Pyrrolizidine alkaloids – a practitioner's perspective

Dr Christopher Etheridge

Pyrrolizidine alkaloids

- Pyrrolizidine alkaloids (PAs) have been of concern to the BHMA and EHTPA since February when the contamination of herbal supplies, particularly St John's wort, was reported by the MHRA.
- However, another unrelated concern is the use by herbal practitioners of a small number of herbs that naturally produce and contain significant levels of PAs.

Pyrrolizidine alkaloids

- PAs are naturally occurring, nitrogen containing chemicals found mainly in 3 plant families: Boraginaceae (all genera), Asteraceae (Senecioneae and Eupatorieae tribes) and Fabaceae (Crotalaria genus).
- More than 6,000 plant species are known to contain PAs and over 600 different types of PAs have been identified.
- PAs are produced by plants as antifeedants - as a defence against herbivores.

Pyrrolizidine alkaloids

- Some PAs have been found to have hepatotoxic, mutagenic and carcinogenic effects – accounting for the toxicological relevance to humans (Chen et al. 2010; Li et al. 2011; Stewart and Steenkamp 2001). The main area of concern is the toxicity of 1,2-unsaturated PAs.
- A number of herbs with medicinal uses contain are described as containing toxic PAs e.g.:

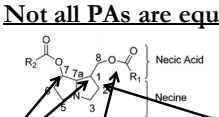
Pyrrolizidine alkaloids

Herb	Average PA content (dried weight)
Arnebia euchroma	0.0006%
Borago officinalis	< 0.001%.
Crotalaria asamica	2-3%
Crotalaria sessiliflora	Not stated
Cynoglossum officinale	<1%
Delairea odorata (syn *Senecio scandens*)	0.0007%
Eupatorium cannabinum	Not stated
Eupatorium fortunei	0.0095%
Eupatorium japonicum	0.0422%
Gynura segetum	Not stated
Jacobaea officinalis (syn *Senecio vulgaris*)	0.2-0.3%
Liparis japonicus	Not stated
Lithospermum erythrorhizon	0.02%
Packera aurea (syn *Senecio aureus*)	0.02%
Petasites hybridus	0.007%
Senecio kirilowii	Not stated
Senecio vulgaris	0.16%
Symphytum officinale herba	0.02-0.18%
Symphytum officinale radix	0.25-0.29%
Tussilago farfara	0.015%

Chen and Huo 2010, Dharmananda 2001, Roeder 1995, Roeder and Wiedenfeld 2009, Roeder and Wiedenfeld 2013.

Pyrrolizidine alkaloids

Not all PAs are equal

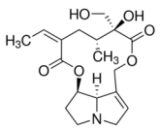


- For maximum toxicity 3 criteria essential:
- 1) Double bond (unsaturated) at 1,2 position of necine base.
- 2) Hydroxymethyl group at C1 and preferably hydroxyl group at C7.
- 3) Esterification of C1 hydroxymethyl group with branched mono- or di-carboxylic acid containing at least 5 carbon atoms (necic acid structure).

Mechanisms of antibacterial herbal action

Not all PAs are equal

- For example, one of the most toxic PAs known is retrorsine (found in Senecio species), which contains all 3 criteria for toxicity:

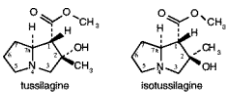


7

Pyrrolizidine alkaloids

Not all PAs are equal

- However, some PAs such as tussilagine and isotussilagine are not toxic (Bauer and Wagner, 1991).



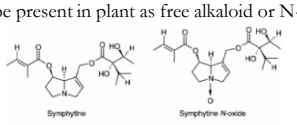
1,2-saturated

- These PAs are saturated at the 1,2 position (no double bond) and lack the C7 hydroxyl group.

8

Pyrrolizidine alkaloids

- PAs can be present in plant as free alkaloid or N-oxide:



- PA N-oxides cannot be directly absorbed in GIT and are therefore non toxic.
- However, they are rapidly converted by commensal bacteria in colon into free alkaloid which is then absorbed and transferred to the liver where it can cause toxic effects.

