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Jared T. Seale

Boise State University

Xinzhu Pu

Boise State University

Owen M. McDougal

Boise State University

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***Veratrum parviflorum* Poisoning: Identification of Steroidal Alkaloids in Patient Blood and Breast Milk**

Jared T. Seale
Boise State University

Joseph E. Carpenter
Emory University School of
Medicine
and
Georgia Poison Center

Matthew D. Eisenstat
Emory University School of
Medicine,
Georgia Poison Center,
and
University of Louisville

Emily A. Kiernan
Emory University School of
Medicine
and
Georgia Poison Center

Brent W. Morgan
Emory University School of
Medicine
and
Georgia Poison Center

Daniel P. Nogee
Emory University School of
Medicine
and
Georgia Poison Center

Xinzhu Pu
Boise State University

Colin A. Therriault
Emory University School of
Medicine,
Georgia Poison Center,
and
University of Illinois College
of Medicine at Peoria

Michael Yeh
Emory University School of
Medicine
and
Georgia Poison Center

Owen M. McDougal*
Boise State University
owenmcdougal@boisestate.edu

Abstract

Introduction

The *Veratrum* genus is composed of plants containing a diverse set of steroidal alkaloids. *Veratrum* plant material has been utilized for centuries as herbal medicines, however the alkaloids have such a low therapeutic index that they are not used in modern medicine. Here we report an incident of inadvertent ingestion of *V. parviflorum* by hikers in Georgia that allowed detection, and in several instances identification of alkaloids from the plant, and correlated their presence within patient blood and breast milk specimens.

Case History

Eight patients, three male and five female, presented in the spring of 2020 and 2021 with symptoms requiring emergent medical attention after ingestion of *Veratrum parviflorum*. All patients believed the plants to be a local native species of wild leek, *Allium tricoccum*, locally known as ramps. Plants were identified using photographs as well as fresh and cooked plant material provided by patients, in consultation with botanists at the University of Georgia Herbarium. Written consent was obtained from all patients for collection of blood and breast milk specimens for laboratory identification of *Veratrum* alkaloids.

Methods

V. parviflorum plant material, and patient serum and breast milk were analyzed by high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (HPLC-QTOF) to identify steroidal alkaloids.

Results

The *V. parviflorum* extract was confirmed to contain cyclopamine, veratramine, jervine, and muldamine. Two out of the eight patients had detectable concentrations of *Veratrum* alkaloids. Of the alkaloids identified in the plant, cyclopamine and jervine were detected within patient serum, and cyclopamine and veratramine were observed to be present in breast milk.

Discussion

Toxicity resulting from *Veratrum* steroidal alkaloids has primarily been reported from *V. album* and *V. viride*. This is the second report of *V. parviflorum* poisoning. The present work reports for the first time the presence of muldamine and jervine within *V. parviflorum*. This work provides the first instance of identification of *Veratrum* alkaloids in breast milk. Thus, the findings presented herein add to literature record causative agents contributing to the toxicity of *V. parviflorum* when ingested and potential for secondary poisoning through breastfeeding.

Conclusion

V. parviflorum toxicity was observed to cause nausea, vomiting, hypotension, bradycardia, abdominal pain, light-headedness, blurred vision, and tingling in the arms. Patients experiencing mild symptoms improved with supportive care, IV fluids, and antiemetics, but hemodynamically unstable patients required atropine and vasopressors. This study demonstrated that more lipophilic *Veratrum* alkaloids can be passed along in breast milk, which suggests additional precautions may be critical to limit further poisonings.

Keywords: *Veratrum*, steroidal alkaloid, ramps, cyclopamine, Bezold-Jarisch, breast milk, blood, poisoning

Introduction

The *Veratrum* genus is comprised of 14-45 distinct species of woodland and alpine perennial herbs located throughout temperate regions of the northern hemisphere [1-3]. Nine species, or species complexes, of *Veratrum* may be found in North American meadows, swamps, alpine forests, and riverbanks, including *V. album*, *V. californicum*, *V. viride*, *V. parviflorum*, and *V. tenuipetalum* [1-4]. These plants have been incorporated into traditional medicines for centuries due to powerful physiological effects resulting from their diverse mixture of steroidal alkaloids. Over 200 unique steroidal alkaloids have been identified in *Veratrum* spp. and can be separated into two distinct structural skeletons: C-nor-D-homosteroidal skeleton and cyclopentanophenanthrene skeleton (Figure 1) [2,4].

