Toxicity of Pyrrolizidine Alkaloids – a Serious Health Problem

Helmut Wiedenfeld

Pharmaceutical Institute, University of Bonn

ÖZET
Pyrrolizidine alkaloidlerinin toksisitesi - ciddi bir sağlık problemi


Anahtar sözcükler: Pyrrolizidine alkaloidler, metabolik toksikasyon, detoksifikasyon, insanlarda zehirlenme

ABSİTRACT
Toxicity of pyrrolizidine alkaloids - a serious health problem

Pyrrolizidine alkaloids (PAs) are toxic for human and livestock. The PAs undergo a metabolic toxication process in the liver which is the first target organ for PA poisoning. World-wide many incidents of PA intoxications in humans have been reported. This intoxication is not only related to the amount and duration of the exposure but also to age and gender. Besides the metabolic toxication, detoxication processes are also important. The paper discusses the toxication and detoxication processes and gives an overview about PA poisoning cases in humans.

Key words: Pyrrolizidine alkaloids, metabolic toxication; detoxication; poisoning in humans

GİRİŞ

Pyrrolizidine alkaloids (PAs) have been described to be hazardous to humans and livestock for a long time already. In 1903/1904 Gilruth established that tansy ragwort (Senecio jacobaea, L.) produced chronic liver disease in livestock (1,2). In 1956 Bull and Dick could prove this and they showed furthermore that also another PA-containing genus (Crotalaria) led to similar diseases (3). But also human intoxications by PAs were reported: In 1920 a widespread liver disease in South Africa was shown to be caused by the consumption of bread contaminated with seeds from Senecio species (4,5). In the late 1940s it was established that a similar episode which became manifest in an endemic liver disease happened in the former USSR and was caused by the consumption of PA-contaminated bread. The plant source for this contamination was Heliotropium lasiocarpum (6,7). The severest cases of intoxication took place in Afghanistan and Tadjikistan: In 1975/76, 8000 people were affected after having consumed cereals which were contaminated with Heliotropium popovii subsp. gillianum; 3000 of them were seriously poisoned and died (8,9) and, similarly, 4000 people were hospitalized in 1992 on account of grain intoxication due to Heliotropium lasiocarpum (10-12).

In 1989 the International Program on Chemical Safety (IPCS), a joint agency of WHO, FAO and ILO, summarized the then current knowledge with the statement that “consumption of contaminated grain or the use of PA-containing plants as herbal medicine, beverages, or food by man, or grazing on contaminated pastures by animals, may cause acute or chronic disease” (13). In the last three decades, different incidents of human intoxication by PA-containing medicinal plants have been reported (14,15) and in these cases, children in particular were affected due to their higher susceptibility to PA intoxication (13). Intensive investigations were done on herbal teas or other extracts from medicinal plants which were suspected to contain toxic PAs, especially after it became obvious that liver diseases in Jamaica and the West Indies were caused by so-called “bush-teas” which contained PAs (16,17).
While PA poisoning used to be mainly a problem in developing countries because there the use of traditional medicine is normal practice, within the last 25 years, especially in industrialized countries the use of herbal medicine has become more and more common due to an increasing interest of people in alternative medicine, hand in hand with a greater influence of the “green wave”.

In Western countries like the EU, UK and USA, many alternative medical practitioners claim that traditional medicine show only benefits without undesired side effects. This attitude has led to increasing fatal intoxications being reported caused by the use of herbal products that contain toxic PAs.  

