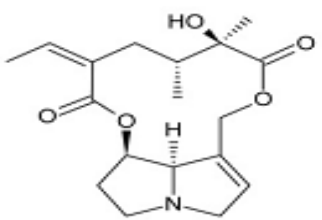
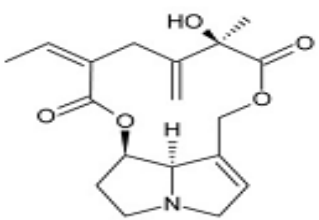
Pyrrolizidine Alkaloids in Western Herbal Medicine



Senecionine



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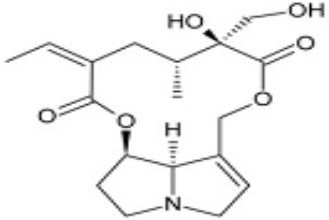

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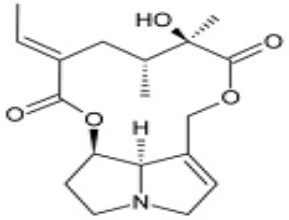

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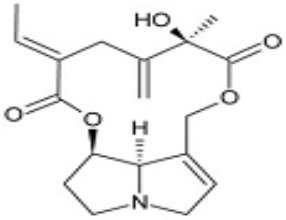
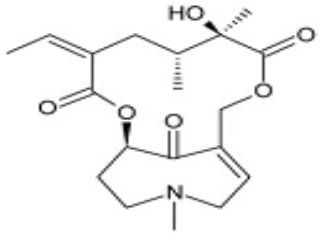


Spartiodine



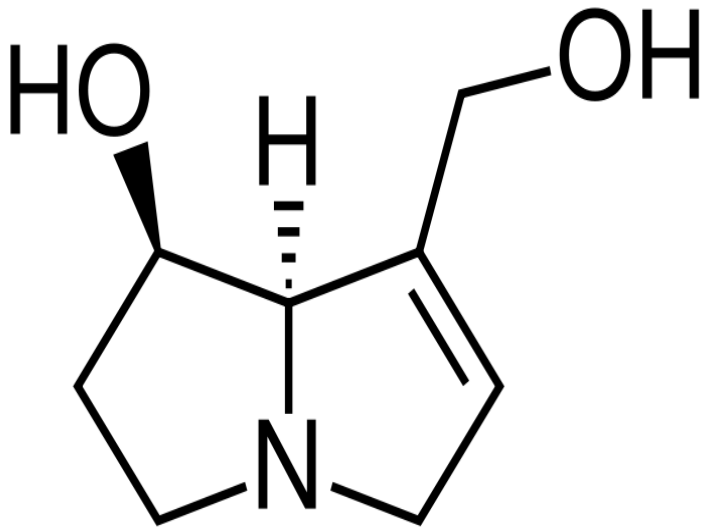
Senkirkine

What are Pyrrolizidine Alkaloids?

Pyrrolizidine Alkaloids (PAs) are a group of secondary metabolites produced by around 3% of all plant species. To date PAs have been recorded in over 6000 plants belonging to 13 plant families including the Asteraceae, Convulvulaceae, Orchidaceae, Poaceae, Fabaceae and Boriginaceae families in particular (Smith & Culvenor, 1981).

The Chemistry of PAs

- PAs are derived from the amino acid ornithine.
- PAs are composed of 2 fused, 5-membered rings with a nitrogen atom (N) common to both rings.
- PAs consist of an amino alcohol (called the necine base) and necic acid.
- PAs are esters of either hydroxylated 1-methylpyrrolizidine or otonecine-type necine bases.



PAs are NOT all the Same!

- Different types of PAs form in plants due to varying combinations of pools of **necine bases** and pools of **necic acid**, as well as the formation of **monoesters** at different positions and open or cyclic **diesters** (Figure 1).
- There are two forms of pyrrolizidine alkaloids: N-oxide and tertiary base types (EFSA, 2011). Over 660 types of pyrrolizidine alkaloids have been discovered so far falling into two main categories – saturated PAs and unsaturated PAs (Robertson & Stevens, 2017). Of these, only 100 types have been associated with any toxicity (Roeder, 1995).