Figure 1. Representative steroidal alkaloids from *Veratrum* spp. categorized by structure template.

Veratrum steroidal alkaloids are primarily recognized for their propensity to bind voltage gated sodium channels in vertebrate organisms [8]. German physiologists Albert von Bezold and Ludwig Hirt first observed the hypotensive and bradycardic effects of veratrine administration on the heart of a rabbit in 1867, however it took until 1939 before Jarisch and Richter confirmed the mechanism by which *Veratrum* steroidal alkaloids act on heart muscle [2,5,6]. In their definitive work, Jarish and Richter identified that the effects of veratrine, a mixture of *Veratrum* derived alkaloids, resulted from reflex action in the ventricles of the heart and transmission by the afferent and efferent pathways of the vagus nerve [6]. The symptoms of bradycardia, vasodilation, and hypotension resulting from cardiac receptor stimulation is now termed the Bezold-Jarisch reflex [2,7,8].

Indigenous communities of North America are reported to have developed medicines utilizing *Veratrum* plant material. Most notably, *V. viride* has been used to treat a variety of conditions including boils, ulcers, pain, rheumatism, and venereal diseases [2,9]. Interestingly, a non-medicinal application of *Veratrum* plants was observed by Charles Osgood in 1835. As part of the election of Native American leaders, the rhizome of *V. viride* was made into a concoction that was ingested by candidates and those who resisted the extract's emetic effects the longest were considered most fit to lead [10].

Despite recognition as an ingredient in potent traditional medicines, the history of *Veratrum* spp. is rife with accidental poisoning. *Veratrum* intoxication is most often due to inadvertent consumption as a result of misidentification during foraging [11-19]. Cases of *V. viride* poisoning are reported to have occurred due to

mistaking the plant for *Symplocarpus foetidus* (skunk cabbage), *Phytolacca americana* (pokeweed), *Allium ampeloprasum* (wild onion), and *Allium tricoccum* (ramps) [12,13,15]. Likewise, *V. album*, a species most prominently found in northern Eurasia and localized regions of Alaska, is reported to have been misidentified as *Allium ursinum* (wild onion) and *Gentiana lutea* (yellow gentian) [16,19]. Most cases of poisoning manifest with symptoms including diarrhea, nausea, vomiting, and a Bezold-Jarisch reflex (bradycardia, hypopnea, and hypotension). Patients with mild symptoms typically respond well to symptomatic and supportive treatments with intravenous fluids and antiemetics, but those with symptomatic bradycardia or hypotension may require treatment with atropine or vasopressors [11-19].

V. parviflorum (Appalachian Bunchflower) may be found in the moist, deciduous forested slopes (800-2000 m) of the southern Appalachian Mountains. Characteristics of this plant include shortened rhizome (0.3-6 cm), narrow stem with lengths of 0.5-1.5 m, broadly oblanceolate to obovate leaves, and green-yellow to dark green flowers (Figure 2) [11,20,21].

Figure 2. In situ *Veratrum parviflorum* (left) and plant material collected in April 2020 for steroidal alkaloid analysis (right) [22].

In April 2015, two patients presented to a Georgia hospital with *Veratrum* poisoning resulting from erroneous identification of *V. parviflorum* as *Allium tricoccum* [11]. In addition to a Bezold-Jarisch reflex, neurological symptoms including taste disturbance, vertigo, dysarthria, and vision changes were observed. These neurological symptoms have not been reported for previous cases of toxicity with *V. viride* or *V. album* [12-16,18,19]. Analysis of *V. parviflorum* plant biomass identified verazine, veratramine, veratridine, and cycloamine, all of which have been previously observed in *Veratrum* species [11]. Due to the appearance of atypical neurological symptoms and a lack of information regarding the phytochemical profile of *V. parviflorum*, it was hypothesized that additional steroidal alkaloids, beyond those previously detected from patients, may be observed. The present study investigated eight cases of *V. parviflorum* poisoning resulting from the misidentification of plant material. Patient serum and breast milk was collected over the course of inpatient treatment and analyzed using high performance liquid chromatography-quadrupole time of flight mass spectrometry (HPLC-QTOF).

