Detection of pyrrolizidine alkaloids in German licensed herbal medicinal teas

M. Schulza, J. Meinsb, S. Diemerta, P. Zagermann-Muncke a, R. Goebela, D. Schrenkc, M. Schubert-Zsilaveczb,d, M. Abdel-Tawabb,∗

a Drug Commission of German Pharmacists (AMK), Jaegerstrasse 49/50, 10117 Berlin, Germany
b Central Laboratory of German Pharmacists, Carl-Mannich-Strasse 20, 65760 Eschborn, Germany
c Department of Food Chemistry and Toxicology, Technische Universität Kaiserslautern, Erwin-Schrödinger-Strasse, 67663 Kaiserslautern, Germany
d Department of Pharmaceutical Chemistry, Goethe-University Frankfurt, Max-von-Laue Strasse 9, 60438 Frankfurt am Main, Germany

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A B S T R A C T

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).

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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 neonic bases) and aliphatic mono- or dicarboxic acids (referred to as neonic 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
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 mono-oxygenases 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 distinction 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 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.
Material and methods

Chemicals and solvents

All chemical reagents were purchased from Merck, Roth or Sigma–Aldrich and were of analytical grade. All solvents used were of HPLC–MS grade purity. The standard substances for the tested PAs echimidine, erucifoline, erucifoline-N-oxide, erupoine, erupine-N-oxide, heliotrine, heliotrine-N-oxide, intermedine, jacobine, jacobine-N-oxide, lasiocarpine, lasiocarpine-N-oxide, lycopsamine, monocrotaline, monocrotaline-N-oxide, retorsine, retorsrine-N-oxide, seneconine, seneconine-N-oxide, seneciphylline, seneciphylline-N-oxide, senkirkine, and trichodesmine were acquired from PhytoLab, Germany. Propranolol hydrochloride (100.5% purity), used as control standard was purchased from Fagron, Germany. Blank plant material was donated by BfR, Germany, which is gratefully acknowledged.

Samples

For this study, 169 different commercially available medicinal teas licensed or registered in Germany including 60 single-ingredient herbal tea products as well as 109 tea mixture products were purchased from the wholesaler Alliance Healthcare Deutschland AG, Frankfurt am Main, Germany. The investigated assortment comprised 19 single-ingredient medicinal tea products containing dried nettle leaves (Urtica dioica subsp. dioica), 12 single-ingredient products containing dried fennel fruits (Foeniculum vulgare subsp. vulgare (Mill.) var. vulgare (Mueller) Thellung), 14 single-ingredient products containing dried chamomile flowers (Matricaria recutita subsp. recutita (L.) Rauschert), 11 single-ingredient products containing dried peppermint leaves (Mentha × piperita var. officinalis (Sole.) Sore.) in addition to the investigated tea mixtures. All plant names have been checked with the plant list on http://www.theplantlist.org, produced by the Royal Botanic Gardens, the Missouri Botanical Garden and other collaborators worldwide. Depending on the indication, tea mixtures contained one of the above mentioned herbs selected for the single-ingredient products in addition to for example valerian, hop cones, lavender flowers in case of sleeping teas or in addition to for example caraway, anise, coriander in case of gastrointestinal teas.

The choice of samples was meant to reflect the huge variety of medicinal teas containing the aforementioned herbal ingredients. Further, it was intended to incorporate at least one sample from each manufacturer of medicinal teas. Being licensed medicinal products the manufacturer is responsible for ensuring the traceability and reproducible quality of every processed herbal material. All tested tea products were assigned voucher numbers and representative voucher specimen have been deposited in the Central Laboratory of German Pharmacists, Eschborn, Germany.

