REPORT ON THE SAFETY OF THE ORAL CONSUMPTION OF THE PYRROLIZIDINE ALKALOID CONTAINING HERBS *SYMPHYTUM OFFICINALE*, *TUSSILAGO FARFARA* AND *BORAGO OFFICINALIS*

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ABBREVIATIONS

ANZFA	Australia New Zealand Food Authority
ADR	Adverse drug reaction
CEBM	Centre for Evidence-Based Medicine
CIOMS	Council for International Organizations of Medical Sciences
COT	Committee on Toxicity
DILI	Drug induced liver injury
EMA	European Medicines Agency
HILI	Herb induced liver injury
HSS	Homospermidine synthase
HVOD	Hepatic veno-occlusive disease
ICSR	Individual case safety report
LFT	Liver function test
NIMH	National Institute of Medical Herbalists
PA	Pyrrolizidine alkaloid
PANO	Pyrrolizidine alkaloid N-oxide
QCRAEI	Quality of Case Reports of Adverse Events instrument
RP	Relative Potency
RUCAM	Roussel Uclaf Causality Assessment Method
TLC	Thin layer chromatography
UMC	Uppsala Monitoring Centre
VOD	Veno occlusive-disease
WHO	World Health Organization
WHO-IPCS	World Health Organization International Programme on Chemical Safety

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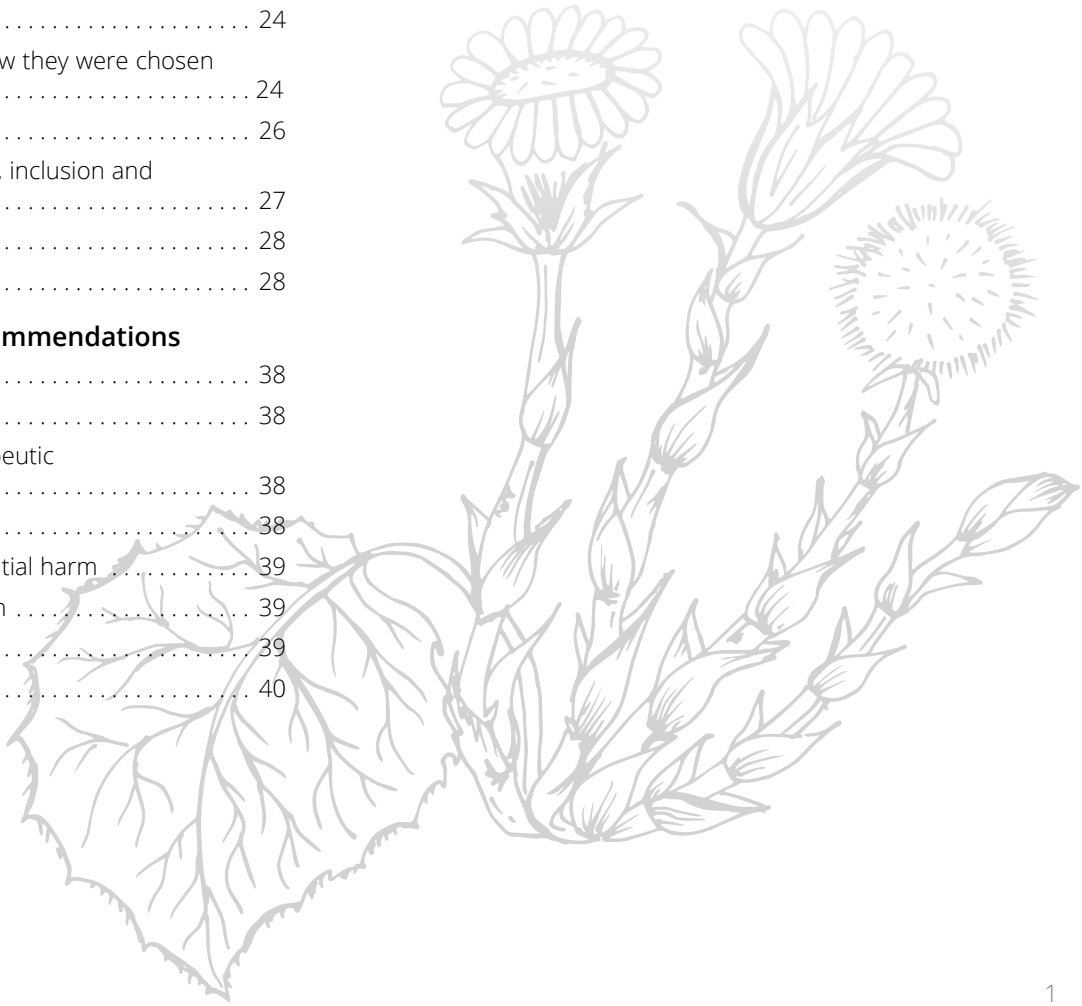

EXECUTIVE SUMMARY

Symphytum officinale (comfrey), ***Tussilago farfara*** (coltsfoot) and ***Borago officinalis*** (borage) have a long history of therapeutic use, but have been implicated in safety issues due to the presence of unsaturated pyrrolizidine alkaloids (PAs). These substances are increasingly seen as a public health issue due to their potential role in the development of chronic health issues, especially hepatic veno-occlusive disease and liver cancer. Consequently, a range of regulatory restrictions on their use have been put in place in many of those jurisdictions where these plants are used.

However, many herbalists are unconvinced about the toxicity of these plants which are highly valued for their established therapeutic use. The animal studies and case histories on which the initial concerns about these plants were raised were of poor quality and traditional texts rate comfrey, coltsfoot and borage as safe. The collective clinical experience of herbalists is at variance with suggestions of toxicity, and they question the need for restriction. The National Institute of Medical Herbalists (NIMH) has therefore requested expert opinion to be provided in relation to their safety for oral use.

This group has undertaken multifaceted analysis of the current evidence, which is presented below. What has been established beyond doubt is there is very little evidence from human studies of harm caused by the consumption of comfrey or coltsfoot and none was found concerning borage, as a whole herb. The best evidence is all low level, based on case studies. A systematic review details an exhaustive search and critical appraisal of the published case studies documenting harm associated with these plants. This examination included approaching the original authors of these studies to provide further information on which we could assess the potential of a causal relationship between the plants and the adverse events. While most of these cases involved patients' with pathologies characteristic of poisoning by PAs, direct causality to the herbs of interest could not be established. Neither could it be ruled out. In some cases, substitution or mistaken identity appear to have played a part and the poor quality of reporting was a feature in several reports. Two cases stand out; these involved exposure to PAs in utero, possibly comfrey in one case and coltsfoot in the other. What is certain in these cases was exposure to PA containing plants and the outcome – in each case the infant died.

Post-market surveillance data from the WHO adverse events database was also examined for incidences of potential human harm associated with use of these plants. The incidence rate based on oral consumption was extremely low.