This has led to regulations in many countries: In the USA, FDA banned all PA-containing comfrey/symphytum preparations from the market, based on the scientific data available, the FDA saw no possibility to specify a level of PAs where a possible human risk could be excluded (18). In the EU, the EFSA determined that the uptake of toxic PAs produces veno-occlusive disease (VOD) and that the carcinogenic potential of PAs is demonstrated in rodents but not yet proven in humans. For food and other sources of a PA contamination and especially for milk, with respect to its use in high amounts in the food of neonates and children, more scientific data are required (19). In Germany, the German Federal Department of Health established regulations in 1992: For all herbs or preparations from them, it has to be ensured that the daily uptake of PAs is less than 1 µg and the use is restricted to 6 weeks. In case of a longer use the daily limit is reduced to 0.1 µg. During breast-feeding and pregnancy the use of these preparations is not allowed; these regulations are also applied to homeopathic descriptions up to D6 (20). Austria has banned all PA-containing products from the market (21). In The Netherlands, it has been decided that all herbal preparations and extracts of PA-containing plants are limited to 1 µg of PAs/kg or 1 µg of PAs/l in the final product and this limit also applies to food (22). 

Apart from that, other possibilities for a human uptake of PAs have been found: milk can contain toxic PAs in case the milk-producing animals have access to PA-containing plants (23-30). Human milk has also caused liver diseases in neonates and infants (31). Honey was shown to contain PAs by pollen transferred by bees into the honey (14,15,32-37). Eggs from poultry with access to PAs in PA-contaminated grain were also shown to be a possible source of PA exposure to humans (38). It was shown in Germany that salads can sometimes be contaminated with PA-containing plants (39). It was found that, especially in supermarkets, ready-packed rocket salads and also salad mixtures were contaminated by Senecio vulgaris.

Since most recently, tansy ragwort (Senecio jacobaeae) has been under discussion: Its extensive expansion into pastures and meadows has led to a great number of intoxicated in livestock (mainly horses), especially in Germany [http://www.jacobskreuzkraut.de; http://www.izn.nieder-sachsen.de/servlets/download?C=39412784&L=20].

As well as the hazard to grazing animals (i.e. direct toxicity), the possibility of contaminated hay and silage and transfer of PAs into food such as milk and milk products is under investigation and considered to be a serious problem.

PAs occur in about 3% of all flowering plants (40); until now they are analyzed in 13 plant families. If the PAs possess a double-bond in their basic moiety (necine) and a free position adjacent to their nitrogen atom, they are hepatotoxic, carcinogenic, genotoxic, teratogenic and sometimes pneumotoxic. The toxicity of PA-containing plants from many plant genera, mainly of the families Asteraceae, Fabaceae and Boraginaceae, is well known.

**Toxicity of pyrrolizidine alkaloids**

**Metabolic toxification of PAs**

PAs are ester alkaloids derived mainly from the necines retronecine and onotene (Fig. 1). They are carcinogenic, mutagenic, genotoxic, fetotoxic and teratogenic. 

![Figure 1: Structures of necines occurring in PA](http://example.com/figure1.png)
PAs themselves show a more or less low acute toxicity but in vivo they undergo a metabolic toxication process in the liver, which is, as a result, the first target organ for the toxicity.

This toxication process is well investigated (13,41-46).

After oral uptake and absorption of the PAs (Fig. 2: Ia and Ib), a hydroxyl-group is introduced adjacent to the nitrogen-atom in the necine (position 3 or 8) by the cytochrome P-450 monoxogenase enzyme complex in the liver (Fig. 2: Ila and IIb).

These hydroxyl PAs (OHPAs) are unstable and undergo a rapid dehydration to the dehydropyrrolizidine alkaloids (DHPAlk; Fig. 2: III). This dehydration results in a second double-bond in the necine followed by spontaneous rearrangement to an aromatic pyrrole system III.

PAs occur mainly as their N-oxides in the plants and these cannot be directly converted to the OHPAs, but on oral ingestion they are reduced by the gut enzymes or the liver microsomes and NADH or NADPH to the free bases and therefore they show equal toxicity to that of the free bases (47-52).

Otonecine-type PAs (Fig. 2: Ib) are metabolized to the OHPAs (45,53,54). These otonecine-PAs possess a methyl-function at the nitrogen and a quasi keto-function at the bridge-carbon 8. After hydroxylation of the N-methyl-group, it is split off as formaldehyde leaving a NH-function which undergoes condensation with the C8 keto group to produce product IIb (Fig. 2) which spontaneously dehydrates to the DHPAlk III.