Sample preparation

The analysis of PAs was performed according to the analytical method applied by the BfR in its study on PA content in tea products marketed as food with minor modifications. In brief, it is based on acidic extraction, clean-up and enrichment of PAs by means of solid phase extraction (SPE) followed by liquid chromatographic separation and mass spectrometric detection (LC–MS/MS (BFR 2013)). Using diluted aqueous acid as extraction solvent allows simultaneous profiling of both the free bases and the N-oxides from the herbal product in contrast to other extraction procedures like Soxhlet extraction described for the determination of PAs (Crews et al. 2010). Moreover the combination of SPE with LC–MS/MS represents a powerful and sensitive procedure for generating a complete PA profile of the tested products compared to other methods like HPLC–UV (Ndjoko et al. 1999), LC–MS (Lin et al. 1998), and ELISA (Lee et al. 2001).

Based on that background, 2.5 g of each herbal product were ground and sieved through a sieve of 500 µm mesh, then through a sieve of 300 µm mesh. Afterward 2 × 1 g of the ground herb were extracted with 10 ml 0.05 M sulfuric acid for 15 min on a vertical shaker at 200 rpm, followed by sonication for 15 min in an ultrasonic bath. Afterward the samples were centrifuged at 4618 g at -4 °C. The supernatant was removed and the residue was extracted again with 10 ml 0.05 M sulfuric acid as mentioned before. Both supernatants were combined and adjusted to pH 6–7 with 6.3% ammoniac solution.

After filtration through a Rotilabo®-Fibre glass syringe filter (Roth, Germany), 10 ml of the neutralised combined extract was subjected to SPE using DSC-C18 SPE cartridges (500 mg, Supelco, Germany) preconditioned with 5 ml methanol followed by 5 ml water. Then the cartridges were washed twice with 5 ml water, and dried under vacuum for 5–10 min. Elution was performed with 2 × 5 ml methanol. The eluate fraction was dried under a gentle stream of nitrogen at 50 °C and redissolved by shaking with 1 ml methanol/water (5/95, v/v). Finally, 100 µl of control standard solution (1 mg/ml propranolol hydrochloride in methanol/water; 5/95, v/v) were added and the samples centrifuged for 15 min at 4000 rpm (3345 g) and 15 °C.

The standard stock solutions for calibration were prepared by dissolving 15 mg of each PA reference standard in 25 ml acetonitrile yielding a concentration of 0.6 mg/ml. The standard working solution (PA-Mix) representing a mix of all PA reference standards at a concentration of 1 µg/ml each was prepared by pipetting the respective volume of each PA stock solution into a volumetric flask and filling up to mark with acetonitrile.

In order to compensate for possible matrix effects, calibration solutions were prepared by spiking reconstituted blank herbal extracts (free of PAs) with the respective volume of PA-Mix covering a concentration range of 10–300 µg/kg and 100 µl of the internal standard solution. For that purpose blank plant material mix composed of equal amounts of peppermint, chamomile, caraway and fenel was processed exactly as described before for the medicinal teas.

LC–MS/MS analysis

Liquid chromatography was performed on an Agilent 1200 series HPLC system equipped with a gradient pump with vacuum degasser, an autosampler and a column oven. A Thermo Hypersil Gold C18 column (150 × 2.1 mm; 1.9 µm; Thermo scientific, Germany), and an upstream VICI-InLine Filter, ID 0.75 µm with frit (VICI, Switzerland) were used for chromatography. Mobile phase A was prepared by dissolving 315 mg ammonium formate and 1 ml formic acid (98–100%) in 999 ml water. Mobile phase B was prepared by dissolving 315 mg ammonium formate, 5 ml water, 1 ml formic acid (98–100%) in 994 ml methanol. After injection of 10 µl, separation was achieved using a gradient program starting with 95% mobile phase A and 5% mobile phase B for 0.5 min, changing to 65% mobile phase A within 11.6 min. This gradient was held constant for 4.4 min and was then changed to 20% mobile phase A within 0.5 min, which was kept constant again for 3 min. Afterward the gradient was changed to 0% mobile phase A within 0.2 min and was kept 9.8 min at this level. Finally, mobile phase A was increased to 95% within 0.1 min and kept constant for 9.9 min till the end of the run. The total run time was 40 min at a flow rate of 0.3 ml/min. The column oven was set to 40 °C and the autosampler was cooled to 20 °C.