A review of the phytochemistry of the relevant unsaturated PAs in these three plants and in related species reveals the complexity of this area of phytochemistry; there are numerous PA containing plants and numerous examples of the dangerous unsaturated PAs, a few of which occur in the plants of interest, ranging from the most toxic at very low levels in coltsfoot to the least toxic in higher concentrations in comfrey. Consistent with the human evidence, borage appears relatively benign.

The mechanism of toxicity has been established by *in vitro* and some *in vivo* studies, with many, but not all studies using isolated PAs. This involves a complex metabolic pathway leading to secondary metabolites, the dehydropyrrolizidine alkaloids. The extent to which these metabolites are formed varies species to species and within individuals of the same species. These studies suggest that dietary factors and the concurrent consumption of herbs or medications that act as cytochrome P4503A enzyme inducers may make individuals particularly susceptible to the potential toxicity of PA-containing herbs.

Reports of toxicity arising from misidentification, and the ongoing issues of adulteration in medicinal plant products as related to these plants are discussed. Examples of regulatory restrictions are provided and the evidence on which these decisions are based is discussed.

Recommendations made based on this body of evidence are necessarily cautious and they are in line with current NIMH advice to their members.

Certain populations should avoid using these plants, in particular pregnant and lactating women, those with poor hepatic glutathione status or selenium deficiency, and those with pre-existing liver conditions. Additionally, patients taking herbs or medicines that induce the cytochrome P4503A enzyme system or those found, when genotyped for the CYP3A4*22 allele, to be extensive metabolisers should not consume these plants. It is further suggested that adverse events arising from ingestion of these plants is a rare harm, and that a patient-specific risk:benefit analysis should always be conducted before these plants are prescribed.

There are many gaps in current knowledge base which impact our understanding regarding the safety of these herbs. These questions require further investigation and specific recommendations for a targeted program of research are made.

Symphytum officinale (comfrey)

Borago officinalis (borage)

Tussilago farfara (coltsfoot)

INTRODUCTION

The review

This review was commissioned by the United Kingdom's National Institute of Medical Herbalists (NIMH) for two general purposes: to provide advice regarding recommendations to members about safe herbal practice, and to inform the Institute's position regarding the regulation of UK herbalists' use of comfrey (*Symphytum officinale*), borage (*Borago officinalis*) and coltsfoot (*Tussilago farfara*).

1.1 Introduction

Comfrey, borage and coltsfoot have long been valued by herbalists and the public for their therapeutic benefits. However, they are also subject to scrutiny within regulatory bodies, as they are known to contain the phytochemicals pyrrolizidine alkaloids (PAs). Since the 1970s, concerns have been raised about possible serious adverse events consequent to the ingestion of plants containing PAs, particularly the development of veno-occlusive disease.

PAs are found in common foodstuffs. The European Medicines Agency (EMA) has identified milk, eggs, honey, pollen products, grain and meat as well as herbal products, including borage and comfrey consumed in salads, as sources of PAs. The EMA states that 'according to the published literature, it is possible that the average dietary daily intake might already be more than the amounts of toxic, unsaturated PAs which are seen to be safe' (European Medicines Agency, 2014). For these reasons they are the focus of ongoing international concern, with regulatory authorities now attempting to determine safe levels of PA intake.

The charge has been made that the PAs in these three plants have the potential to cause serious and long-term harm to patients, such that they should not be prescribed or taken. The suggestion is that this is a Type C adverse event, where the reaction is uncommon and a result of cumulative dose. This type of event is recognised as a major pharmacoepidemiological challenge, requiring consideration of a full range of evidence (Meyboom et al., 1997).

Current NIMH advice is that these herbs should not be used with pregnant/breastfeeding women, babies, children, teenagers (anyone under 18), or anyone with, or suspected of having, liver disease. Further, attention is drawn to glutathione status.

The question we have been asked to answer is whether the current assessment about these herbs is reasonable. When suspected adverse reactions to the plants are appropriately evaluated, are restrictions to their use required, or are they unnecessary? Is there a genuine risk to patients in using these herbs, and can that risk be managed or mitigated, or should they be banned? What is the evidence?

The review will be used to inform the advice provided by the NIMH to their members.

1.2 Background

It has been estimated that up to three percent of all flowering plants may contain pyrrolizidine alkaloids, and the vulnerability of grazing animals to some of these plant species has been known since the 18th century. Not all plants containing PAs are toxic to livestock. Those which have been identified as problematic include species from the *Senecio* (*S. jacobaea, S. plattensis*) and *Crotalaria* (*C. sagittalis*) *genera*. Their management is an ongoing agricultural problem in parts of Europe, the Americas and Australia (Culvenor, 1985; Edgar et al., 2011).

Episodes of acute human toxicity ascribed to the ingestion of PAs are not common, but they have been identified in developing countries including Afghanistan, Ethiopia, India and Jamaica. These cases have involved plants mostly from the *Heliotropium*, *Crotalaria* and *Senecio genera*. Such instances are sporadic, often occurring when food staples have been contaminated with seeds of plants with high levels of unsaturated PAs (Dharmananda, 2001; Edgar et al., 2011; Mohabbat, 1976; Tandon et al., 1976).

In 1988 a World Health Organization report recommended that member states should be aware of the toxicity of PAs and the hazards to human health of ingesting plants containing them, especially in the form of cereals or medicinal plants. The same report recommended assaying milk and honey where a transfer of PAs via feed may occur, for example in areas where weeds with a high PA content are common (IPCS, 1988).

The long-term effects of the consumption of PAs has been labelled an 'iceberg' problem, as it is understood that symptoms may take decades to appear. Human data regarding long-term exposure is difficult to gather, and so much of this understanding has been derived by extrapolation from animal data.

Low et al. (2017) point out that methods used for risk assessment are highly conservative in that they utilise 'worst case scenarios' in relation to the assumptions made for lifetime daily exposure of these foodstuffs. However some authors suggest that the low levels found in these common foodsuffs may be unrecognised causes of a range of serious health problems including hepatic veno-occlusive disease, cirrhosis, pulmonary arterial hypertension and cancer (Edgar et al., 2011).

Uncertainty is rife in estimating the exposure of populations to PAs, particularly in relation to herbal teas, and authorities err on the side of caution. Habs et al. (2017) suggest that PA-containing teas are likely to be safe, a perspective not shared by Colegate (2018). Not only are there variations among the PA content of plants of the same species, but the process of herb-tea making by the consumer may affect PA extraction. Factors such as water temperature, the length of time a plant is infused, water-to-tea ratio, and whether or not the tea is loose leaf are understood to affect extraction (European Food Safety Authority, 2016).

Of particular concern in the context of herbal medicine is that increased sensitivity and specificity of analytic techniques means that very low levels of PAs can now be identified and quantified (Cramer et al., 2013). This increased sensitivity of detection is likely to extend to plants not previously understood to contain PAs, as has recently occurred with low levels of intermedine and lycopsamine. These unsaturated PAs have been identified in *Eupatorium perfoliatum* (boneset), which has resulted in recommendations that its use be suspended due to the presence of dehydropyrrolizidine alkaloids (Colegate et al., 2018).

