

Detection of karakin poisoning using a targeted mass spectrometric workflow

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Treatment and management of plant toxicosis is made more difficult when an alien plant species is ingested, as identification of the toxin may pose a challenge. High-resolution mass spectrometers are required for the toxicological analysis of samples in these cases owing to their ability to scan large mass ranges and accurately identify mass features. We present this case to highlight the value of this technology in clinical toxicology. A middle-aged woman reported visual impairment, dizziness and numbness of her mouth and tongue following the ingestion of a berry. Over time her condition deteriorated, warranting toxicological analysis. The tree the berry came from was identified as *Cornynocarpus laevigatus*, which is known to produce the karakin neurotoxin. The patient's samples and the husk and pulp of the berries were analysed using a high-resolution mass spectrometer. This resulted in the identification of the toxin in the berry kernel and husk and patient's hair, suggesting that karakin could have contributed to the patient's condition.

S Afr Med J 2022;112(12):901-903. <https://doi.org/10.7196/SAMJ.2022.v112i12.16667>

The high number of toxic endemic and alien plants in South Africa (SA) represents a health hazard, and plant toxicosis is common.^[1-3] Misidentification of toxic plants and ingestion of toxic components by pets or children can lead to severe toxicity.^[1] While most cases are treated symptomatically, if the plant or toxin is correctly identified, available antidotes may be considered.^[4] For example, the delirium of the anticholinergic syndrome caused by belladonna alkaloids can be treated by physostigmine.^[4] Poisoning by alien plant species is especially dangerous as the lack of local knowledge makes timeous identification and treatment more difficult.^[5] In cases involving rare toxins, toxicological analysis using low-resolution mass spectrometers is ineffective, as reference standards and full product ions spectra for multiple reaction monitoring are required to identify a compound.^[6] These reference standards are either not available or difficult to obtain if the compounds in question are non-endemic toxins. High-resolution mass spectrometers, however, can obtain spectral data of compounds over a wide mass range without prior knowledge of the compounds present.^[6] These spectral data can then be compared with global databases to identify molecules present in patient samples.

Using a high-resolution mass spectrometer, we were able to show that the neurotoxin karakin likely contributed to a patient's toxicosis in SA.

Case report

We describe a case of a 46-year-old female who presented with visual impairment, dizziness, numbness in her mouth and tongue and an elevated heart rate following the ingestion of a berry from a tree in her garden. The patient was initially diagnosed as having a panic attack and was prescribed the benzodiazepine lorazepam. Over the next few days her condition deteriorated, and she developed 'para-seizures' in her arms, and intense headaches, as described by her general practitioner (GP). The patient sought further medical care, and investigations including electrolytes, calcium, as well as her renal and liver function were all normal. A computed tomography scan of her head showed no abnormalities. Despite symptomatic therapy, her condition continued to worsen. The patient's GP was concerned that

the patient had ingested a toxin when tasting the berry, and that the toxin may still have been present in her system.

A cutting of the tree was identified by a horticulturist at Stark Ayres Garden Centre, Rosebank, Cape Town, as *Cornynocarpus laevigatus*, more commonly known as the karaka tree, which is endemic to New Zealand.^[7]

The patient's GP then contacted the toxicology unit housed in the Division of Clinical Pharmacology, Department of Medicine, Faculty of Health Sciences at the University of Cape Town, and submitted urine, blood, saliva and hair samples from the patient for toxicological analysis.

The karaka tree is known to produce a potent neurotoxin called karakin.^[8] Karakin contains the 3-nitropropionic acid (3-NPA) subunit that interferes with adenosine triphosphate synthesis by inhibiting succinate dehydrogenase.^[7,9] Limited human data suggest that it causes excitotoxic effects on the brain leading to paralysis and convulsions^[10] similar to our patient's presenting symptoms. Glutamate antagonists, if given early post ingestion, may reverse the toxic effects of 3-NPA containing compounds.^[7]

The patient's samples (blood, hair, urine and saliva) and the karaka berry husk and pulp from the tree were analysed by independent data acquisition using a Sciex X500R QTOF high-resolution mass spectrometer in the negative ion mode. The raw spectra were then processed using a targeted workflow through the MSConvert (ProteoWizard, USA) and MZMine 2.5 (MZMine, Czech Republic) programs.^[11,12] These programs process spectra by converting the raw spectra into a standard format, detecting mass features, building chromatograms, removing background contaminants and aligning the results. Karakin was identified, using the database Pubchem, in the patient's hair and the berry's husk and pulp (Fig. 1, Table 1).^[8] Karakin was not detected in the patient's blood, urine or saliva. Blank solvents were injected between runs to ensure there was no carry-over between samples.

Our patient fully recovered from the toxicosis 8 weeks post ingestion. There are limited data on the long-term sequelae from karakin ingestion, and we will continue to follow her regularly to assess for any relapse.