MS analysis was performed in the positive multiple reaction monitoring (MRM) mode on an Agilent Triple Quadrupole LC/MS 6410 series (Agilent Technologies, Germany) equipped with an Electro Spray (ESI) Ionization source. Dwell time was chosen to be 20 ms.
In total, 23 PAs which are commercially available as reference substances were determined in this study. Each PA was identified by comparing the retention time with that of the standard substance and the detection of two substance-specific fragment ions (see Table 1). Due to the large number of samples and analytes all PA concentrations determined in this study are unique values generated by a single analysis of the respective sample. Based on the validation results mentioned below a maximum variability of ±27.3% may be assumed. The Mass Hunter software (Agilent, Germany) was used for data acquisition and processing.

A representative chromatogram is shown in Fig. 2.

Quantification was carried out using the external standard method by comparing the peak areas obtained for the individual PA in data acquisition and processing.

### Table 1

<table>
<thead>
<tr>
<th>PA</th>
<th>Precursor ion</th>
<th>Product ion</th>
<th>Retention time [min]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocrotaline</td>
<td>326.2</td>
<td>121.2</td>
<td>5.11</td>
</tr>
<tr>
<td>Erucifolin</td>
<td>350.3</td>
<td>120.2</td>
<td>6.54</td>
</tr>
<tr>
<td>Monocrotaline-N-oxide</td>
<td>342.2</td>
<td>137.2</td>
<td>7.19</td>
</tr>
<tr>
<td>Jacobin</td>
<td>352.3</td>
<td>120.2</td>
<td>7.81</td>
</tr>
<tr>
<td>Erucifolin-N-oxide</td>
<td>366.2</td>
<td>94.1</td>
<td>11.9</td>
</tr>
<tr>
<td>Intermedine</td>
<td>300.3</td>
<td>139.1</td>
<td>7.99</td>
</tr>
<tr>
<td>Europine</td>
<td>330.3</td>
<td>138.2</td>
<td>8.02</td>
</tr>
<tr>
<td>Lycopsamine</td>
<td>300.3</td>
<td>157.1</td>
<td>8.32</td>
</tr>
<tr>
<td>Jacobin-N-oxide</td>
<td>368.3</td>
<td>296.3</td>
<td>8.46</td>
</tr>
<tr>
<td>Europine-N-oxide</td>
<td>346.3</td>
<td>172.2</td>
<td>8.82</td>
</tr>
<tr>
<td>Trichodesmin</td>
<td>354.3</td>
<td>121.2</td>
<td>10.63</td>
</tr>
<tr>
<td>Retrorsine</td>
<td>352.3</td>
<td>120.2</td>
<td>10.78</td>
</tr>
<tr>
<td>Retrorsine-N-oxide</td>
<td>368.3</td>
<td>136.2</td>
<td>11.07</td>
</tr>
<tr>
<td>Seneciphylline</td>
<td>334.3</td>
<td>120.1</td>
<td>11.43</td>
</tr>
<tr>
<td>Heliotrine</td>
<td>314.3</td>
<td>138.2</td>
<td>11.73</td>
</tr>
<tr>
<td>Seneciphylline-N-oxide</td>
<td>350.3</td>
<td>136.1</td>
<td>12.12</td>
</tr>
<tr>
<td>Heliotrine-N-oxide</td>
<td>330.3</td>
<td>172.2</td>
<td>12.70</td>
</tr>
<tr>
<td>Senecionine</td>
<td>336.3</td>
<td>138.2</td>
<td>13.73</td>
</tr>
<tr>
<td>Senecionine-N-oxide</td>
<td>352.3</td>
<td>118.1</td>
<td>14.25</td>
</tr>
<tr>
<td>Echimidine</td>
<td>398.4</td>
<td>120.2</td>
<td>16.68</td>
</tr>
<tr>
<td>Senkirkine</td>
<td>366.2</td>
<td>168.2</td>
<td>17.30</td>
</tr>
<tr>
<td>Lasiocarpine</td>
<td>412.3</td>
<td>120.2</td>
<td>19.42</td>
</tr>
<tr>
<td>Lasiocarpine-N-oxide</td>
<td>428.3</td>
<td>255.2</td>
<td>19.61</td>
</tr>
<tr>
<td>Propranolol</td>
<td>260.1</td>
<td>183.0</td>
<td>19.64</td>
</tr>
</tbody>
</table>

The control standard propranolol hydrochloride was added in order to control the chromatographic system.

