ORIGINAL ARTICLE

Yew poisoning of olive baboons (*Papio anubis*) in captivity: laboratory diagnosis

Marketa Kominkova [1,2,3], Maja Stanisavljevic [1], Ondrej Zitka [1,2,3], Stanislav Mazanek [4], Jana Sedlackova [3], Hana Bandouchova [3], Jiri Pikula [3], Miroslava Beklova [3], Rene Kizek [1,2]

1 Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University in Brno, Czech Republic
2 Central European Institute of Technology, Brno University of Technology, Brno, Czech Republic
3 Department of Ecology and Diseases of Game, Fish and Bees, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic
4 Brno City Zoological Garden, Brno, Czech Republic

Correspondence to: Prof. Ing. Rene Kizek, PhD.
Department of Chemistry and Biochemistry, Mendel University in Brno, Zemedelska 1, 613 00 Brno, Czech Republic
tel: +420-5-4513-3350; fax: +420-5-4521-2044; e-mail: kizek@sci.muni.cz

Submitted: 2013-06-21 Accepted: 2013-08-30 Published online: 2013-11-10

Key words: **Zoo animals; fatality; European yew; *Taxus baccata*; toxoids**

Neuroendocrinol Lett 2013; **34**(Suppl.2):130–133 **PMID:** 24362105 NEL341013A20

Abstract

OBJECTIVES: Toxic effects of the yew have been known since ancient times. Yew toxicity is due to the content of cyanogenic glycosides and a mixture of alkaloids known as taxines. Taxine B is probably responsible for the most part of adverse effects in poisoned organisms. This particular taxoid is common in body fluids of the yew-poisoned. The present study is engaged with laboratory examination to confirm substances that lead to fatality of a pair of olive baboons (*Papio anubis*) following ingestion of yew seeds. When both cage mates (male and female) died suddenly, poisoning was suspected because many berries had fallen into the cage from a nearby fruiting yew tree (*Taxus baccata*) during the windy night before.
METHODS: The analysis was performed using electrospray ionization quadrupole time-of-flight mass spectrometry. A flow injection analysis/mass spectrometry setting was prepared for this purpose.
RESULTS: The above mentioned mass spectrometry analysis of taxoids confirmed poisoning by taxanes. The presence of taxin B/isotaxin B was confirmed in all investigated samples. Apparently in urine and bile there were concentrations ranging 150–220 ng.mL^{-1} and in blood serum concentrations 25–30 ng.mL^{-1}.
CONCLUSION: It follows from the results obtained that we confirmed that baboons were deadly intoxicated by yew fruits.

Abbreviations:

ESI	- Electrospray ionization
MS	- Mass spectrometry
Q-TOF	- Quadrupole time-of-flight
FIA	- Flow injection analysis
SPE	- Solid-phase extraction

INTRODUCTION

There are 9 species of yews (*Taxus spp.*) planted for their ornamental qualities in parks worldwide. The most common ones include *Taxus baccata*, *T. canadensis*, *T. brevifolia*, *T. media*, *T. capitata* and *T. cuspidata* (Vaningen *et al.* 1992). Apart from the ornamental usage, yews are also used in pharmaceutical industry (DeSmet, 1997; Eisenhauer & Vermorken, 1998; Gut *et al.* 2006; Li *et al.* 2009). Signs of toxicity include dizziness, nausea, abdominal pain, cardiotoxicity (tachycardia later followed by bradycardia), dyspnea, muscle tremors, convulsions and unconsciousness. Severe toxicity often results in a fatal outcome (Handeland, 2008; Panzeri *et al.* 2010). The toxicity is due to the content of cyanogenic glycosides and a mixture of alkaloids known as taxines. Taxine B (monohydroxy-monoacetyl-taxine) or isotaxine B is probably responsible for the most part of adverse effects in poisoned organisms (Kite *et al.* 2000; Wilson *et al.* 2001; Ruha *et al.* 2002). These effects are to a lesser degree accompanied by action of 1-deoxytaxine B, 1-deoxyisotaxine B and pseudoalkaloids of taxine B (13-deoxo-13α-acetyloxy-taxine B, 13-deoxo-13α-acetyloxy-1-deoxytaxine B and 13-deoxo-13α-acetyloxy-1-deoxy-nortaxine B). Taxine A is another important toxin present in yew plants (Wilson *et al.* 2001). Paclitaxel (taxol A) and its analogues, employed in chemotherapy, are not less important from the toxicological point of view (Jenniskens *et al.* 1996). Apart from the above-mentioned substances, it is possible to quantify 10-deacetyltaxol, baccatin III, 10-deacetylbaccatin III, cephalomannine (taxol B), 3,5-dimethoxypheno- and triacetyl-taxine in body fluids of yew-poisoned animals and humans (Grobosch *et al.* 2012, 2013). Metabolites of these substances can be found in blood serum, bile, faeces and urine (Monsarrat *et al.* 1998; Shibasaki *et al.* 2012).