Case History

Eight patients were reported to the regional poison center in the spring of 2020 and 2021 with symptoms requiring emergent medical attention after ingestion of *Veratrum parviflorum*. All patients believed the plants to be a local native species of wild leek, *Allium tricoccum*, locally known as ramps. Plants were identified using photographs as well as fresh and cooked plant material provided by patients, in consultation with botanists at the University of Georgia Herbarium. Written consent was obtained from all patients for collection of blood and breast milk specimens for laboratory identification of *Veratrum* alkaloids. Clinical findings are summarized in Table 1.

A 34-year-old man (patient 1) and 34-year-old woman (patient 2) presented to a community emergency department (ED) in April 2020 after ingesting plants thought to be ramps (*Allium tricoccum*) that they foraged in Union County, Georgia and sauteed in oil. Patient 1 developed abdominal cramping with nausea and profuse vomiting approximately one hour after ingesting a half cup of cooked plants. He also reported visual disturbances, including a yellow tint to his vision and halos. His blood pressure on arrival was 78/40, and heart rate was 43 beats per minute, occasionally dropping to the 30s. A 12-lead EKG showed sinus bradycardia with a rate of 54.

Patient 2 developed nausea and vomiting approximately two hours after ingestion of approximately one tablespoon of cooked plants. Her initial blood pressure was 90/52, and heart rate was 60 beats per minute. Her 12-lead EKG showed sinus bradycardia with heart rate of 56, with incomplete right bundle branch block.

Both patients were treated with IV fluid resuscitation and antiemetics. Their clinical presentation was concerning for cardiac glycoside ingestion, and the plant leaf material was initially noted to resemble lily of the valley (*Convallaria majalis*). Both patients were treated empirically with five vials of digoxin immune Fab (DIF) (200 mg) IV. Serum digoxin levels were undetectable in both patients.

Patient 1 did not improve after receiving DIF. He was unable to receive more DIF as the hospital pharmacy had no more in stock. He received atropine 0.4 mg IV and a norepinephrine infusion for persistent symptomatic bradycardia and hypotension. His hemodynamics improved with vasopressor support, and his nausea and vomiting resolved. He was transferred to an intensive care unit (ICU) at a tertiary care hospital, where he was observed overnight. He remained hemodynamically stable and norepinephrine was weaned off. All symptoms resolved approximately 12 hours after arrival, and he was discharged home.

Patient 2 responded well to ondansetron, IV fluids, and DIF with full resolution of her symptoms. She was observed in the ED for approximately five hours and remained hemodynamically stable. She was discharged home. As she was breastfeeding at the time, she agreed to provide breast milk samples from the time of exposure for further analysis, in addition to blood samples collected at the hospital.

Patients 3-6 were a family of four who presented in April 2020, consisting of a 50-year-old man (patient 3), 53-year-old woman (patient 4), 15-year-old daughter (patient 5), and 18-year-old son (patient 6). They foraged and ate plants thought to be ramps found along the Benton MacKaye Trail on Flat Top Mountain in Epworth, Fannin County, Georgia. Approximately six to seven whole plants including the leaves, stem, and root material were sauteed with olive oil and consumed. All family members developed symptoms within 3.5 hours of ingestion and presented to an ED approximately 14 hours after ingestion.

Patient 3 developed nausea with multiple bouts of profuse vomiting with epigastric pain 3.5 hours after ingestion of two plants. He also developed generalized weakness and lightheadedness that was worse with exertion. Initial vitals showed blood pressure of 117/88 and heart rate 52. EKG showed sinus bradycardia with rate of 44 and first degree block. Labs were significant for elevated serum creatinine of 1.92 mg/dL (reference range 0.7 – 1.3), total bilirubin of 1.3 mg/dL (reference range 0.3-1), and undetectable digoxin level. He was treated symptomatically with ondansetron and fluids. He remained stable and symptoms resolved. Serum creatinine improved to 1.52 mg/dL the following day, although total bilirubin increased to 1.6 mg/dL. He was discharged home approximately 36 hours after ingestion.

