Toxicity of Pyrrolizidine Alkaloids – a Serious Health Problem

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ÖZET
Pirolizidin alkaloidlerin toksisitesi - ciddi bir sağlık problemi

Pirolizidin alkaloidleri (PA) insanlar ve çiflik hayvanları için toksiktir. Karaciğer, PA'ların toksik etkiyi gösterdikleri ilk hedef organdır ve burada metabolik toksikasyon işlemi başlar. Dünya çapında, insanlarda PA intoksikasyonları rapor edilmiştir. Bu intoksikasyonlar sadece maruz kalınan miktar ve süreye bağlı değildir. Aynı zamanda yaş ve cinsiyetle de ilişkilidir. Metabolik toksikasyonun yanısıra detoksifikasyon işlemi de önemlidir. Bu çalışma, PA'ların toksikasyonu ve detoksifikasyonu proseslerini tartışmakta ve PA'larla ilgili olarak insanlarda zehirlenme vakaları hakkında genel bilgi vermektedir.

Anahtar sözcükler: Pirolizidin alkaloidleri, metabolik toksikasyon, detoksifikasyon, insanlarda zehirlenme

ABSTRACT
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 preparation 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 jacobaea) has been under discussion: Its extensive expansion into pastures and meadows has led to a great number of intoxications 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 toxication of PAs

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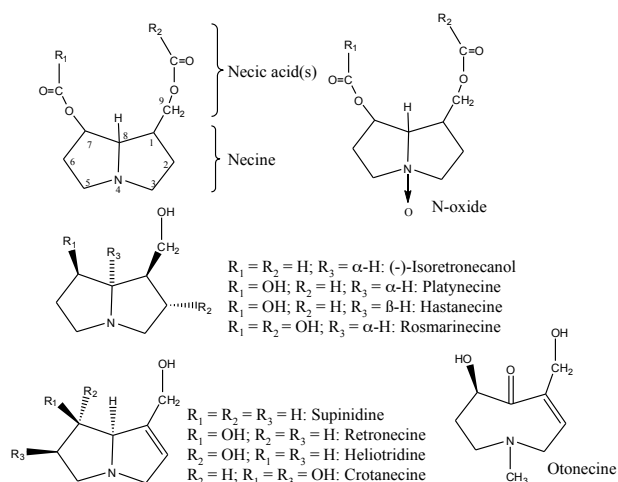

Figure 1: Structures of necines occurring in PA

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: IIa and IIb).
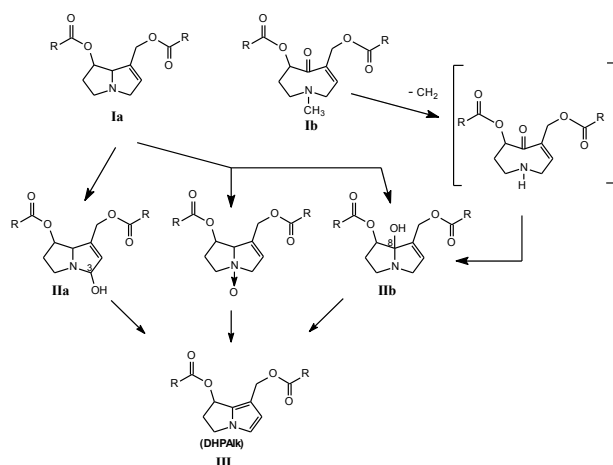
**Figure 2:** Enzymatic hydroxylation and dehydropyrrolizidine products

