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Case report

Two cases of lysergamide intoxication by ingestion of seeds from Hawaiian Baby Woodrose

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ABSTRACT

We describe two cases of human consumption of seeds from *Argyreia nervosa* (Hawaiian Baby Woodrose), which resulted in one fatality due to falling from a building and one surviving witness. The principal psychoactive constituent of the seeds, lysergamide (LSA), was recovered from blood and urine samples by mixed-mode cation exchange solid-phase extraction and quantified by ultra performance liquid chromatography–time of flight mass spectrometry (UPLC–ToF/MS). The LSA concentrations were determined by UPLC–ToF/MS to be 4.9 μg/L in blood and 1.0 mg/L in urine in the dead person and 1.8 μg/L in blood and 0.50 mg/L in urine in the living person. These analytical findings were found to be in accordance with the case story, which indicated that seeds had been ingested and also noted psychological reactions, i.e. the will to jump out of the window. Other findings in the dead person were 22 μg/L THC in blood, 0.71 g/L ethanol in blood and 1.0 g/L ethanol in vitreous humor. Constituents originating from the seeds of *A. nervosa*, i.e. LSA, ergonovine, lysergic acid α-hydroxyethylamide were also identified in the biological samples. The 2-hydroxy-3-oxo metabolites of LSA and ergonovine were identified in the urine sample of the deceased.

1. Introduction

Since ancient times humans have been fascinated by the effects of ingesting hallucinogenic material from plants. Lysergamide, also known as lysergic acid amide (LSA) or ergine, is the non-alkylated amide-analogue of the potent and widely known hallucinogen LSD (lysergic acid diethyl amide), and is an ergot (syn: ergoline) alkaloid found in the seeds of *Convolvulaceae* members *Argyreia nervosa* (Hawaiian Baby Woodrose), *Ipomoea violacea* (Morning Glory) and *Rivea corymbosa* (Ololiuhqui). There is mounting evidence that some of these *Convolvulaceae* members are infected with clavicipitaceous fungi and that these fungi are responsible for ergoline alkaloid biosynthesis [1,2]. The seeds of Hawaiian Baby Woodrose have the highest percentage of ergoline constituents among all *Convolvulaceae* members [3].

In general, 2–5 mg of LSA is sufficient to produce hallucinations, providing a 4–8 h intoxication that reportedly has quantitative as well as qualitative differences from LSD [4]. Seeds are eaten whole or crushed or, frequently, consumed as an extract after being soaked in water. Five to 10 seeds of Hawaiian Baby Woodrose or 150–200 seeds (3–6 g) of Morning Glory yield commonly used doses of LSA [5,6].

Following Schultes' reports on native, ceremonial use of Morning Glory and Ololiuhqui [7] and the subsequent isolation and identification of ergot alkaloids in these plants by Hofmann and Tscherter [8], recreational abuse surged. Only few older [9–12] and some more recent [5,6,13,14] scientific reports exist on the effects of human consumption of LSA-containing seeds. Patients complained of anorexia, nausea, anxiety, suicidal ideation, a fear of going insane, a sense of derealization, distortions in sense of time and memory loss. Some, but not all, experienced hallucination. Findings upon examination included tachycardia, hypertension, pupil dilation, flushing and polyuria. A suicide has been reported after intake of Morning Glory seeds [9].

The Internet is abundant with accounts of personal experiences with LSA as well as modes of extraction (http://www.erowid.org, http://www.drugs-forum.com). Moreover, LSA-containing seeds are easily obtained from internet-based stores, marketed by some under purportedly legitimate uses (i.e. ornamental), others explicitly for their hallucinogenic properties.

LSA is classified as a LSD precursor, Schedule III drug in the DEA Controlled Substances Act in the USA and a Misuse of Drugs Act Class A drug in the United Kingdom.