Patient 4 ate one leaf and developed nausea with mild epigastric discomfort approximately one to two hours after ingestion. Her symptoms persisted for approximately 16 hours before resolving spontaneously. On arrival at the ED, blood pressure was 134/75, heart rate 105. EKG showed normal sinus rhythm with a rate of 75. Lab studies showed no acute abnormalities, with undetectable digoxin level.

Patient 5 reported eating one plant that included three leaves, stalk, and roots. She developed nausea and diffuse abdominal pain 2.5 hours after ingestion, but had no vomiting or other symptoms. Initial blood pressure in the ED was 139/66, with heart rate of 84. EKG showed sinus bradycardia with a ventricular rate of 53. Blood lab analyses revealed a positive digoxin assay with a reported level of 0.4 ng/mL (reference <2 ng/mL).

Patient 6 developed nausea two hours after ingesting six leaves, followed by three episodes of profuse vomiting. He also reported palpitations with lightheadedness, diaphoresis, and blurred vision as well as one bout of loose stool. Initial blood pressure in the ED was 104/51, and heart rate was 58. EKG showed normal sinus rhythm, rate 63. Lab studies showed slightly elevated total bilirubin of 1.7 mg/dL, but otherwise normal with negative digoxin assay. He received ondansetron and IV fluid hydration, with subsequent resolution of nausea and lightheadedness.

Patient 7 is a 57-year-old female who picked *V. parviflorum* plants mistaken for ramps in Hiwassee, Cherokee County, North Carolina. She ate above-ground leafy material from two plants that were blanched in water and seasoned with a dressing. She developed nausea, vomiting, diarrhea, and cramping epigastric pain within four hours of ingestion. She received oral promethazine and ondansetron from her primary care physician, but symptoms persisted. She presented to the ED eight hours after ingestion and was hypotensive, with blood pressure of 84/49, heart rate 91. Labs showed mild hypokalemia with potassium of 3.3 mEq/L, and undetectable digoxin. She continued to have intractable vomiting with persistent hypotension, with lowest blood pressure of 78/41, but no bradycardia. She was admitted and treated with IV fluids and metoclopramide, but did not require vasopressors. EKG showed normal sinus rhythm with rate of 96 and normal intervals. Her symptoms resolved with supportive care and she was discharged 42 hours after ED presentation.

Patient 8 is a 58-year-old female who also consumed false hellebore leaves boiled in water. She developed vomiting, lightheadedness, chest discomfort, generalized weakness, and a sensation of pressure with tingling in the right arm 1.5 hours after the meal. She initially presented to an urgent care clinic where she was noted to be hypotensive, and transferred to an ED. Blood pressure on arrival was 80/50, with a heart rate of 46. She received atropine 0.5 mg IV with transient improvement of her heart rate to 80, and blood pressure of 130/70. Her heart rate decreased to the 40s again in the ED and blood pressure was 80/50. She received another dose of atropine 0.5 mg IV and a 1 L IV fluid bolus. Labs showed no acute abnormalities, with undetectable digoxin. EKG revealed sinus bradycardia. She was started on a dopamine infusion at 15 mcg/min and admitted to the ICU. She remained hemodynamically stable with heart rate in the 60s and 70s with normal blood pressure overnight. The dopamine drip was weaned off over 24 hours, with full resolution of all symptoms.

Table 1. Summary of patients and clinical presentation

Patient	Age/Gender	Clinical Effects	Time to Symptom Onset	Lab Findings*	EKG	Treatment
1	34M	Nausea, vomiting, abdominal cramping, hypotension, bradycardia, visual disturbances	1 hour	No acute abnormalities	Sinus bradycardia, rate 54	Digoxin immune Fab, IV fluids, promethazine, atropine, norepinephrine
2	34F	Nausea, vomiting, hypotension, bradycardia	2 hours	No acute abnormalities	Sinus bradycardia, rate 56, incomplete right bundle branch block	Digoxin immune Fab, IV fluids, ondansetron
3	50M	Nausea, vomiting, epigastric pain, generalized weakness, lightheadedness, bradycardia	3.5 hours	Creatinine 1.92 mg/dL (ref 0.7-1.3), total bilirubin 1.6 ng/dL (ref 0.3-1)	Sinus bradycardia, rate 44, 1st degree AV block	IV fluids, ondansetron
4	53F	Nausea, mild epigastric discomfort	1-2 hours	No acute abnormalities	Normal sinus rhythm, rate 75	None