Table 1. PubChem ID of karakin in samples^[8]

PubChem ID	Common name	Formula	Accurate mass (Da)	Mass difference (ppm)
100274	Karakin	C15H21N3O15	483.0972	0.0464

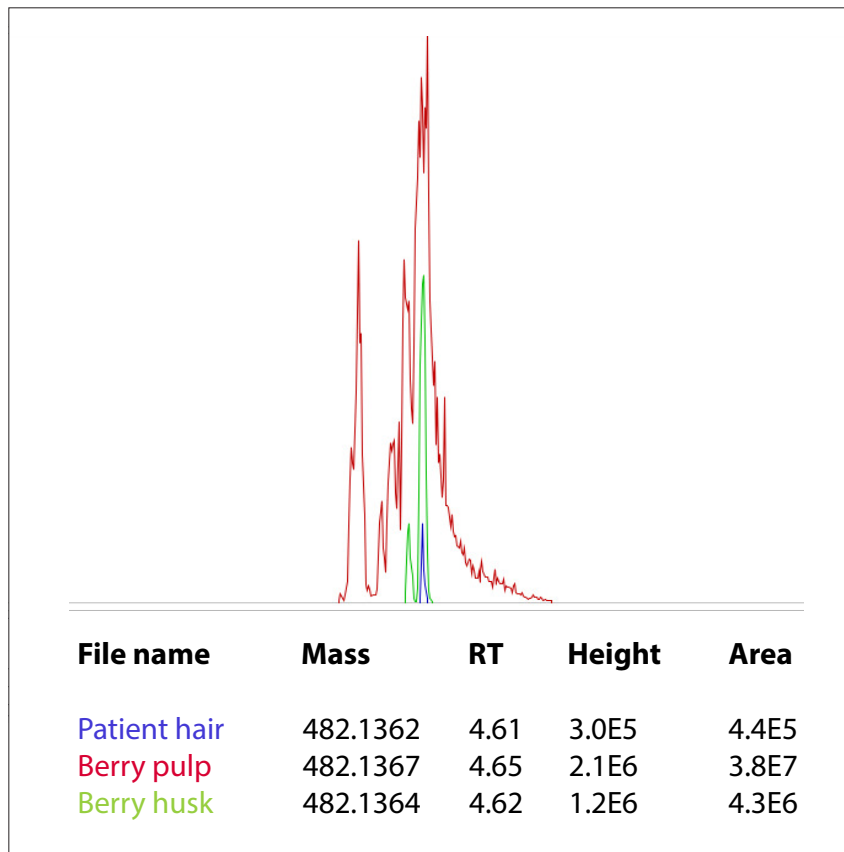


Fig. 1. Mass spectrum showing m/z 482.1340 (karakin) in the negative ionisation mode ($M-H^+$) in berry pulp (red), berry husk (green) and patient hair sample (blue). The mass detected, retention time (RT), relative height and area for each sample are presented.

Discussion

The analysis and detection of karakin in the patient’s hair and the berry husk and pulp suggest that the patient did ingest the toxin, and that it likely led to her clinical presentation. Exogenous compounds such as toxins or drugs are often incorporated into hair.^[13] Hair is therefore an extremely useful matrix to analyse in toxicology as it has a larger surveillance window of days to weeks, compared to that of blood or urine, which is usually only a few hours to days.^[13] Regarding our patient, this was beneficial, as samples were submitted for analysis weeks post ingestion, thus it was unlikely that karakin would still be present in the other samples.

Importantly, the parent compound is known to be incorporated into hair and not its metabolites, simplifying analysis, as very little is known about the metabolism of karakin in humans.^[13]

In conclusion, the results obtained suggest that karakin likely contributed to the patient’s condition. This case report highlights the

power and clinical utility of high-resolution mass spectrometry in the management of toxic ingestions. With these instruments and analyses a wide range of compounds in varying matrixes can be identified effectively without standards and extensive previous knowledge.

Teaching points

- One must have a high index of suspicion of a toxic ingestion when a patient presents with symptoms and signs unexplained by clinical examination and routine investigations.
- A standard toxicology screen would not have identified karakin, as there are no regular assays for it, and to our knowledge it has never been previously reported in a poisoning case in SA.
- This case study highlights the power of high-resolution mass spectrometry in clinical toxicology, as we were able to accurately identify karakin in the berry components and patient sample without

prior knowledge and reference standards. High-resolution mass spectrometry has steadily become an all-in-one tool for clinical and forensic toxicology as it can be used for targeted screening (library searches), untargeted screening, metabolomic studies and quantitation.^[14,15]

- Being able to identify karakin in the berry and the patient’s hair greatly increased the confidence in our analysis. This emphasises the importance of correctly identifying the source of the toxicosis.
- The MSConvert and MZMine workflows are easily available, free of charge, well described and straightforward to use, making them extremely accessible.^[11,12]

Patient consent. Informed consent was obtained from the patient. The Health Research Ethics Committee of the University of Cape Town approved this case report (ref. no. 286/2022).

Acknowledgements. The authors would like to acknowledge and thank Promolab (Pty) Ltd, trading as Separations, South Africa for their contribution to the X500R QTOF instrument. We would also like to thank the patient for their co-operation.

Author contributions. AE processed the patient samples and berry components on the high-resolution mass spectrometer. DJW analysed the data and identified karakin. DJW wrote the first draft. All authors were involved in editing and approving the final version of the manuscript.

Funding. None.

Conflicts of interest. None.

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Accepted 22 August 2022.