The metabolites III are able to generate stabilized carbonium ions (Fig. 3: IV and VI) by loss of hydroxy groups or ester functions as hydroxyl or acid anions. These carbonium ions can react rapidly with nucleophiles (Fig. 3: VII).

In the case of necine-diesters (as shown in Ia and Ib, Fig. 2), typical of Senecio species, the formation of the reactive carbonium ions is facilitated because the necic acid groups provide good leaving groups that facilitate rapid formation of the carbonium ions III in high yield. When one of the hydroxy groups at C7 or C1of the necine is not esterified, formation of the carbonium ions is not so spontaneous. In these cases the carbonium ions are most readily formed after protonation of the hydroxyls and loss of H2O (55).

The metabolites IV and VI react rapidly with nucleophilic groups of proteins and the amino groups of the bases in nucleosides like DNA and RNA which leads to abnormal functions showing finally the veno-occlusive disease (VOD) in which the veins are narrowed. Typical macrocyclic diester PAs (like senecionine, seneciphylline, retrorsine and senkirkine which are PAs commonly found in Senecio species) have been shown to produce liver damage due to cross-linking of DNA (49,56-65). In case of PA monoesters (e.g. derived from the necine supinidine which lacks a C7 hydroxyl, Fig. 1) cross-linking is not possible and they show a lower toxic potential. It has also been shown that the
nucleophilic activity at C7 is higher than at C9 resulting in the primary nucleophilic attack at C7 followed by an attack at C9 (Fig. 3) (66).

As shown in Figure 3, the DHPAlks can also react with SH groups found in more soluble components like glutathione and cysteine (Fig. 3: VIII). High levels of glutathione and cysteine therefore reduce the toxic potential of PAs (53,67-69).

Furthermore, hydrolysis can take place where the DHPAlks (Fig. 3) yield dehydronecine alcohols (DHNecs) (Fig. 3: IX) which are more water-soluble and less reactive than the DHPAlks but still display a moderate level of alkylating activity (70,71). The higher water-solubility and lower reactivity can lead to the escape from the liver tissue and subsequent reaction in other organs (13,72,73). DHNecs like dehydroretronecine and dehydroheliotridine have also been shown to produce rhabdomyosarcoma, skin, liver and lung tumours (26,74-77).

**Detoxication of PA**

As well as the metabolic activation, detoxication of PAs also occurs in vivo: hydrolysis of the ester bonds in PAs from type Ia or Ib by esterases leads to necic acids and to the free necines. Both are non-toxic products and - on account of their higher water-solubility - can be renally excreted. The rate of hydrolysis is dependent on the level of steric hindrance of the ester linkages; and it has been shown that the more highly branched the necic acids are, the more resistant they are to hydrolysis (66,78). This means that macrocyclic diesters (Ia and Ib) with more complex acid moieties are more hazardous on account of their lower rate of hydrolytic detoxication.

The N-oxides of PAs (the form occurring most commonly in plant sources) are highly water-soluble and can therefore be renally excreted. Besides their natural occurrence, N-oxidation of PAs also takes place in the liver and can be seen as a detoxication process (Fig. 2) (41,43,47,79,80). However, it has been shown that the N-oxides - besides excretion - can be converted by dehydration or by acetylation followed by elimination of acetic acid to the DHPAlk (Fig. 2: III) (55,66).

In conclusion it can be stated that the toxicity level of different PAs in non-ruminants is dependent on three aspects:

- The efficiency of metabolic activation to form the key-intermediate III (Fig.2)
- The efficiency of ester hydrolysis to form non-toxic and water soluble necines and necic acids
- The efficiency of N-oxidation and excretion via urine.