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).
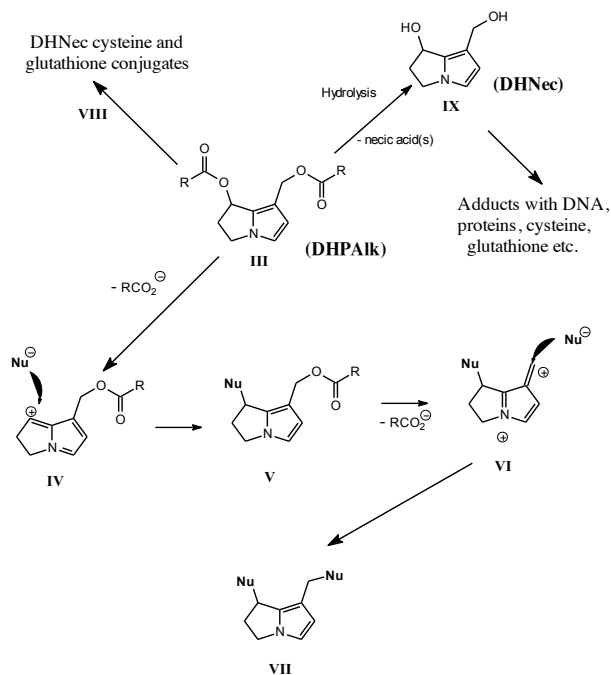
Figure 3: DHPAlk and carbonium ion building

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 C1 of 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 H_2O (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 1: Human intoxications

Location and year	Affected people	Observed damage	Source of PA	Lit.
South Africa, 1920	11 adult people	Abdominal pain, vomiting, cirrhosis	Senecio illicifolius, S, burchelli	5
Jamaica, 1954	23 adults	VOD	Bush-teas with Crotalaria fulva	16, 17
South Africa, 1968	15 children; 10 died	VOD	Bush-teas; Crotalaria sp.?	91
Venezuela, 1969	5 years old girl	VOD	Crotalaria anagyroides, C. pumila consumed as infusion and as vegetable soup	92
Kuweit, 1970	adults	Liver carcinoma	Heliotropium ramosissimum ("Ramram")?	93
Jamaica, 1970	6 children	VOD	Bush-tea from Crotalaria and Senecio sp.	94
Iraq, 1970	9 children	VOD	food contaminated by a Senecio spec.	95
Afghanistan , 1970-72	7200 people	VOD	Wheat contaminated with Heliotropium popovii, ssp. gillianum	9, 10
India, 1973	486 people	VOD	Cereals contaminated with Crotalaria spp.	96
Ecuador, 1973	female	VOD	Herbal tea with Crotalaria juncea	97
India, 1973, 1975	4 males	Endemic ascites	Millet contaminated with Crotalaria spp.	98
China, 1973, 1978	2 adults	VOD	Gynura segetum	99
India, 1974-1977	6 people	VOD	Heliotropium eichwaldii	100
Martinique, 1975	2 children	VOD	Bush-teas with Crotalaria retusa and/or Heliotropium sp.	101
USA, 1976, 1977	4 children	Vein congestion and necrosis of liver	Senecio longilobus	102
UK, 1976	female	VOD	Maté (Paraguay tea) contaminated with PA of unknown origin	103
USA, 1984	49 year old female	VOD	Food supplement containig Symphytum spp. root	104
China, 1985	4 females	VOD	Herbal tea containig Heliotrpopium lasiocarpum	105
Switzerland, 1985	59-year- old male and 27-years-old son	VOD	Herbal tea consisting of Senecio spp.	106
Switzerland, 1986	5-day-old baby	VOD	Herbal tea containing Tussilago farfara consumed during whole pregnancy	31
UK, 1986	13-year- old boy	VOD	Herbal tea containing Symphytum spp.	107
Tadjikistan, 1992, 1993	3906 people	Abdominal pain, hepatomegaly, ascites, alteration of consciousness	Heliotropium lasiocarpum	10, 11, 12
Peru, 1994	38-year-old female	VOD	Herbal tea from Senecio tephrosioides	108
Spain, 1995	73-year- old male	VOD	Senecio vulgaris	109
Austria, 1995	18-month-old boy	VOD	Herbal tea with Adenostyles alliariae	110
Argentina; 1999	23-year-old female	VOD	Herbal tea containing Senecio vulgaris	111
Germany, 2002	foetus	VOD	Symphytum spp.	84

REFERENCES

1. Gilruth JA. Hepatic cirrhosis affecting horses and cattle (so-called "Winton disease"). New Zealand Department of Agriculture, 11th annual report. pp., 1903: 228-279.

