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Evaluation of Ricinine, a Ricin Biomarker, from a Non-Lethal Castor Bean Ingestion

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Abstract

A case of attempted suicide of a 58-year-old man using castor beans is presented. The patient came to the emergency room complaining of nausea, vomiting and diarrhea for nine hours following ingestion of six castor beans. Urine samples were taken throughout the hospital stay and submitted to the Centers for Disease Control and Prevention for analysis of ricinine, a castor bean component. The samples were found to be positive for ricinine with a maximum concentration of 674 μg/g-creatinine excreted around 23 hours post-exposure. Subsequent samples demonstrated lower ricinine concentrations with the final sample taken at 62 hours post-exposure at a concentration of 135 μg/g-creatinine of ricinine. The estimated urinary excretion half-life was around 15 hours and the recovery of ricinine in the urine over the three days was estimated to be less than 10%. The patient fully recovered with supportive care and was discharged from the hospital six days after admission.

Introduction

Ricin is a highly toxic protein found in the castor bean plant, *Ricinus communis* [1]. These large red and green leaved plants can be found in tropical and subtropical climates throughout the world. *Ricinus communis* is grown as both an ornamental plant and a commercial source of castor oil, since the seeds contain about 40% oil [2]. Castor oil is produced worldwide for industrial uses such as lubricants, pharmaceuticals [2], cosmetics and plastics [3]. Additionally, ricin itself has also been evaluated as a potential treatment for cancer [4–6] and AIDS [5].

Many poisonings from ricin have been reported, including accidental castor bean ingestion as well as suicide and homicide attempts with both the intact seeds and extracted ricin [7–10]. The notorious assassination of Georgi Markov, an outspoken Bulgarian defector [11] and the attempted poisoning of Soviet dissident, Alexander Solzhenitsyn [12], were both attributed to ricin. Exposure to this poison can occur from inhalation, injection or ingestion, and the most commonly reported form of ricin poisoning is through ingestion. The toxicity of ricin varies greatly according to the route of exposure and decreases from injection to ingestion by approximately three orders of magnitude [10].

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Previous documents have reported non-lethal poisonings from ingestion of multiple castor beans. An infant ate two castor beans, experienced vomiting and diarrhea and subsequently developed hepatotoxicity, but was recovered one month following the incident [13]. Another accidental poisoning of a young adult by10–15 castor beans resulted in non-lethal dosage with recovery within 3 days of exposure [14]. Even ingestion of up to thirty or these seeds for an adult did not result in a lethal dosage [8]. Mastication is thought to be critical for sufficient ricin exposure since the seed casing prevents any release of the toxin through the digestive track. It is also postulated that ingested ricin may have low toxicity due to potential inactivation by gastrointestinal tract enzymes [7]. Overall, the mortality rate for ingestion of castor beans is estimated to be about 0.4% with adequate supportive care [9].

Ricinine, a small alkaloid present in the castor bean can be used to assess exposure to castor beans and unpurified ricin, since this biomarker is readily excreted in urine [15–17]. The advantages of quantitating ricinine as opposed to historical ELISA and radioimmunoassay methods that detect ricin itself [18–21] are rapid turn-around and ability to process large number of samples. The structure of ricinine is presented in Figure 1. Quantification of ricinine in urine is performed using solid phase extraction coupled with liquid chromatography and tandem mass spectrometry. The additional use of isotopically labeled internal standards reduces matrix issues and recovery biases [16]. This method has been applied to a case of multiple-organ failure due to castor oil injection [22] as well as direct injection of ricin for suicidal purposes [16].

Case History

A 58-year-old male presented to an emergency department following an attempted suicide by castor bean ingestion. The patient complained of nausea, vomiting, diarrhea and chills. He confessed to ingesting six castor beans nine hours prior to arrival at the hospital. Due to his prior research into ricin poisoning, the patient intentionally well-masticated the castor beans to maximize the bioavailability of the toxin. Five hours following ingestion, the patient experienced six bouts of vomiting and two bouts of diarrhea with continued abdominal cramping; he had noted castor bean residue in the vomitus [23].

The patient was a homeless male. He was afebrile, normotensive with a heart rate of 65, respiration of 15 and oxygen saturation of 99% on room air. On physical exam brisk bowel sounds were heard and tenderness to palpation in left lower and right lower quadrants was noted.