The analytical method applied in this study had been extensively validated by BfR. The validity of the analytical method had been further established in the frame of an international collaborative trial carried out by BfR based on the ISO/IUPAC/AOAC protocol involving 24 different laboratories testing six tea samples purchased from retail markets in Berlin containing PAs and one spiked recovery sample. The relative repeatability standard deviation (RSDr) indicating the repeatability of the first and second measurement of a sample in one laboratory ranged between 7.7% (retrorsine-N-oxide) and 9.8% (seneciphylline) and the relative reproducibility standard deviation (RSDR) comparing analytes and test samples tested by different laboratories ranged between 18.4% (lasiocarpine-N-oxide) and 27.3% (seneciphylline). In addition the Horwitz Ratio (HorRat value) describing the ratio between the reproducibility standard deviation and the predicted reproducibility standard deviation (PRSD), which is calculated from the Horwitz equation, was determined.

\[
\text{HorRat} = \frac{\text{RSDR}}{\text{PRSD}}
\]

whereas PRSD = \(g(1-\log \text{MR}/2)\) and MR is the normalized mean value.

This performance parameter is used as measure to evaluate the acceptability of analytical methods with respect to among-laboratory precision (reproducibility) (Horwitz 1995). Values ≤0.5 point out, that method reproducibility may be in question due to lack of study independence, values >2.0 are a sign for a problematic method reproducibility, and values <1.5 reflect a method reproducibility as normally would be expected. The HorRat values determined in the frame of the collaborative study ranged between 0.8 for lasiocarpine-N-oxide and 1.2 for seneciphylline indicating sufficient precision and a good inter-laboratory comparability of the applied method for the detection of PAs. The recovery rate was determined by spiking a PA-free herbal mixture with a PA-mix of known concentration. The obtained recoveries ranged between 76% for lasiocarpine and 125% for senkirkine. According to the AOAC-International Guidelines for Standard Method Performance Requirements (2012) they are considered to be sufficient for reliable analysis.

Based on the excellent HorRat values and the above mentioned results for recovery and precision the method revealed to be applicable for the determination of PAs in herbal products.

### Preparation of herbal infusions

Following the manufacturer’s instruction one teabag of the five most contaminated medicinal tea products was extracted by adding 150 ml of boiling water and leaving to brew for 15 min. Then 10 ml of the tea preparation was subjected to SPE as described under section “Sample preparation” and quantified by LC–MS/MS as described under section “LC–MS/MS analysis”.

### Results and discussion

An overview of the PA content determined in nettle, fennel, chamomile, melissa, peppermint and in the tea mixtures is presented
in Fig. 3a, b, c, d, e and f, respectively. A great variety of the PA content within the same species can be noticed. This is mainly attributed to the fact, that the samples originate from different manufacturers and consequently have experienced different cultivation, harvesting, storage and transport conditions.

Both, the free PA bases and their corresponding N-oxides were detectable with the N-oxides present in larger amounts. In general, the total PA contents ranged from 0 to 5668 μg/kg, being <300 μg/kg in most products. Nevertheless, 30% of the tested single-ingredient tea products and 56.9% of the tested medicinal tea mixtures were found to contain PA concentrations above the LOQ of 10 μg/kg. In eleven products PA contents >300 μg/kg were determined and three additional products 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. This is an indication that the PAs detected in the tea mixtures may not necessarily result primarily from the five herbs nettle, fennel, melissa, chamomile or peppermint, and that they rather result from the other herbal components of the mixture that have not been subject to PA analysis in this study but were often present in even larger amounts.