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2. Case

We report the voluntary ingestion of Hawaiian Baby Woodrose seeds in two males aged 29 (deceased) and 25 (surviving witness). The seeds were soaked in water for approximately 2.5–3 h and subsequently ingested. The witness ingested 6 seeds. He could not recall how many seeds the deceased had ingested. Shortly thereafter, both males smoked cannabis. Approximately 40 min after ingestion, the witness experienced a sense of wellbeing as well as losing track of time. Approximately 3 h after ingestion, the deceased became severely agitated and jumped out of a window, falling 4 floors. He was pronounced dead upon arrival of emergency medical staff.

In conjunction with the autopsy, samples for chemical–toxicological analysis were taken 13 h after ingestion. The main autopsy findings were: conquassation of the skull, multiple rib and pelvic fractures, lacerations of the right lung, cardiac contusion, haemopericardium (50 mL), bilateral haemothorax (right: 650 mL, left: 250 mL), total rupture of the thoracic aorta, subarachnoideal haemorrhage and atherosclerosis of the aorta and the coronary arteries.

The witness' blood and urine was sampled 9 h after ingestion.

3. Materials and method

3.1. Materials

Lysergamide (LSA) was synthesized from ergotamine according to the procedure described below: Ergotamine tartrate (LGC standards, UK) was subjected to alkaline hydrolysis to liberate lysergic acid. Following cooling to 10 °C, lysergic acid was precipitated as its sulfate and left overnight. The precipitated solids were isolated by filtration and washed with diethyl ether. The solid material was purified by extraction with 15% ammonia in absolute ethanol, removal of solvent, re-dissolution in 1% aqueous ammonia and re-precipitation with sulfuric acid. Filtration and drying gave the crude lysergic acid hydrate. By conversion of the carboxylic group to its acid chloride and reaction with gaseous ammonia, lysergamide (LSA) was formed. LSA was then purified in three steps by solid-phase extraction on aluminium oxide (methanol/ethyl acetate), by preparative chromatography on silica gel (2-propanol/2,2,4-trimethylpentane) and finally by reversed-phase chromatography on LC8-DB (Supelco Inc., PA, USA) using 50% methanol. The identity of the synthesized LSA was verified by H^1 NMR. The purity of LSA in the 100 mg/L standard solution was found to be 90% by two-dimensional thin layer chromatography (HPTLC no indicator, Merck, Darmstadt) using two different solvent systems. The dried plate was sprayed with 10% sulfuric acid in ethanol which results in dark red spots of ergot alkaloids. The thin layer plate was subsequently heated on a hot plate causing charring in order to visualize non-ergot compounds. Since it is possible to detect about 10 ng of a compound using this technique and applying at least 1 μg on the plate, impurities down to about 1% can be detected.

Lysergic acid beta-propanol amide maleate (ergonovine) according to US Pharmacopeia (MD, USA) was obtained from LGC Standards (Middlesex, UK). Internal standard LSD-D3 was obtained from Cerilliant (Texas, USA). The solvents methanol (LC–MS grade), acetonitrile (LC–MS grade) and ethyl acetate were obtained from Fisher Scientific (Leicestershire, UK). β-Glucuronidase/arylsulfatase (*Helix pomatia*) was obtained from Roche Diagnostics (Copenhagen, Denmark). Other chemicals were of analytical grade and from Fluka or Merck.

Pools of reference matrix, human urine ($n > 3$) and whole blood ($n > 200$), were verified to be blank by in-house full toxicological screening for medicinal drugs and narcotics.

The 100 mg/L stock solutions of LSA in ethanol and LSD-D3 in acetonitrile were stored in amber glass vials at −20 °C. The mixed-mode cation exchange 3 mL column was Isolute HCX-3, 130 mg (Argonaut, Mid Glamorgan, UK).