Two olive baboon cage mates (a ten-year old male and a six-year old female) were found dead on 30th October 2006 in The Zoological Garden of Brno City (Brno, Czech Republic). The night before was windy and the floor was full of scarlet-aril-covered seeds that had fallen down from a tree (*Taxus baccata*) growing above the cage. Accidental yew poisoning was suspected because of the sudden death associated with the presence of yew seeds in the cage. Considering the history of the case and the suspicion of yew poisoning in captive baboons, necropsy and laboratory analysis of body fluids was performed to diagnose the mortal cause in the present study.

MATERIALS AND METHODS

Biological materials

Two olive baboon (*Papio anubis*) cage mates (a ten-year old male and a six-year old female) were found dead on 30th October 2006 in The Zoological Garden of Brno City (Brno, Czech Republic). Blood serum, bile and urine were collected during necropsy of both olive baboons and stored frozen at –20 °C until analysis. Blood was obtained using cardiac puncture.

Chemicals

All chemicals used in the study were obtained from Sigma Aldrich (St. Louis, USA) in ACS purity unless noted otherwise. When preparing buffers for the analysis, pH values were measured using InoLab pH 730 WTW (Weilheim, Germany).

Preparation of samples

A previously described method was employed in this study (Green *et al.* 2006). Diethylether (1 mL) was added to the analysed volume of 100 µL of body fluids (urine, bile, blood serum). This mixture was treated using a vortex for 5 minutes and then centrifuged (Eppendorf 5417R, USA) at 20 °C, 4,000 *g* for another 5-minute period. Supernatant was collected into a 2 mL-microtube and 1 mL of diethylether was added to the precipitate. Again, the mixture was vortexed for 5 minutes and then centrifuged at 20 °C, 4,000 *g* for 5 minutes. Supernatant was added to the supernatant from the previous step. It was mixed and transferred into a 96-well plate and evaporated using a nitrogen vaporiser (Ultravap™, UK) at 40 °C for 10 minutes. The dried sample was diluted using 500 µL 0.2 mol.L^{-1} ammonium acetate buffer with pH 5.

Solid-phase extraction (SPE)

SPE (solid phase extraction) columns (Chromabond Multi 96, Macherey-Nagel, Germany) were washed gradually using EpMotion 5075 (Eppendorf, USA) and 1 mL methanol first and then 1 mL 0.01 mol.L^{-1} ammonium acetate buffer with pH 5. It was followed by addition of the sample (total volume and again washing using 1 mL 0.01 mol.L^{-1} ammonium acetate buffer with pH 5 and 1 ml of a mixture of methanol with ammonium acetate buffer with pH 5 (ratio 0.5 : 9.5). The next step was elution by 1 ml of acetonitrile with triethylamine (1000:1). The resulting extract was dried using a nitrogen vaporiser at 40 °C. The sample was then diluted in 1 mL acetonitrile (MS purity) for the purpose of the electrospray ionization mass spectrometry (ESI-MS) analysis.

ESI-MS

For measurements Bruker Maxis Impact quadrupole time-of-flight detector (Q-TOF) mass spectrometer was employed. ESI source was operated in positive mode. Voltage of electrospray capillary was set to 3,500 V with nebulizing gas (N_2) flow rate of 4 L.min^{-1} and drying gas temperature was set on 350 °C. The flow of the injected sample was 3 µL.min^{-1} (KDS 100, KD Scientific, USA). Scanning was carried out in the range of 100–1000 m/z. Table 1 provides the list of measured taxoids. Due to the fact that structure of taxoids is similar, baccatin III was used as a calibration standard. Based on this, we determined concentration of other taxoids.