5	15F	Nausea, diffuse abdominal pain, bradycardia	2.5 hours	Digoxin level 0.4 ng/mL (ref <2)	Sinus bradycardia, rate 53	None
6	18M	Nausea, vomiting, palpitations, light- headedness, diaphoresis, blurred vision, loose stool	2 hours	Total bilirubin 1.7 ng/dL (ref 0.3-1)	Normal sinus rhythm, rate 63	IV fluids, ondansetron
7	57F	Nausea, vomiting, diarrhea, epigastric pain, hypotension	4 hours	Potassium 3.3 mEq/L (ref 3.5-5)	Normal sinus rhythm, rate 96	IV fluids, metoclopramide
8	58F	Nausea, vomiting, light-headedness, chest discomfort, generalized weakness, arm tingling, hypotension, bradycardia	1.5 hours	No acute abnormalities	Sinus bradycardia	Atropine, IV fluids, dopamine

* All patients had lab tests including serum electrolytes, BUN/creatinine, liver enzymes, complete blood count, and digoxin level. Normal results are not listed in this table

Materials and Methods

Root and Rhizome Extraction

Whole *V. parviflorum* plants were collected by the treating medical toxicology physicians at the same site where patients 3, 4, 5, and 6 gathered the plants they consumed. Roots and rhizomes were separated from the plant, diced, and lyophilized for 24 hrs before being ground into a fine powder. Ethanol (100 mL of 95%, Decon Laboratories, Inc.) was added to 10 g of dried *V. parviflorum* powder to form a slurry that was sonicated for 30 min and stirred for 24 hrs at room temperature. The mixture was filtered to produce a clear and brown supernatant that was then concentrated via rotary evaporation. Removal of solvent resulted in a viscous dark brown extract to which 2 mL of 100% ethanol (Decon Laboratories, Inc.) was added. This extract was filtered through a 0.45 µm nylon syringe filter and placed in an autosampler vial for steroidal alkaloid characterization via HPLC-QTOF.

Serum Extraction

Blood samples from all eight patients were spun down and serum decanted. Chloroform was used to extract the steroidal alkaloids from the serum. A biphasic mixture containing 1 mL patient serum and 0.4 mL chloroform was vortexed for 1 min then centrifuged for 5 min at 14000 rpm. A 0.3 mL aliquot of the chloroform layer was removed and evaporated to dryness under a stream of nitrogen. The remaining residue was dissolved in 0.1 mL ethanol (100%) for HPLC-QTOF analysis. A calibration curve consisting of 5, 1, 0.5, 0.1, and 0.05 µg/mL of standard was formed using commercially available cyclopamine (≥95%, PhytoLab GmbH & Co. KG), veratramine (>98%, TCI America), veratridine (≥98%, Tocris Bioscience), muldamine (99%, Logan Natural Products), and jervine (≥95%, PhytoLab GmbH & Co. KG) to quantify steroidal alkaloids in patient serum.

Breast Milk Extraction

A 0.5 mL aliquot of patient breast milk was diluted with 0.5 mL ammonium hydroxide (29%) and loaded onto a supported liquid extraction (SLE) column (Agilent Chem Elut S) and allowed to adsorb onto the solid phase for 10 min. Following adsorption, 10 mL of chloroform was added to the column and eluted on a vacuum manifold with -0.2 bar applied vacuum for 30 sec. The eluted solvent was filtered through a 0.45 µm PVDF syringe filter and evaporated to dryness under a stream of nitrogen. Remaining residue was dissolved in 0.1 mL ethanol (100%) and analyzed via HPLC-QTOF. Quantification of alkaloids in breast milk was performed using a calibration curve consisting of 5, 1, 0.5, 0.1, and 0.05 µg/mL of alkaloid standards.