2. Gilruth JA. Hepatic cirrhosis due to ragwort (Senecio jacobaea). Div. Vet. Sci. New Zealand, Dept. Agric., Bull. 1904; No. 9. 252-254.

3. Bull LB, Dick AT. The chronic pathological effects on the liverof the rat of the pyrrolizidine alkaloids heliotrine, lasiocarpine, and their N-oxides. J. Pathol. Bacteriol. 1959; 78: 483-502.

4. Willmott FC, Robertson GW. Senecio disease or cirrhosis of the liver due to Senecio poisoning. Lancet 1920; 23 October: 848-849.

5. Steyn DG. Poisoning of human beings by weeds contained in cereals (bread poisoning). Onderstepoort J. Vet Sci Anim. Ind. 1933; 1: 219-266.

6. Bourkser GV. On the question of the aetiology and pathogenesis of toxic hepatitis with ascites (heliotrope toxicosis). Hyg. Sanit.1947; 6: 24-26.

7. Milenkov SM, Kizhaikin Y. Toxic hepatitis with ascites. Proc. Sympos. V. M. Molotov Med. Inst., Tashkent. 1952.

8. Tandon HD, Tandon BN. Epidemic of liver disease – Gulran District, Herat Province, Afghanistan, Alexandria, World Health Organization, Regional Office for the Eastern Mediterranean (Assignment report No. EM/AFG/OCD/001/RB). 1975.

9. Mohabbat O, Srivastava RN, Younos MS, Sediq GG, Menzad AA, Aram GN. An outbreak of hepatic veno-occlusive disease in north-western Afghanistan. Lancet 1976; 7 August: 269-271.

10. Chauvin P, Dillon J-C., Moren A. (1994) Épidémie d' intoxication alimentaire á l' héliotrope, Tadjikistan, Novembre 1992-Mars 1993. Cahiers Santé 4: 263- 268.

11. Chauvin P., Dillon J-C, Moreu A, Talbak S, Barakaev S. Heliotrope poisoning in Tadjikistan. Lancet 1993; 341: 1633.

12. Mayer F, Lüthy J. Heliotrope poisoning in Tadjikistan. Lancet 1993; 342: 246-247.

13. International Programme on Chemical Safety.(IPCS) Pyrrolizidine Alkaloids Health and Safety Guide. Health and Safety Guide No. 26. Geneva: WHO, 1989.

14. Roeder E. Medicinal plants in Europe containing pyrrolizidine alkaloids. Pharmazie 1995; 50: 83-98.

15. Roeder E. Medicinal plants in China containing pyrrolizidine alkaloids. Pharmazie 2000; 55: 711-726.

16. Bras G, Jelliffe DB, Stuart KL. Veno-occlusive disease of liver with nonportal type of cirrhosis, occurring in Jamaica. Arch. Pathol. 1954; 57: 285-300.

17. Bras G, Brooks SEH, Watler DC. Cirrhosis ofLiver in Jamaica. J. Pathol. Bacteriol. 1961; 82: 503-11.

18. FDA. FDA Advises Dietary Supplement Manufacturers to Remove Comfrey Products From the Market. 2001.

19. EFSA. Opinion of the Scientific Panel on contaminants in the Food Chain of the European Food Safety Authority (EFSA) on a request from the European Commission related to pyrrolizidine alkaloids as undesirable substances in animal feed. EFSA J. 2007; 447:1-51.

20. Bundesanzeiger, June 17, 4805; cited by: Dtsch. Apoth. Ztg. 1992; 132, 1406-1408.

21. Bundesgesetzblatt Verordnung des Bundesministers für Gesundheit und öffentlicher Dienst vom 5. Mai 1989 betreffend Arzneimittel, die nicht in Verkehr gebracht werden dürfen. 555/1994 Wien, Österreich.

22. Staatsblaad Besluit van 19 januari 2001, houdende vaststelling van het Warenwetbesluit Kruidenpreparaten. Staatsblad van het Koninkrijk der Nederlanden. 2001; 56:1–12.

