

A toxic self-made beverage: Analytics of alkaloids from *Veratrum album* by LC-MS/MS

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Abstract

A 49-year-old man consumed two glasses (approx. 2 x 20 ml) of a beverage containing yellow gentian (*Gentiana lutea*). Shortly after ingestion, he developed nausea, vomiting and oral paraesthesia. On admission to the hospital he suffered from severe bradycardia and hypotension and he was treated with activated charcoal, antiemetics, atropine and i.v. volume. The patient completely recovered from the symptoms within 24 hours and was discharged from the hospital. The initial suspicion of *Veratrum* poisoning was confirmed by identifying protoveratrin A (ProA) and protoveratrin B (ProB) in a sample from the beverage as well as in the patients serum by LC-(ESI)-MS-MS.

Introduction

Gentiana lutea (Great Yellow Gentian) or “Yellow Gentian” is a herbaceous perennial plant, growing to 1-2 m tall, with broad lanceolate to elliptic leaves approx. 10-30 cm long and 4-12 cm broad and yellow flowers. Gentian root has a long history in the treatment of digestive disorders and is an ingredient in a variety of medicines. It contains some of the most bitter compounds known (e.g. amarogentine) (1).

Veratrum album commonly known as the *White Hellebore* which is similar to *Gentiana lutea*. Both species are alpine plants and when not in flower, the leaves have a similar morphology at first sight. Various reports describe the ingestion of fermented tea or wine decoctions containing *Veratrum album*, which was mistaken for *Gentiana lutea* (2-5). In comparison to *Gentiana lutea*, the plant is highly toxic. For example, the rhizome of *Veratrum album* (*veratri rhizoma*) contain a range of alkaloids (1), which can be classified into different groups: e.g. with a typical steroidal skeleton (jervine, pseudojervine, veratramine) or with a cevanine skeleton (ProA, ProB) (6, 7). In contrast, the ester alkaloids ProA and ProB represent the largest group in *Veratrum album* (1).

Some confusion can occur, because related alkaloids are found in other plants like *Veratrum californicum*, *Veratrum viride*, *Veratrum nigrum*, *Amianthium muscitoxicum*, *Zigadenus* and *Schoenocaulon officinale* (8-12).

Veratrum alkaloids act by increasing the permeability of sodium channels of excitable cells, causing them to fire prematurely and then leaving them refracted. The onset of symptoms occurs between 30 minutes and 4 h after the ingestion of the *Veratrum* plant (beverages containing *Veratrum* plant in traces). The symptoms are vomiting, nausea and abdominal pain. They are followed by cardiovascular effects such as severe bradycardia, hypotension and in severe cases cardiac conduction abnormalities and death (4). With prompt supportive care, patients usually make a full recovery within the first day. The use of atropine is of major importance to treat severe bradycardia.

Case history

A 49-year-old man reported an ingestion of two glasses (approx. 2 x 20 ml) of a self-made alcoholic root beverage containing what he thought was yellow gentian (*Gentiana lutea*). Shortly after ingestion, he developed nausea, vomiting and oral paraesthesia. On admission to the hospital he suffered from severe bradycardia (35 beat/min) and hypotension (50/30 mmHg). Laboratory results were normal (except Glucose/P: 158 (range: 55-110) mg/dL). For primary detoxification he orally received activated charcoal. Further medication consisted of atropine, metoclopramide, ondansetron and i.v. volume for symptomatic treatment. With suspicion of an intoxication with *Veratrum* alkaloids, the beverage and a serum sample were sent to our institute for investigation.

Material and methods

Because an intoxication with *Veratrum* alkaloids was suspected, a serum sample and the beverage were analysed using the described LC-MS-MS method. This method enables the identification and quantitation of five *Veratrum* alkaloids (protoveratrine A (ProA) and B (ProB), veratridine, cevadine and jervine (figure 1). The quantitation of ethanol was performed by headspace-gas chromatography (HS-GC).