Steroidal Alkaloid Identification

Veratrum steroidal alkaloids were identified in blood, breastmilk, and root/rhizome extracts using HPLC-QTOF and commercially available standards. Analysis was performed on a Bruker maXis ESI Q-TOF mass spectrometer (Bruker Corporation, Billerica, MA) coupled with a Dionex Ultimate 3000 LC system (Thermo Scientific, Waltham, MA). The samples were injected onto a Waters Xterra MS C₁₈ column (5 µm, 2.1 x 150 mm) maintained at 40 °C at an injection volume of 5 µL. A gradient elution consisting of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B) was used with a 300 µL/min flowrate. The elution method began at 10% solvent B before increasing to 20% solvent B after 1 min. Between minute 1 and 16, solvent B was increased to 40%. After 16.1 min, solvent B was increased to 100% and maintained until minute 20. Solvent B was decreased to 5% at 20.1 min and held constant until minute 25. The electrospray ionization (ESI) source was operated under the following conditions: positive ion mode, 4000 to -500 V voltage between capillary and end plate offset, 10.0 L/min flow rate of N₂ drying gas at 200 °C.

Results

To identify the presence of *Veratrum* steroidal alkaloids within patient blood samples, commercially available human serum was spiked at 1, 10, and 50 mg/L with five steroidal alkaloid standards: jervine, veratramine, veratridine, cyclopamine, and muldamine. The spiked serum underwent the described extraction procedure to not only determine the retention time and ions produced, but to also assess the limit of detection (LOD) and limit of quantification (LOQ) for the alkaloids. The LOD for jervine, veratramine, veratridine, cyclopamine, and muldamine was 44, 16, 63, 45, and 38 ng/mL, respectively, while the LOQ were 134, 48, 191, 136, and 115 ng/mL, respectively. Figure 3 shows the mass spectrum base peak for human serum extracts and an overlay with commercial steroidal alkaloid standards to validate qualitative assignment.

Figure 3. Base peak chromatogram for human serum extracts. Base peak chromatogram for patient #1 overlaid with extracted ion chromatograms for commercially available steroidal alkaloids that were spiked to 10 mg/L into and extracted from a human serum standard. Peaks 1-5 were identified as jervine, veratramine, veratridine, cyclopamine, and muldamine, respectively.

The retention time for cyclopamine in the standard and patient extracts were 12.0 and 11.9 minutes, respectively (Figure 4). An $[M+H]^+$ parent ion for cyclopamine (412.32 ± 0.02 m/z) was identified in the patient #1 extract (Figure 4C). Jervine, veratramine, veratridine, and muldamine were not detected in the blood extract of patient #1.

Figure 4. Serum extracts for patient #1 were compared to a cyclopamine standard. A) Extracted ion chromatogram for cyclopamine (412.32 ± 0.02 m/z) extracted from commercially available human serum. B) Extracted ion chromatogram for 412.32 ± 0.05 m/z. C) Mass spectrum of the peak (RT: 12.0 min) in chromatogram A where the observed $[M+H]^+$ ion for cyclopamine (412.32 ± 0.02 m/z) has been identified. D) Mass spectrum of the peak (RT: 11.9 min) in chromatogram B where the observed $[M+H]^+$ ion for cyclopamine (412.32 ± 0.02 m/z) has been identified.

Qualitative analysis of the blood extract of patient #4 recognized jervine and cyclopamine (Figures S1 and S2). The retention time for jervine was 9.1 minutes (Figures S1A and S1B) and the $[M+H]^+$ parent ion (426.30 ± 0.05 m/z) was identified (Figure S1C). Cyclopamine was identified in the blood of patient #4 by the presence of the $[M+H]^+$ parent ion (412.32 ± 0.05 m/z) (Figure S2C). Veratramine was identified in the blood extract of patient #7 by the observation of the $[M+H]^+$ parent ion (410.31 ± 0.05 m/z) (Figure S3) and retention time of 10.0 min. *Veratrum* alkaloids were not detected in blood extracts of patients 2, 3, 5, 6, and 8. The alkaloids identified within the serum extracts of patients 1, 4, and 7 were present in concentrations below the LOQ.