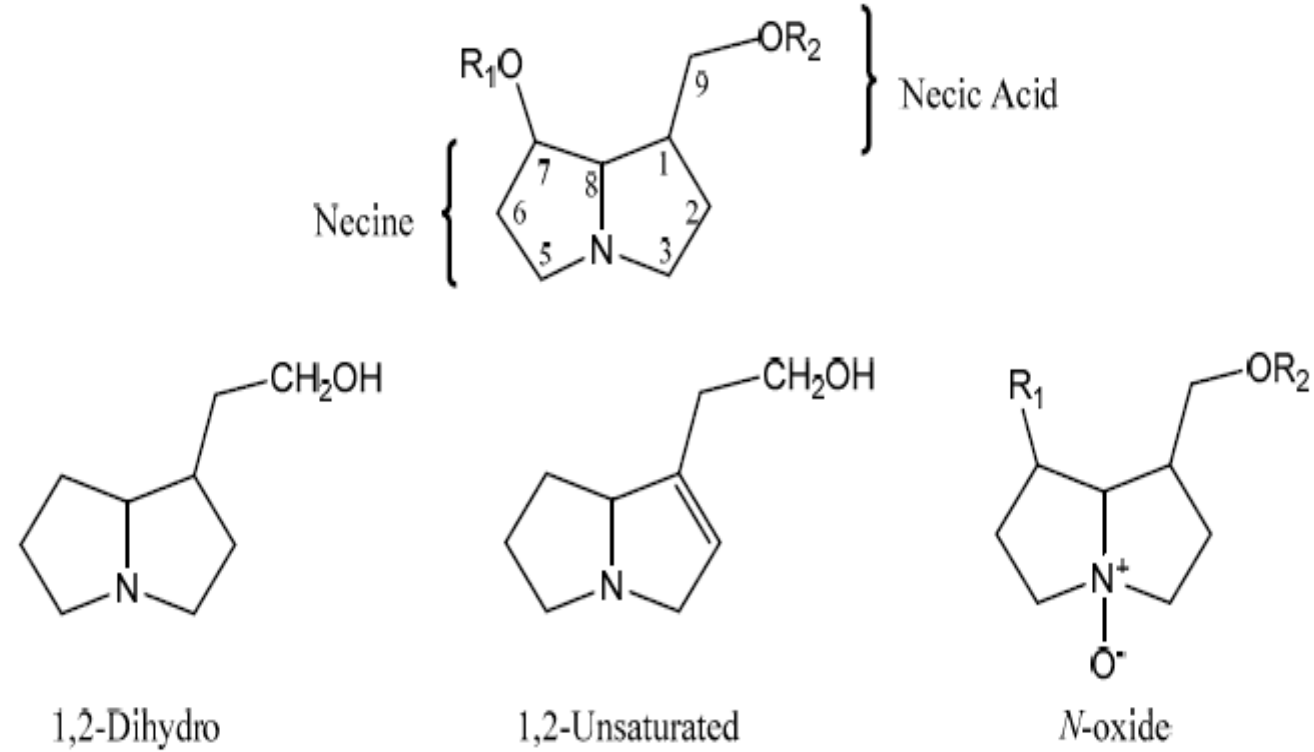


Figure 1. Structure of a PA and its different forms. R_1 and R_2 correspond to different necic acids.

Some PAs, such as Retronecine, Heliotridine and Otonecine types, display a double bond at the 1,2 position (Figure 2), which may result in increased toxicity (Moreira et al., 2018). There are three pathways involved in the metabolic activation of PAs: hydrolysis to produce necines and necic acids; N-oxidation to form PANO; and oxidation that leads to the formation of pyrrolic esters or dehydropyrrolizidine alkaloids (DHPA). Cytochrome P-450 (**CYP450**) monooxygenases (CYP3A and CYP2B in particular) are involved. The activity of these enzymes can partly explain the distinct susceptibility of different species to PAs.

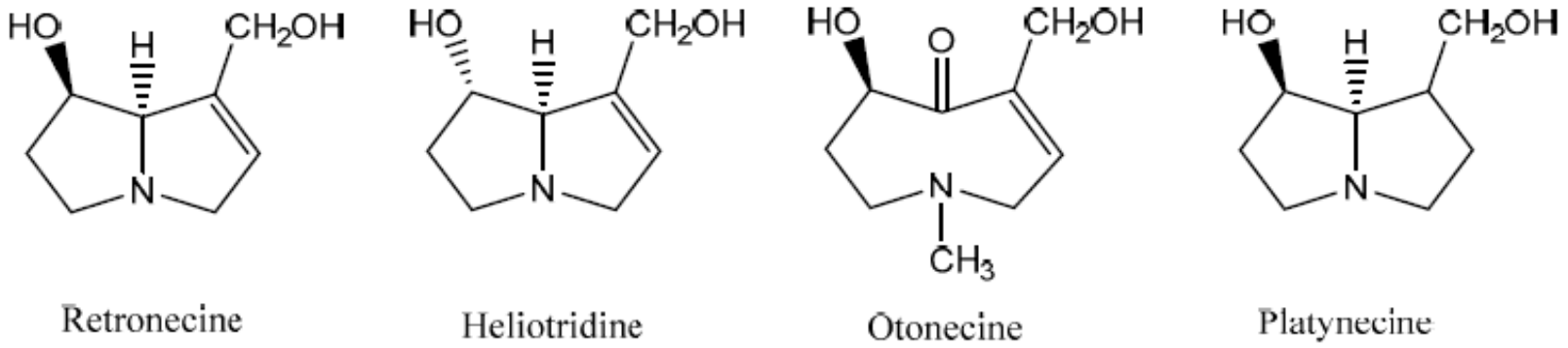


Figure 2. Groups of PA, according to the necine base.

