltration. Liver: intense congestion, signs of a hepatitis in a cirrhotic evolution. Brain: micro-haemorrhages in the white matter. Kidney and suprarenal glands: intense hyperaemia and congestion, micro-haemorrhages in the perirenal fat.

3. **Experimental**

3.1 Chemicals and reagents

Mephedrone hydrochloride solution (1.0 mg/mL) and deuterated internal standard mephedrone-d3 (1.0 mg/mL), sodium carbonate, methanol, ethyl acetate, β-glucuronidase (from H. pomatia) and MSTFA (N-Methyl-N-(trimethylsilyl)-trifluoroacetamide were provided by Sigma-Aldrich (Milan, Italy). Acetate buffer was prepared combining two solutions (50:50): solution A (1M) was prepared by dissolving sodium acetate in deionized water; solution B (1M) contained glacial acetic acid with deionized water. The carbonate buffer was prepared by dissolving potassium carbonate in deionized water with the addition of potassium bicarbonate to reach a basic pH.

3.2 Sample preparation

The urine sample was screened with immunochemical kits for a comprehensive preliminary toxicology investigation of most drugs of abuse (Screen Italia srl).

Then, 50 µL of a working solution mixture of deuterated drugs of abuse at 10 µg/mL were added to 1 mL aliquots of samples. Later, samples were buffered at pH 4-5 with 100 µL of 1M acetate buffer and urine and bile samples were deconjugated with the addition of 10 µL of β-glucuronidase prior to incubating the mixture at 45°C overnight, as this is our routine protocol for all investigations. Samples were subsequently extracted with 3 mL of ethyl acetate by automated shaking for 30 minutes and centrifuged for 5 minutes at 4500 rpm. The supernatant organic layer was then transferred and evaporated under a gentle stream of nitrogen to dryness. Further extraction was performed under alkaline conditions (pH 8-9) on the same aliquot by adding 100 µL of a 1M carbonate buffer and then 3 mL of ethyl acetate. After automated shaking of the samples for 30 minutes, the supernatant was separated and dried under nitrogen. The resulting residue was derivatised with MSTFA ~~BSTFA 1% TMCS~~ in ethyl acetate 1:1 for 30 minutes at 80 °C. Then 2 µL were injected into the GC-MS system.

For HPLC-MS/MS analysis an aliquot of 10 µL of urine, in 90 µL of ultra-pure water and 100 µL of the internal standard working solution (IS) was poured into the vial. Following an intense vortex mixing for 1 min, 10 µL of the mixture was injected into the LC-MS/MS system. An aliquot of 50 µL of blood samples (with 100 µL of the IS) was mixed in amber tubes. Then, a protein precipitation with 500 µL of acetonitrile was performed. After vortex mixing and centrifugation (5 min, 12000 rpm, 4°C), the supernatant was transferred to a clean amber tube. The mixture was evaporated under nitrogen stream until 100 µL circa was left. The concentrated mixture was transferred to the injection vial and 10 µL was injected into the HPLC-MS/MS system.

3.3 Analytical equipment

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Preliminary screening analysis for MDMA, GHB, methadone, morphine, metamphetamine, cocaine, THC, benzodiazepines, oxycodone, ketamine, synthetic cannabinoids and tramadol were performed on urine by the multi-drug test. Quantitative analysis was performed on a 7820 A GC-MS apparatus (Agilent Technologies, Milan - Italy) equipped with a 30 m capillary column (J&W DB-5% phenylmethylsilicone) with 0.25 mm diameter and 0.25 µm film thickness. Helium was the carrier gas with constant flow of 40 mL/min. The liner temperature was held at 270 °C, splitless mode. The oven temperature started from 100 °C, then 250 °C for 10 min with a 20 °C/min heating rate, to 280 °C for 5 min with a 20 °C/min heating rate and to 320 °C at 20 °C/min for 4 min. The total run time was 32 min. Full scan (*m/z* 50–500) and selected ion monitoring (SIM) modes were applied: quantitative determination of mephedrone was performed by monitoring the ions in SIM mode at *m/z* 119 (quantifier ion), 130, 131 and 234 (qualifiers ions). Deuterated internal standard ions for mephedrone-d3 were *m/z* 122 and 133. Mass detector voltage at 70eV Data analysis was performed using the Agilent GC–MS software (MassHunter).