Table 2. Identity of *Veratrum* Alkaloids Identified in Patient Serum Extracts

Patient #	Alkaloids Identified
1	Cyclopamine
4	Cyclopamine
	Jervine
7	Veratramine

Veratramine and cyclopamine were the only steroidal alkaloids identified in an extract of the breast milk of patient #2 (Figures S4-S6). Veratramine was identified to have a retention time of 9.4 min and a $[M+H]^+$ parent ion of 410.31 ± 0.02 m/z. Cyclopamine was identified with a retention time of 11.6 min and a $[M+H]^+$ parent ion of 412.32 ± 0.02 m/z. The concentration of veratramine (LOD = 11 ng/mL; LOQ = 34 ng/mL) in the milk was observed to decrease in the second sample, then increase in the third sample to a lower concentration than initially observed. The concentration of cyclopamine (LOD = 11 ng/mL; LOQ = 32 ng/mL) was below the LOQ in all samples. The fourth breast milk sample collected on April 9 at 17:00 did not contain a detectable level of veratramine or cyclopamine (Table 3).

Table 3. Identity and Concentration of Steroidal Alkaloids in Patient Breast Milk Extracts

Patient #	Date/Time of Collection	Alkaloids Identified	Concentration (ng/mL)
2	April 8 2020 at 11:30	Veratramine	405
		Cyclopamine	-
	April 8 2020 at 16:20	Veratramine	136
		Cyclopamine	-
	April 9 2020 at 8:00	Veratramine	303
		Cyclopamine	-
	April 9 2020 at 17:00	-	-

The root and rhizome extract of *V. parviflorum* yielded a chromatogram with over 53 peaks representing unique constituents (Figure S7). Commercially available steroidal alkaloid standards for jervine, veratramine, veratridine, cyclopamine, and muldamine were used to identify known alkaloids within the *V. parviflorum* extract. All steroidal alkaloid standards were present in the plant extract with the exception of veratridine (Figures S8-S11).

Discussion

Veratrum steroidal alkaloids are recognized for their toxicity and have been implicated in multiple cases of inadvertent poisoning [11-19]. An ethanolic extract of the roots and rhizomes of *V. parviflorum* resulted in the identification of four common *Veratrum* steroidal alkaloids: cyclopamine, veratramine, jervine, and muldamine. Contrary to the results presented by Anwar et al., where cyclopamine, veratramine, verazine, and veratridine were identified, veratridine was not identified in the present extract of *V. parviflorum* [11]. A standard of verazine could not be obtained, however, an EIC for 398.3553 m/z presented several peaks suggesting that the previously identified alkaloid may still be observed given the appropriate reference material. Variation in the presence of veratridine in *V. parviflorum* may be due to differences in growth stage, harvest time, or harvest location [23]. The growth stage, time of harvest, harvest location, and part of the plant for *V. californicum* has shown to influence the alkaloid composition in plant material [23]. Although the poisoning described by Anwar et al. also occurred in April, it is possible that the *V. parviflorum* used in that study was harvested at an earlier date, in a different location, or at a different stage of growth.

This study provides the first evidence for the presence of muldamine and jervine within *V. parviflorum*. Jervine has previously been isolated from *Veratrum* spp. including *V. nigrum* L., *V. californicum*, *V. album*, and *V. viride*, whereas muldamine has only been isolated from *V. californicum* [23-28]. Symptoms exhibited by the patients in the present study, including nausea, vomiting, hypotension, and bradycardia, were consistent with those commonly observed for *Veratrum* toxicity. Additionally, the time between ingestion and the appearance of symptoms was consistent with prior cases [11-19].

The identification of veratramine and cyclopamine in breast milk suggests that indirect exposure of an infant via ingestion is possible, although the clinical significance is unknown. The alkaloid profiles and concentrations observed in breast milk may differ from blood. In samples from patient 2, *Veratrum* alkaloids were only detected in the breast milk, but not blood, even though the first milk sample was collected the morning after her ED visit. Veratramine and cyclopamine were identified, although the cyclopamine concentrations were below the LOQ. Lipophilicity may account for the presence of these alkaloids in breast milk even after they are no longer detectable in the blood. SwissADME was used to predict the partition coefficients for each of the *Veratrum* steroidal alkaloids. As predicted by SwissADME, veratramine is the most lipophilic of the three alkaloids we identified ($\text{LogP}_{o/w} = 4.30$), followed by cyclopamine ($\text{LogP}_{o/w} = 4.16$), with jervine being the least lipophilic ($\text{LogP}_{o/w} = 3.53$).