**PA toxicity in humans**

PA poisoning of humans can be described by three dose-related levels: acute, sub-acute and chronic. These levels can be progressive resulting in irreversible chronic toxic effects (13,65,73,77,81,82,83).

On account of the low toxicity of the PAs themselves, acute poisoning has been reported only in very rare cases; it occurs only in infants and neonates due to their higher susceptibility to a PA poisoning. It is characterised by haemorrhagic necrosis, hepatomegaly and ascites; death is caused by liver failure (13,73,77,82).

Sub-acute levels are characterised by hepatomegaly and recurrent ascites; endothelial proliferation and medial hypertrophy leading to an occlusion of hepatic veins, resulting in the so-called veno-occlusive disease (VOD) which can be seen as a characteristic histological sign for PA poisoning (13,65,73,77,82). The VOD causes centrilobular congestion, necrosis, fibrosis and liver cirrhosis, the end-stage of chronic PA intoxication.

As well as the liver VOD, other organs can be affected by PAs. It has been shown that the pyrrolic metabolites (DHPAlks and DHNecs) can escape from the liver into pulmonary arterioles where they can produce damage similar to the VOD changes in the liver (82). It could be shown that from 62 tested PAs all can produce (dose-dependent) lung lesions (78) and it is speculated that pulmonary damage results from long-term and low-level exposure to PAs (13,82).

PA intoxication in humans is not only related to the amount and the duration of the exposure but also to age and gender: males react more sensitive than females, and foetuses and children (especially neonates or infants) show the highest sensitivity to PA poisoning: In 2003 it was shown that the daily uptake of ~ 7 µg PA (from a herbal spice containing comfrey) during pregnancy did not show a toxic effect in the mother’s liver but damaged the foetal liver in such a way that the new-born child died after 2 days (84). It has also been observed that cofactors can exacerbate the PA poisoning: liver damaging agents, bacterial or viral
infections but also medical drugs like barbiturates or metals like copper or mycotoxins like aflatoxins can increase the severity and likelihood of PA liver damage (85-90).

There is a large number of reports in the literature about different liver diseases (mainly VOD) possibly connected with PA poisoning. But in most cases the connection could not be proven because the outbreak of the liver disease and a possible ingestion of PA-containing material were often separated by a long time period.

The following table therefore lists only those cases where a source of PAs was identified and the liver disease was therefore undoubtedly caused by PA intoxication:

<table>
<thead>
<tr>
<th>Location and year</th>
<th>Affected people</th>
<th>Observed damage</th>
<th>Source of PA</th>
<th>Lit.</th>
</tr>
</thead>
<tbody>
<tr>
<td>South Africa, 1920</td>
<td>11 adult people</td>
<td>Abdominal pain, vomiting, cirrhosis</td>
<td>Senecio illicifolius, S. burchelli</td>
<td>5</td>
</tr>
<tr>
<td>Jamaica, 1954</td>
<td>23 adults</td>
<td>VOD</td>
<td>Bush-teas with Crotalaria fulva</td>
<td>16, 17</td>
</tr>
<tr>
<td>South Africa, 1968</td>
<td>15 children; 10 died</td>
<td>VOD</td>
<td>Bush-teas; Crotalaria sp.?</td>
<td>91</td>
</tr>
<tr>
<td>Venezuela, 1969</td>
<td>5 years old girl</td>
<td>VOD</td>
<td>Crotalaria anagroیدs, C. pumila consumed as infusion and as vegetable soup</td>
<td>92</td>
</tr>
<tr>
<td>Jamaica, 1970</td>
<td>adults</td>
<td>Liver carcinoma</td>
<td>Heliotropium rossissimum (&quot;Ramram&quot;)?</td>
<td>93</td>
</tr>
<tr>
<td>Iraq, 1970</td>
<td>6 children</td>
<td>VOD</td>
<td>Bush-tea from Crotalaria and Senecio sp.</td>
<td>94</td>
</tr>
<tr>
<td>Afghanistan, 1970-72</td>
<td>7200 people</td>
<td>VOD</td>
<td>food contaminated by a Senecio spec.</td>
<td>95</td>
</tr>
<tr>
<td>India, 1973</td>
<td>486 people</td>
<td>VOD</td>
<td>Cereals contaminated with Crotalaria spp.</td>
<td>96</td>
</tr>
<tr>
<td>Ecuador, 1973</td>
<td>female</td>
<td>VOD</td>
<td>Herbal tea with Crotalaria juncea</td>
<td>97</td>
</tr>
<tr>
<td>India, 1973, 1975</td>
<td>4 males</td>
<td>Endemic ascites</td>
<td>Millet contaminated with Crotalaria spp.</td>
<td>98</td>
</tr>
<tr>
<td>India, 1974-1977</td>
<td>6 people</td>
<td>VOD</td>
<td>Heliotropium eichwaldii</td>
<td>100</td>
</tr>
<tr>
<td>Martinique, 1975</td>
<td>2 children</td>
<td>VOD</td>
<td>Bush-teas with Crotalaria retusa and/or Heliotropium sp.</td>
<td>101</td>
</tr>
<tr>
<td>USA, 1976, 1977</td>
<td>4 children</td>
<td>Vein congestion and necrosis of liver</td>
<td>Senecio longilobus</td>
<td>102</td>
</tr>
<tr>
<td>UK, 1976</td>
<td>female</td>
<td>VOD</td>
<td>Maté (Paraguay tea) contaminated with PA of unknown origin</td>
<td>103</td>
</tr>
<tr>
<td>USA, 1984</td>
<td>49 year old female</td>
<td>VOD</td>
<td>Food supplement containing Symphytum spp. root</td>
<td>104</td>
</tr>
<tr>
<td>China, 1985</td>
<td>4 females</td>
<td>VOD</td>
<td>Herbal tea containig Heliotropium lasiocarpm</td>
<td>105</td>
</tr>
<tr>
<td>Switzerland, 1985</td>
<td>59-year-old male and 27-years-old son</td>
<td>VOD</td>
<td>Herbal tea consisting of Senecio spp.</td>
<td>106</td>
</tr>
<tr>
<td>Switzerland, 1986</td>
<td>5-day-old baby</td>
<td>VOD</td>
<td>Herbal tea containing Tussilago farfara consumed during whole pregnancy</td>
<td>31</td>
</tr>
<tr>
<td>UK, 1986</td>
<td>13-year-old boy</td>
<td>VOD</td>
<td>Herbal tea containing Symphytum spp.</td>
<td>107</td>
</tr>
<tr>
<td>Tadjikistan, 1992, 1993</td>
<td>3906 people</td>
<td>Abdominal pain, hepatomegaly, ascites, alteration of consciousness</td>
<td>Heliotropium lasiocarpm</td>
<td>10, 11, 12</td>
</tr>
<tr>
<td>Peru, 1994</td>
<td>38-year-old female</td>
<td>VOD</td>
<td>Herbal tea from Senecio tephrooides</td>
<td>108</td>
</tr>
<tr>
<td>Spain, 1995</td>
<td>73-year-old male</td>
<td>VOD</td>
<td>Senecio vulgaris</td>
<td>109</td>
</tr>
<tr>
<td>Austria, 1995</td>
<td>18-month-old boy</td>
<td>VOD</td>
<td>Herbal tea with Adenostyles alliariae</td>
<td>110</td>
</tr>
<tr>
<td>Argentina, 1999</td>
<td>23-year-old female</td>
<td>VOD</td>
<td>Herbal tea containing Senecio vulgaris</td>
<td>111</td>
</tr>
<tr>
<td>Germany, 2002</td>
<td>20-year-old female</td>
<td>VOD</td>
<td>Symphytum spp.</td>
<td>84</td>
</tr>
</tbody>
</table>

Table 1: Human intoxications
REFERENCES


Toxicity of pyrrolizidine alkaloids - a serious health problem