Saturated or Unsaturated?

- No concerns have been raised in the literature about saturated PAs. However, **unsaturated PAs** may be toxic depending on their form, their quantity, and the presence or absence of other phytochemicals.
- Some forms of unsaturated PAs are thought to be more toxic than others. For example, riddelliine, lasiocarpine and monocrotaline are among the more toxic forms (Field et al., 2015).
- Not all PAs lead to the synthesis of toxic metabolites. Only the esters of 1,2-unsaturated retronecine and otonecine types, which are bioactivated by CYP450 enzymes to dehydropyrrolizidines (DHP) are toxic (Prakash et al., 1999).

Good Science?

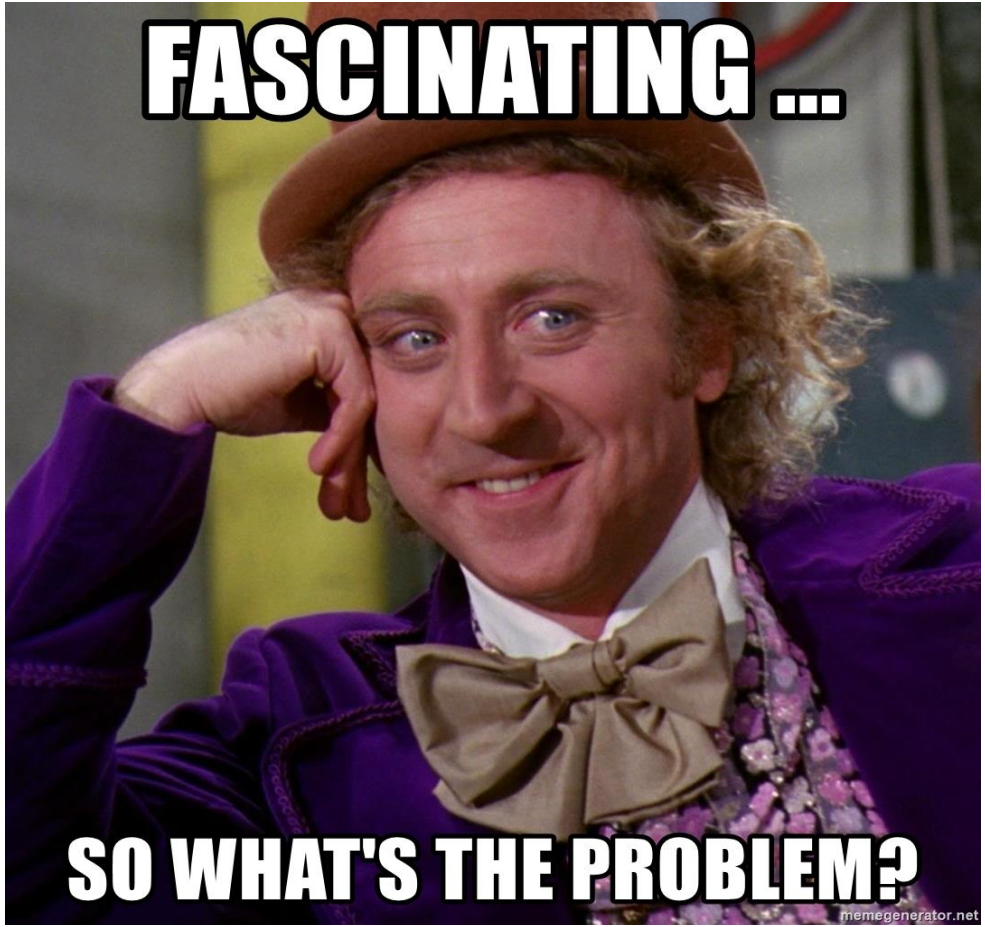
- To study unsaturated PAs it makes sense to appreciate that there are different types and variable levels of toxicity associated with each type. For example, only riddelliine has been classified as a potential human carcinogen by the National Toxicology Program in the US (Field et al., 2015).
- However, there has been a tendency in the literature to consider all unsaturated PAs to be equally toxic and to use the most toxic forms as a benchmark for establishing safety levels in all types. This is not sound science. Proper differentiation is required between each type of unsaturated PA.

