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Analytical Aspects of Diterpene Alkaloid Poisoning with Monkshood

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A sensitive and specific method for aconitine extraction from biological samples was developed. Aconitine, the main toxic alkaloid from plants belonging to *Aconitum* species (family Ranunculaceae), was determined in plant material by an external standard method, and by a standard addition calibration method in biological fluids. Described here is one fatal case and five intoxications of accidental aconitine poisoning following the ingestion of aconite mistaken for an edible grass, *Aruncus dioicus* (Walt.) Fernald, "mountain asparagus", and *Cicerbita alpina* (L.) Wallroth. The aconitine content in urine was in the range 2.94 μg/mL (dead patient) – 0.20 μg/mL (surviving patients), which was almost two to four times higher than that in plasma.

Keywords: Diterpene alkaloids, aconitine, plant material, biological fluids, HPLC analysis.

Monkshood or aconite (*Aconitum napellus* and related species, family Ranunculaceae) is a well known plant because it is extremely toxic and there is no known antidote. *Aconitum* species grow wild in temperate regions of the northern hemisphere. *A. napellus* specifically is found in mountainous regions of Europe [1a,1b]. Toxicologists say aconite is the "perfect" drug to mask a murder [2a-2d]. Aconite is a fast-acting poison, the active principles of which are aconitine and related alkaloids. A light touch of the juice causes a numb, tingling sensation; any more has a similar effect to cyanide, paralysing the breathing and stopping the heart [3a,3b].

In traditional Asian medicine, extracts of the root are typically mixed with other ingredients (e.g. licorice, ginger) for ailments ranging from sciatica to nephritis [3c,3d]. In this paper we describe efficient techniques for rapid aconitine extraction from plant material and from biological fluids of patients involved in a poisoning case. The aconitine analyses were developed for a lethal food poisoning case that occurred in northern Italy, due to wild grass ingestion. The initial clinical picture registered in the patients was generalized paresthesia, nausea, diarrhea, vertigo, thoracic pain, dyspnea, and dyschromatopsia. Within one hour, patients presented ventricular tachycardia and fibrillation with different levels of severity. The intoxication was caused by *Aconitum* leaves cooked together with other edible wild vegetables known as "mountain asparagus": *Aruncus dioicus* (Walt.) Fernald (goat's-beard, Asteraceae) shoots, and *Cicerbita alpina* (tall blue-lettuce, Asteraceae) leaves.

Aconitine, widely perceived as the major toxin in *A. napellus* and other *Aconitum* species, was the screening target. After botanical identification, the cooked vegetables (3 batches) that had been partially eaten and ingested were analyzed by RP-HPLC. The aconitine levels in the cooked vegetables were 0.009 ± 0.0011, 0.028 ± 0.0015, and 0.065 ± 0.0012 mg/g, evaluated by means of an external standard method. The botanical results and aconitine contents of the cooked wild grasses were highly related to the toxic symptoms of the patients and to those induced by *Aconitum* plant ingestion [3d,3e]. The aconitine level in serum, urine, bile and stomach contents was evaluated by a standard addition calibration method. This procedure involves the preparation of several solutions, each of which contained the same mass of unknown alkaloid (solution X in Table I). Increasing

Table 1: Standard addition of increasing quantities of aconitine standard to biological matrices (sample volume).

Solution	sample volume µL MeOH	µL aconitine 0.01µg/µL	µL MeOH	aconitine µg/mL
Sx	100	0	400	---
S1	100	100	300	2
S2	100	200	200	4
S3	100	300	100	6

quantities of known standard (solutions 1, 2, and 3 in Table 1) were added to each vessel and MeOH was used in order to maintain the same final volume. The vessels were analyzed separately for the quantification of the alkaloid under study.

Aconitine levels of the serum, plasma, bile and stomach contents of the dead and intoxicated patients are reported in Table 2. The content in urine was in the range 2.94 µg/mL (dead patient) and 0.20 µg/mL (surviving patients), which was almost two to four times higher than that in plasma. Urine was a useful biological fluid in which to identify the toxicants in the case of aconite intoxication [3f,4]. A calibration curve was produced for the standard addition analysis of the plasma sample of an intoxicated patient (Patient 2 in Table 2). The x-intercept (y=0) value was the aconitine concentration in the biological samples.

Experimental*:* The plant material was thinly minced, extracted two times with 0.1 N HCl (1:10 w/v) for 1 h at r.t. and filtered. The acidulated aqueous phases were collected, saturated KCl solution was added and the mixture was extracted (1:1 v/v) with $CHCl_3$ x 3, concentrated and stored at +4°C.

Aconitine analysis was performed in serum, urine, stomach contents, and bile. Four aliquots (1 mL each) of each biological sample was treated as plant material and the residue was dissolved in MeOH (100 µL). One aliquot was maintained unaltered, and to 3 were added increasing quantities of aconitine. Then each vessel was made up to a given volume with MeOH, according to data reported in Table I.

HPLC aconitine analysis: Alkaloids were monitored by reversed phase HPLC using a Jasco instrument, equipped with a UV 975 Intelligent UV/VIS Detector, a PU-980 Intelligent HPLC Pump, Rheodyne mod. 7725, and a LC-8 SupelcoSIL 25 cm x 4 mm, 5 µm column. The eluent was H_2O:CH_3CN: 1,4-dioxan (265 : 150 : 75 v/v) + $HClO_4$ 0.01 M, to pH = 2.6 with NH_4OH at 10%. Injection volume: 20 µL; flow rate 1 mL/min; detection λ = 235 nm.

Table 2: Aconitine content in biological fluids of dead and intoxicated people.

Patient	Clinical picture	Plasma µg/mL	Urine µg/mL	Bile µg/mL	Stomach content µg/g
1	ventricular tachycardia fibrillation intensive care unit death	1.14±0.002	2.94±0.002	4.21±0.002	1.78±0.002
2	ventricular tachycardia fibrillation intensive care unit kidney transplantation	0.63±0.003	1.37±0.002	unavailable sample	
3	ventricular tachycardia fibrillation intensive care unit	0.35±0.003	0.81±0.004	unavailable sample	
4	ventricular tachycardia fibrillation intensive care unit	0.42±0.002	0.64±0.003	unavailable sample	
5	ventricular tachycardia fibrillation	0.33±0.003	0.77±0.002	unavailable sample	
6	ventricular tachycardia fibrillation	0.052±0.003	0.20±0.003	unavailable sample	

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