9

Pyrrolizidine alkaloids

Not all PAs are equal

- Some PAs are more toxic than others e.g. retrorsine over 40 times more toxic than lycopsamine.
- Therefore it is not just the amount of PAs found in a plant that is important, but the type of PA as well: a small amount of a very toxic PA could be as damaging to health as a large amount of a less toxic PA.

10

Pyrrolizidine alkaloids

1,2-unsaturated pyrrolizidine alkaloid	LD50 (mg/kg body weight)
Retrorsine	34
Senecionine	50
Heliosupine	60
Lasiocarpine	72
Seneciphylline	77
Jacobine	77
Riddelliine	105
Symphytine	130
Heleurine	140
Jaconine	168
Monocrotaline	175
Echimidine	200
Spectabiline	220
Senkirkine	220
Heliotrine	300
Echinatine	350
Supinine	450
Europine	1000
Heliotridine	1200
Intermedine	1500
Lycopsamine	1500

COT Statement of Pyrrolizidine Alkaloids in Food, 2014

11

Pyrrolizidine alkaloids

Not all PAs are equal

- PA toxicity is also affected by factors such as:
  - Age (children are far more prone to PA toxicity).
  - Exposure to aflatoxins (synergistic effect).
  - Exposure to high levels of alcohol.
  - Exposure to high levels of copper (synergistic effect).
  - Genetics of the individual.
  - Infection with liver damaging viruses and bacterial endotoxins (synergistic effect).
  - Gender.

http://digitalcommons.usu.edu/cgi/viewcontent.cgi?article=5551&context=etd

12

Pyrrolizidine alkaloids

Not all PAs are equal

- Until such time as an official analytical method is available the HMPC advises use of solid-phase extraction-liquid chromatography–tandem mass spectrometry (SPE-LC-MS/MS) as published by BfR (Federal Institute for Risk Assessment: BfR-PA-Tea-2.0/2014) – which is a very sensitive method able to pick up the tiny amounts of PAs in samples.
- This test method allows quantification of the following 28 key toxic PAs:

13

Pyrrolizidine alkaloids

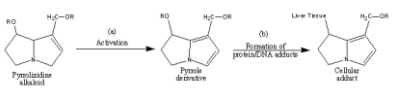
2. Echimidine-N-oxide	12. Jacobine-N-oxide	22. Senecionine-N-oxide
3. Erucifoline	13. Lasiocarpine	23. Seneciphylline
4. Erucifoline-N-oxide	14. Lasiocarpine-N-oxide	24. Seneciphylline-N-oxide
5. Europine	15. Lycopsamine	25. Senecivernine
6. Europine-N-oxide	16. Lycopsamine-N-oxide	26. Senecivernine-N-oxide
7. Heliotrine	17. Monocrotaline	27. Senkirkine
8. Heliotrine-N-oxide	18. Monocrotaline-N-oxide	28. Trichodesmine
9. Intermedine	19. Retrorsine	
10. Intermedine-N-oxide	20. Retrorsine-N-oxide	

14

Pyrrolizidine alkaloids

Mechanisms of toxicity

- Unsaturated PAs are not themselves toxic – they are **protoxins**.
- Absorbed in jejunum and ileum and transported to liver, where biotransformed by mixed function oxidases (particularly CYP3A4), into reactive pyrroles that rapidly combine with proteins and DNA in the liver to form adducts:



Rode, 2002

15

Pyrrolizidine alkaloids

Mechanisms of toxicity

- Protein binding can alter cell functions and cause cell damage and death. Cross-linking to DNA may cause carcinogenesis.
- Chronic PA exposure known to damage liver (due to liver being main production site), lung or blood vessels.
- Kidney, GI tract, pancreas and bone marrow damaged to lesser extent.
- Venous occlusions in liver and lung, megalocystosis, inhibition of cell division (mitosis) and liver cirrhosis are all signs of PA toxicity. Genotoxic effects are seen as well (Mattocks 1986, Fu et al. 2004).