Hospital lab results were unremarkable, except for an alkaline phosphatase level of 133 international units/liter. Toxicology report was negative for aspirin and acetaminophen, but positive for cannabinoids. In response to this information the patient reported medical marijuana use for chronic pain.

The patient was admitted and followed closely and continued to improve without evidence of organ dysfunction based on symptomatology or lab results. He was cleared for release by medical staff at three days post admission and by psychiatry staff at six days post admission.

Experimental

Materials

The analytical standards were prepared by Cerillant (Round Rock, TX) at concentrations of 0.0830, 0.125, 0.333, 0.624, 0.830, 1.66, 8.32, 83.2, 333, and 832 ng/mL in synthetic urine. Two quality control samples were also prepared by Cerilliant at a low and high level in synthetic urine. The isotopically labeled internal standard, ricinine $^{13}C_6{}^{12}C_2{}^{1}H_8{}^{14}N_2{}^{16}O_2$ (170 g/mol), was obtained from Cerilliant in an aqueous solution of 11 ng/mL. Deionized water (18-MΩ) was generated by a purification system purchased from Aqua Solutions (Jasper, GA). High-performance liquid chromatography-grade methanol and acetonitrile were purchased from Tedia (Fairfield, OH).

Sample preparation

The standards, quality control samples and patient samples were prepared for analysis in the same manner. Solid phase extraction (SPE) using Strata-X SPE cartridges (200 mg/6 mL) by Phenomenex (Torrance, CA) were conditioned using 6 mL of methanol followed by 6 mL of deionized water. A premixed solution of one milliliter of sample combined with 100 microliters of internal standard solution was loaded onto the SPE bed. To remove unwanted urine components, six milliliters of 5% methanol in 95% water was washed through the cartridge. Finally, the cleaned sample was eluted from the SPE using 6 mL of acetonitrile. This eluent was taken to dryness using a Turbovap LV Evaporator (Caliper, Hopkington, MA) at 70 °C with continuous nitrogen flow. The dried sample was then reconstituted with 200 μL of deionized water, mixed well and transferred to 300–μL autosampler vials in preparation for LC/MS/MS analysis.

Apparatus and methods

The liquid chromatography system used for separation was an Agilent 1100 HPLC complete with degasser, column oven, autosampler and gradient pump (Palo Alto, CA). A Polar RP chromatography column, a C18 column with embedded polar groups by Phenomenex, with the dimensions of 4 × 20 mm with 2 μm particle size was used for the isocratic separation. The mobile phase consisted of 70% solvent A (10% methanol in 90% water) and 30% solvent B (neat methanol).

An Applied Biosystems (Foster City, CA) API 4000 MS equipped with turbo-ionspray source was used for the detection following the chromatographic separation. The system was operated in positive ion multiple reaction monitoring mode identifying two MS transitions for ricinine (165 to 82 m/z (quantification ion); 165 to138 m/z (confirmation ion)) and one transition for the isotopically labeled internal standard (171 to 85 m/z). All ions were detected in the lowest standard (0.0830 ng/mL) in human urine. A ratio of the confirmation ion to the quantitation ion (1:3) was used to reduce the possibility of false positives in this difficult matrix. Additional parameters have been specified previously [16].

Results and Discussion

The patient attempted suicide by thoroughly chewing and ingesting six castor beans to achieve a lethal dosage. Urine samples were obtained from the patient following exposure, corresponding with approximately 14, 27, 37, 62 and 86 hours post exposure. These samples were analyzed using the method described above along with standards and quality control samples (Table 1). One sample contained quantities of ricinine above the upper limit of the calibration range; thus, this sample was diluted with deionized water to fall within the method range for accurate quantitation as previously done [16]. The urinary results reported here were corrected for creatinine level and charted according to approximate time post-exposure in Figure 2. This graph indicates a peak in excretion of ricinine around 27 hours post exposure followed by a rapid decrease of ricinine excretion. This data were used to estimate the urinary excretion half-life of ricinine at 15 hours.

Imprecision and accuracy for this method ranged from 2–4% and 96–98% respectively, as determined for the quality control samples at concentrations of 0.49 and 51.0 ng/mL. These specifications qualify this ultra-trace analysis as a high precision and accuracy method [24]. The method was characterized with 20 independent analyses of quality control materials from which the limits were established and runs were judged. All standard curves were linear with a correlation coefficient greater than 0.990. The quality control samples and standard correlation coefficient were confirmed to be within the specifications for this analysis.