3.2. Sample preparation

The biological samples were stored at −10 °C prior to analysis. The urine samples were centrifuged for 10 min, 20 °C at 3600 × *g*, and the upper layer was amended for further analysis. An aliquot of 500 μL urine, 1.5 mL water, 1 mL 1 M acetate buffer, pH 5.5, 25 μL β-glucuronidase/arylsulfatase and internal standard was mixed and incubated for enzymatic hydrolysis, 18 h at 40 °C. Whole blood samples, 500 μL, were diluted with 2.7 mL 0.1 M KH_2PO_4 buffer pH 6.0, internal standard and homogenized by sonication for 10 min. The urine and blood samples were centrifuged 10 min at 3600 rpm, and the supernatants were amended for extraction. For calibration, matrix- and recovery experiments, blank whole blood and urine samples were spiked with LSA and LSD-D3. The internal standard LSD-D3 was added to all types of samples at a fixed level (100 μg/L).

3.3. Extraction procedure

Gilson ASPEC XL4 (Gilson, Viliers-le-Bel, France) was used for automated solid-phase extraction. A previously described procedure was modified for the extraction of LSA [15]. Extractions were performed in duplicate. The SPE columns were conditioned with 1.5 mL methanol and 1.5 mL 0.1 M KH_2PO_4 buffer, pH 6. The sample (2.6 mL) was loaded, followed by washing steps consisting of 1 mL 0.1 M KH_2PO_4 buffer, 1 mL 1 M acetic acid, and 1 mL methanol in consecutive order. After drying for 10 min, the analytes were eluted with a freshly prepared solution of ammonium hydroxide (25% aq.)–acetonitrile–ethyl acetate (2:10:88, v/v). Evaporation was carried out at 40 °C. The extracts were reconstituted by sonication in 30% acetonitrile in water, 100 μL for blood and 500 μL for urine.

3.4. UPLC–ToF analysis

The UPLC (Acquity, Waters, Milford, MA, USA) was operated with a gradient elution with water–formic acid (0.05%) and methanol (90:10) to (0:100) on an Acquity HSS $T3C_{18}$ column (2.1 mm × 100 mm, 1.8 μm) at 35 °C [16]. The ToF/MS (LCT Premier XE, Waters Corporation, Manchester, UK) was operated in electrospray positive (ESI+), W-mode with dynamic range enhancement. Full scan mass spectra (m/z 50–1000) were collected at high resolution (>10,000, full-width half-maximum). Each sample was injected in duplicate (5 μL). The MassLynx software module QuanLynx (Waters Corporation, Manchester, UK) was used for data analysis. The area of the extracted ion chromatogram at a 0.02 Da mass window was recorded for LSA at m/z 268.1449 and for the internal standard LSD-D3 at m/z 327.2264.

3.5. Method validation

The calibrators were duplicate extracted standards, 0, 5, 10 μg/L for blood, and 0, 10, 100, 500, 1000 μg/L for urine. Calibration curves were linear ($r^2 > 0.99$) with $1/x$ weighting. Due to the limited amount of reference compound available, validation was performed in triplicate determinations including recovery, matrix effect, LOD and LOQ in blank urine and whole blood [17]. The average recovery was determined as the amount of extracted standard compared to standards in pure solvent. Matrix effect was performed by spiking samples before and after extraction compared with a non-extracted standard solution in mobile phase at the same nominal concentration.

3.6. Other findings

Quantification of THC in blood was performed by liquid/liquid extraction using n-pentane followed by UPLC–MS/MS by a validated in-house method. Quantification of ethanol in blood and vitreous humor was performed using head-space GC [18].

3.7. Extraction of A. nervosa seeds

The whole seeds were weighed and crushed in a mortar and 50 mg was extracted for 30 min in 5 mL methanol. The extract was centrifuged to precipitate solids, and the supernatant was diluted with solvent for UPLC–ToF analysis.

4. Results and discussion

The chromatograms of LSA in standard and blood samples are shown in Fig. 1. The validation parameters are shown in Table 1. The extraction recoveries were 73% and 50% in urine and blood, respectively. The matrix effects were 36% and 46% in urine and blood, respectively. The uncertainty of LSA measurement in both cases and matrices was 10–20%. The blood concentration of LSA (Table 2) was lower in the surviving person (1.8 μg/L) than the dead person (4.9 μg/L). LSA is the non-alkylated amide of lysergic acid, and due to its structural similarity to LSD it may have similar pharmacokinetics. The half-life of LSD is estimated to be 3 h [19], and the blood of the witness was sampled 9 h after ingestion. Hence the analytical results may suggest that the two people ingested approximately the same amount of seeds, i.e. if LSA has a half-life similar to LSD. The concentration of THC in the blood of the deceased, 22 μg/L, is in the normal range of abuse. The ethanol concentrations were 0.71 g/L in blood and 1.0 g/L in vitreous humor. The presence of THC and ethanol may have aggravated the LSA intoxication. THC and ethanol were not detected in the surviving witness, which may be due to the later time of sampling.

