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Detection of pyrrolizidine alkaloids in German licensed herbal medicinal teas

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ABSTRACT

Background: Because of the hepatotoxic, mutagenic, and cancerogenic effects of pyrrolizidine alkaloids (PAs) the German Federal Institute for Risk Assessment (BfR) recommends not to exceed a daily PA intake of 0.007 μg/kg body weight (0.42 μg/60 kg adult). In a recent study conducted by the BfR, up to 5647 μg PA/kg dried herbal material were detected in tea products marketed as food.
Purpose: The present study aimed at elucidating whether medicinal teas licensed or registered as medicinal products contain PAs as well.
Study design: One hundred sixty-nine different commercially available medicinal teas, i.e. 19 nettle (*Urtica dioica* L.), 12 fennel (*Foeniculum vulgare* Mill.), 14 chamomile (*Matricaria recutita* L.), 11 melissa (*Melissa officinalis* L.) and 4 peppermint (*Mentha piperita* L.) teas as well as 109 tea mixtures were analyzed for the presence of 23 commercially available PAs.
Method: LC/MS was used for the determination of the PAs
Results: In general, the total PA contents ranging 0–5668 μg/kg. Thirty percent of the tested single-ingredient tea products and 56.9% of the tested medicinal tea mixtures were found to contain PA concentrations above the limit of quantification (LOQ) of 10 μg/kg. In 11 medicinal teas PA contents >300 μg/kg dry herb were determined thus exceeding the recommended limit for PA intake by BfR. In addition three products of the investigated tea mixtures revealed extremely high PA contents of 4227, 5137, and 5668 μg/kg. Generally, single-ingredient tea products contained much less or even no detectable amounts of PAs when compared to the tea mixtures. PAs in the range between 13 and 1080 μg/kg were also detected in five analyzed aqueous herbal infusions of the medicinal tea mixture products with the highest PA content. Two out of the five investigated herbal infusions exceeded the recommended BfR limit for PA intake.
Conclusion: This study demonstrates clearly that also medicinal teas licensed as medicinal products may partly contain high amounts of PAs exceeding current recommendations. For that reason manufacturers are advised to carry out more rigorous quality control tests devoted to the detection of PAs. This is very important to minimize PAs in medicinal teas accounting for possible additional exposure of the consumer to PAs from other food sources (e.g. honey).

Introduction

Pyrrolizidine alkaloids (PAs) constitute a group of heterocyclic compounds naturally occurring in a wide variety of plants, mostly *Asteraceae, Boraginaceae* and *Fabaceae* (Roeder 1995). They are esters of hydroxylated methylpyrrolizidines (referred to as necine bases) and aliphatic mono- or dicarbonic acids (referred to as necine acids) (BfR 2013). Up to date, more than 600 different PAs have been described (Cramer and Beuerle 2012).

Several PAs have been found to cause hepatotoxic, mutagenic and cancerogenic effects – accounting for the toxicological relevance of PAs to humans (Chen et al. 2010; Li et al. 2011; Stewart and Steenkamp 2001). It is widely accepted, however, that only PAs which meet certain structural requirements have toxic effects. These are all alkaloids derived from 1-hydroxymethyl-1,2-dehydropyrrolizidine with the primary hydroxymethyl group being esterified with one branched mono- or dicarboxylic acid containing at least 5 C-atoms (Fu et al. 2004). The toxic effects are generally enhanced if a second hydroxyl

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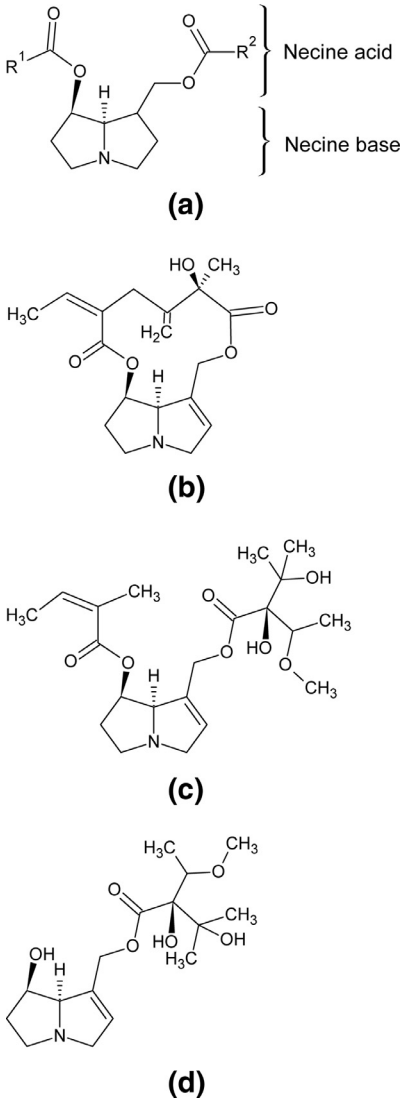

Fig. 1. Chemical structures of PAs showing (a) structural requirements for toxicity and exemplary structures of (b) seneciphylline as example of PA with cyclic diester, (c) lasiocarpine as example of a diesterified PA and (d) europine as example of a monoesterified PA.

group is present in position C-7 (Fig. 1a). Exemplary structures for diesterified and monoesterified PA are shown in Fig. 1b, c and d, respectively (Roeder 1995).