The only natural source for ricinine is from castor beans [25]; therefore, exposure to castor beans, extracted ricin or other castor bean products, such as castor oil would result in the presence of ricinine in urine. Evaluation of thirty individual non-exposed urine samples determined the ricinine concentration to be below the detection limit for this method [16]. Subsequent studies have indicated that ricinine will be present in urine following ingestion of castor oil, resulting in positive responses [22].

In this case report, the estimated ingestion of ricinine was 25 mg from six seeds. This approximation assumes a ricinine content of 0.77% [26] and a seed weight of 0.54 g. Of this amount, 1.7 mg of ricinine was recovered based on concentrations of ricinine found in urine and expected creatinine excretion. A standard value of creatinine excretion used was 1.7 g/day for a male patient [27]. The creatinine-corrected ricinine levels were averaged and multiplied by the daily creatinine output to estimate the ricinine output per day (Table 2). The sum of the daily excreted ricinine results was 1.7 mg. This value divided by the estimated ricinine from the six seeds indicates that 8% of the ingested amount of ricinine was detected in the urine for the three days following exposure.

Conclusions

Analysis of ricinine in urine is an indirect means of detecting exposure to ricin. This method has been successfully applied to a person no longer exhibiting symptoms following ingestion of six castor beans, a source of ricin, confirming the detection of ricinine to be a reasonable determination of exposure to castor beans. This method may be applied to even

lower level exposures and/or utilized for longer times post exposure for additional confirmation of ricin poisoning. Low concentrations of ricinine may be present due to other castor bean sources including castor oil and cosmetics; therefore, the interpretation of the results of this method should consider the patient symptoms and history.

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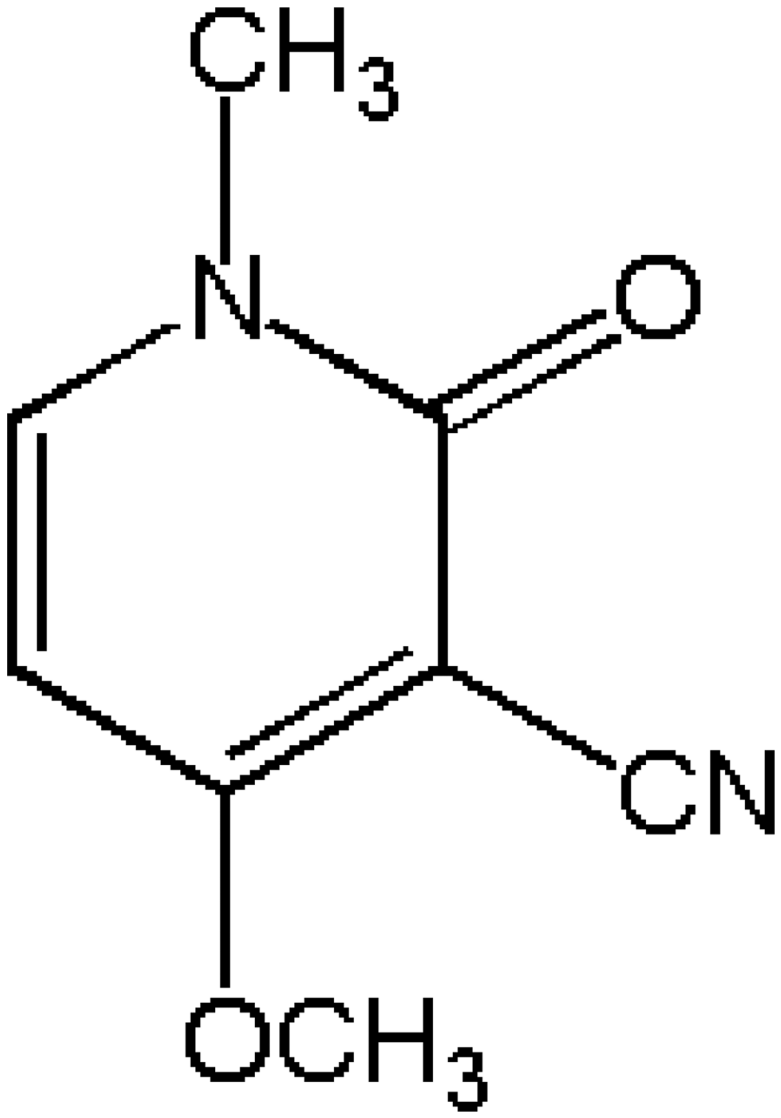

