Int J Legal Med (2007) 121: 417–422
DOI 10.1007/s00414-006-0099-5

CASE REPORT

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A comparative study of five fatal cases of *Taxus* poisoning

Received: 3 February 2006 / Accepted: 12 April 2006 / Published online: 6 May 2006

Abstract The study presents five fatal cases of poisoning with *Taxus spp*., all of which were suicides of young people aged between 16 and 26 years. Yew leaves were consumed in four fatalities, whereas a mash from *Taxus* was ingested in one case. No relevant concentrations of alcohol, narcotic drugs, and pharmaceuticals were determined in postmortem toxicological screening. At forensic autopsy, a widely dilated pupil was found in two decedents. Furthermore, autopsy showed unspecific findings of intoxication in all cases: acute blood congestion of lungs, liver, kidney, and brain as well as dilated cardiac ventricles. No signs of violence could be found in any of the fatalities. Yew leaves were identified in four cases in the stomach and duodenum. 3,5-Dimethoxyphenol, the aglycon of the *Taxus* ingredient taxicatine, was determined as toxicological evidence for the absorption of yew ingredients. *Taxus* intoxication could be confirmed by 3,5-dimethoxyphenol concentrations in cardiac blood between 31 and 528 ng/ml for all cases. 3,5-Dimethoxyphenol was also detected in stomach contents as well as in urine, liver, kidneys, and brain samples. Based on the different concentrations of 3,5-dimethoxyphenol determined in the cardiac blood samples, it was concluded that the form of ingestion plays a decisive role in the process of poisoning. Finally, a toxic range for *Taxus* poisoning based on 3,5-dimethoxyphenol as marker substance is proposed as orientation.

Keywords *Taxus spp.* · Fatalities · 3,5-Dimethoxyphenol · HPLC/PDA

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Introduction

The toxicity of *Taxus spp.* (yew), an evergreen shrub, has been known since antiquity; however, many web sites are dealing with the application of parts of yew as a classical way of committing suicide to date [1]. In addition, the application of *Taxus* ingredients as psychoactive drug and hallucinogens is described elsewhere [2, 3].

Taxine-derived alkaloids (e.g., taxine A and B, isotaxine B, paclitaxel), taxane-derived substances (e.g., taxol A and B), and glycosides (e.g., taxicatine) seem to be responsible for the toxicity of *Taxus spp.* because they cause dizziness, nausea, vomiting, diffuse abdominal pain, tachycardia (initially), and convulsions followed by bradycardia, respiratory paralysis, and death [4, 5]. Whereas the seeds, bark, and leaves of *Taxus* contain toxic compounds, the sweet tasting red aril can be eaten without danger [4].

The postmortem evaluation of acute poisoning is usually unremarkable, visible abnormalities are often absent, and microscopic lesions are rarely observed [5]. A lot of fatal intoxications in man [6–16] and livestock [17–20] have been reported, previously. However, toxicological data from biological materials have seldom been published [1, 13–15].

Lethal oral doses (LD_{min}) of yew leaves in human and animals have been published by Wilson et al. [5], varying from 0.2 to 0.4 g/kg body weight (bw) for the sensitive horses, 0.6–1.3 g/kg bw for humans to the less sensitive chicken (LD_{min}=16.5 g/kg bw). Estimating that 1 g of yew leaves contains approx. 5 mg of taxines [21, 22], the minimal toxic dose for humans was calculated to be 3.0–6.5 mg taxines/kg bw [5].

Many methods for the analysis of the manifold *Taxus* ingredients have been described: immunoassay [23–25],

thin-layer chromatography [15, 26, 27], gas chromatography (GC) [10], and GC/mass spectroscopy (MS) [1, 15, 18, 28], as well as high-performance liquid chromatography (HPLC) combined with UV [15, 25, 29–34] and MS [13, 14, 17, 35, 36]. However, most of the papers deal with the detection of extracts of *Taxus spp*, whereas analytical procedures of forensic relevance were seldom reported.

The study in hand presents five cases of fatal intoxication with *Taxus spp*. to collect more data for the estimation of the toxicity of *Taxus*. Due to the lack of a sophisticated HPLC/MS system and a commercially available taxine standard 3,5-dimethoxyphenol, the aglycon of the *Taxus* ingredient taxicatine [37–40], was determined as toxicological evidence for the absorption of yew ingredients.