1.2.1 The PA-containing plants: comfrey, coltsfoot and borage

In the UK, all three herbs are highly valued by herbal practitioners for their medicinal use. While some herbalists avoid the use of these three herbs, others are wary of the research on which safety concerns have been raised. This group of authors questions the extrapolation of data from animal studies to humans. They also question the applicability of research into the activity of isolated PAs to their activity of whole plant extracts, which have a multiplicity of constituents (Whitelegg, 1996). They reject attempts to equate the action (and potential toxicity) of a specific isolated constituent with the action (and potential toxicity) of the whole plant. As (Whitelegg, 1992) comments:

> ***…one of the central tenets of herbal medicine [is] that an isolated chemical of a plant, while useful for certain indications, cannot define the action of the whole herb, where the herb is more than the sum of the individual parts, its constituents working synergistically to create its healing effects. (p.1)***

Two species of comfrey, *Symphytum officinale* and *S. x uplandicum*, have been implicated in this ongoing controversy. *S. officinale* has been a highly valued medicinal plant for many generations, and questions about its safety had not been raised until the 1980s. *S. x uplandicum* is a more recently introduced plant associated with a range of benefits in agriculture, food and medicine. The story of its development and introduction to the UK and the controversies that surrounded it from the 1970s are provided in Appendix 1.

The unique ability of comfrey particularly in tissue healing is widely recognised. Research continues into the development of non-PA containing variants (Schmidt, 2012) while a body of evidence continues to develop for its therapeutic application in this context (Sowa et al., 2018; Staiger, 2012).

Coltsfoot and borage have less popular and unique applications than comfrey, and have been less consistently researched. Borage leaves contain unsaturated PAs, raising concerns in some parts of Europe where these are used as a salad vegetable (European Medicines Agency, 2014). However research also indicates that its ingestion may be useful in gastric cancer prevention (Gonzalez et al., 1993; Lozano-Baena et al., 2016). Basar et al. (2013) suggest that the phenolic content in the leaves of *Borago officinalis* is more beneficial than it is harmful to human health and may mitigate against the PA content. This concept seems congruent with the assertions made by Whitelegg (1992) above.

1.2.2 International Regulation

International regulation of herbal products is inconsistent and fragmented. In the EU, India, New Zealand and the USA, herbs are regulated as food; in Australia and Canada they are regulated as medicines and in China and Japan they are regulated as health foods (Low et al., 2017). Concerns about ingestion of PA-containing plants have led to the prohibition of comfrey, coltsfoot and borage in Australia (Poisons Standard, 2018), the restriction of sale of these plant products when they contain PAs in Canada and Austria, and limits in other parts of Europe (Canada, 2018; Low et al., 2017). Additionally a 2001 request by the FDA requested that herbal manufacturers in the US cease selling comfrey (Packer-Tursman, 2001).

1.2.3 Outline of this report

Initially, current understandings of the phytochemistry and toxicology of pyrrolizidine alkaloids are reviewed, before considering reports of adverse events from VigiBase, the WHO-UMC repository of suspected adverse event reports. A detailed examination of the case histories involving these plants is then undertaken.

The aim of the detailed examination is to assess whether the causation of adverse effects can be attributed to these plants or the PAs they contain. Risks to patients of the oral consumption of these herbs is reviewed utilising the data from relevant adverse event reports which have been published as case reports in peer-reviewed journals. The evidence is re-examined with the application of a validated and hepato-specific assessment of the original published and unpublished records. This provides a depth to our understanding of these events and builds a better picture of the safety issues, particularly around dose and duration of exposure.

Note:

Throughout this document, the plants are largely referred to by their common names – common comfrey (*Symphytum officinale*), Russian comfrey (*Symphytum x uplandicum*), coltsfoot (*Tussilago farfara*) and borage (*Borago officinalis*). 'Comfrey' refers to common comfrey.

This report considers borage leaf only. Borage oil, popularly used as a source of γ-linoleic oil, is not included in this discussion as it does not contain PAs (Dodson & Stermitz, 1986; Langer & Franz, 1997).

INTRODUCTION

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2.1 Phytochemistry

Pyrrolizidine alkaloids (PAs) are a diverse group of alkaloids with a characteristic necine base structure. This necine base is a fused 5/5 ring structure with a nitrogen atom in the fourth position and is thus a tertiary nitrogen base. In most naturally occurring PAs the C-1 position carries a hydroxymethyl group and the C-7 position carries a hydroxyl group. Commonly there is esterification of these hydroxyl and hydroxymethyl groups, resulting in various monoesters, open chain diesters and macrocyclic diesters (Roeder, 1999).

2.1.1 Structural Characteristics

Of particular relevance to the toxicology of PAs is the bond configuration in the C1,2 position of the necine base. A double bond in this position, combined with esterification with a minimum of one branched five carbon carboxylic acid, is required for the documented hepatotoxic, pneumotoxic, mutagenic and carcinogenic activities (Roeder, 1999). Diesters and the macrocyclic diesters have been reported as being more toxic than the monoesters (Stegelmeier et al., 2016).

Almost all PAs can also naturally form N-oxides, and most plants will contain a mixture of free base and N-oxide forms. This is particularly relevant to the pharmacokinetics of PAs as N-oxides are readily water soluble, as opposed to the free base forms (ibid).

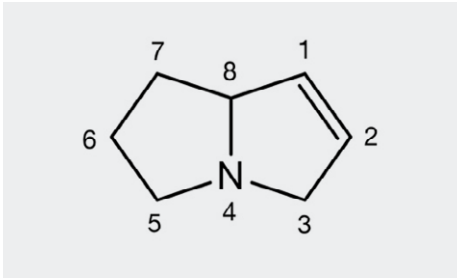

Figure 2.1 The 1,2-unsaturated necine base

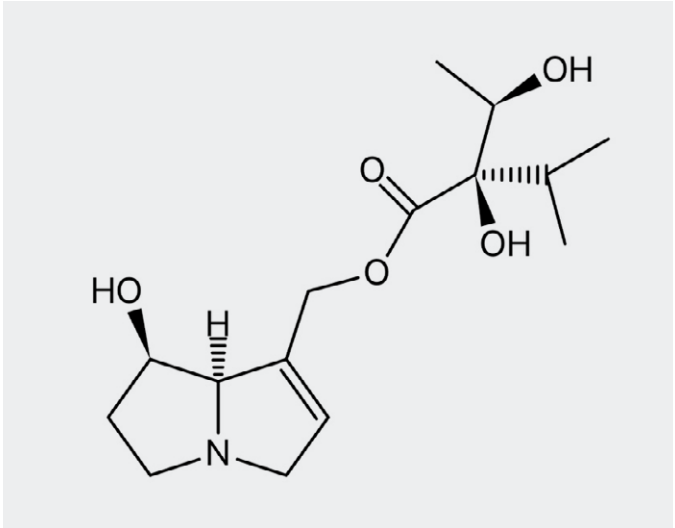

Figure 2.2 A 7*R* monoester (intermedine)