23. Schoental R. The chemical aspects of seneciosis. Proc. Roy. Soc. Med. 1959; 53: 284-288.

24. Dickinson J.O, Cooke MP, King RR, Mohamed PA. Milk transfer of pyrrolizidine alkaloids in cattle. J. Am. Vet. Med. Assoc. 1976; 169: 1192-1196.

25. Dickinson JO. Release of pyrrolizidine alkaloids into milk. Proc. West. Pharmacol. Soc. 1980; 23: 377-379.

26. Johnson WD, Robertson KA, Pounds JG, Allen JR. Dehydroretronecine-induced skin tumours in mice. J. Natl. Cancer Inst. 1978; 61: 85-89.

27. Goeger DE, Cheeke PR, Schmitz JA, Buhler DR. Toxicity of tansy ragwort (Senecio jacobaea) to goats. Am. J. Vet. Res. 1982; 43: 252-254.

28. Lüthy J, Heim TH, Schlatter CH. Transfer of [3H] pyrrolizidine alkaloids from Senecio vulgaris L. and metabolites into rat milk and tissues. Toxicol. Lett. 1983; 17: 283-288.

29. Candrian U, Lüthy J, Graf U, Schlatter CH. Mutagenic activity of the pyrrolizidine alkaloids seneciphylline and senkirkine in Drosophila and their transfer into rat milk. Food Chem. Toxicol. 1984; 22: 223-225.

30. Molyneux RJ, James LF. Pyrrolizidine alkaloids in milk: Thresholds of intoxication. Vet. Hum. Toxicol. 1990; 32: S94-103.

31. Roulet M, Laurini R, Rivier L, Calarme A. Hepatic veno-occlusive disease in newborn infant of a woman drinking herbal tea. J. Pediatr. 1988; 112: 433-36.

32. Deinzer ML, Thomson PA, Burgett DM, Isaacson DL. Pyrrolizidine alkaloids: their occurrence in honey from tansy ragwort (Senecio jacobaea L.). Science 1977; 195: 497-499.

33. Culvenor CCJ, Edgar JA, Smith LW. Pyrrolizidine alkaloids in honey from Echium plantagineum L. J. Agric. Food Chem. 1981; 29: 958-960.

34. Edgar JA, Roeder E, Molyneux RJ. Honey from plants containing pyrrolizidine alkaloids: A potential threat to health. J Agric. Food Chem. 2002; 50: 2719-30.

35. Beales KA, Betteridge K, Colegate SM, Edgar JA. Solid phase extraction and LCMS analysis of pyrrolizidine alkaloids in honeys. J Agric Food Chem. 2004; 52: 6664-72.

36. Boppré M, Colegate SM, Edgar JA. Pyrrolizidine alkaloids of Echium vulgare honey found in pure pollen. J Agric. Food. Chem. 2005; 53: 594-600.

37. Betteridge K, Cao Y, Colegate SM. An improved method for extraction and LCMS analysis of pyrrolizidine alkaloids and their N-oxides in honey: Application to Echium vulgare honeys. J. Agric. Food. Chem. 2005; 53: 1894 -1902.

38. Edgar J.A, Smith LW. Transfer of pyrrolizidine alkaloids into eggs: food safety implications. In Natural and Selected Synthetic Toxins Biological Implications; Tu, A. T., Gaffield, W., Eds., ACS Symposium Series 745; American Chemical Society: Washington, DC. 1999; Chapter 8, pp 118-128.

39. Bundesamt für Risiokobewertung. Salatmischung mit Pyrrolizidinalkaloid-haltigem Greiskraut verunreinigt; Stellungnahme Nr.028/2007 des BfR vom 10. Januar 2007.

40. Smith LW, Culvenor CCJ. (1981) Plant sources of hepatotoxic pyrrolizidine alkaloids. J. Nat. Prod. 1981; 44: 129-152.

41. Mattocks AR. (1968) Toxicity of pyrrolizidine alkaloids. Nature (London) 1968; 217: 723-728.

42. Culvenor CCJ, Downing DT, Edgar JA, Jago MV. Pyrrolizidine alkaloids as alkylating and antimitotic agents. N.Y. Acad. Sci. 1969; 163: 837-847.