Quality & Quantity

- As well as appreciating the potential toxicity of each unsaturated PA; it is also important to consider the quantity of each type in a given plant species. Some plant species have relatively higher quantities than others, and so some species can be regarded as being more toxic than others.
- However, what is even more fundamental to herbalists, is that plants are not merely vehicles for PAs. They contain many other primary and secondary compounds as well, some of which may mediate or serve as a check to the potential toxicity of other compounds, such as PAs.

Herbalists do not use PAs!

- Herbalists do not use isolated PAs. We use whole plant preparations or complex extractions which contain multiple constituents often in combination with other plants such as in polyherbal tincture formulations.
- This is an important consideration as much research data used to establish PA “safety margins” is gleaned from animal experiments using isolated PAs in their particularly toxic forms, and often at unrealistic dosages. Furthermore, some species have greater susceptibility than others so consolidating data from among multiple models is flawed.



The question is, how appropriate is it to rely on data using isolated PAs in their most toxic forms fed to susceptible animals at unrealistic doses in contrived conditions which lack external validity as a benchmark for any plant that happens to contain unsaturated PAs in any quantity used by humans?

The Context of Risk

- It has been proposed that some unsaturated PAs may provoke Sinusoidal Obstruction Syndrome (aka Veno-Occlusive Disease) potentially leading to Hepatocellular carcinoma and other genotoxicities. This theory was first proposed following several outbreaks of mass poisoning thought to be caused by consumption of plants containing high levels of especially toxic types of PAs (Schoentel, 1968).

- Reports of alleged PA poisoning in livestock were recorded as far back as 1903. Reports of PA poisoning in humans were first suspected in the 1920s. The plants involved in these cases were from Senecio and Heliotropium spp. (Wiedenfeld & Edgar, 2010).
- Schoentel (1968) was the first to suggest that regular consumption of PA-containing plants, such as Senecio and Heliotropium spp., could lead to liver cancer, but he thought that a "ripening process" was necessary, implying repeated and regular consumption of such plants was an important factor in potential mutagenicity. Cases in impoverished countries were often attributed to regular consumption of "bush teas" for example.

- *There are 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 cannot be proven because the outbreak of the liver disease and a possible ingestion of PA-containing material is often separated by a long time period* (Wiedenfeld & Edgar, 2010).

Contamination of Foodstuffs

- Many common foods such as milk, honey, meat, eggs, salad crops and herbal teas can become contaminated with known toxic species (Wiedenfeld & Edgar, 2010). In some cases the highly mechanised and undifferentiated harvesting of monoculture crops can be the source of the problem. Despite contamination of herbal teas and foods no cases of hepatotoxicity have been correlated, and risk-assessment is difficult.
- Attention turned to herbal medicines in the late 80s when PAs became a topic of concern for herbalists (IPCS, 1988). The problem for herbalists was that the species confirmed as toxic were not used by herbalists. Herbs such as Comfrey, Borage and Coltsfoot on the other hand had no history of toxicity at this time.

Mechanisms of Toxicity

- If Schoentel is correct, that a ripening process is necessary to generate hepatotoxicity, then short term use would be less problematic than long term use.
- The mechanism of toxicity was proposed by Mattocks (1986). Unsaturated PAs are initially harmless until they are metabolically activated by CYP450 pathways in the liver.
- Once PAs pass through Phase I of liver detoxification pathways they form **Pyrroles**, an unstable intermediary which is potentially toxic. Essentially, pyrroles are reactive oxygen species capable of generating oxidative stress.

Animal Research?

More recent research has established that on metabolic activation certain PAs can exhibit a variety of genotoxicities, including DNA binding, DNA cross-linking, DNA-protein cross-linking, sister chromatid exchange, chromosomal aberrations, mutagenicity, teratogenicity, and carcinogenicity (Fu et al., 2004). However, in this review only those PAs exhibiting the highest toxicity in animal experiments were considered. Such research has been used to determine margins of exposure levels in humans.

Questioning Animal Research

- There are multiple problems with using animal models to determine human responses. Animals are a different species, and some animals have greatly varying metabolisms. Some animals, such as cows, are very susceptible to PAs while others, such as Guinea pigs, have greater tolerance. Extrapolation from one species to another is deeply flawed, while summarizing data from multiple species is similarly contentious.
- Lab animals are inbred and more susceptible than their wild counterparts. Experiments are carried out in conditions which lack external validity and do not reflect the living world. Lab animals are sometimes fed doses that are above and beyond the levels commonly found in herbs and foods. Inducing cancer by such artificial means is not a reflection of how cancer is generated in the living world with all its multiple variables.