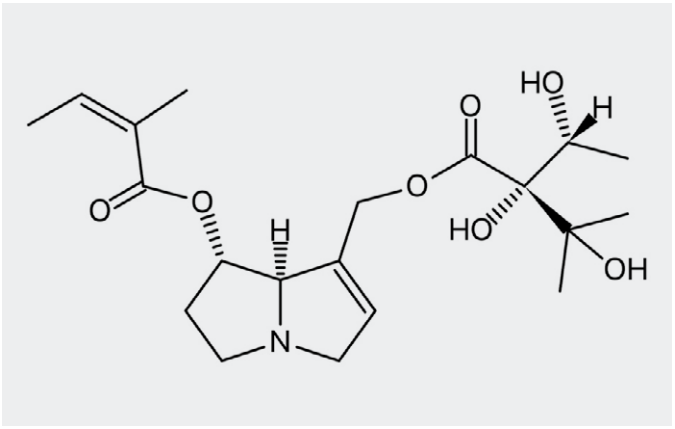

Figure 2.3 A 7*S* diester (heliosupine)

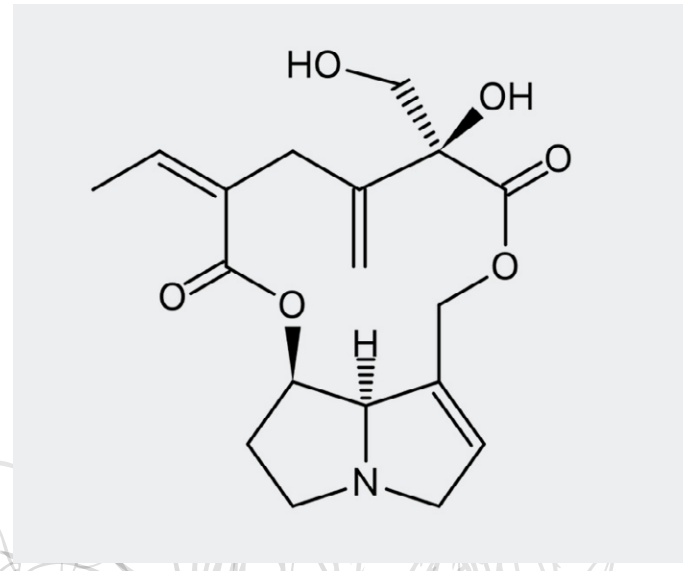

Figure 2.4 A 7*R* macrocyclic diester (riddelliine)

2.1.2 Plant Distribution

Pyrrolizidine alkaloids occur widely across a number of botanical families and genera. However, it is important to realise that not all of these genera produce the potentially toxic unsaturated pyrrolizidines, and thus the continued food and medicinal use of many of these is not a primary safety concern.

2.1.3 Biosynthesis

Extensive work has been conducted over the last four decades to determine the mechanisms of plant synthesis of PAs. Much of this work has been summarised by Herbert in a series of review papers on alkaloid biosynthesis published in *Natural Product Reports* between 1984 and 2003.

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Both the necine ring structure and the necic acids owe their origin to amino acid metabolism in the plant. L-arginine and L-ornithine are decarboxylated by arginine and ornithine decarboxylase, leading to the formation of the intermediate putrescine (Herbert, 1984). Homospermidine synthase subsequently catalyses the synthesis of homospermidine from two molecules of putrescine (Herbert, 2003), although spermidine can substitute for one of the putrescine molecules in this reaction (Herbert, 2001). Homospermidine is then cyclised to produce the iminium ion, with further reduction and cyclisation yielding a group of 1-hydroxymethylpyrrolizidines (Roeder, 1999). The 1,2 double bond in the unsaturated pyrrolizidines is created upon further hydroxylation and dehydration (ibid).

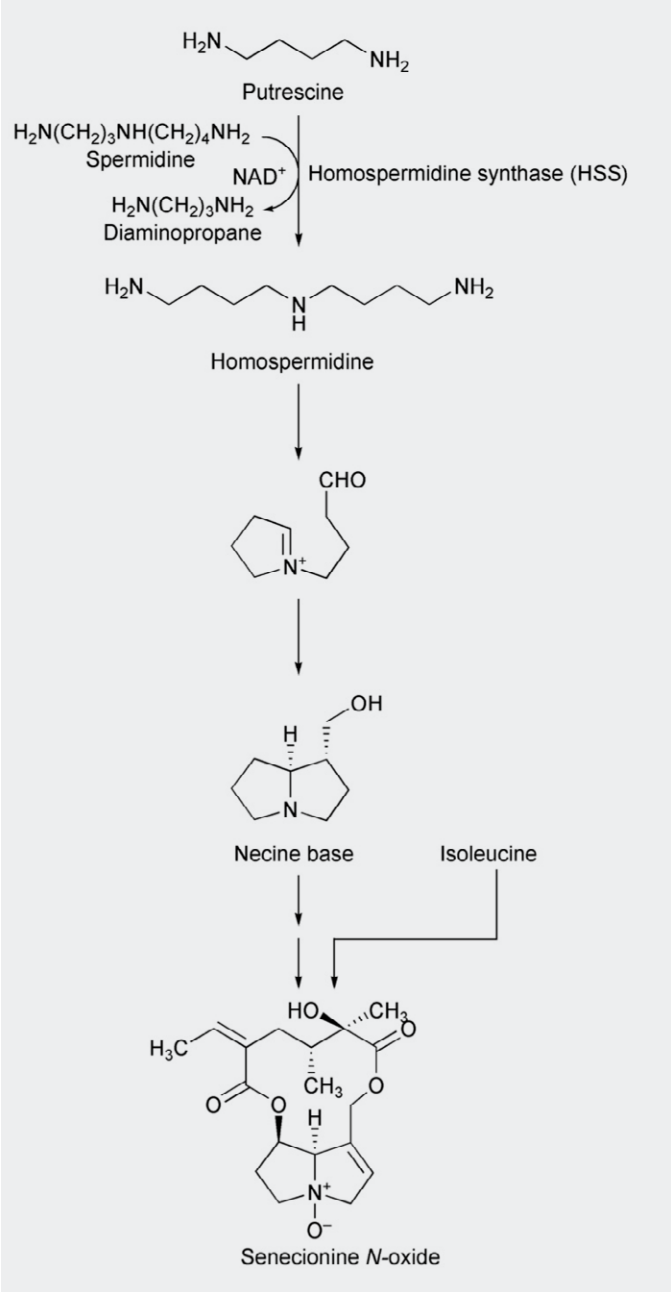

Figure 2.5 The biosynthesis of pyrrolizidine alkaloids using senecionine N-oxide as an example (Herbert, 2003)

The biosynthetic origin of the esterified necic acid moieties is less clear (Herbert, 2003). However the amino acids valine, isoleucine and threonine appear to be important precursors (Herbert, 1985; Roeder, 1999; Herbert, 2003).