Case histories

Case 1

A 23-year-old woman who had been in long-term psychiatric therapy was found dead in her flat. Previous suicide attempts were known and information material about toxic plants was also discovered in the flat. Police investigation showed that the young female took medical advice 2 weeks before death due to *Taxus*-like symptoms such as dizziness and arrhythmia. However, she refused to be referred to a hospital.

Restricted by advanced decay, neither specific anatomic changes nor signs of violence could be observed at the forensic autopsy carried out 1 day after discovery. Some older, probably self-inflicted, skin-deep injuries on the extremities confirmed the psychiatric disease. The duodenum and colon contained approx. 200 g of green-brown plant particles (Fig. 1), identified as whole leaves as well as fragments of leaves of *Taxus baccata*.

No alcohol and drugs were detected by general toxicological analysis; therefore, a *Taxus* poisoning was diagnosed.

Fig. 1 Leaves of *Taxus baccata* identified in the duodenum content of case 1

Case 2

A 20-year-old man was found dead in a park, unseasonably dressed in an early morning of September. There were no signs of violence on the body, but a widely dilated pupil was obtained. Findings by the police identified a distance of 200 m between the places of ingestion and retrieval. It is surprising to note that it could be traced back by a sniffer dog that the young man had covered a total of approx. 1,000 m around the park for this short distance, demonstrating unmistakable signs of confusion. In addition, no preexisting diseases were known from the decedent.

Forensic autopsy revealed blood congestion of the lungs and the brain as well as dilated cardiac ventricles. No other anatomical or histological abnormalities could be observed. However, in the stomach and duodenum, approx. 150 g of green leaves, identified as parts of *T. baccata*, were found leading to the hypothesis of *Taxus* intoxication.

In the general toxicological analysis, a low level of cannabinoids (THC, 2.9 ng/ml; THC–COOH, 14.7 ng/ml) was determined in the femoral blood. No further narcotic drugs, sedatives, or other violent drugs and alcohol could be detected in the autopsy material.

Case 3

A 26-year-old man was found dead on the top floor of his parents' house on a December day. The decedent was lying completely dressed in an untidy room on his bed, and the face was on a pillow. Beside the door a mixer containing residues of a green mash and a bowl with an adherence of the green pulp were identified. No preexisting diseases were known from the decedent.

Forensic autopsy of the decayed body resulted in nonspecific signs of intoxication: high-grade swelling of the brain (1,750 g) and acute blood congestion of the viscera. Besides curly kale fragments of yew needles were identified in the greenish plant material of the stomach content by their characteristic stoma in microscopic analysis (Fig. 2) leading to the hypothesis of a fatal *Taxus* ingestion.

General toxicological screening produced a blood alcohol concentration of 0.38 g/kg and negative findings for narcotic drugs and pharmaceuticals at the time of death.

Case 4

A 23-year-old male student was found dead on a stairway of a congress center at lunch time. The decedent had felt sick and dizzy on the morning before his death, and he was last seen drinking tea 2 h before death. In addition, the landlord of the decedent's flat found a small plastic bag with yew leaves in a backpack and reported a conversation about *Taxus* intoxication of cattle weeks before the death of his tenant.

Forensic autopsy revealed signs of chronic asthma, known as preexisting disease, in the lungs as well as a

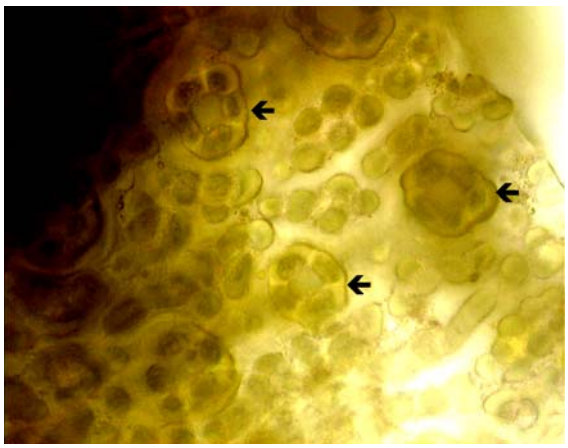

Fig. 2 Microscopic analysis of fragments of leaves of *Taxus baccata* identified in the stomach content of case 3 (*arrows* mark the characteristic stoma)

general blood congestion of organs (brain, liver, spleen, kidney) including cyanosis, but no pathological cause of acute death. No signs of violence could be found on the body. However, 200 ml of stomach content containing plant particles were obtained at autopsy.