As can be seen in Fig. 4, the PAs identified in the samples of the present study included also PAs with cyclic diesters, which are thought to be most toxic and carcinogenic (Roeder 1995). Hence, seneciphylline, retrorsine, seneconine, and/or their corresponding N-oxides represent the cyclic diesters most often detected in the present study. The highest total amount of these cyclic diesters was detected in a tea mixture and was found to be 3891 μg/kg. The other cyclic diesters like monocrotaline, monocrotaline-N-oxide and trichodesmine tested in this study could not be detected in any investigated products with the exception of two fennel products containing 16 μg/kg monocrotaline-N-oxide and 16 μg/kg trichodesmine, respectively. In addition lasiocarpine-N-oxide, euripinin-N-oxide and heliotrine-N-oxide were most frequently detected.

In general the tea mixtures revealed the highest percentage of products containing PAs. With regard to the single-ingredient tea products fennel and chamomile revealed the lowest share and melissa, and peppermint the largest share of samples with PA findings. It becomes also apparent that melissa teas tend to have higher PA contents on average compared to the other single-ingredient tea products (Table 2).

Our study coincides with the BfR study “Pyrrolizidine alkaloids in herbal teas and teas” carried out on 221 nettle, fennel, chamomile and peppermint teas which is part of a research project on the “Determination of pyrrolizidine alkaloids in food and feed” (BfR 2013). As it is the case in the present study, very high PA concentrations were measured by the BfR only in a few individual herbal tea samples. Also in the BfR study, melissa teas were affected most by PA contamination. In contrast, however, to the present study 91.7% of the 12 investigated nettle teas were contaminated with PAs being thus comparable to peppermint teas with 86.2% of 29 products and chamomile with 87.1% of 31 products being affected. Notably, 56.7% of the 30 investigated fennel teas in the BfR study were also contaminated with PAs.

For the assessment of possible health risks to consumers it is generally assumed that PAs migrate completely from the dry material into the finished beverage (BfR 2013). However, with the exception...
of one study carried out by Oberlies et al. (2004) on the content of symphitine and echimidine in teas prepared from comfrey leaves, no data have been published until a short time ago, which support this assumption. Only recently Mathon et al. (2014) demonstrated on the example of nine PAs that PA concentrations in tea beverages prepared with boiling water were similar to the PA concentrations determined in the herbal material following acidic extraction indicating complete PA migration into the herbal infusion. In this study the sum of the nine targeted PA concentrations ranged between 0.021 and 0.954 μg per cup of tea in 24 out of 70 tested teas, herbal teas and instant teas purchased in Swiss supermarkets and teashops. Nevertheless the knowledge of PA migration from the herbal drug into the aqueous herbal infusion is still very limited. To address this limitation the present study determined PAs in tea preparations. Herbal infusions were prepared from five tea products, which had been found to contain the highest PA content (1127–5137 μg/kg), according to the manufacturer’s instruction. The total PA content determined in the aqueous herbal infusions ranged between 13 and 1080 μg/kg. For
better comparability, the PA concentrations in the aqueous herbal infusions have been converted from μg/L to μg/kg.

In this context, it has to be pointed out, that the procedure for sample preparation and the LC–MS/MS method have not been validated for their applicability to aqueous herbal infusions. As, however, the dilute aqueous acid extract of the tea products is being neutralized to yield the same pH as the aqueous herbal infusion, no great differences in the matrix composition following the preparation of aqueous herbal infusions and dilute aqueous acid extraction of the dry tea products should be expected, as demonstrated before by Mathon et al. (2014). For that reason the results in the aqueous herbal infusion provide a good preliminary estimate on PA migration from the dry herbal tea product into the aqueous infusion. Based on these results it may be assumed that the PAs contained in medicinal tea products in fact migrate into the aqueous herbal infusion being thus ingested after consumption. As in the case of the medicinal tea products, mostly the more hydrophilic N-oxides of the PAs were detected in the aqueous herbal infusions. In general, the total PA content in the aqueous herbal infusion was found to be relatively smaller than that of the respective dry herbal product. Moreover the N-oxides of the highly toxic and carcinogenic PAs with cyclic diesters (seneciphylline-N-oxide, senecionine-N-oxide, retrorsine-N-oxide) have been also detected in the aqueous infusions. This finding is of particular relevance, since orally ingested N-oxides of PAs have similar hepatotoxic properties as their parent alkaloids, following their reduction to the corresponding free base in the human intestine (Wiedenfeld 2011; Mattocks 1986).