2.1.4 Role in the Plant

Recent investigations into the biosynthesis of PAs in *Symphytum officinale* have pointed to a role in protecting plant reproductive structures from herbivore attack (Kruse et al., 2017). Previous investigations involving localisation of homospermidine synthase (Niemüller et al., 2012), as well as tracer experiments involving radiolabeling of putrescine (Frölich et al., 2007), suggest that the roots of *S. officinale* are the primary site of PA biosynthesis. However Kruse et al. (2012) demonstrate that homospermidine synthase (HSS) is expressed in young leaves directly below the inflorescence, with levels of HSS in the leaves decreasing as the distance of the leaves from the inflorescence increases. Additionally, they show that HSS expression was highest in earlier stages of flower bud development and decreased to lower levels in later stages of bud growth and flowering. Finally, they show that PA levels also increase in these subtended leaves from undetectable levels at the earliest stage of bud development to concentrations of approximately 180 micrograms/g of dry weight in later stages of flowering.

Kruse et al. (2012) also examine the PA content of the flowers across their lifespan, and show that at the earliest stage of bud development, PAs were undetectable. They show that PA content increased to a concentration of 3344 micrograms/g dry weight once all flowers have opened, and dropped to approximately 500 micrograms/g dry weight as the flowers withered and fruits developed. The high PA levels in the inflorescences did not correspond with increased HSS expression – in fact HSS expression within the inflorescence remained very low at all stages of development, suggesting efficient translocation of PAs from the young subtending leaves to the inflorescences.

The authors suggest that this report, combined with previous investigations into PA production in the flowers of *Phalaenopsis spp.* and the independent evolution of HSS in *Symphytum* and *Phalaenopsis*, indicate an adaptive response to protect reproductive tissues against attack by herbivores (Kruse et al. 2012).

Furthermore a symbiotic role for PAs has been demonstrated between the plants and various insects. Some insect species which feed on PA-containing plants have well-developed enzyme systems to maintain ingested PAs in their N-oxide state (thus eliminating toxicity to invertebrates), and some sequester PAs and accumulate them in exocrine defensive secretions (Lindigkeit et al., 1997).

Interestingly Honda et al. (2018) showed a significant role for ingested PAs in the reproductive activity of male danaine butterflies (*Parantica sita*). Adult male butterflies of this species have been observed massing on dead or decaying flowers of PA-containing plants, especially from the Asteraceae, Boraginaceae and Fabaceae families, and utilise ingested PAs in the biosynthesis of two PA-derived pheromones (danaidone and 7*R*-hydroxydanadial). Males of this species which were fed on intermedine, lycopsamine and retronecine (three unsaturated PAs) also had significantly higher rates of copulation than those fed only retronecine, indicating that specific structural features of some PAs are desirable (Honda et al., 2018).

2.1.5 Specific Alkaloid Occurrence and Species Variation

The table below is a compilation of PAs with an unsaturated 1,2-necine backbone, reported in *Borago officinalis*, *Symphytum officinale* and *Tussilago farfara*. In addition, other species have been included if they have been reported in the literature as possible adulterants of, or substitutes for, any of the aforementioned plants.

It should be noted that the table does not specifically name N-oxide variants of the named alkaloids as it is widely understood that interconversion occurs within plants.

Table 2.1 Specific alkaloid occurrence and species variation

Listed alkaloids all have unsaturated 1,2-necine backbone. Includes N-oxide variations as plants commonly interconvert. Where spp. is indicated, the alkaloids listed will not occur in all members of that genus. Genera other than *Borago*, *Symphytum* and *Tussilago* are reported adulterants or substitutes.	***Adenostyles alliariae, A. glabra***	***Borago officinalis***	***Crotalaria spp.***	***Heliotropium spp.***	***Petasites hybridus***	***Senecio spp.***	***Symphytum officinale***	***Symphytum asperum***	***Symphytum caucasicum***	***Symphytum tuberosum***	***Symphytum x uplandicum***	***Tussilago farfara***
adonifoline						5						
amabiline		4, 6, 8, 13, 14										
anacrotine			9			4						
anadoline										13, 14		
trans-anacrotine			5									
anonamine						5						
asperumine								14	13, 14			
boveinine				8								
croaegyptine			6									
crosemperine			6									
crotalarine			3, 6									
crotalarine lactone			6									
cynaustine												
doronenine						13						
echimidine							10, 14	2, 13, 14	13, 14	13, 14	2, 13, 14	
acetylechimidine								14				
echinatine				3			14		13, 14			
7-epidesacetylsenaetnine						5						
7-episenaetnine						5						
erucifoline						5, 13						
europine				3, 4, 7, 9								
floridanine						13						
florosenine						3, 13						
heleurine				7, 8								
heliosupine ** heliosupine is a 7S stereoisomer of echimidine, and it's reported occurrence in Symphytum is controversial*							14 *	14 *				

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Listed alkaloids all have unsaturated 1,2-necine backbone. Includes N-oxide variations as plants commonly interconvert. Where spp. is indicated, the alkaloids listed will not occur in all members of that genus. Genera other than *Borago*, *Symphytum* and *Tussilago* are reported adulterants or substitutes.	***Adenostyles alliariae, A. glabra***	***Borago officinalis***	***Crotalaria spp.***	***Heliotropium spp.***	***Petasites hybridus***	***Senecio spp.***	***Symphytum officinale***	***Symphytum asperum***	***Symphytum caucasicum***	***Symphytum tuberosum***	***Symphytum x uplandicum***	***Tussilago farfara***
heliotrine				1, 3, 4, 7, 8					14			
2'-acetylheliotrine				8								
ilamine				9								
indicine				13								
integerrimine					1, 11, 13	1, 4, 5						11
intermedine		8, 13, 14					2, 13, 14	13			2, 13, 14	
7-acetylintermedine		8, 13, 14					2, 13, 14	13			2, 13, 14	
isopterophorine						3						
isosenaetnine						3						
jacobine						5, 13						
jacoline						4, 13						
jaconine						13						
jacozine						13						
lasiocarpine				3, 4, 7					13, 14			
lycopsamine		4, 8, 13, 14					2, 13, 14	13		14	2, 13, 14	
7-acetyllycopsamine		8, 13, 14					2, 13, 14	13, 14		14	2, 13, 14	
madurensine			5									
monocrotaline			6									
myoscorpine							13	13			13	
neopetasitenine					11, 13							
neosenkirkine						3						
otosenine						1, 13						
petasitenine					11, 13							
retroisosenine						8						
(13*R*)-13-hydroxyretroisosenine						8						
(12*S*)-12-hydroxyretroisosenine						8						
retronecine				1, 6								
7-angeloylretronecine										14		
retrorsine						1, 3, 4, 5, 13						
riddelliine			3			1, 4, 13						
sceleratine						3						
senaetnine						3						
senecionine	13, 15				1, 11, 13	1, 3, 4, 5, 12, 13						1, 6, 11, 13
seneciphylline	13, 15					1, 3, 4, 13						11
acetyl-seneciphylline	15											
senecivernine	15					4, 12, 13						
senkirkine					1, 11, 13	1, 3						6, 11, 13