RESULTS AND DISCUSSION

Necropsy of both baboons revealed a lot of yew seeds chewed to pieces in the stomach and cranial part of duodenum, gastritis and dilated heart chambers. Body fluids (blood serum, bile and urine) were subjected to analysis to confirm the preliminary diagnosis. Procedures employed solid-phase extraction (van Tellingen *et al.* 1999; Green *et al.* 2006; Persico *et al.* 2011) and direct detection using electrospray ionization quadrupole time-of-flight mass spectrometry (Hodek *et al.* 2011; Pelclova *et al.* 2011) . Using these procedures, the presence of the individual taxoids specific for the yew poisoning was evaluated (van Tellingen *et al.* 1999).

As it is shown in Figure1, the analysis of body fluids ((A) bile, (B) blood serum and (C) urine) confirmed taxine B and isotaxine B, respectively, which amount to the highest concentrations in *Taxus baccata* plants (Wilson *et al.* 2001; Ruha *et al.* 2002). Other taxines could not be confirmed using the flow injection analysis and mass detection with sufficient reliability. The highest signal intensities were observed in urine and bile, while up to six times lower signal intensity was measured in blood serum. Based on calibration curves the total concentration of sum of taxine B and isotaxine B, was determined. I.e. 170 ng.mL^{-1} in bile, 25 ng.mL^{-1} in blood serum and 210 ng.mL^{-1} in urine for male and 150 ng.mL^{-1} in bile, 30 ng.mL^{-1} in blood serum and 220 ng.mL^{-1} in urine for female were gained. The concentration of the taxoids differs when intoxication of the taxus by human and animals occurs in body fluids but it is in the level of ng.mL^{-1} (Frommherz *et al.* 2006; Pietsch *et al.* 2007; Beyer *et al.* 2009; Froldi *et al.* 2010; Panzeri *et al.* 2010; Grobosch *et al.* 2013), that corresponds with concentrations in body fluids of dead baboons.

Yew poisoning is often acute and the suspicion can be based on the history of sudden death of animals with some access to yew. Pathological findings at necropsy are non-specific. However, when careful inspection of gastrointestinal contents provides evidence for the fact that the animal ingested yew (i.e., foliage, seeds, clippings), the diagnosis can be made. In some cases, necropsy reveals findings such as gastrointestinal congestion and lung oedema (Casteel, 2003), congestion of liver, kidney and dilated heart chambers (Vaningen *et al.* 1992). In doubt, laboratory measurements of taxine B/isotaxine B can be employed to confirm the diagnosis. Interestingly, there were no differences in the intensity of signal in samples of bile and urine collected from baboons, while much lower levels were found in blood serum. The lower taxine concentration found in blood against the higher concentrations found in urine and bile is in good agreement with the previously published data (Frommherz *et al.* 2006; Grobosch *et al.* 2013). However, taxine B was confirmed in all types of examined samples and it is clear that both baboons ingested a considerable amount of yew, some of which has already been absorbed and metabolised (Vaclavikova *et al.* 2004). Effective measures are necessary to prevent such cases of poisoning in zoo animals (Pikula *et al.* 2013).

Tab. 1. Taxoids and their mass (m/z) analysed by flow injection analysis with mass detection using the Q-TOF analyser (Grobosch *et al.* 2013; Kite *et al.* 2013).

Taxoid	m/z
10-deacetylbaccatin III	544.6
Monoacetyltaxine	568.4
Taxine B/Isotaxine B (Monohydroxymonoacetyltaxine)	584.2
Baccatin III	586.6
Triacetyltaxine	652.3
Monohydroxytriacetyltaxine	668.4
Cephalomannine (Taxol B)	832
Paclitaxel (Taxol A)	854.3

CONCLUSION

The present results document that the pair of olive baboons (*Papio anubis*) died of yew (*Taxus baccata*) poisoning. This is evidenced by the following facts:

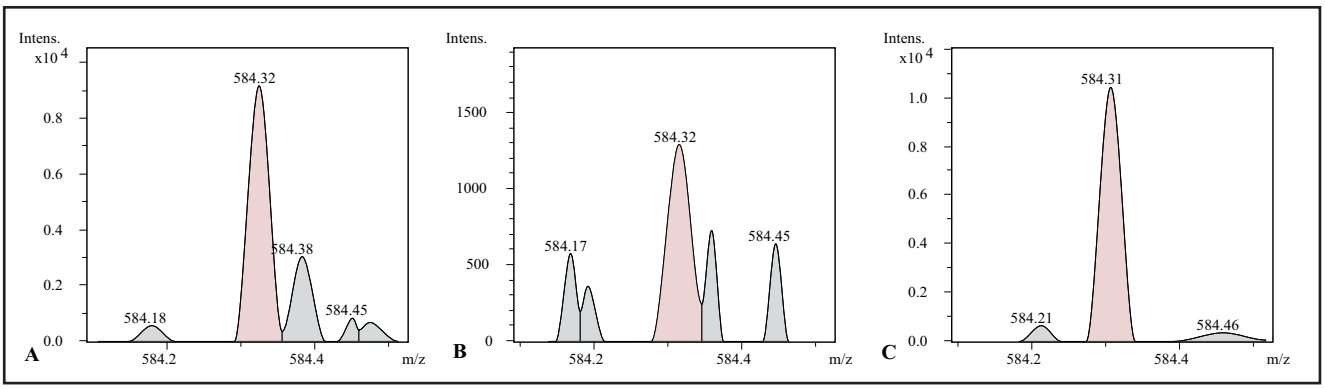

Fig. 1. ESI-MS spectra of taxine B/isotaxine B (584.2 m/z) obtained by analysis of the samples and confirming yew poisoning. Spectra were recorded in different body fluids of male of baboon (**A**) bile, (**B**) blood serum (**C**) urine.

many aril-covered seeds of yew were found on the cage floor, pieces of yew seeds were recognized in the gastrointestinal tract of baboons at necropsy, and toxic substances such as taxine B/isotaxine B were confirmed by laboratory analysis in body fluids of baboons. The accident was a management failure because the toxicity of yew is well known. Therefore, the decision to let a fruiting yew tree grow right behind the cage with curious baboons ready to inspect and test new food items is hard to understand. Hence a compliance of the basic, effective measures is necessary to prevent such cases of poisoning in zoo animals (Pikula *et al.* 2013).

ACKNOWLEDGEMENTS

This work was supported by MSMT 6215712402. The text of the manuscript was corrected by a native speaker.