Alcohol and drugs could not be detected by general toxicological screening.

Case 5

A 16-year-old girl, who was mentally disordered and depressive, was found dead in the bathroom of her parents flat. The parents reported ailment (dizziness, nausea, abdominal pain) of their daughter hours before death; however, they did not attach any importance to the symptoms. No preexisting diseases relevant for the cause of death were known.

There were no signs of violence on the body, but a widely dilated pupil was found at forensic autopsy. In addition, pulmonary edema and acute blood congestion of the liver, spleen, and kidney could be identified. Fragments of *Taxus* leaves were found in the fauces, esophagus, stomach, and duodenum as well as in the trachea at postmortem examination.

No alcohol, narcotic drugs, or pharmaceuticals were determined by general toxicological analysis; therefore, a *Taxus* poisoning was diagnosed.

Materials and methods

Reagents and chemicals

Acetonitrile, dichloromethane, 1-chlorobutane, HCl, phosphate buffer (Merck, Darmstadt, Germany), and bidistilled water (J.T. Baker, Deventer, The Netherlands) were of analytical reagent or gradient grade for HPLC. 3,5-Dimethoxyphenol (purum) was purchased from Fluka Chemie (Buchs, Switzerland).

Extraction procedure

A liquid–liquid extraction procedure was used for sample preparation. The samples were analyzed twice: pure and spiked with 0.50 μg/g 3,5-dimethoxyphenol for quantification by standard addition procedure.

Two milliliters of blood and urine, 2 g of intestines (brain, liver, kidney), and 2 g of stomach or duodenum content, respectively, were acidified with 400 μl of 0.1 N HCl. Samples were extracted three times with 3 ml of a mixture of 1-chlorobutane and dichloromethane (80:20, v/v). The combined organic eluates were evaporated to dryness in a stream of nitrogen at 50°C, redissolved in 100 μl (blood, urine, intestines) or 500 μl (stomach and duodenum content) of acetonitrile/phosphate buffer pH 2.3 (1:1), and injected for HPLC/photodiode array detection.

Chromatographic conditions

Analytical measurements were carried out by using an Agilent 1100 Series HPLC system (Agilent Technologies, Waldbronn, Germany) consisting of a binary pump, an autosampler, a column oven, and a photodiode array detector (PDA).

The chromatographic separation was achieved at 30°C on a ZORBAX Eclipse XDB-C8 HPLC column (150×4.6 mm, 5 μm i.d.) combined with an analytical guard column Eclipse XDB-C8 (12.5×4.6 mm, 5 μm i.d.) both from Agilent Technologies. A mixture of acetonitrile and 0.05 M phosphate buffer pH 2.3 (33:67, v/v) was used as mobile phase. The injection volume was 20 μl and the flow rate was maintained at 1.5 ml/min, isocratically. The effluent was monitored by photodiode array detection (190–400 nm).

Identification was done by comparison of the UV spectra of the extracts with the standard 3,5-dimethoxyphenol, and wavelengths of 205, 220, and 250 nm were chosen for quantification.

Results and discussion

Based on the analytical approach published by Musshoff et al. [15], an indirect evidence of *Taxus* intoxication has been applied by the identification of 3,5-dimethoxyphenol, easily formed from taxicatine by cleavage of the glycosidic bond and absorbed into the blood [15].

The results of 3,5-dimethoxyphenol detected by HPLC/PDA analysis (limit of quantification, 20 ng/ml) in the acidic extracts of the various samples are summarized in Table 1 for the five cases. However, the investigations of Cases 3, 4, and 5 were carried out retrospectively; therefore, not all body fluids and viscera were available for analysis. In addition, femoral blood and urine samples could not be obtained at autopsy in three cases.