Listed alkaloids all have unsaturated 1,2-necine backbone. Includes N-oxide variations as plants commonly interconvert. Where spp. is indicated, the alkaloids listed will not occur in all members of that genus. Genera other than *Borago*, *Symphytum* and *Tussilago* are reported adulterants or substitutes.	***Adenostyles alliariae, A. glabra***	***Borago officinalis***	***Crotalaria spp.***	***Heliotropium spp.***	***Petasites hybridus***	***Senecio spp.***	***Symphytum officinale***	***Symphytum asperum***	***Symphytum caucasicum***	***Symphytum tuberosum***	***Symphytum x uplandicum***	***Tussilago farfara***
spartioidine	13, 15					13						
supinidine				1								
supinine		8, 13, 14		4, 7								
symlandine							10, 13	13		13	13, 14	
symphytine							2, 10, 13, 14	2, 13, 14		14	2, 13, 14	
symviridine							13, 14	13, 14			13, 14	
triangularine						4						
trichodesmine			6									
uplandicine											13, 14	
uspalatine						3,4						

1 Robins DJ (1984). Pyrrolizidine alkaloids. Natural Product Reports, 1(3), 235-243.

2 Huizing HJ, Gadella ThWJ, Kliphuis E (1982). Chemotaxonomical investigations of the *Symphytum officinale* polypoid complex and *S. asperum* (Boraginaceae): The pyrrolizidine alkaloids. Plant Systematics and Evolution, 140(4), 279-292.

3 Robins DJ (1987). Pyrrolizidine alkaloids. Natural Product Reports, 4, 577-590.

4 Robins DJ (1989). Pyrrolizidine alkaloids. Natural Product Reports, 6(3), 221-230.

5 Robins DJ (1993). Pyrrolizidine alkaloids. Natural Product Reports, 10(5), 487-496.

6 Robins DJ (1995). Pyrrolizidine alkaloids. Natural Product Reports, 12(4), 413-418.

7 Liddell JR (1998). Pyrrolizidine alkaloids. Natural Product Reports, 15(4), 363-370.

8 Liddell JR (1999). Pyrrolizidine alkaloids. Natural Product Reports, 16(4), 499-507.

9 Liddell JR (2001). Pyrrolizidine alkaloids. Natural Product Reports, 18(4), 441-447.

10 Liddell JR (2002). Pyrrolizidine alkaloids. Natural Product Reports, 19(6), 773-781.

11 Nedelcheva A, Kostova N, Sidjimov A (2015). Pyrrolizidine alkaloids in *Tussilago farfara* from Bulgaria. Biotechnology & Biotechnological Equipment, 29(S1), S1-S7.

12 Robertson J, Stevens K (2014). Pyrrolizidine alkaloids. Natural Product Reports, 31(12), 1721-1788.

13 Roeder E (1995). Medicinal plants in Europe containing pyrrolizidine alkaloids. Pharmazie, 50, 83-98.

14 El-Shazly A, Wink M (2014). Diversity of Pyrrolizidine Alkaloids in the Boraginaceae Structures, Distribution, and Biological Properties. Diversity, 6, 188-282.

15 Chizzola R (2015). Pyrrolizidine alkaloids in *Adenostyles alliariae* and A. glabra from Austrian Alps. Natural Product Communications, 10(7), 1179-1180.

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2.2 Adulteration, substitution and misidentification

Broadly speaking an adulterated herbal product can be defined as 'one in which the customer does not receive what he or she is led to believe to be purchasing' (Foster, 2011). This may be intentional, negligent or accidental. Substitution occurs when one plant is replaced by another, for example due to unavailability or price considerations. This may occur with the customer's knowledge and agreement; the problem arises when this is not the case. Misidentification occurs through a lack of appropriate botanical knowledge by the individuals harvesting the plants. Once misidentified species have been dried and chopped or powdered, it may be very difficult to establish misidentification without microscopic examination of the plant. If extraction has already occurred, misidentification may only be determined through broad phytochemical fingerprinting or screening for characteristic phytochemicals.

Adulteration has been, and remains, a significant challenge in herbal medicine, with initiatives such as the ABC-AHP-NCNPR Botanical Adulterants Prevention Program attempting to systematically address this issue in relation to herbal products (http://cms.herbalgram.org/BAP/index.html). For the three plants discussed (*Borago officinalis*, *Symphytum officinale* and *Tussilago farfara*) there are various reports of adulteration and substitution discussed in the literature.

2.2.1 Misidentification of *Borago officinalis*

Misidentification of foxglove (*Digitalis purpurea*) for both borage and comfrey has been recorded. While the flowers of these plants are distinct, the young leaves before flowering are similar. Symptoms of foxglove poisoning include hyperkalaemia, with patients often presenting with nausea, vomiting, diarrhoea and dizziness. Cardiac symptoms include sinus bradycardia and in more serious cases, heart block. Where symptoms are recognised in time and appropriate medications available, patients can make a full recovery. However, this is not always the case and fatalities have been recorded.

In a case report published in 2009, a 62-year-old father, 58-year-old mother, and their 35-year-old daughter were all unintentionally poisoned when they ingested potato dumplings flavoured with what they believed to be the leaves of borage grown in their garden. Upon presentation all three individuals presented with symptoms consistent with *digitalis* toxicity, and were treated accordingly, with subsequent recovery. Three samples taken from the family's garden and evaluated by the Medicinal Plant Biology Laboratory, Department of Drug and Science Technology, School of Pharmacy of the Turin University, confirmed that the plants were in fact *Digitalis purpurea* and not *Borago officinalis* (Maffè et al., 2009).

The leaves of *Borago officinalis* (left) and *Digitalis purpurea* (right) are easily confused before flowers appear.

2.6.2 Misidentification of *Symphytum officinale*

There are four additional species of comfrey discussed in the literature which have been either misidentified as, or used as a substitute for, *Symphytum officinale*. They include *S. asperum*, *S. caucasicum*, *S. tuberosum*, and *S. x uplandicum* (Roeder, 1995).

S. x uplandicum is a sterile hybrid of *S. officinale*, and their appearance is very similar. Differences are often apparent to those who cultivate the two species, with *S. officinale* having more narrow leaves, *S. x uplandicum* often growing more vigorously, as well as *S. officinale* producing viable seeds while *S. x uplandicum* needs to be propagated by root division. However, variation between plants is such that this may not be easily apparent.

Whilst there are some variations in the type of unsaturated pyrrolizidine alkaloids occurring in different species as illustrated in the previous table, there are also significant similarities. Another factor to be considered is total alkaloid concentration variations between species, between different parts of the same species (Roeder, 1995) and different harvest times (Kruse et al., 2012). The level of variation is such that it has been suggested that some comfrey species should not be used, whilst also suggesting *S. tuberosum* as a suitable alternative to other species for medicinal usage (Roeder, 1995).