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Standard calibration curves were prepared using a blank matrix samples spiked at increasing concentration levels in triplicates. Further confirmatory analyses were carried out by means of HPLC-MS/MS equipment: Agilent 1100 series, column C18 Agilent Pursuit XRs ultra 2.8 micron, 100x2.0 mm; MS: ABSciex API 4000 with turbo V ion source; mobile phase A: 10 mM formic acid, pH 3.75; mobile phase B: MeOH and ACN, 95:5. Analytes were determined with a selected reaction monitoring (SRM) method by acquiring ~~three~~ two or more transitions for each compound and optimum cone voltage and collision energy, in ESI positive mode, 10 microliter injection volume (SRM transitions for mephedrone 178→ 160, 145, 119, mephedrone d3 →181 → 91, 163).

3.4 Method validation

The method was validated by investigating linearity, selectivity, ~~identification limit~~limit of detection (LOD), ~~quantitation limit~~limit of quantitation (LOQ), precision and accuracy in blood and urine as required from guidelines **[25]**. The linear calibration models were checked by analyzing (three replicates) blank samples spiked with mephedrone standard solution at final concentrations of 0, 0.10, 0.25, 1, 10, 20, 40 µg/ml. If the concentration exceeded the calibration range, the samples were diluted to fit the quantitation interval considered in the curve. The calibrators were processed as reported above. The dilution integrity was evaluated by spiking each matrix at a mephedrone concentration 1.5 times the highest calibration point and diluting the resulting solution twice and ten times with blank matrix. These samples were analyzed along the standard calibration curve, and the accuracy was considered satisfactory within the interval ±20% the expected value. The LOD was estimated as the analyte concentration whose response provided a signal-to-noise (S/N) ratio of 3, as determined from the least abundant qualifier ion. The S/N ratio at the lowest concentration was used to extrapolate the theoretical LOD. The calculated LOD for both matrices was experimentally confirmed by analyzing urine and blood samples spiked with mephedrone at the LOD concentration. The LOQ value was estimated as the analyte concentration whose response provided an S/N value equal to 10. For each matrix, ten different blank samples were prepared, as described above, in order to test the selectivity of the whole analytical procedure. No interferences from endogenous substances were detected by monitoring the S/N for the characteristic selected-ion chromatograms at the expected retention time of mephedrone. Within-batch precision (expressed as variation coefficient, CV%) and accuracy (expressed as bias %) were assessed by extracting and analyzing, for each tested matrix, a series of five samples fortified at 0.10 and 1.0 mg/L.

4. Results and discussion

4.1 Toxicological analysis

Toxicological analysis of urine, bile and blood samples were carried out following the routine protocol of the toxicology laboratory of our Unit.

The LOD (10 ng/ml) and LOQ (20 ng/ml) was evaluated as detailed above. The regression coefficients were~~as~~ both r^2 >= 0.995~~74~~ in urine and blood, also ~~-~~demonstrating a good linearity and satisfactory repeatability (CV% 1.5–3.6% for mephedrone spiked in blood and urine at both ~~the~~ 0.10 and 1.0 mg/L); accuracy varied from −2.1 to +15.9%, expressed as percent bias, showing all experimental values below the acceptable bias limit of ±20% at 0.10 and 1.0 mg/L in blood and urine.

A preliminary screening analysis on urine was performed for the detection of the most common illicit substances (cocaine, delta9-THC, GHB, buprenorphine, methadone, amphetamine, benzodiazepines, tramadol, ketamine, opiates), which resulted positive for metamphetamine. Subsequently, qualitative and quantitative analyses of urine, bile and blood samples were performed by means of liquid-liquid extraction and GC-MS and HPLC/MS-MS: ~~-~~these were negative for methamphetamine and 3-MMC; on the other hand, m~~M~~ephedrone was confirmed in urine, bile and blood samples in SIM mode in the following concentrations: 2.0 mg/L in urine sample, 1.1 mg/L in bile and 1.0 mg/L in central blood sample while 0.8 mg/L in peripheral blood samples, respectively. None of the other most common drugs of abuse, in particular mephedrone's isomer 3-MMC, could be detected neither in GC-MS or HPLC/MS-MS analyses by retention time and SRM transitions.

4.3 Discussion

The past few years have seen an increase in the reporting of serious adverse events, particularly in respect of severe and fatal poisonings, sometimes manifesting as outbreaks. Although drugs and alcohol have often been used in sexual contexts throughout history, mephedrone provides a particular sexually-disinhibiting "high", which represents a different public health concerns than those associated with traditional drugs of abuse. In this paper, the first case of mephedrone acute intoxication in our area, after a recreational use, is described.