16

Pyrrolizidine alkaloids

Mechanisms of toxicity

- Doses associated with acute and short-term toxicity in humans are in the region of greater than or equal to 1 mg of PA/kg body weight (bw) per day.
- The lowest known dose known to be directly associated with long-term toxicity is reported to be 15 µg of PA/kg bw per day.

17

Pyrrolizidine alkaloids

Toxic effects of comfrey

- In 1993 report by Committee on Toxicity of Chemicals in Food (COT) to Food Advisory Committee and t Ministry of Agriculture, Fisheries and Food looking through latest research on potential acute toxicity by PAs, led to voluntary withdrawal from sale of all products containing **comfrey root** (*Symphytum officinale* radix – which contains large amounts of toxic PAs) for internal use from sale in UK, and advice issued that preparations of root and leaves should be labelled with warnings against ingestion.
- At the time, it was considered by COT that comfrey tea contained relatively low concentrations of PAs and did not need warning labels.

18

Pyrrolizidine alkaloids

Toxic effects of butterbur

- In January 2012, the MHRA asked for the voluntary withdrawal of all products containing butterbur (*Petasitis hybridus* – a herb that contains small amounts of toxic PAs).
- The reason given was: "...it is the Agency's view that herbal products such as butterbur (*Petasites hybridus*) which contain unsaturated pyrrolizidine alkaloids (PAs) and are intended for internal use in humans may pose a serious risk to public health and it would not be appropriate for individuals to continue to use such products.

19

Pyrrolizidine alkaloids

Toxic effects of butterbur

- The MHRA advice continued: "... Butterbur products have been associated with cases of liver toxicity; a total of 40 cases have been reported in the literature. Of these cases, nine cases were of acute hepatitis and two of the nine cases resulted in liver failure requiring transplantation. The cases of liver toxicity appear to have occurred with extracts of butterbur where the PAs had been removed and only small amounts remained."
- "There is some evidence that other constituents found in Butterbur such as the sesquiterpene constituents such as petasin may be implicated in the liver toxicity."

20

Pyrrolizidine alkaloids

BfR

- The German Federal Institute for Risk Assessment (BfR) published their opinion on PAs in herbal teas and teas in 2013 (BfR opinion no. 018/2013 of 5 July 2013. Pyrrolizidine alkaloids in herbal teas and teas: http://www.bfr.bund.de/cm/349/pyrrolizidine-alkaloids-in-herbal-teas-and-teas.pdf).
- The BfR stated that short-term intake of some herbs that contain low levels of PAs (up to 14 days) may not pose a health risk, but that this has not been sufficiently tested.

21

Pyrrolizidine alkaloids

BfR

- However, the report stated that there is a greater risk for:
  - Long-term consumption of herbal products with high PA content.
  - Frequent consumption of large quantities of herbal tea made from herbs that naturally contain PAs.
  - Patients who have PAs in their diet from food sources (which will include many people in the UK).
  - Children, pregnant women and breast feeding mothers.

22

Pyrrolizidine alkaloids

BfR

- The recommendations from BfR were commented on in a recent paper (Schulz et al. 2015) suggesting that maximum long-term **daily** exposure should be less than 0.007 µg/kg bw or 0.42 µg/62 kg bw, in order to avoid possibility of liver cirrhosis and pulmonary hypertension
- Cirrhosis and pulmonary hypotension may not appear until many years after exposure, so may not be picked up by yellow card notification schemes.

23

Pyrrolizidine alkaloids

BfR

- Unfortunately, long-term follow-up data or epidemiological studies to assess whether exposure to 1,2-unsaturated PAs can cause in cancer in humans are not currently available. However, animal models show a clear causative link which is of major concern.
- As for cirrhosis, liver cancer caused by liver damage would not be expected to manifest for many years (possibly 20-30 years) after initial exposure. This again makes a clear correlation difficult to find.