REFERENCES

1 Beyer J, Drummer OH, Maurer HH (2009). Analysis of toxic alkaloids in body samples. Forensic Sci Int. **185**: 1–9.
2 Casteel SW (2003). Taxine alkaloids. In Plumlee K (ed): Clinical Veterinary Toxicology. Elsevier Health Sciences. 379–381.
3 DeSmet PAGM (1997). The role of plant-derived drugs and herbal medicines in healthcare. Drugs. **54**: 801–840.
4 Eisenhauer EA, Vermorken JB (1998). The taxoids – Comparative clinical pharmacology and therapeutic potential. Drugs. **55**: 5–30.
5 Froldi R, Croci PF, Dell'Acqua L, Fare F, Tassoni G, Gambaro V (2010). Preliminary gas chromatography with mass spectrometry determination of 3,5-dimethoxyphenol in biological specimens as evidence of *Taxus* poisoning. J Anal Toxicol. **34**: 53–56.
6 Frommherz L, Kintz P, Kijewski H, Köhler H, Lehr M, Brinkmann B, et al (2006). Quantitative determination of taxine B in body fluids by LC-MS-MS. Int J Legal Med. **120**: 346–351.
7 Green H, Vretenbrant K, Norlander B, Peterson C (2006). Measurement of paclitaxel and its metabolites in human plasma using liquid chromatography/ion trap mass spectrometry with a sonic spray ionization interface. Rapid Commun Mass Spectrom. **20**: 2183–2189.
8 Grobosch T, Schwarze B, Felgenhauer N, Riesselmann B, Roscher S, Binscheck T (2013). Eight cases of fatal and non-fatal poisoning with *Taxus baccata*. Forensic Sci Int. **227**: 118–126.
9 Grobosch T, Schwarze B, Stoecklein D, Binscheck T (2012). Fatal poisoning with *Taxus baccata*. Quantification of paclitaxel (taxol A), 10-deacetyltaxol, baccatin III, 10-deacetylbaccatin III, cephalomannine (taxol B), and 3,5-dimethoxyphenol in body fluids by liquid chromatography-tandem mass spectrometry. J Anal Toxicol. **36**: 36–43.
10 Gut I, Ojima I, Vaclavikova R, Simek P, Horsky S, Linhart I, et al (2006). Metabolism of new-generation taxanes in human, pig, minipig and rat liver microsomes. Xenobiotica. **36**: 772–792.
11 Handeland K (2008). Acute yew (*Taxus*) poisoning in moose (*Alces alces*). Toxicon. **52**: 829–832.
12 Hodek P, Krizkova J, Frei E, Singh R, Arlt VM, Stiborova M (2011). Impact of beta-naphthoflavone on genotoxicity of food-derived carcinogens. Neuroendocrinol Lett. **32**: 25–34.
13 Jenniskens LHD, van Rozendaal ELM, van Beek TA, Wiegerinck PHG, Scheeren HW (1996). Identification of six taxine alkaloids from *Taxus baccata* needles. J Nat Prod. **59**: 117–123.
14 Kite GC, Lawrence TJ, Dauncey EA (2000). Detecting Taxus poisoning in horses using liquid chromatography/mass spectrometry. Vet Human Toxicol. **42**: 151–154.
15 Kite GC, Rowe ER, Veitch NC, Turner JE, Dauncey EA (2013). Generic detection of basic taxoids in wood of European Yew (*Taxus baccata*) by liquid chromatography-ion trap mass spectrometry. J Chromatogr B. **915**: 21–27.
16 Li SM, Fu YJ, Zu YG, Zu BS, Wang Y, Efferth T (2009). Determination of paclitaxel and its analogues in the needles of *Taxus* species by using negative pressure cavitation extraction followed by HPLC-MS-MS. J Sep Sci. **32**: 3958–3966.
17 Monsarrat B, Chatelut E, Royer I, Alvinerie P, Dubois J, Dezeuse A, et al (1998). Modification of paclitaxel metabolism in a cancer patient by induction of cytochrome P450 3A4. Drug Metab Dispos. **26**: 229–233.
18 Panzeri C, Bacis G, Ferri F, Rinaldi G, Persico A, Uberti F, et al (2010). Extracorporeal life support in a severe *Taxus baccata* poisoning. Clin Toxicol. **48**: 463–465.
19 Pelclova D, Navratil T, Fenclova Z, Vlckova S, Kupka K, Urban P, et al (2011). Increased oxidative/nitrosative stress markers measured non-invasively in patients with high 2,3,7,8-tetrachlorodibenzo-p-dioxin plasma level. Neuroendocrinol Lett. **32**: 71–76.
20 Persico A, Bacis G, Uberti F, Panzeri C, Di Lorenzo C, Moro E, et al (2011). Identification of Taxine derivatives in biological fluids from a patient after attempted suicide by ingestion of Yew (*Taxus baccata*) leaves. J Anal Toxicol. **35**: 238–241.
21 Pietsch J, Schulz K, Schmidt U, Andresen H, Schwarze B, Dressler J (2007). A comparative study of five fatal cases of *Taxus* poisoning. Int J Legal Med. **121**: 417–422.
22 Pikula J, Hajkova P, Bandouchova H, Bednarova I, Adam V, Beklova M, et al (2013). Lead toxicosis of captive vultures: case description and responses to chelation therapy. BMC Vet Res. **9**: 1–11.
23 Ruha AM, Tanen DA, Graeme KA, Curry SC, Miller MB, Gerkin R, et al (2002). Hypertonic sodium bicarbonate for *Taxus* media-induced cardiac toxicity in swine. Acad Emerg Med. **9**: 179–185.
24 Shibasaki H, Okamoto S, Inoue R, Okita M, Yokokawa A, Furuta T (2012). Separation and quantitative determination of 6 alpha-hydroxycortisol and 6 beta-hydroxycortisol in human urine by high-performance liquid chromatography with ultraviolet absorption detection. Anal Bioanal Chem. **402**: 2945–2952.
25 Vaclavikova R, Soucek P, Svobodova L, Anzenbacher P, Simek P, Guengerich FP, et al (2004). Different in vitro metabolism of paclitaxel and docetaxel in humans, rats, pigs, and minipigs. Drug Metab Dispos. **32**: 666–674.
26 van Tellingen O, Beijnen JH, Verweij J, Scherrenburg EJ, Nooijen WJ, Sparreboom A (1999). Rapid esterase-sensitive breakdown of polysorbate 80 and its impact on the plasma pharmacokinetics of docetaxel and metabolites in mice. Clin Cancer Res. **5**: 2918–2924.
27 Vaningen G, Visser R, Peltenburg H, Vanderark AM, Voortman M (1992). Sudden unexpected death due to *taxus* poisoning - a report od 5 cases, with review of the literature Forensic Sci Int. **56**: 81–87.
28 Wilson CR, Sauer JM, Hooser SB (2001). Taxines: a review of the mechanism and toxicity of yew (*Taxus spp.*) alkaloids. Toxicon. **39**: 175–185.