All around Europe, mephedrone was reported to be implicated in several fatalities, but only a few have actually resulted in acute intoxication **[8,9]**. In general, mephedrone seems to have stimulant effects, although unlike other stimulants, some users report that an associated euphoric effect is not associated. Limited data are available on toxicological analyses: papers on the detection of isomers and various mephedrone derivatives, including GC-MS, NMR and IR spectra of possible metabolites have been published recently **[13, 24].**

~~Mephedrone is a semi-synthetic compound included in the class of synthetic cathinones and sold as a white powder defined "meow meow" ,"4-MMC", "bubbles", "crab" or "M-Cat [37].~~

As in the present case, cross-reactivity with methamphetamine test on urine sample at screening level is given by their similar chemical structures, however more selective and accurate confirmatory analysis on the same sample could actually distinguish between mephedrone and metamphetamine. Since few cases related to the toxicity of mephedrone have been reported, reference toxic and lethal concentrations in body fluids and organs are not available yet, however, comparing with previously published data on the few acute intoxication case-reports, the concentrations calculated in our case report suggest that the deceased consumed the substance before the death (hence the presence of the compound in bile and urine), while the high concentration found in blood specimens confirms that at the time of death the mephedrone had just been injected and still had not been metabolised. This hypothesis is corroborated from time elapsed between the injection witnessed from the decedent's friend and the declaration of death from medical staff. Considerations on the levels of mephedrone calculated can be drawn on the basis of the data recently published. Cases of mephedrone non-fatal intoxication from living individuals were reported in which the drug in the plasma was in the range 0.01 mg/L to 0.74 mg/L alone or with other substances **[14].** Mephedrone was detected within the range of 0.13–0.50 mg/L in blood

samples in cases where other drugs (GHB, morphine or methadone) played a key-role in the death due to a multidrug intoxication. The blood concentration was between 1.94 mg/L and 22 mg/L in six cases where mephedrone acute intoxication was reported as the cause of death **[30-35]**. In two cases, mephedrone was the only drug detected in the blood, at levels of 3.3 and 5.5 mg/L**;** in one case where the death was attributed to adverse effect of mephedrone on an atherosclerotic coronary artery disease, mephedrone was detected alone in peripheral blood at 0.98 mg/L **[31],** close to the concentrations found in the case here reported (1.0 mg/L in central blood sample while 0.8 mg/L in peripheral blood). In an extreme case of fatal excited delirium after mephedrone abuse, an exceptional concentration of 5.1 mg/L was found in the femoral blood, along with traces of cocaine, MDMA and benzodiazepines; in another mephedrone-related fatality, the blood and urine concentrations were 1.33 mg/L and 144 mg/L, respectively **[14-15]**.

Nevertheless, in the case hereby presented it is difficult to extrapolate from the toxicological results the exact amount of mephedrone used, due to the dilution operated from the decedent or the quantity injected. Taking into account tolerance phenomena and inter-individual variability, however these concentrations seem to be comparable to those already reported in literature and therefore the sudden death could be linked to the toxicity of the substance. In the present case, with particular reference to mephedrone peripheral blood concentration, the compound concentration was slightly lower than in the cases previously reported, where no other drugs were found to play a role in the intoxication. On the other hand, the mephedrone central blood concentration determined is higher than in the reported non-fatal mephedrone intoxications, suggesting a post-mortem redistribution based on the ratio between the central and peripheral concentrations (C/P=1.25). The presence in urine and in bile to some extent confirms that the subject was a habitual user of the substance, as further confirmed from anamnestic data and witnesses reports. Mephedrone, similarly to all cathinones, is characterized by instability in blood matrices at neutral and basic pH conditions [41,50] and in urine [22], however the addiction of preservatives to the samples, the storage at -20°C and the analyses rapidly performed prevent significative loss of the analyte. In fact, the storage of whole blood, urine and plasma at −20 °C revealed no significant decrease of concentration during the period of 14 days [41,50].

Regarding the potential mephedrone-addiction, accumulating evidence suggests that it has a clear addiction potential comparable to cocaine or methamphetamine. Among regular users, about 50% reported an addiction to the compound and about 25% admitted its craving with proper withdrawal effects **[17]**.