43. Jago MV, Edgar JA, Smith LW, Culvenor CCJ. Metabolic conversion of heliotrine based pyrrolizidine alkaloids to dehydroheliotridine. Mol. Pharmacol. 1970; 6: 402-406.

44. Mattocks AR, White INH. Pyrrolic metabolites from non-toxic pyrrolizidine alkaloids. Nat. New Biol.1971; 231: 114-115.

45. Culvenor CCJ, Edgar JA, Smith LW, Jago MV, Peterson JE. Active metabolites in the chronic hepatotoxicity of pyrrolizidine alkaloids, including otonecine esters. Nature New Biol. 1971; 229: 255-256.

46. Mattocks AR. Acute hepatotoxicity and pyrrolic metabolites in ratsdosed with pyrrolizidine alkaloids. Chem. Biol. Interact. 1972; 5: 227-242.

47. Mattocks AR, White INH. The conversion of pyrrolizidine alkaloids to dihydropyrrolizine derivatives by rat-liver microsomes in vitro. Chem. Biol. Interact. 1971; 3: 383-396.

48. Powis G, Ames MM, Kovach JS. Metabolic conversion of indicine-N-oxide to indicine in rabbits and humans. Cancer Res. 1979; 39: 3564-3570.

49. Chou MW, Wang YP, Yan J, Yang YC, Berger RD, Williams LD, Doerge DR , Fu PP. Riddelliine N-oxide is a phytochemical and mammalian metabolite with genotoxic activity that is comparable to the parent pyrrolizidine alkaloid riddelliine. Toxicol. Lett. 2003; 145: 239-247.

50. Wang Y-P, Yan J, Fu PP, Chou M.W. Metabolic activation of the tumorigenic pyrrolizidine alkaloid, retrorsine, leading to DNA adduct formation in vivo. Int. J. Environ. Res. Public Health 2005; 2: 74-79.

51. Wang Y-P, Yan J, Beger RD, Fu PP, Chou MW. (2005b) Metabolicactivation of the tumorigenic pyrrolizidine alkaloid, monocrotaline, leading to DNA adduct formation in vivo. Cancer Lett. 2005; 226: 27-35.

52. Wang Y-P, Yan J, Fu PP, Chou MW. Human liver microsomal reduction of pyrrolizidine alkaloid N-oxides to form the corresponding carcinogenic parent alkaloid. Toxicol. Lett. 2005; 155: 411-420.

53. Lin G, Cui Y, Hawes EM. Microsomal formation of a pyrrolic alcohol glutathione conjugate of Clivorine firm evidence for the formation of a pyrrolic metabolite of an otonecine-type pyrrolizidine alkaloids. Drug Metabol. Disposit. 1998; 26: 181-184.

54. Lin G, Cui Y, Hawes EM. Characterization of rat liver microsomal metabolites of clivorine, an hepatotoxic otonecine-type pyrrolizidine alkaloid. Drug Metabol. Dispos. 2000; 28: 1475-1483.

55. Culvenor CCJ, Edgar JA, Smith LW, Tweeddale HJ. Dihydropyrrolizines. III. Preparation and reactions of derivatives related to pyrrolizidine alkaloids. Aust. J. Chem. 1970; 23: 1853-1867.

56. Curtain CC, Edgar JA. The binding of dehydroheliotridine to DNA and the effect of it and other compounds on repair synthesis in main and satellite band DNA. Chem. Biol. Interact. 1976; 13: 243-256.

57. Hincks JR, Kim HY, Segal HJ, Molyneux RJ, Stermitz FR, Coulombe RA. DNA cross-linking in mammalian cells by pyrrolizidine alkaloids: structure-activity relationships. Toxicol. Appl. Pharmacol. 1991; 111: 90-98.

58. Kim H-Y, Stermitz FR, Coulombe RA. Pyrrolizidine alkaloid-induced DNA-protein cross-links. Carcinogenesis 1995; 16: 2691-2697.