Researcher Comments

- *"Our experience … illustrates that it is not possible to assess the toxicity of a plant from the toxicity of the isolated alkaloid"* (Schoentel, 1968).
- *"The findings highlight that direct comparison between animal and human results is not always possible. For example, the PA-induced tumourigenicity previously reported for animals has not, to this day, been demonstrated in humans"*(Moreira et al., 2018).

- *“Based on these data it is not possible to establish a concrete lethal dose of particular PAs for each ruminant species, the dosages and especially the duration of administration are too variable. On the other hand these experiments give clear evidence for the fact that indeed the susceptibility for PA intoxication is different in individual species: in case of ruminants it is decreasing from cattle (which seem to show the highest sensitivity) to goats and sheep which are the most resistant animals discussed in this overview presumably due in large part to their different rumen microflora”* (Wiedenfeld & Edgar, 2010).

In Vitro Human Research

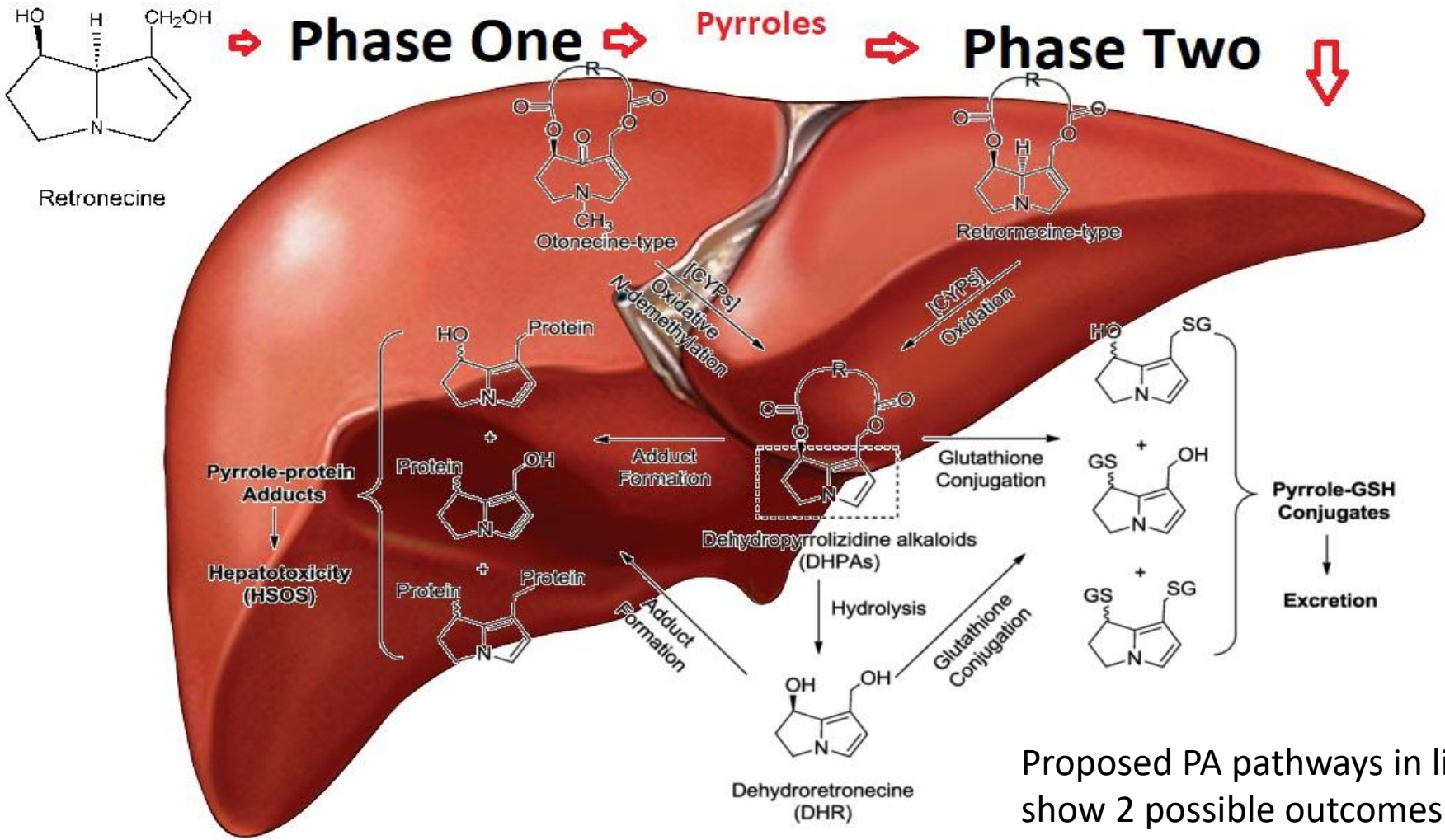
- In vitro research using human umbilical vein endothelial cells determined that lasiocarpine and senecionine resulted in significant cytotoxic effects when metabolically activated. However, no effect was observed for the PAs echimidine, heliotrine, lasiocarpine, senecionine, senkirkine and platyphylline without metabolic activation. This confirms that toxicity is metabolism-dependent in vitro (Ebmeyer et al., 2019). Extrapolation from in vitro to in vivo is as deeply problematic as extrapolation from animal research.