The mechanism of action is very similar to that of amphetamines, characterized by a predominant action on plasma membrane catecholamine transporters. Such compounds have shown to bind to noradrenalin, dopamine and serotonin transporters **[5,23,26,38]**.

As per literature, methamphetamine-like substances give dose-dependent hypertension and tachycardia due to adrenergic stimulation, sudden cardiac death, arrhythmias, accelerated atherosclerosis, vasospasm, acute coronary syndrome, coronary, carotid and aortic dissections, and circulatory collapse, as well as cardiomyopathy **[37, 39,40]**. One of the frequent histopathological pattern is the focal myocyte as well as contraction bands necrosis, like those here reported. Moreover, in emergency room, when acute catecholamine syndromes arise, a CT scan is highly recommended **[40]** for cerebral micro-haemorrhages, which are documented in our samples.

As the case here presented shows, mephedrone can be injected either intramuscularly or intravenously, at one half or two thirds of the oral dose. Due to the capability of the drug to rapidly induce tolerance upon administration of repeated doses, an increasing number of users reported a quick progression to either regular drug use and/or uncontrolled bingeing ~~behaviour~~behavior with 1–4 g of mephedrone consumed in a session to prolong the duration of its effects, up to 11.16 g for each consumer **[27-29]**.

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It is known that, when similar compounds are taken parenterally, the peak vasopressor effects show up within about 30 minutes, as plausible in our case, while if taken orally the peaks are observed after 1-2 hours **[40]**, as well as effects on cardiovascular district such as spikes in high blood pressure, heart arrhythmia, coronary artery spasms, coronary thrombosis **[17-19]**.

It is frequent, although not observed in our case, that intravenous injections can result in vein blockages, leading to localized infections, abscesses, lumps, gangrenous tissue, blood clots, and large necrosis at the injection site probably due to the formulation or excipients used.

Objective assessment and the screening for the presence of principal drugs of abuse and psychoactive pharmaceuticals in blood and urine excluded the implication of any other pharmacologically active substance in this case report, categorizing it as a mephedrone-correlated death. Moreover, in particular the subject with cardiac hypertrophy and a moderate aortocoronary sclerosis, after the mephedrone injection might have suffered the effects of peripheral vasoconstriction with pressure increase and consequent increased cardiac work, determining the necessity for more blood supply to the heart and acute cardiac decompensation. The heart suffering is documented by the contraction bands necrosis and micro-haemorrhages, while acute hypertension can be supposed thanks to the micro-haemorrhages in the brain.

Facies edematosa, oedema and polyvisceral congestion, interstitial petechiae findings are compatible elements with death from acute cardio-respiratory failure, with peri-mortem agony of a few minutes in which the cardiac hypertrophy, the moderate aortocoronary sclerosis and mephedrone injection have played a substantial role in the evaluation of the sudden death cause.

5. CONCLUSIONS

In the case here described, the investigation was effectively guided by the circumstantial data reported by the survivor: they provided useful information on the substance taken by the decedent.

Pharmacodynamics and pharmacokinetic studies are currently ongoing and further data from peer-reviewed reports are needed, however the reported effects of mephedrone are generally similar to those of MDMA and cocaine, as documented by the histopathological findings too.

Mephedrone therefore may present mainly stimulant-like effects, including mood enhancement and alertness, but possesses as well both hallucinogenic and empathogenic properties at higher dosages. Only a few related mephedrone acute toxicity case reports have been published to date, and there is a distinctive lack of information about the acute and chronic toxicity of mephedrone. The ruggedness and reproducibility of the analyses were secured by the use of a properly selected isotope-marked analogue as internal standard.

This case study also underlines the importance of reporting histopathological changes obtained from autopsies together with toxicological results for a more comprehensive data interpretation.