59. Kim H-Y, Stermitz FR, Li JK, Coulombe RA. (1999) Comparative DNA cross-linking by activated pyrrolizidine alkaloids. Food Chem. Toxicol. 1999; 37: 619-625.

60. Pereira TN, Webb RL, Reilly PE, Seawright AA, Prakash AS. Dehydromonocrotaline generates sequence-selective N-7 gyanine alkylation and heat and alkali stable multiple fragment DNA crosslinks. Nucleic Acids Res. 1998; 26: 5441-5447.

61. Coulombe RA, Drew GL, Stermitz FR. Pyrrolizidine alkaloids crosslink DNA with actin. Toxicol. Appl. Pharmacol. 1999; 154:198-202.

62. Yan J, Nichols J, Yang Y-C, Fu PP. Detection of riddelliine-derived DNA adducts in blood of rats fed riddelliine. Int. J. Mol. Sci. 2002; 3: 1019-26.

63. Fu PP, Xia Q, Lin G, Chou MW. Genotoxic pyrrolizidine alkaloids mechanisms leading to DNA adduct formation and tumorigenicity. Int. J. Mol. Sci. 2002; 3:948-64.

64. Fu PP, Xia Q, Lin G. Pyrrolizidine alkaloids – genotoxicity, metabolism enzymes, metabolic activation, and mechanism. Drug Metabol. Revs. 2004; 36: 1-55.

65. Xia Q, Chou MW, Edgar JA, Doerge DR, Fu PP. Formation of DHP-derived DNA adducts from metabolic activation of the prototype heliotridine-type pyrrolizidine alkaloid, lasiocarpine. Cancer Lett. 2006; 231: 138-145.

66. Mattocks AR. Chemistry and Toxicology of Pyrrolizidine Alkaloids, London, New Yorck, Academic Express. 1986.

67. Cheeke PR, Gorman GR. Influence of dietary protein and sulphur amino-acid levels on the toxicity of Senecio jacobaea (tansy ragwort) to rats. Nutr. Rep. Int. 1974; 9: 197-207.

68. Nigra L, Huxtable RJ. Hepatic glutathione concentrations and the release of pyrrolic metabolites of the pyrrolizidine alkaloid, monocrotaline, from the isolated perfused liver. Toxicon 1992; 30: 1195-1202.

69. Reed RL, Miranda CL, Kedzierski B, Henderson MC, Buhler DR. Microsomal formation of a pyrrolic alcohol glutathione conjugate of the pyrrolizidine alkaloid senecionine. Xenobiotica 1992; 1: 1321-1327.

70. Peterson JE, Jago MV. Comparison of the toxic effects of dehydroheliotridine and heliotrine in pregnant rats and their embryos. J. Pathol. 1980; 131: 339-355.

71. Robertson KA. Alkylation of N2 in deoxyguanosine by dehydroretronecine, a carcinogenic metabolite of the pyrrolizidine alkaloid monocrotaline. Cancer Res. 1982; 42: 8-14.

72. Peterson JE, Samuel A, Jago MV. Pathological effects ofdehydroheliotridine, a metabolite of heliotridine-based pyrrolizidinealkaloids in the young rat. J. Pathol. 1972; 107: 107-189.

73. Prakash AR, Pereira TN, Reilly PEB, Seawright AA. (1999) Pyrrolizidine alkaloids in human diet. Mutation Res. 1999; 443: 53-67.

74. Allen JR, Hsu I-C, Carstens LA. (1975) Dehydroretronecine induced rhabdomyosarcomas in rats. Cancer Res. 1975; 35: 997–1002.

75. Shumaker RC, Robertson KA, Hsu IC, Allen JR. Neoplastic transformation in tissues of rats exposed to monocrotaline or dehydroretronecine. J. Natl. Cancer Inst. 1976; 56: 787-789.

76. Mattocks AR, Cabral JRP. Carcinogenicity of some pyrrolizidine alkaloid metabolites and analogues. Cancer Lett. 1982; 17: 61-66.