A case of accidental *digitalis* poisoning was reported in 1985, involving a 70-year-old previously healthy man who ingested a tea made from what was thought to be comfrey leaves gathered locally. His symptoms were consistent with *digitalis* toxicity, and he recovered completely 10 days after admission to a hospital. Subsequent examination of a sample of the plant harvested confirmed that it was *Digitalis* and not *Symphytum spp*. (Bain, 1985).

Colls (1999) reports on three flatmates sharing a salad made from a plant in their back garden which one believed was Russian comfrey, but in fact was foxglove. They fell ill within six hours, and the severity of their symptoms correlated with the amount of the salad they reported consuming, with the patient who consumed most becoming most ill. All three were discharged after 6-12 days, again in correlation to the amount of salad consumed.

In a case reported by Flanagan and Harruff (2016) in the Annual General Meeting of the United States and Canadian Academy of Pathology, a previously healthy 69-year-old woman died after mistakenly ingesting *Digitalis purpurea*. She was including what she thought was *Symphytum officinale* leaves in smoothies and tea, which she had personally gathered in its fresh state, and botanically misidentified. The symptoms upon presentation were consistent with acute *digitalis* toxicity, and despite resuscitation efforts and administration of a *digitalis* antidote, she subsequently died, and her autopsy concluded that the cause of death was acute *digitalis* intoxication.

Another somewhat similar case of a 63-year-old woman was also reported in 2016. The woman had for the first time consumed a hot water infusion of what she believed to be comfrey leaves to assist with insomnia. The leaves had been selected by the patient from a local market. Upon presentation the next day she had symptoms of acute *digitalis* toxicity, and this was confirmed through blood analysis. Over the course of 5 days of hospitalisation she was successfully treated (in part through the use of a digoxin antidote) with no lasting problems. The authors conclude that the patient had accidentally ingested a species of *digitalis* rather than *Symphytum officinale* (Vithayathil and Edwards, 2016).

In 2017 the fatal case of a 55-year-old woman was reported. She had consumed a juice of what she believed to be raw comfrey leaves. Approximately 4 hours after ingesting the juice the patient presented to hospital with nausea and vomiting and was treated then discharged. She returned to the hospital due to continuation of her symptoms, and investigations revealed acute *digitalis* toxicity. Unfortunately this delay, as well as a lack of appropriate pharmacy supplies when *digitalis* poisoning was diagnosed, meant that despite undergoing aggressive treatment the patient died after 7 days of hospitalisation. Subsequent botanical identification of a sample of the leaves misidentified as comfrey confirmed that they were in fact *Digitalis purpurea* leaves (Wu et al., 2017).

The leaves of *Symphytum officinale* (left) and *Digitalis purpurea* (right) are easily confused before flowers appear.

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Table 2.2 Total alkaloid content in different *Symphytum* species and different plant parts, as summarized in Roeder, 1995.

Symphytum asperum		***Symphytum caucasicum***	***Symphytum officinale***		***Symphytum tuberosum***	***Symphytum x uplandicum***
Dried leaves	Dried roots	Unknown	Dried leaves	Dried roots	not stated	Dried aerial parts
0.13%	0.14-0.37%	0.48%	0.02-0.18%	0.25-0.29%	0.02%	0.2%

2.6.3 Misidentification of *Tussilago farfara*

Adulteration and misidentification of *Tussilago farfara* with *Petasites hybridus* (Liddel, 2001) and with *Adenostyles alliariae* has been reported, with cut dry leaf material of *Petasites spp*. being difficult to distinguish from *Tussilago farfara* macroscopically, although they are distinguishable microscopically (Nedelcheva et al., 2015).

In 1995 the case of an 18-month-old boy presenting with portal hypertension and severe ascites was reported. The infant was regularly given a herbal tea mixture since 3 months of age, which the mother thought contained peppermint and coltsfoot, gathered fresh by the parents. Investigations revealed the infant to be suffering from hepatic veno-occlusive disease (he recovered completely within two months), and analysis of the tea mixture revealed the presence of unsaturated PAs. Subsequent macroscopic and microscopic analysis revealed that the plant material thought to be *Tussilago farfara* was in fact *Adenostyles alliariae* (Sperl et al., 1995).

Foxglove poisoning, while serious, has a clearly identified profile. However the same cannot be said for coltsfoot, as unsaturated PAs are found in both *Petasites spp* and *Adenostyles alliariae*. This may lead to case reports of hepatic veno-occlusive disease incorrectly linking consumption of *Tussilago farfara* to the aetiology of the condition, due to issues of adulteration with these and potentially other plants

An example of such a situation examined in Roulet et al. (1988). The case, discussed in depth elsewhere in this report, involved the death of a newborn infant who upon autopsy was shown to have hepatic veno-occlusive disease. The mother revealed daily consumption of a herbal tea during pregnancy which was purchased at a pharmacy and used as an expectorant. The tea was tested and revealed the presence of unsaturated PAs (specifically senecionine), and the ingredients were declared in the report as 10 different plants including *Tussilago farfara* (Roulet et al., 1988). In a subsequent letter to the editor, Spang (1988) reported that research on the tea in question showed that it also contained the roots of *Petasites officinalis*, another plant containing unsaturated PAs. This reported adulteration makes the original case report linkage of *Tussilago* to the death of the infant questionable at best.

The leaves of *Tussilago farfara* (l) and various species of *Petasites* (here *P. hybridus* (r)) are easily misidentified.

2.3 Toxicology

As previously detailed, PAs are synthesised by many plant species throughout the world. For the purposes of this report, however, the focus of this report is exclusively on three plant species (*Borago officinalis*, *Symphytum officinale*, and *Tussilago farfara*) and the PAs found within these species.

Borago officinalis – intermedine, lycopsamine, 7-acetyllycopsamine, 7-acetylintermedine, amabiline, supinine; total alkaloid amount is less than 0.001% (<10µg/g) relative to dry weight (Roeder, 1995).

Symphytum officinale – echimidine (Liddell, 2002), echinatine (Liddell 2002; El-Shazly & Wink 2014), intermedine, lycopsamine, 7-acetyllycopsamine, 7-acetylintermedine, symlandine, symviridine, myoscorpine, symphytine; total alkaloid amount is 0.02-0.18% (~1000 µg/g) in the leaves and 0.25-0.29% (~2700µg/g) in the roots (Roeder, 1995). Note: *Symphytum officinale* was previously believed to contain heliosupine, based on data using techniques unable to differentiate this alkaloid from its stereoisomer echimidine. Recent research using more accurate analysis techniques has failed to find heliosupine in this species – suggesting that what was previously reported as heliosupine was in fact echimidine (Avula, Sagi et al., 2015; Mudge, Jones et al., 2015).

Tussilago farfara – senkirkine, senecionine; integerrimine, seneciphylline; total alkaloid content is 0.0055% relative to dry weight (55 µg/g) (Roeder, 1995; Nedelcheva, Kostova et al., 2015).