Theories of Protection

- Research is clear that the potential of PAs to be hepatotoxic is strongly influenced by individual metabolic activity. Given the absence of toxicity in the herbal historical record, is there any evidence to suggest that there are other factors which may prevent reactivity? Let us consider the proposed mechanisms of toxicity, pharmacodynamics and pharmacokinetics.

Polyphenols & DNA Cross Linking

- The proposed mechanism of toxicity begins with DNA cross linking, however the body has mechanisms to check DNA cross linking, such as Nucleotide Excision Repair (NER). NER can be enhanced by polyphenols at low concentrations (Azqueta & Collins, 2016).
- Comfrey (Trifan et al, 2018), Borage (Segovia et al., 2014) and Coltsfoot (Dobravalskytė et al., 2013) contain polyphenols, however further research is required to elucidate this protective mechanism.



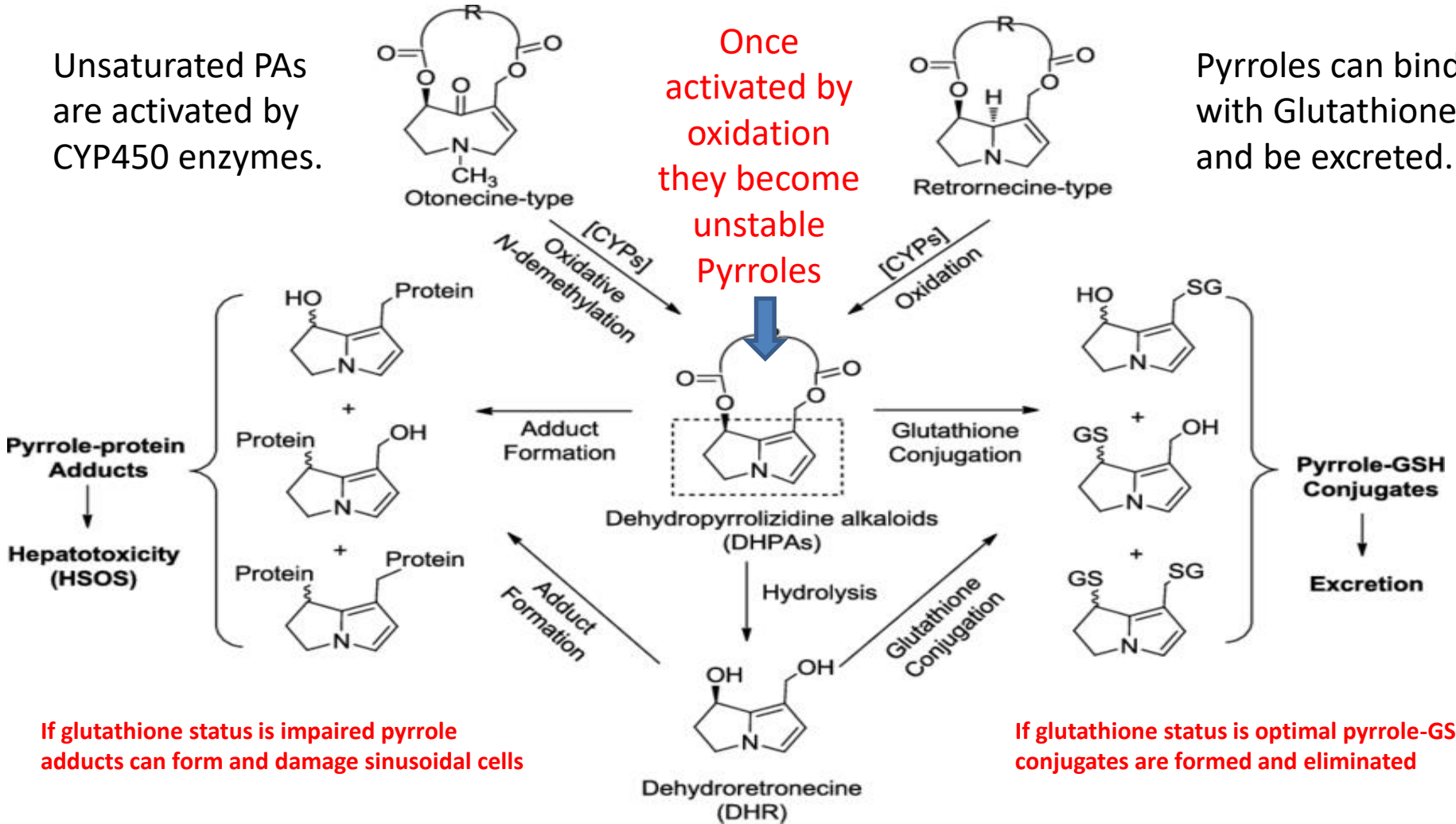
Proposed PA pathways in liver show 2 possible outcomes