77. Peterson JE, Culvenor CCJ. Plant and fungal toxins. In: Keeler, R.F. & Tu, A.T., ed. Handbook of natural toxins, New York, Marcel Dekker, Vol. 1. 1983; pp. 637-681.

78. Culvenor CCJ, Edgar JA, Jago MV, Outteridge A, Peterson JE, Smith LW. (1976) Hepato- and pneumotoxicity of pyrrolizidine alkaloids and derivatives in relation to molecular structure. Chem.-Biol. Interact. 1976; 12: 299-324.

79. Williams DE, Reed RL, Kedziersk B, Dannan GA, Guengerich FP, Buhler DR. Bioactivation and detoxication of the pyrrolizidine alkaloid senecionine by cytochrome P-450 enzymes in rat liver. Drug Metab. Dispos. 1989; 17: 387-392.

80. Miranda CL, Chung W, Reed RE, Zhao X, Henderson MC, Wang JL, Williams DE, Buhler DR. Flavin-containing monooxygenase: a major detoxifying nzyme for the pyrrolizidine alkaloid senecionine in guinea pig tissues. Biochem. Biophys. Res. Commun. 1991; 178: 546-552.

81. McLean EK. The toxic actions of pyrrolizidine (Senecio) alkaloids. Pharmacol. Rev. 1970; 22: 429-483.

82. Huxtable RJ. Human health implications of pyrrolizidine alkaloids and herbs containing them. In: Cheeke PR, ed. Toxicants of Plant Origin, Vol 1. 1989; pp. 42-86 Boca Raton: CRC Press.

83. Stegelmeier BL, Edgar JA, Steven M, Colegate SM, Gardner DR, Schoch TK, Coulombe RA jr. Pyrrolizidine alkaloids plants, metabolism and toxicity. J. Nat. Toxins 1999; 8: 5-116.

84. Rasenack R, Müller C, Kleinschmidt M, Rasenack J, Wiedenfeld H. Veno-occlusive disease in a foetus caused by pyrrolizidine alkaloids of food origin. Fetal Diagn. Ther. 2003; 18: 223-25.

85. Yee SB, Kinser S, Hill DA, Barton CC, Hotchkiss JA, Harkema JR, Ganey PE, Roth RA. Synergistic hepatotoxicity from coexposure to bacterial endotoxin and the pyrrolizidine alkaloid monocrotaline. Toxicol. Appl. Pharmacol. 2000; 166: 173-185.

86. Newberne .M, Rogers AE. Nutrition, monocrotaline and aflatoxin B1 in liver carcinogenesis. Plant Food Man. 1973; 1: 23-31.

87. White INH, Mattocks AR, Butler WH. The conversion of thepyrrolizidine alkaloid retrorsine to pyrrolic derivatives in vivo andin vitro and its acute toxicity to various animal species.Chem. Biol. Interact. 1973; 6: 207-218.

88. Tuchweber B, Kovacs K, Jago MV, Beaulieu T. (1974) Effect of steroidal and nonsteroidal microsomal enzyme inducers on the hepatotoxicity of pyrrolizidine alkaloids in rats. Res. Commun. Chem. Pathol. Pharmacol. 1974; 7: 459-480.

89. Lin JJ, Liu C, Svoboda DJ. (1974) Long-term effects of aflatoxin B1 and vocal hepatitis on marmoset liver: a preliminary report. Lab. Invest. 1974; 30: 267-278.

90. Bull LB, Culvenor CCJ, Dick AT. The pyrrolizidine alkaloids, Amsterdam, 1968; North Holland Publishing Co.

91. Freiman I, Schmaman A, Zamit R, Appleberg M. Veno-occlusive disease of the liver – some new aspects. S. Afr. Med. J. 1968; 42: 126-129.

92. Grases PJ, Beker SG. Veno-Occlusive Disease of the Liver – A Case from Venezuela. Am. J. Med. 1972; 53: 511-16.

93. Macksad A, Schoental R, Coady A. The Hepatotoxic Action of a Traditionl Bedu Plant Remedy "Ramram". J. Kwt. Med. Assoc. 1970; 4: 297-299.