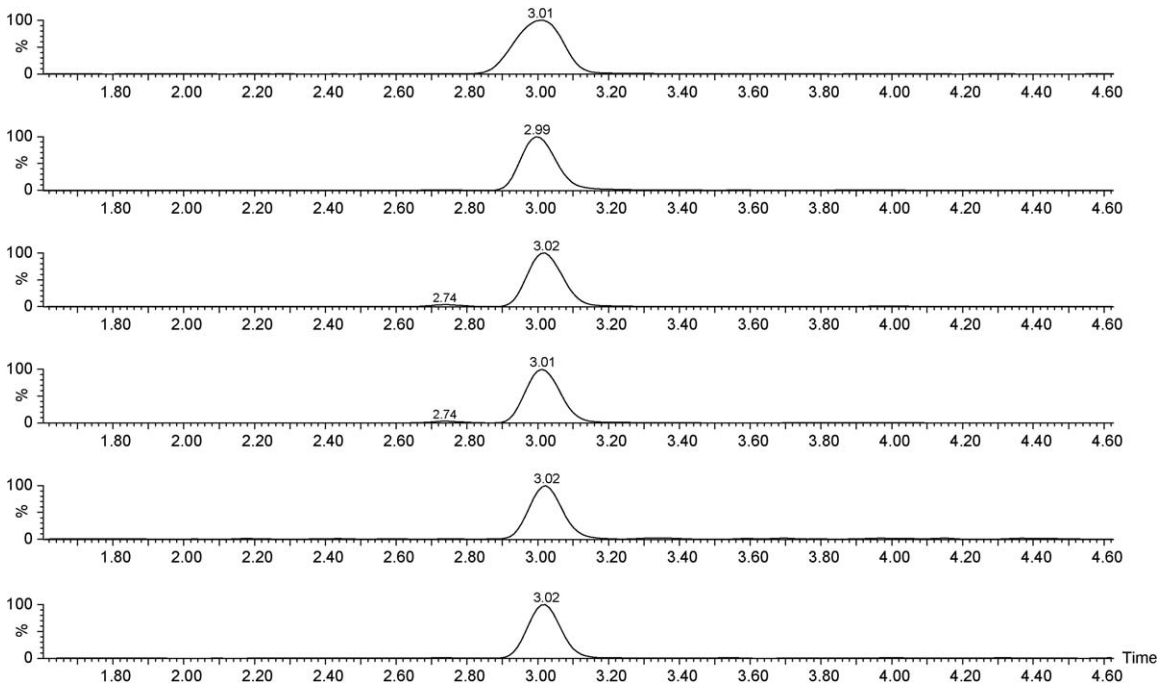

Fig. 1. Lysergamide (LSA) extracted ion chromatograms at *m/z* 268.1449. From top to bottom: standard, seed extract, urine sample of living witness, urine sample of the deceased, blood sample of living witness and blood sample of the deceased.

Table 1
Validation parameters for LSA ($n = 3$).

	Urine	Blood
LOD	5.0 μg/L	1.3 μg/L
LOQ	10 μg/L	1.6 μg/L
Recovery[a]	73%	50%
Matrix effect[a]	36%	46%

[a] Tested levels of LSA were 100 μg/L in urine and 10 μg/L in blood.

Table 2
Results of chemical–toxicological analysis.