Acknowledgments

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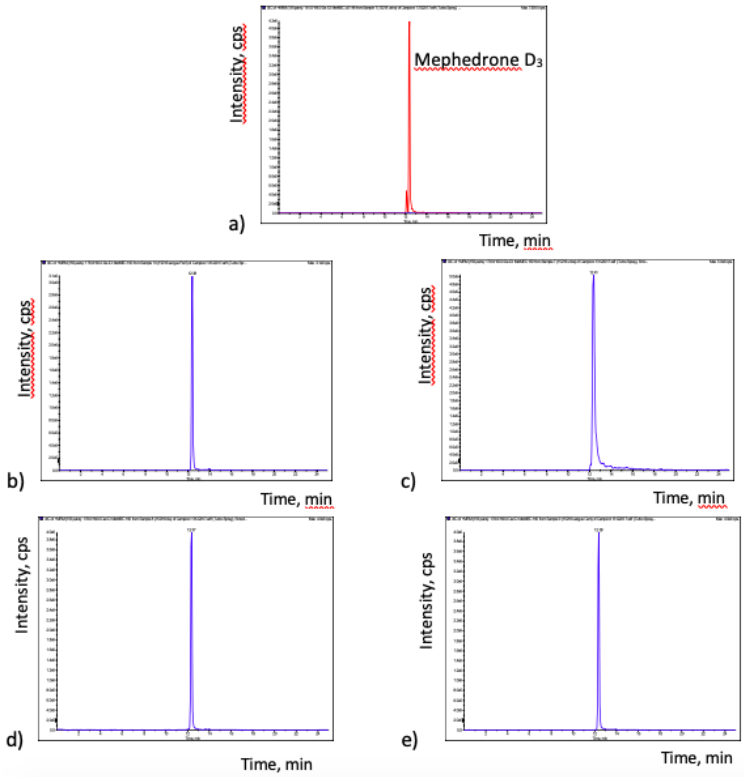

Fig. 1. HPLC-MS/MS chromatogram of the internal standard (mephedrone D_3 181→148) in urine sample (a). Chromatograms of mephedrone (178→160) in peripheral blood sample (b), urine sample (c), bile sample (d) and central blood sample (e).

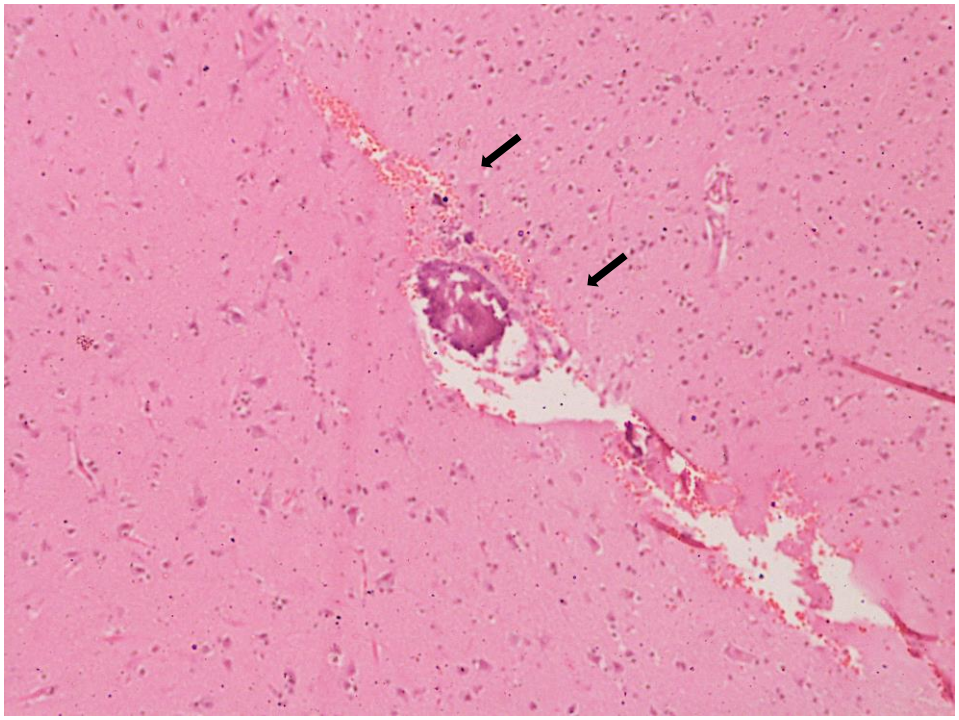

Fig. 2. HE brain : Haematoxylin and eosin staining showing cerebral micro-haemorrhages histopathology (Object 10x).