HPLC/UV chromatograms of cardiac blood extracts of the decedent of Case 2 are presented in Fig. 3. Because the recovery of the extraction could not be calculated for each

Table 1 Results of the HPLC/PDA analysis of 3,5-dimethoxyphenol in blood, urine, tissue samples and stomach content calculated to a standard addition procedure

	Cardiac blood (ng/ml)	Femoral blood (ng/ml)	Urine (μg/ml)	Brain (ng/g)	Liver (ng/g)	Kidney (ng/g)	Stomach content (μg/g)
Case 1	47	na	8.7	<30	161	275	7.8[a]
Case 2	97	29	na	35	512	382	13.4
Case 3	528	na	na	na	918	418	118
Case 4	110	217	na	na	175[b]	na	1.4
Case 5	31	na	2.7	na	na	na	0.6

na Not available
[a]Duodenum content
[b]Bile

individual sample in the study, standard addition proved to be a well-suited procedure for identification and quantification of 3,5-dimethoxyphenol. The concentration in the stomach contents of the Case 2 and 3 was calculated by an external standard.

The preliminary diagnosis of *Taxus* intoxication made at autopsy could be confirmed by the positive results of 3,5-dimethoxyphenol in the cardiac blood samples in all cases (Table 1). It was not surprising that 3,5-dimethoxyphenol was also detected in the stomach contents as well as in femoral blood, urine, liver, and kidneys if samples had been available. However, the detection of 3,5-dimethoxyphenol in the stomach contents is only a qualitative indication of the presence of *Taxus* ingredients because its concentration in stomach content can extremely change after death. It is remarkable that 3,5-dimethoxyphenol could also be detected in the brain (Case 2).

On detailed consideration of the obtained concentrations, it is noticeable that 3,5-dimethoxyphenol was determined in the cardiac blood samples between 31 and 110 ng/ml in four cases compared to 528 ng/ml in Case 3 (Table 1). In addition, the exceptional position of Case 3 is underlined by the concentrations measured in the stomach content and liver (Table 1).

In looking for an explanation for these differences of the 3,5-dimethoxyphenol concentrations, the form of the *Taxus* ingestion seems to suggest that it plays a decisive role. In Case 1, 2, 4, and 5, whole leaves and parts of leaves were predominantly consumed. Due to a ceraceous layer on the yew needle surface, the release of the active *Taxus* ingredients into the intestine is relatively slow and the concentration of 3,5-dimethoxyphenol in cardiac blood therefore achieves only amounts less than approx. 100 ng/ml.

In contrast, the decedent of Case 3 had probably ingested a green mash of yew leaves (see "Case histories"). In this case, the plant cells were extracted mechanically before ingestion; therefore, the active *Taxus* compounds were available for a fast absorption into the cardiac blood resulting in a more than fivefold concentration of 3,5-dimethoxyphenol compared to the other cases (Table 1). In the context of the five fatalities presented in this study, the results published by Musshoff et al. [15] fit in well. A 3,5-dimethoxyphenol concentration of 320 ng/ml in cardiac blood [15] clearly underlines that extraction of the plant material before ingestion, in this case