- Rode (2002) summarised the problems with extrapolation from animal experiments, and considered the centrality of hepatic status. She cites Anderson & McLean (1989) who determined the serum concentration of AST, GGT and bilirubin in 29 long-term comfrey users, and AFP in a subgroup of seven comfrey users. Although this cohort was too small to ascertain risk, it was interesting that AST, GGT, bilirubin and AFP were considered normal, even after prolonged consumption of comfrey leaf (0.5–25 g day−1 for 1–30 years). Rode contends that this is where future research should be focused.
- Habs (2017) contends it is obvious that hepatic toxicity of monocrotaline is triggered by an overload of the detoxification systems of the liver. This finding suggests a threshold dose for this type of toxicity. The toxicities of individual PAs are also based on the pattern of expression and the selectivity of the CYP isoforms present. Inter-individual enzyme concentrations of liver CYP3A4 and CYP3A5 vary over a 30-fold range.

Unsaturated PAs are activated by CYP450 enzymes.

Once activated by oxidation they become unstable Pyrroles

Pyrroles can bind with Glutathione and be excreted.

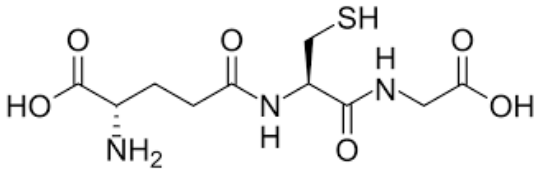


If glutathione status is impaired pyrrole adducts can form and damage sinusoidal cells

If glutathione status is optimal pyrrole-GSH conjugates are formed and eliminated

- This protective ability of the liver stems from the expression of a wide variety of xenobiotic biotransforming enzymes whose common underlying feature is their ability to catalyse the oxidation, reduction and hydrolysis (Phase I) and/or conjugation (Phase II) of functional groups of drug and chemical molecules (Grant, 1991). The toxicity of unsaturated PAs is therefore dependant on Phase II conjugation pathways and glutathione status in particular.

Glutathione (GSH)



- GSH plays a critical role in protecting cells from oxidative damage and the toxicity of xenobiotic compounds, and maintaining homeostasis. If glutathione levels in the liver are appropriate, then sinusoidal cell damage from pyrrole adducts may not occur, or be limited. After these highly reactive metabolites are formed, they can bind to glutathione (GSH) to form GSH conjugates and in doing so, they can be eliminated.
- Glutathione requires the presence of the amino acid cysteine. Cysteine occupies a central position in plant metabolism because it is a reduced sulphur donor molecule involved in the synthesis of essential biomolecules and defence compounds (Romero et al., 2014).

Herbs high in Cysteine

- *Anethum graveolens*
- *Arctium lappa*
- *Artemisia dracunculus*
- *Ballota nigra*
- *Carum carvi*
- *Chicorum intybus*
- *Coriandrum sativum*
- *Equisetum arvense*
- *Euphrasia officinalis*
- *Ocimum bacillicum*
- *Origanum majorama*
- *Pimpinella anisum*
- *Thymus vulgaris*
- *Verbena officinalis*

Using herbs rich in cysteine in formulas with PA-containing herbs may further enhance glutathione production.

The herbs in the list contain modest to high levels of cysteine (USDA, 2019).

Levels can also be boosted with N-acetyl cysteine, selenium, zinc, vitamin C and Vitamin E.

The Alliums are a particularly good source of cysteine.

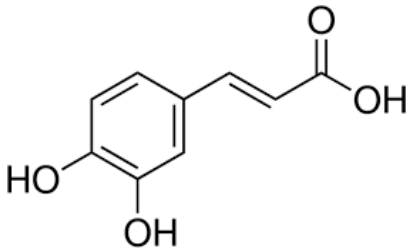
GSH Levels Determine Oxidative Stress

- Glutathione plays important roles in antioxidant defence, nutrient metabolism, and regulation of cellular events (including gene expression, DNA and protein synthesis, cell proliferation and apoptosis, signal transduction, cytokine production and immune response, and protein glutathionylation). Glutathione deficiency contributes to oxidative stress (Wu et al., 2014). Therefore, PA toxicity may be determined by GSH, but antioxidant compounds may also have a role.