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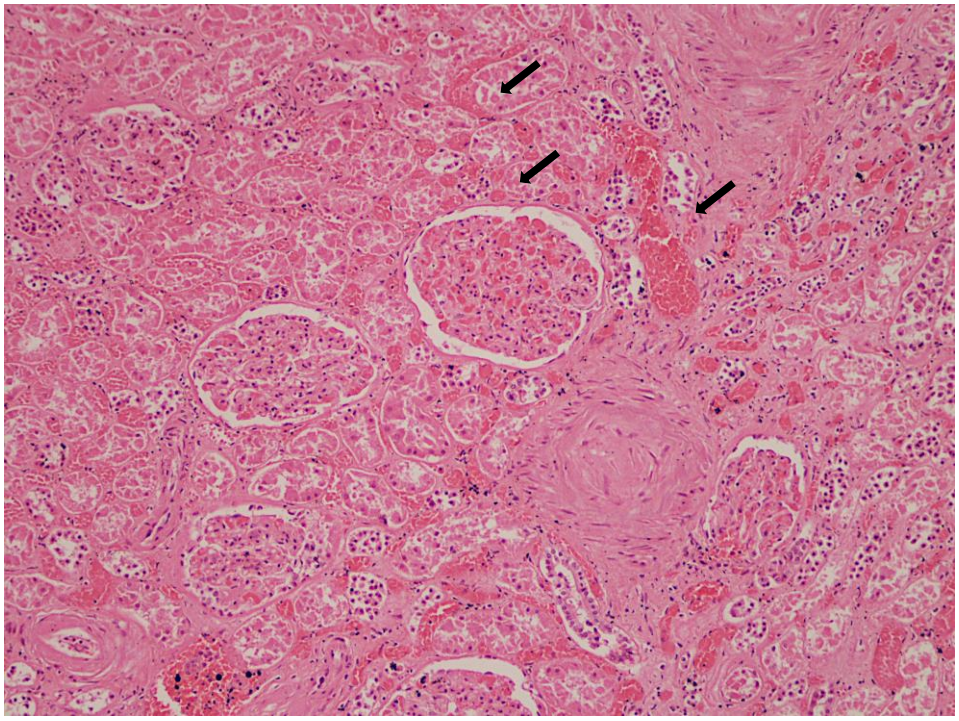

Fig. 3. HE kidney; hemorrhage in kidney parenchyma and hyperemia in glomeruli (object 10x).

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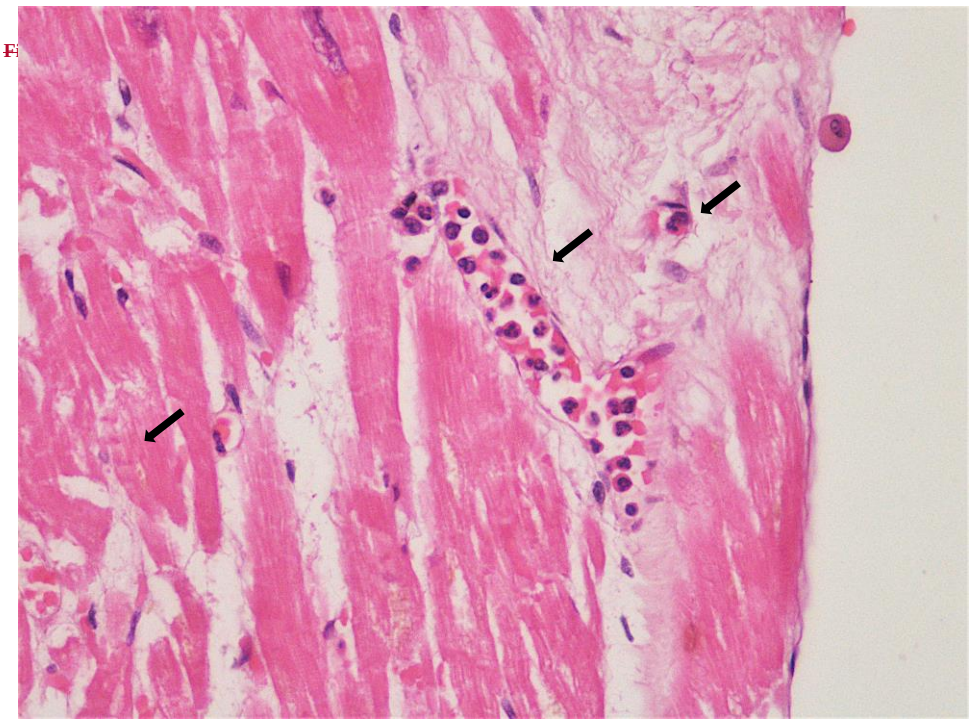

Fig. 4. HE heart: interstitial oedema, signs of sub-endocardial contraction bands necrosis, neutrophil leukocytosis and leukocytes early adhesion to endothelial cells (object 40x).

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