PAs are readily absorbed from the intestine and partly hydrolyzed by esterases. The resulting cleavage products of necine bases and necine acids are relatively non-toxic and believed to be renally excreted. The majority of PAs, however, is metabolized by liver monooxygenases resulting in highly reactive pyrrolic metabolites (Roeder 1995). These metabolites subsequently form adducts with proteins and nucleic acids. Due to the relatively high reactivity of these metabolites, damage is mainly confined to the liver but may also affect extrahepatic blood vessels and the lung, leading to pulmonary hypertension. Other organs less frequently affected by PA toxicity include the kidneys, the gastro-intestinal tract, the pancreas and bone marrow (Edgar et al. 2011).

In man, ingestion of a toxic dose of PAs corresponding to 0.015 mg/kg of body weight per day causes acute veno-occlusive disease (Roeder 1995; WHO 1988). For a 70 kg adult, that would correspond to 1 mg total PAs per day. As a consequence of venous occlusion and restricted blood flow, necrosis of the surrounding tissue, fibrosis, nodular regeneration, cirrhosis and subsequent liver failure may occur (Prakash et al. 1999). Symptoms include colicky abdominal pain, vomiting and diarrhea, ascites (within days), enlargement and induration of the liver (within weeks) and in some cases hematemesis (Roeder 1995). Mortality following PA ingestion occurs due to liver failure or complications arising from cirrhosis like rupture of esophageal varices (Wiedenfeld 2011; EMA/HMPC 2014). Cases of suspected PA poisoning have been reported from both developing and industrialized countries (Roeder 1995). An overview of human case reports has recently been published (Wiedenfeld 2011). Data from *in vitro* and animal studies provide further evidence, that PAs not only cause hepatotoxicity but also possess mutagenic and carcinogenic properties. To the best of our knowledge no data are available with regard to the long-term follow-up of humans exposed to PAs. The frequent occurrence of liver tumors in certain regions of Central and South Africa, however, is ascribed, at least in part, to the consumption of PA containing herbs (Roeder 1995).

Despite the pronounced toxicity of PAs, only little regulatory guidance concerning limits of intake of PAs for medicinal products, food including food supplements exists. Several EU-member states, however, have adopted national regulations on the consumption of PAs. In Germany, for example, a graduated plan set up in 1992 limits the maximum daily intake of PAs for medicines for internal use to 1 μg for a maximum of 6 weeks/year and 0.1 μg for medicines with no limited duration of treatment. Evaluating the non-cancer effects of PAs, the British "Committee on Toxicity of Chemicals in Food, Consumer Products and Environment" (COT) came to the conclusion that doses of PAs below 0.007 μg/kg body weight/day, would unlikely be of concern. Accordingly the German Federal Institute for Risk Assessment (BfR) identified that for 1,2-unsaturated PAs a daily intake of 0.007 μg/kg (0.42 μg/60 kg adult) should not be exceeded. Also the EMA/HMPC permits a daily intake of 0.007 μg PA/kg body weight in its finalized public statement on the use of herbal medicinal products containing toxic, unsaturated PAs released in November 2014 (EMA/HMPC 2014). With regard to the mutagenic effects of PAs, the Dutch National Institute for Public Health and the Environment stated in 2005, that a "Virtually Safe Dose" (VSD) for PAs would be 0.00043 μg/kg body weight/day (BfR 2013).

Recently, the BfR conducted a study to assess the content of PAs in 184 tea products marketed as food as well as in 37 medicinal teas from pharmacies (BfR 2013). Even though none of the plants, which had been used for the tea-products endogenously produces PAs, the BfR found up to 3430 μg PAs/kg dried herbal material. No distinguishment was made by the BfR between medicinal teas and tea products marketed as food in reporting the results. The BfR concluded that consumers drinking tea regularly and with a tendency to stick to a certain (supposedly contaminated) brand product might be at increased risk. As a worst case scenario, the BfR estimated that adults might consume as much as 0.144 μg PA/kg body weight per day, hence greatly exceeding the aforementioned limits. In another study of the BfR on 274 tea samples total PA concentrations up to 5647 μg/kg were detected (Bodi et al. 2014).

As all previous studies focused on herbal tea products sold as food two independent, not-for-profit organizations, the Drug Commission of German Pharmacists (AMK) and the Central Laboratory of German Pharmacists (ZL) carried out the present study on herbal tea products licensed or registered as medicinal products to elucidate whether licensed medicinal teas contain PAs as well. In contrast to herbal teas marketed as food for which good manufacturing practice isn't always guaranteed registered and licensed medicinal herbal teas are subject to intense quality control measures. For that reason they are generally considered to be safe. Similar to the study conducted by the BfR, none of the investigated plants is known to produce PAs on its own.