	LSA mg/L urine	LSA μg/L blood	THC μg/L blood	Ethanol g/L blood	Ethanol g/L vitreous
Dead person	1.0	4.9	22	0.71	1.0
Surviving person	0.5	1.8	0	0	n/a

LSA has previously been identified in seeds from Hawaiian Baby Woodrose [20,21]. To date we have found one study of bioanalytical LC–MS/MS analysis of LSA in two urine samples, resulting in levels of 31–49 μg/L LSA in urine [22,23]. From intake of 6 seeds, corresponding to 825 mg, with a LSA content of 0.324% the estimated intake of LSA is 2.65 mg [3]. In comparison, LSD is administered by drug abusers in oral doses of 25–250 μg, resulting in 1–9 μg/L LSD in blood and 1–55 μg/L LSD in urine [24]. The concentrations of LSA in blood measured here, are thus of the same order of magnitude as reported LSD concentrations. The oral route of administration of whole seeds may result in a slow release of LSA; hence, even if the total content of LSA ingested is about 10 times higher than LSD, the LSA blood levels will not be 10 times higher. The LSA urine concentrations were found to be a factor 10–20 higher than typical LSD concentrations and previously reported LSA concentrations [23]. The stability of LSA in biological matrices was not investigated in this study. However, previous studies of LSD have shown that it is unstable in blood and more stable in urine [24]. The specimens were analyzed one year after sampling, since the LSA

Table 3
Lysergamide and ergonovine type derivatives, identified by UPLC-ToF in the seeds and in the forensic cases.

	Formula	$[M+H]^+$ (Da)	RT (min)	Seeds	Blood	Urine
Lysergamide (LSA)[a]	$C_{16}H_{17}N_3O$	268.1449	3.0	P	P	P
Ergonovine[a]	$C_{19}H_{23}N_3O_2$	326.1869	3.5	P	P	P
iso-Ergonovine[a]	$C_{19}H_{23}N_3O_2$	326.1869	4.2	P	P[c]	P
Lysergic acid beta-propanolamide[a]	$C_{18}H_{21}N_3O_2$	312.1712	3.1[d]	P	P	P
iso-Lysergic acid beta-propanolamide[a]			4.0[d]	P	P	P
Lysergamide, 2-oxo-3-hydroxy[b]	$C_{16}H_{17}N_3O_3$	300.1348	1.5	N	N	P[c]
Ergonovine, 2-oxo-3-hydroxy[b]	$C_{19}H_{23}N_3O_4$	358.1767	2.2[d]	N	N	P
iso-Ergonovine, 2-oxo-3-hydroxy[b]			2.9[d]	N	N	P[c]

P: Positive (verified by examination of isotopic distribution (I-fit)). The retention time for lysergamide and ergonovine could be verified by a reference standard.
N: Not found.
[a] From Argyreia nervosa seeds.
[b] From metabolism.
[c] Not found in living person.
[d] Retention time not assigned to specific analyte.