Veratramine has been identified to exert bradycardic and teratogenic effects through the inhibition of Na⁺ ion channels and Hedgehog signaling, respectively, with an LD₅₀ of 15.9 mg/kg when administered to Kunming mice intragastrically [29,30]. Furthermore, veratramine is a releaser and uptake inhibitor of 5-HT [29,30]. Cyclopamine has also been identified as an inhibitor of the Hedgehog signaling pathway with an LD₅₀ of 43.5 mg/kg when administered to 129S11/Sv1mJ mice intraperitoneally [2,4,31].

Excretion of xenobiotics in breast milk can depend on many factors such as plasma levels and physical properties including lipophilicity, protein binding, and ionization. Concentrations of *Veratrum* alkaloids in milk may also vary with different collection times. We observed higher veratramine concentrations in milk samples collected in the morning compared to the afternoon. The patient reported that she had not nursed or pumped milk for 16 hours prior to collection of the first sample included in our analysis. It is possible that more *Veratrum* alkaloids accumulated overnight due to less frequent nursing and pumping, leading to higher concentrations in milk expressed in the morning compared to other times during the day. Although breast milk samples were collected four times over a 29.5 hr period, additional time points over a longer collection period are required to determine the half-life of veratramine and cyclopamine more accurately. Variation in observed concentrations of veratramine may be due to acute changes in the breast milk composition and chemical properties of the small molecule [32,33]. Additional study regarding the pharmacokinetics of *Veratrum* steroidal alkaloids in relation to concentration in breast milk may be beneficial.

Three of the eight patients had detectable levels of *Veratrum* alkaloids in the blood. Due to the small sample size, we were unable to characterize associations between the presence of certain alkaloids or their concentrations with severity of illness. As the blood specimens were convenience samples left over from routine clinical testing, there were limitations including differences in collection times, storage conditions, and the prolonged time in storage before analysis. This study was further limited by the sensitivity of the liquid-liquid extraction method implemented in the processing of patient serum samples. The LOD (16-63 ng/mL) and LOQ (48-191 ng/mL) ranges for the *Veratrum* alkaloids were not sufficient for detection and quantification in patient serum.

Cross-reactivity with digoxin clinical chemistry assays have been reported in patients who have ingested *Veratrum* species due to the structural similarity of *Veratrum* alkaloids and digoxin [34]. Only one patient in our study had a positive digoxin assay, although she had mild symptoms. There was no discernable association between symptom severity and digoxin assay results.

Acute poisoning due to *Veratrum* alkaloids may be difficult to distinguish clinically from cardioactive steroid toxicity, with common symptoms of nausea, vomiting, generalized weakness, bradycardia, and hypotension. Young *V. parviflorum* plants in the spring may resemble *Convallaria majalis*, which contains convallatoxin. Patients 1 and 2 were initially thought to have ingested *C. majalis* and were treated empirically with DIF, given the structural similarity of convallatoxin to digoxin. In-vitro studies have shown no neutralization of convallatoxin by DIF, suggesting that DIF is unlikely to provide benefit [35].

Conclusion

This study sought to identify toxic steroidal alkaloids in the blood of eight patients and breast milk for one of them that inadvertently ingested *V. parviflorum*. Four alkaloids were identified in an ethanolic extract from the roots of *V. parviflorum*: jervine, veratramine, cyclopamine, and muldamine. An efficient and effective method was developed for the extraction of steroidal alkaloids from patient samples, leading to the identification of cyclopamine, jervine, and veratramine in patient blood, and veratramine and cyclopamine in patient breast milk. The presence of steroidal alkaloids in breast milk suggests that the indirect poisoning of a breastfeeding infant should be considered in cases of suspected *Veratrum* poisonings. Characterization of alkaloid profiles in *V. parviflorum* may lead to development of more sensitive identification methods for plant toxins in human serum to improve clinicians' ability to diagnose acute poisonings. Appropriate treatment for acute *V. parviflorum* poisoning includes supportive care, with IV fluids and antiemetics. Patients with persistent hemodynamic instability may require atropine for bradycardia or vasopressors for hypotension and shock, although there is no evidence to support the use of digoxin immune Fab.

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Disclosure Statement

The authors report no potential conflicts of interest.