24

Pyrrolizidine alkaloids

BfR

- However, all tested 1,2-unsaturated PAs are genotoxic.
- All 1,2-unsaturated PAs that have been tested caused tumours in rodents, especially in the liver.
- Based on the available genotoxicity and carcinogenicity data, it is concluded that all 1,2-unsaturated PAs have the potential to be carcinogenic in rodents, and could also be in humans. Such potential would be expected at low, chronic doses.

25

Pyrrolizidine alkaloids

FSA testing

- Food Standards Agency (FSA) tested a range of honeys, herbal teas and herbal products which were reported in December 2015. The results of testing of two comfrey leaf and one borage tea product are of concern as they show large amounts of PAs present (as would be expected) at a potentially toxic level.
- Current recommendation by FSA is to assume no level of PA ingestion that is without risk, i.e. there is no safe level, and therefore a "tolerable intake level" cannot be established.
- Exposure should be reduced to as low as reasonably practicable (ALARP).

26

Pyrrolizidine alkaloids

EHTPA response

- With the new research published on PA toxicity over the last couple of years and with the recommendations from the FSA for herbal teas, the EHTPA with the support of the BHMA, issued advice to its member practitioner associations in February 2016, advising that all use of PA-containing herbs for internal use should be suspended until more research could be undertaken to assess their safety.
- However, the use of PA-containing herbs for topical use on unbroken skin for a maximum of 21 days was deemed safe, as transdermal PA absorption is believed to be low.

27

Pyrrolizidine alkaloids

EHTPA response

- All EHTPA professional associations (CPP, RCHM, URHP, APA and BATM) agreed to immediately suspend the internal use of all PA-containing herbs until more information regarding toxicity issues could be gathered.
- Although the EHTPA are experts in herbal medicine, we have commissioned independent reviews from academic specialists on the safety of PA-containing herbs. These opinions will be used to construct a permanent position statement.

28

Pyrrolizidine alkaloids

EHTPA response

- Unfortunately the NIMH (which left the EHTPA last year) has issued various bulletins to its members countering the EHTPA statement, advising that PA-containing herbs are still safe to use internally for no longer than 6 weeks (but should not be given to pregnant women or children).
- The EHTPA sees this position as unfortunate, particularly given the latest HMPC public statement on safe PA levels (31st May 2016):

29

Pyrrolizidine alkaloids

EHTPA response

- "A contamination level of herbal medicinal products leading to a daily intake of maximum 1.0 µg PAs/day during a transitional period of 3 years is acceptable from a public health point of view..."
- "During this time period the producers of herbal medicinal products should take actions necessary to reduce the contamination to a level leading to a daily intake not exceeding 0.35µg PAs/day."
- Although HMPC statement refers to contamination issues, we believe that it is essential to provide same limits to use of PA-containing herbs.

30

Pyrrolizidine alkaloids

EHTPA response

- For comparison, a cup of comfrey leaf infusion (2-4 g of dried herb, 0.02-0.18 % PA w/w) would be expected to contain between 0.4-7.2 mg of PAs - this is between **400-7,000 times** interim HMPC daily limit of 1.0 µg.
- Comfrey root infusion (2-4 g of dried root, 0.25-0.29% PA w/w) would be expected to contain between 5.0-11.6 mg of PAs – **5,000-11,600 times** daily HMPC limit!

31

Pyrrolizidine alkaloids

Supply concerns

- This is obviously of huge concern to the EHTPA and BHMA, and the issue of the sale of PA-containing herbs by herbal suppliers in the UK is a key part of the HPSS initiative by the BHMA, which Simon Mills will be talking about later.
- Indeed a practitioner recently bought comfrey root tincture from a UK herbal supplier which was not labelled appropriately (for external use only on unbroken skin). They had the tincture tested for unsaturated PA content, and the results showed the level as 100,000 µg/kg. If taken internally, this would provide a daily dose equivalent to 0.42 mg of PAs (400 times above HMPC recommended levels).

32

Pyrrolizidine alkaloids – a practitioner's perspective

Dr Christopher Etheridge

info@chrisetheridgeherbalist.co.uk

ehtpa
European Herbal & Traditional Medicine Practitioners Association