94. Brooks SEH, Miller CG, McKenzie K, Audretsch JJ, Bras G. Acute Veno-occlusive Disease of the Liver. Arch. Path. 1970; 89: 507-520.

95. Al-Hasany M, Mohamed AS. Veno-occlusive of the Liver in Iraq. Arch. Dis. Childhood 1970; 45: 722-24.

96. Tandon BN, Tandon RK, Tandon HD, Narndranathan M, Joshi JK. An Epidemic Veno-Occlusive Disease of Liver in Central India. Lancet 1976; 7 August: 271-72.

97. Lyford CL, Vergara GG, Moeller DD. Hepativ Veno-Occlusive Disease Originating in Ecuador. Gastroenterology 1976; 70: 105-8.

98. Krishnamachari KAVR, Bhat RV, Krishnamurthi D, Krishnaswamy K, Nagarajan V. Aethiopathogenesis of endemic ascites in Surguja district of Madhya Pradesh. Indian J. Med. Res. 1977; 65: 672-78.

99. Hou JG, Xia YT, Yu CS, An Y, Tang YH. Veno-occlusive disease of the liver –with two fatal cases. Zhonghua Neike Zazhi 1980; 19: 187-191.

100. Datta DV, Khuroo MS, Mattocks AR, Aikat BK, Chhuttani PN. Herbal medicines and veno-occlusive disease in India. Postgraduate Medical Journal 1878; 54: 511-15.

101. Saint-Aimé MM, Ponsar C, Lacombe C, Lacombe W. Maladie Veino-Occlusive du Foie chez L'Enfant Martiniquais. Bordeaux Médical 1977; 10: 665-670.

102. Stillman AE, Huxtable RJ, Fox DW, Hart MC, Bergeson PS, Counts JM. Poisoning associated with Herbal Teas –Arizona, Washington. Morbidity and Mortality Weekly Report 1977; 26: 257-59.

103. McGee JO, Patrick RS, Wood CB, Blumgart LH. A case of veno-occlusive disease of theliver in Britain associated with herbal tea consumption. J. Clin. Path. 1976; 29: 788-794.

104. Ridker PM, Ohkuma S, McDermott WV, Trey C, Huxtable RJ. Hepatic veno-occlusive disease associated with consumption of pyrrolizidine alkaloid containing dietary supplements. Gastroenterology 1985; 88: 1050-1054.

105. Culvenor CCJ, Edgar JA, Smith LW, Kumana CR, Lin HJ. Lancet 1986; 26 April: 978.

106. Margalith D, Heraief C, Schindler AM, Birchler R, Mosimann F, Aladjem D, Gonvers JJ. Veno-occlusive disease of the liver due to the se of tea made from Senecio plants. J. Hepatol. 1985; Suppl., 280.

107. Weston CFM, Cooper BT, Davies JD, Levine DF. Veno-occlusive disease of the liver secondary to ingestion of comfrey. Brit. Med. J. 1987; 295: 183.

108. Tomioka M, Calvo F, Siguas A, Sanchez L, Nava E, Garcia U, Valdivia M, Reategui E. Enfermedad hepatica veno-oclusiva asociada a l ingestion de humanrripa (Senecio tephrosioides). Rev. Gastroenterol. Peru 1995; 15: 299-302.

109. Cansado AO, Valades EC, Blanco PM, de Santamaria JS, Gonsalez JM, Téllez TR. Enfermedad venooclusiva hepatica por ingestion de infusiones de Senecio vulgaris. Gastroenterolog. Hepatol. 1995; 18: 413-416.

110. Sperl W, Stuppner H, Gassner I, Judmaier W, Dietze O, Vogel W. Reversible hepatic veno-occlusive disease in an infant after consumption of pyrrolizidine-containing herbal tea. Eur. J. Pedriatr. 1995; 154: 112-116.

111. Vilar JH, Garcia M, Cabrera P. Enfermedad venooclusiva hepática de causa Tóxica por Senecio vulgaris. Gastroenterol. Hepatol. 2000; 23: 285-6.