Polyphenols as Antioxidants

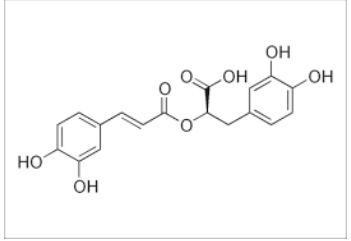
- Polyphenols inhibit certain enzymes involved in reactive oxygen species while they upregulate other endogenous antioxidant enzymes like superoxide dismutase (SOD), catalase, and glutathione (GSH) peroxidase (Px). Furthermore, they inhibit phospholipase A2 (PLA2), cyclooxygenase (COX) and lipoxygenase (LOX) leading to a reduction in the production of prostaglandins (PGs) and leukotrienes (LTs) and inflammation antagonism (Yafoufi et al., 2018).
- Comfrey, for example, contains polyphenols such as rosmarinic acid and also caffeic acid (isomers of salvianolic acid A, B and C) which may upregulate GSH. This suggests that comfrey contains metabolites which may act as a check to pyrroles by upregulating the production of glutathione (Trifan et al., 2018)

Caffeic Acid



- Caffeic acid is found in all plants as it is important in the synthesis of lignin, however levels in comfrey are small. But Comfrey also has modest levels of other antioxidant and anti-inflammatory constituents such as ascorbic acid, asparagine (especially in the roots), carotenes and rosmarinic acid.

Rosmarinic Acid



- Rosmarinic acid, an ester of caffeic acid, is one of several antioxidant polyphenols found in comfrey. The mechanisms by which it affords hepatocellular protection have been recently proposed (Elufiove & Habtemariam, 2019).
- Rosmarinic acid is also commonly found in plants in the Boraginaceae and Lamiaceae families.
- Comfrey also contains p-hydroxybenzoic, chlorogenic and p-coumaric acid which contribute to its overall antioxidant effect (Sowa et al., 2017).

How Do We Determine Safety?

- The only way we can determine whether or not medicinal herbs such as *Symphytum officinale*, *Borago officinalis* or *Tussilago farfara* are safe for medicinal use is to examine their consumption in humans in real world conditions.
- Constructing a database of case series can provide an evidence base that has external validity.
- The use of historical data is not anecdotal but should be considered as an evidence base of far greater importance than has hereto been afforded to it.
- Establishing therapeutic range by establishing LD50 (Lethal Dose in 50%) divided by ED50 (Effective Dose in 50%)

Efficacy & Effectiveness

- Several systematic reviews have determined the efficacy of comfrey as an external remedy (Oltean et al., 2014; Cameron & Chrubasik, 2013; Staiger, 2012). However, there is a gap in the literature for internal uses.
- While future research should concentrate on internal human studies, we need to remind ourselves that comfrey is a valuable therapeutic herb and any risks from internal use should not lead to its being proscribed, given its useful external uses.
- There is a need to epistemologically challenge the current hierarchy of evidence bases. If primary research is flawed so too is secondary and tertiary research. It can be argued that the majority of research is deeply flawed, yet legislation and clinical decision making is informed by it (Ioannidis, 2005).

Protocols

- We have determined that GSH is important, therefore the use of PA-containing herbs in patients with impaired GSH/liver function should be contraindicated, as should use in pregnancy, breastfeeding or in children. Intoxication is not only related to the amount and the duration of the exposure but also to age and gender: males are more sensitive than females, foetuses and children (IPCS 1988).
- If PA toxicity requires a ripening process, short term use may be viable. The German Federal Department of Health restricted the use of these preparations to 6 weeks and in a level of less than 1 g/day; if the use was prolonged in time, the daily limit should be reduced to 0.1 g
- The EMA recommends a maximum daily intake of 0.35 g PA/day for a person with a body weight of 50 kg and life-long exposure.

Cultivation & Preparation

- The Impact of Cultivation Conditions on Levels of Pyrrolizidine Alkaloids in Tussilago farfara (Lebada & Thorball, 2018).
- Cultivation, drying, cooking and other storage methods may lower PA levels in Comfrey and other herbs.

Concluding Remarks

- Requests were made to respective UK and Irish Health Departments in 2018 to ascertain the extent of reported adverse events from comfrey, borage and coltsfoot where a herbalist was involved. In both jurisdictions levels were zero. This may be due to under-reporting but it may also indicate that current recommendations among herbalists are satisfactory.
- A survey was then instigated to determine levels of use among herbalists and doses used as this information could further be helpful in determining safety levels. Independent sampling of tinctures and dried herbs purchased from leading manufacturers could correlate levels that might be used in practice.

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