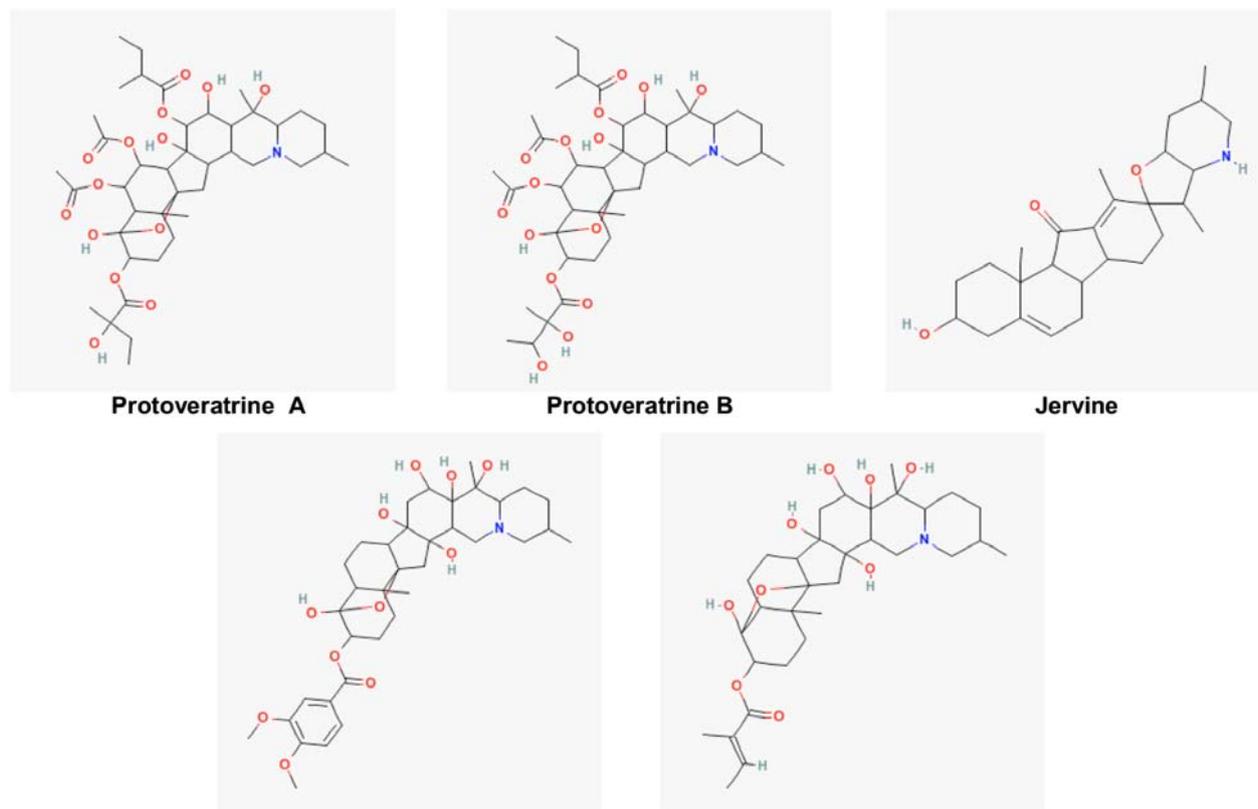


Figure 1. Chemical structures of *Veratrum* alkaloids which are covered by the method

Sample preparation

A total of 1.0 mL serum, 0.4 mL extraction reagent [50 μ L fentanyl- d_5 ($c=0,01$ mg/mL in methanol) dissolved in 50 mL dichloromethane] were mixed in a 1.5-mL eppendorf cup for 2 min. The sample was centrifuged for 2 min at 15,000 x g and 0.3 mL of the organic layer was evaporated to dryness under a stream of nitrogen at 30 °C. The residue was redissolved in 0.1 mL of methanol.

LC-Parameters

A SHIMADZU (Shimadzu, Duisburg, Germany) LC system was used. Analytical separation was carried out using a Varian Pursuit 5 pentafluorophenyl (PFP) column (150 x 3.0 mm, 5 μ m). The oven temperature was 60 °C. The gradient consisted of a mixture of solvent A (methanol:0.1% HAc with 10 mM NH₄Ac (97:3) and solvent B (0.1 % HAc with 5 mM NH₄Ac:methanol (90:10) pumped at a flow rate of 0.55 mL/min.

MS-Parameters

The mass spectrometer ABI 3000-System was obtained from Applied Biosystems/MDS Sciex (Darmstadt, Germany). Compounds were quantitated in the multiple reaction mode (ESI(+), MRM). The following transitions were monitored (*m/z*):

protoveratrine A: 794.3→776.3/676.3/658.3,
 protoveratrine B: 810.4→792.2/676.3/658.3,
 veratridine: 674.3→492.4/456.3/165.1,
 cevadine: 591.7→574.3/456.3/162.3,
 jervine: 426.0→405.2/114.1/84.0,
 fentanyl-d₅ (IS): 342.1→188.1.

Quantitation followed the internal standard method. The drug concentration in the samples was calculated using the peak-area ratios of the base peak ions of the target ions versus IS.

Results and Discussion

Commercial gentian beverage is a clear or pale-yellow coloured liquid containing a minimum of 38% ethanol. In this case, the self-made beverage was intensive yellow and the ethanol concentration was 25%. No ethanol was found in the serum sample (LOQ: < 0,1 g/L). Most published cases related to *Veratrum* poisonings contain no information of blood concentrations. To our knowledge, only one LC-MS method has been reported for the determination of two *Veratrum* alkaloids (veratridine and cevadine) from gastric content and heart blood (13).

The serum sample was collected approx. 6 h after the ingestion of the beverage. Due to less material, the serum sample had to be diluted with drug-free serum (1+1). It is evident from the chromatograms that ProA was clearly identified through its three transitions and their three equal retention times (figure 2). To our knowledge, ProA and ProB could be quantitated for the first time in human serum using LC-MS-MS. The results are summarized in table 1.