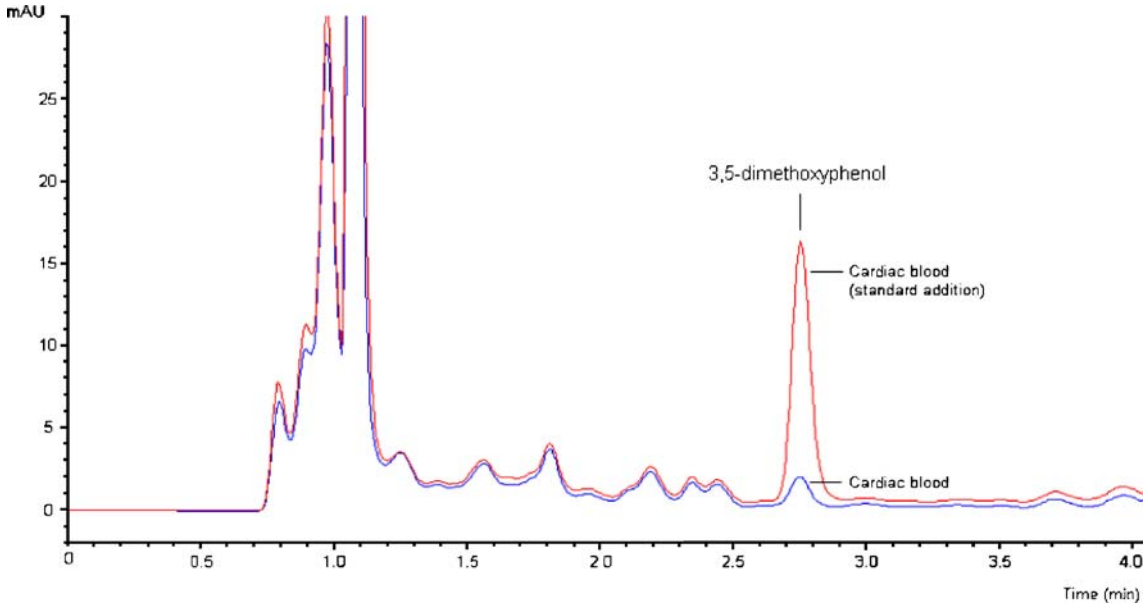

Fig. 3 Superimposed HPLC/UV chromatograms of cardiac blood extracts (pure and spiked with 500 ng/ml 3,5-dimethoxyphenol as standard addition) of the decedent of case 2 at 205 nm

yew leaves were brewed and pressed [15], results in a remarkably higher absorption rate.

A postmortem redistribution of 3,5-dimethoxyphenol to the cardiac blood cannot be completely excluded because unabsorbed drugs in the gastric content at the time of death can generally be redistributed to mediastinal vessels and surrounding organs [41]. In the presented study, a nonuniform result arises from the comparison of cardiac and femoral blood concentrations of 3,5-dimethoxyphenol (Cases 2 and 4, Table 1) underlining that more than the five cases of *Taxus* poisoning presented have to be evaluated to derive a conclusion regarding postmortem redistribution.

Despite the interindividual deviation and the partly unknown postmortem interval of the cases, we tried to interpret the results of the study to provide data for the estimation of fatal *Taxus* poisoning. According to the case histories and the analytical results presented, there are probably acute and subacute fatal cases of *Taxus* poisoning. Acute fatalities seem to be caused by the ingestion of preextracted plant material and result in comparatively high levels of active *Taxus* compounds in the cardiac blood. In contrast, due to the slow toxin release, the ingestion of yew leaves appears to be in a subacute process ranging over a few hours and affected, e.g., by nervousness, dizziness, nausea, vomiting, diffuse abdominal pain, confusion, and bradycardia [5]. The concentrations of the active *Taxus* substances are relatively low; however, when the toxins are absorbed from the intestine, death is relatively sudden, and survival after poisoning is uncommon [42]. Generally, the primary action of the *Taxus* toxins is the production of a block in the distal portion of the conduction system of the heart resulting in fatal arrhythmia [42].

Conclusions

Although seldom reported, the relevance of *Taxus* poisoning seems to be ongoing, especially supported by the Internet, where web sites with advice for suicide, in general, and the application of *Taxus*, in particular, are common.

By means of five cases of fatal *Taxus* poisoning, it could be demonstrated that 3,5-dimethoxyphenol, the aglycon of the *Taxus* ingredient taxicatine, is well-suited as a toxicological marker for the absorption of yew ingredients. However, there are no data available on the toxicity of 3,5-dimethoxyphenol in humans.

Toxicological information for *Taxus* poisoning based on 3,5-dimethoxyphenol as marker substance could be derived from the results of this study to provide data for the estimation of fatal *Taxus* poisoning in future. Accordingly, an acute *Taxus* intoxication caused by the ingestion of preextracted plant material seems to be characterized by a 3,5-dimethoxyphenol concentration in cardiac blood above 300 ng/ml. A concentration of 3,5-dimethoxyphenol less than approx. 100 ng/ml in cardiac blood can indicate a subacute *Taxus* poisoning, predominantly caused by the ingestion of yew leaves.

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