The idea that herbal drugs are generally safe and free from side effects has been disproved for a long time as reflected by the withdrawal of many herbal drugs in Germany from the market including PA-containing plants like comfrey (Keller 1996; Stickel and Seitz 2000). In fact, several cases of veno-occlusive disease (VOD) have been associated with an ingestion of PA-containing plants as herbal teas (Bunchornvatavakul 2012; Bensaude et al. 1998; Ridker and McDermott 1989). Moreover, PAs are also a concern for traditional Chinese herbal medicines (Larry and Faure 2011; Roeder 2000) and traditional Indian medicine including Ayurveda (Roeder and Wiedenfeld 2013). Investigation of targeted PAs in traditional Chinese Medicines and selected herbal teas in Ireland by Griffin et al. (2014) revealed PA contents ranging between 13 μg/kg and 3668 μg/kg in 78% of the investigated Chinese medicines and 10–1733 μg/kg in 50% of tested herbal teas. Whereas the presence of PAs in herbal teas of PA-containing plants developed over time to a matter of public health concern (Alali et al. 2008; Farsam et al. 2000), research on PA contamination was mainly focused in the past on honey (Edgar et al. 2011) and herbal teas marketed as food (BfR 2013). The present study focused on determining the PA content in licensed medicinal teas which meet the requirementsofpharmacopoeias and are frequently believed to be of higher quality than herbal teas marketed as food. The large number of PA-positive medicinal teas identified in the present study including some samples with extremely high PA contents is striking, as all of the investigated medicinal teas are not known to inherently contain PAs. Hence, the high PA content in medicinal teas may be rather attributed to contamination of the medicinal herbs with one.

Table 2
Overview of the maximum, and mean amount of total PAs determined in medicinal herbal teas and the relative number of affected samples.

<table>
<thead>
<tr>
<th>Total number of samples</th>
<th>Number of samples with PA</th>
<th>Relative number of samples with PA [%]</th>
<th>Maximum amount [μg/kg]</th>
<th>Mean [μg/kg]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nettle</td>
<td>19</td>
<td>6</td>
<td>31.6</td>
<td>66.3</td>
</tr>
<tr>
<td>Fennel</td>
<td>12</td>
<td>3</td>
<td>25.0</td>
<td>26.6</td>
</tr>
<tr>
<td>Peppermint</td>
<td>4</td>
<td>2</td>
<td>50.0</td>
<td>20.6</td>
</tr>
<tr>
<td>Chamomile</td>
<td>14</td>
<td>2</td>
<td>14.3</td>
<td>53.0</td>
</tr>
<tr>
<td>Melissa</td>
<td>11</td>
<td>5</td>
<td>45.5</td>
<td>1595.6</td>
</tr>
<tr>
<td>Tea mixture</td>
<td>109</td>
<td>62</td>
<td>56.9</td>
<td>5667.9</td>
</tr>
</tbody>
</table>

Fig. 4. Overview on the PAs detected in medicinal tea products.
or several PA-containing plants during cultivation, harvesting, storage and/or transport (Bodi et al. 2014; Mathon et al. 2014). This is not surprising as many PA-containing plants like Senecio jacobaea (tansy ragwort) are foreign invasive weeds that invade pastures and fields or expand on field edges and thus may accidentally contaminate medicinal herbs. This might also have an impact on the milling and homogenization process during the sample preparation of the medicinal tea products for analysis. Hence depending on the physical properties of the contaminating plant or part of the plants in the tea samples, the distribution of PAs may vary, which might be associated with a great variation in the PA content within different samples of the same batch. In future studies it may be thus useful to optimize the homogenization process and sampling step for tea samples in order to reduce the variability in the analytical results. For example it might be useful to increase the used amount of sample and the extraction volume, keeping the same sample/extraction volume ratio. Another possibility might be the reduction of the particle size, leading to a more homogenous distribution of PAs in the tea sample. Yet though this assumption seems reasonable it has to be verified in separate studies. Nevertheless manufacturers are advised to carry out more rigorous quality control tests for the presence of PAs in their products in order not to expose consumers to unneeded risks.