Table 1. Alkaloid concentrations

| Compound | Beverage (mg/L) | Serum (μ g/L) |
|-------------------------|------------------|--------------------|
| Protoveratrine A | 20.4 | 1.16 |
| Protoveratrine B | 13.7 | 0.40 |
| Jervine | < 0.01 | < 0.10 |
| Veratridine | < 0.01 | < 0.10 |
| Cevadine | < 0.01 | < 0.10 |

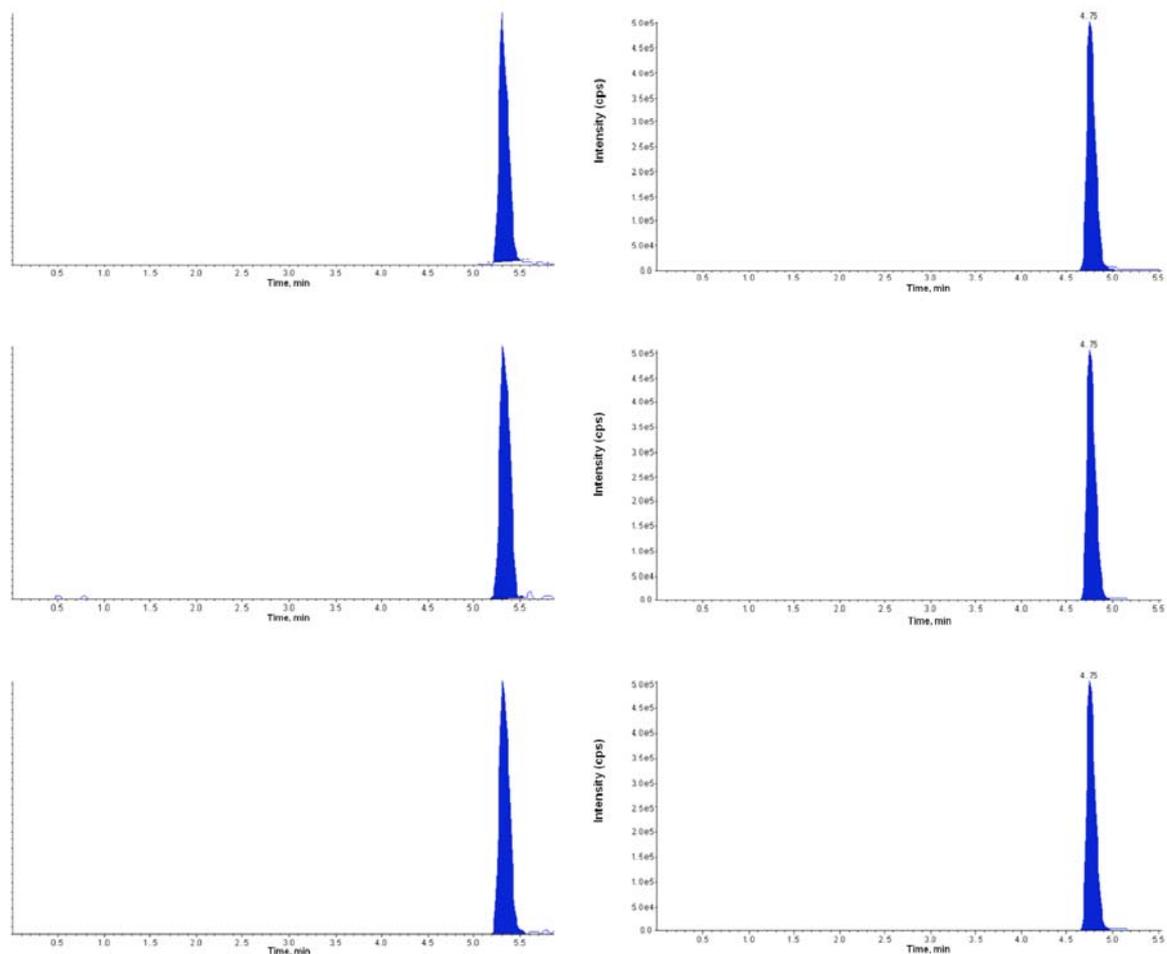


Figure 2. Ion chromatogram of the serum sample containing protoveratrine A ($c_{\text{Pro A}} = 1.16 \mu\text{g/L}$) (left, 3 transitions) and the internal standard=fentanyl- d_5 (right)

The patient was continuously monitored in hospital (e.g. heart rate, blood pressure). Heart rate returned to normal within eight hours after admission. After symptomatic treatment the patient completely recovered from symptoms within 24 hours.

Severe *Veratrum album* poisoning is a rare event. In most cases the plant was mistaken for the yellow gentian. Quatrehomme et al. (4) summarized 32 cases from the medical literature (period: 1912 – 1987) where *Veratrum album* was involved. In eight of approx. 25,000 cases of exposure to toxic plant material -reported to the Swiss Toxicological Centre (period: 1966-96)- *Veratrum album* was identified (14). The leading symptoms in these eight cases were nausea, abdominal discomfort and bradycardia (no deaths).

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