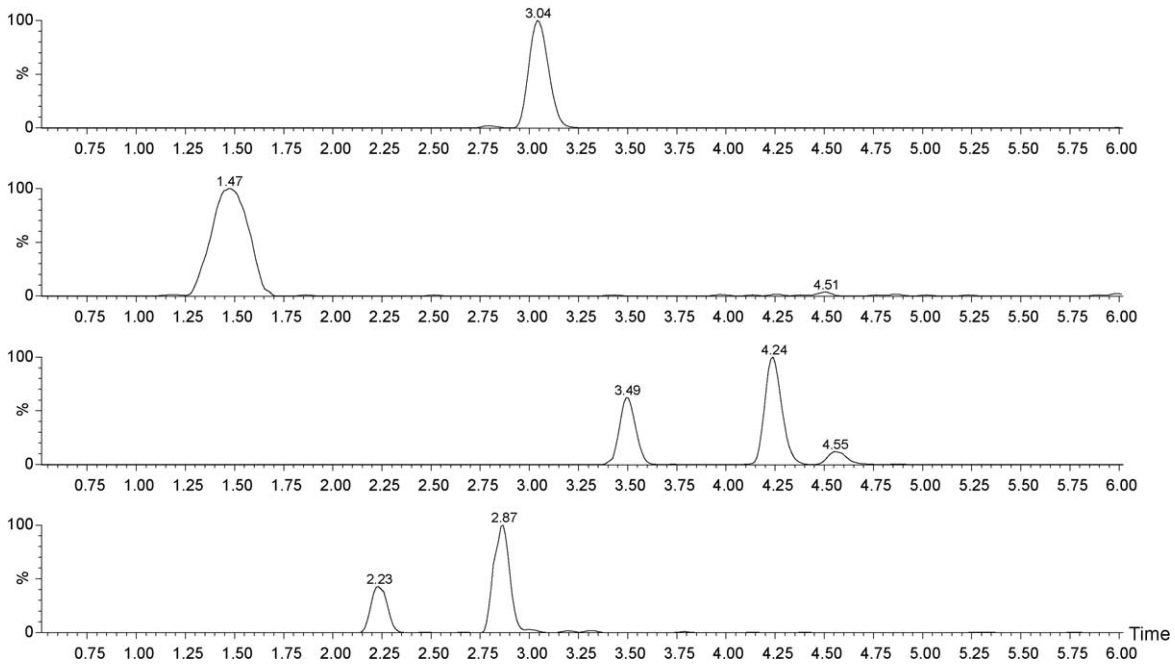

Fig. 2. Extracted ion chromatograms from top to bottom: LSA, 2-oxo-3-hydroxy-LSA, ergonovine (2 isomers), 2-oxo-3-hydroxy-ergonovine (2 isomers), from UPLC–ToF analysis of the urine sample of the deceased.

standard was not available until then. Thus, we suspect that the concentrations of LSA, especially in blood, were higher originally.

By means of their exact mass and isotopic fit by UPLC/ToF, several other analytes were tentatively identified in addition to LSA, both within the biological specimens and the seeds (Table 3). A reference of ergonovine was obtained and verified by retrospective data analysis, however no other reference compounds were available for this study. LSA, ergonovine (i.e. ergometrine or lysergic acid beta-propanolamide) and D-lysergic acid α-hydroxyethylamide, were identified in the seeds of *A. nervosa*, consistent with previous reports [3]; they were also identified in the blood and urine samples. Two isomers of ergonovine and D-lysergic acid α-hydroxyethylamide, respectively, were observed in seeds, urine and blood. No *iso*-LSA was observed. Except for ergonovine, these peaks could not be assigned to a specific isomer, due to the lack of analytical references. Analytes from LSD-type biotransformations [24] were identified in the urine sample of the deceased; 2-oxo-3-hydroxy lysergamide and 2-oxo-3-hydroxy ergonovine (2 isomers), Fig. 2. Due to their limited time window of detection, metabolites of LSA and ergonovine were identified in the urine sample from the deceased person only.

The hallucinogenic properties of LSA have been contradicted by Shulgin [25], who states that ergonovine, also used in obstetrics as an oxytocin [26], is a potent hallucinogen at 10 times therapeutic concentrations. Thus, the quantification of ergonovine is suggested to be included in future chemical toxicological analysis of intoxications by *Argyreia* spp. and other ergot alkaloid-containing plants.

5. Conclusion

This article describes two cases of ingestion of seeds from *A. nervosa* (Hawaiian Baby Woodrose). One of the consumers died due to a fall from a building. The surviving witness provided only few details regarding the effects of consumption of the seeds. Seeds from the crime scene were analyzed and LSA was identified. The main alkaloid, LSA, was extracted by mixed-mode cation exchange solid-phase extraction, then identified and quantified by UPLC–ToF/MS in urine and blood samples. This is the second report on a fatality, apparently due to ingestion of LSA-containing seeds. A synthesis of LSA from ergotamine is described in this article for obtaining this reference compound. Retrospective data analysis of full scan high resolution mass data of ergot alkaloids in biological samples and seeds was found to be useful, where no analytical standards were available. This analytical approach may be applied to other cases of intoxications with plant material of known or unknown origin.

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