Figure 1.
Chemical structure of ricinine

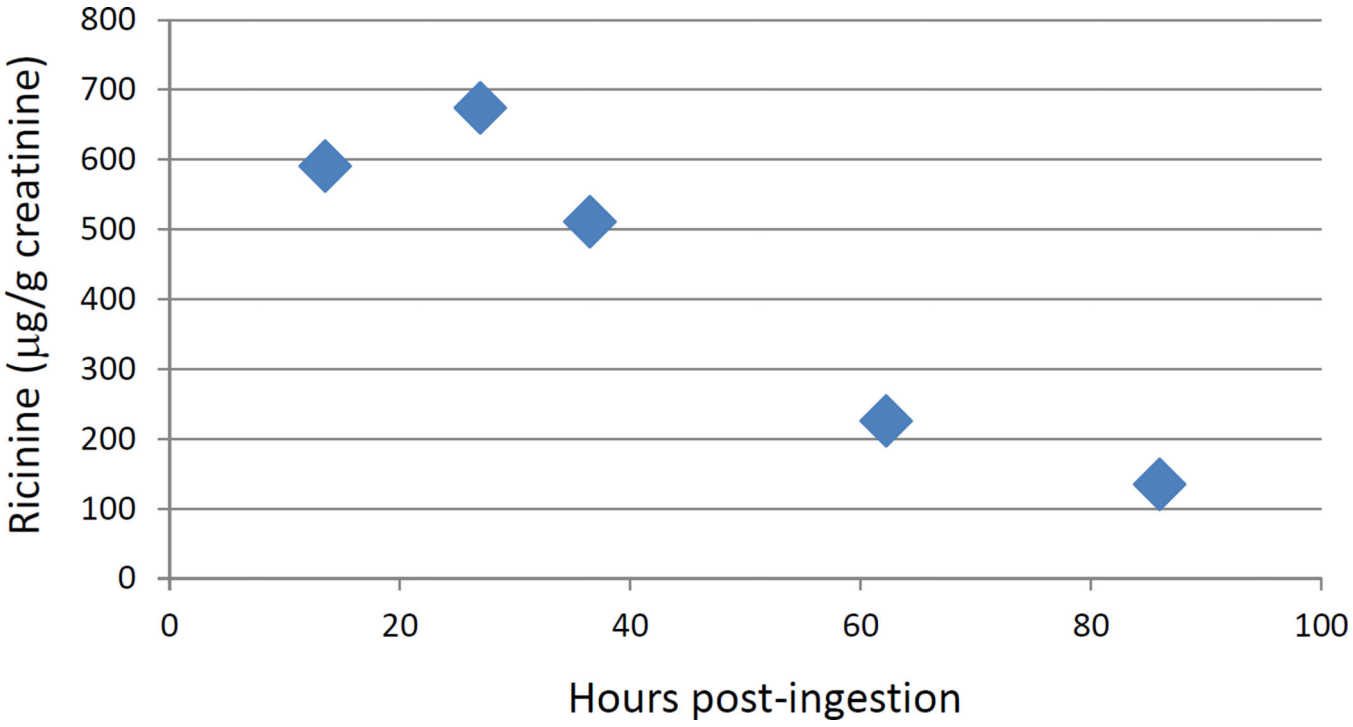

Figure 2.
Excretion profile of ricinine following castor bean exposure

Table 1

Urinary ricinine following castor bean exposure

Time post ingestion (hours)	Ricinine (ng/mL)	Creatinine Corrected Ricinine (μg/g-cr)
14	1400	591
27	190	674
37	550	511
62	330	226
86	130	135

Table 2

Estimated excretion of ricinine

Day	Averaged Creatinine Corrected Ricinine concentration (μg/g-cr)	Estimated Daily Excreted Ricinine (μg/day)
1	632	1074
2	368	626
3	135	230
Total		1.9 mg