Taking into consideration that in case of a total PA content of 300 μg/kg dry herb a tea bag weighing in average 2 g contains 0.6 μg PAs, the consumption of one cup of tea by an adult is sufficient to exceed the recommended limit for PA intake of 0.007 μg/kg body weight/day (corresponding to 0.42 μg/kg/60 kg). The limit of exposure of 0.007 μg/kg/day recommended by BfR is derived from the same point of departure applying a margin of exposure (MoE) of 10 000 as recommended by EFSAn for the safety assessment of impurities which are both genotoxic and carcinogenic (EFSA 2012). In the present study, 16 medicinal teas revealed PA contents >300 μg/kg dry herb. Moreover, two out of five tea infusions prepared from the mostly contaminated medicinal teas were found to contain >300 μg/kg PAs thus exceeding the recommended maximal intake. These measured concentrations of PAs correspond to a MoE <117, calculated by dividing the BMDL10 value for lasiocarpine (70 μg/kg bw per day) by the exposure value of one cup of herbal infusion. Beating far below 10,000, the determined MoE value gives cause for great concern. Especially children are at increased risk because their PA intake relative to their body weight is higher than that of adults when consuming such high quantities of PAs. Keeping in mind that the PA content may vary widely even within the same tea brand, it may be assumed that even higher PA amounts may be ingested.

Nevertheless it should be pointed out in this context that the toxicity of PAs has been mainly demonstrated for purified PAs and their metabolites. Taking into consideration the complexity of herbal extracts, it cannot be excluded in principle that synergistic and antagonistic actions of the various ingredients in herbal extracts may fortify or weaken the toxicity of PAs when taken in form of the whole extract. However Chou and Fu (2006) reported that toxic DNA adducts were neither formed after the administration of the isolated PA riddelline-glucoside nor detected pyrrolizidine alkaloids in traditional Chinese medicines and selected herbal plant extracts containing carcinogenic pyrrolizidine alkaloids. Toxicol. Ind. Health 22, 321–327. Moreover it should be pointed out in this context that the toxicity of PAs depends to some extent on the presence of toxic, unsaturated pyrrolizidine alkaloids (PAs). EMA/HMPC/893108/2011. http://www.ema.europa.eu/docs/en_GB/document_library/Public_statement/2014/12/WC500179559.pdf (last accessed 25.02.15).


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EFSA. 2012. Statement on the applicability of the Margin of Exposure approach for the safety assessment of impurities which are both genotoxic and carcinogenic in substances added to food/feed. EFSA J. 10, 2578.


Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Conclusion

Several previous studies highlighted the problem of PA contamination in honey, pollen, traditional Chinese as well as Indian medicine and herbal tea products marketed as food in drugstores and supermarkets. The present study focused on herbal tea products licensed as medicinal teas and revealed that also high quality medicinal teas generally believed to be safe may partly contain high amounts of PAs exceeding current safety recommendations, resembling thus tea samples marketed as food. This is an important insight for the consumer, who might switch to those high quality tea products to minimize the risk of PA exposure arising from herbal tea products marketed as food. Based on that background additional more rigorous quality control tests should be implemented to detect and minimize possible PAs in medicinal teas, so that the exposure of the consumer to PAs becomes as low as practically achievable. This is also necessary since possible additional exposure to PAs from other food sources (e.g. honey) may occur. For that reason the permitted daily intake of 0.007 μg/kg body weight recommended by various scientific organizations includes herbal tea products and food as well.

M. Schulz et al./Phytomedicine 22 (2015) 648–656