Recently, there has been an attempt made to rank the toxic effects and potencies of different PA congeners. The greatest risk of toxicity has been observed with alkaloids that are cyclic diesters and open-chained diesters with 7*S* configurations. These were given a Relative Potency of 1.0. When compared to this standard, for monoester PAs with the 7*S* configuration, the RP is 0.3. For open-chained diesters with a 7R configuration the RP is 0.1 and for monoesters with 7*R* configuration the RP is 0.01. Hence the capacity of unsaturated PAs vary substantially in their toxicity based on their structure. Consequently, herbal medicines containing only PAs of the mildest potency would have far fewer chances of causing harm when compared to those containing the most potent class of PAs (assuming equal doses). Note: the RP is a logarithmic scale (Merz and Schrenk 2016).

Table 2.3 – PAs in *Borago officinalis*, *Symphytum officinale*, and *Tussilago farfara* and their relative potency.

Most Potent PA (RP=1.0)	Moderate Potency PA (RP=0.3)	Mild Potency PA (RP=0.1)	Mildest Potency PA (PR=0.01)
cyclic diesters and open-chained diesters with 7*S*	monoesters with 7*S*	open-chained diesters with 7*R*	monoesters with 7*R*
senkirkine	echinatine	symphytine	lycopsamine
seneciphylline		symlandine	intermedine
senecionine		echimidine	7-acetyllycopsamine
heliosupine		symviridine	7-acetylintermedine
integerrimine		myoscorpine	

Note: amabiline and supinine are monoesters, however both lack stereochemistry at the 7-carbon position of the pyrrolizidine structure.

2.3.1 Pharmacology of PAs

Ingested PAs appear to be well-absorbed from the gastrointestinal tract. Approximately 80% of ingested PAs (particularly the highly hydrophilic PAs and PA N-oxides) are excreted unchanged via the urine within 24 hours of ingestion. The other 20% undergo bioactivation in the liver – a process necessary for ingested PAs to produce toxic effects (see section below). Those PAs of higher lipophilicity are able to pass through the placenta and into breastmilk (Authority, 2001; Moreira et al., 2018). It is for these latter reasons that the German Federal Ministry of Health has specifically prohibited the use of PA-containing herbal medicines during pregnancy and lactation (Bundesinstitut für Risikobewertung 2013).

2.3.2 Pathogenesis of PA Induced Toxicity

Unsaturated PAs display no toxicity in their native form. Metabolic activation is a necessary step to exert toxicities. As biotransformation of PAs occurs mostly in the liver, this organ is the most affected by the toxic PA metabolites. Three main pathways are involved in the bioactivation of PAs – N-oxidation to produce PANOs; hydrolysis to create necines and necic acids; and oxidation to form either pyrrolic esters or dehydropyrrolizidine alkaloids (DHPAs). The metabolism of PAs is illustrated in Figure 1. (Fu et al., 2004,; Moreira et al., 2018).

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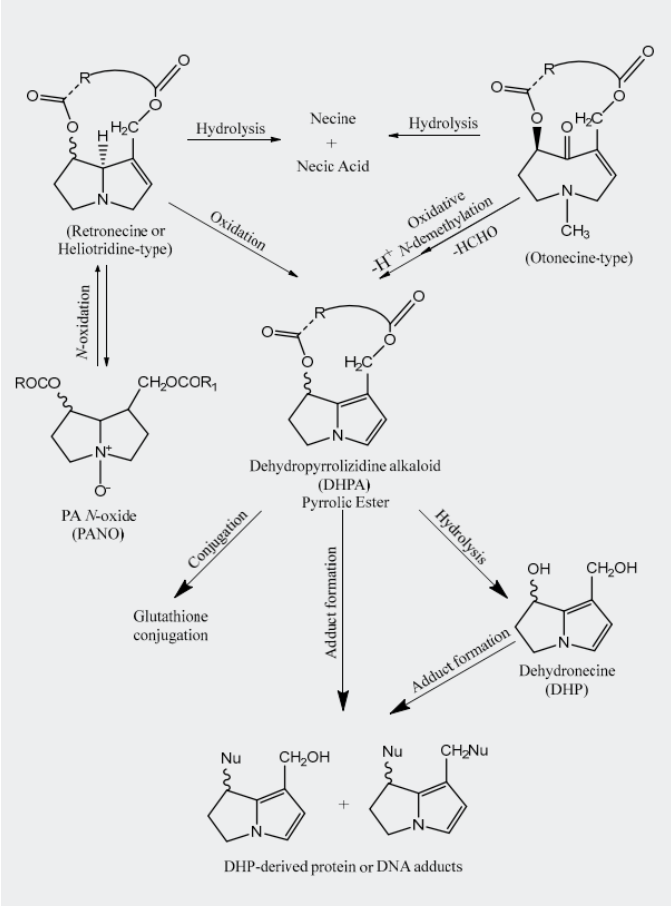

Figure 2.6. Pyrrolizidine alkaloid metabolism.
From: (Moreira et al., 2018)

After the formation of the highly reactive DHPAs (via oxidation), they can subsequently be eliminated from the body via the formation of glutathione conjugates. DHPAs can also undergo hydrolysis to be transformed into dehydronecines (DHP). These latter compounds are also considered toxic metabolites but are significantly less reactive than DHPAs. Pyrrolic esters, DHPAs, and DHPs can bind to proteins and deoxyribonucleic acid (DNA) and, consequently, form adducts. It is these adduct compounds that are believed responsible for the hepatoxic and carcinogenic consequences of PA ingestion (Fu et al.; 2004, Yang et al., 2016, Moreira et al., 2018).

Thus, the main detoxication routes are initial hydrolysis of the ingested PA and N-oxidation to create PANOs. It is worth noting, however, the PANOs can revert back into PAs and suffer oxidation into DHPAs. This conversion is carried out mostly by cytochrome P-450 (CYP450) monooxygenases, specifically CYP3A and to a lesser degree CYP2B. Once pyrrolic esters or DHPAs are created (again through oxidation catabolised by CYP3A and CYP2B), glutathione conjugation is considered an important detoxification route, promoting the harmless elimination of DHPAs (Fu et al., 2004; Moreira et al., 2018).

Different species display differing sensitivities to the toxic effects of ingested unsaturated PAs. This is due to species differences in the biotransformation of PAs resulting in varying levels of formation of toxic DHPAs and their detoxification compounds, such as necines, necic acids, glutathione conjugates and PANOs (Moreira et al., 2018).

Similarly, one would expect substantial differences in PA metabolism amongst different people, owing to vast differences in CYP3A activity (determined by genetic polymorphisms) between individuals, as well as dietary and medication factors which can impact both CYP3A activity and levels of hepatic glutathione (Lamba et al., 2002; Klein and Zanger, 2013). The latter is required to facilitate DHPA conjugation.

Consumption of agents capable of inducing CYP3A activity concurrently with PAs would be expected to substantially increase the production of highly reactive DHPAs and pyrrolic esters. Such agents would include *Hypericum perforatum* and a range of medications including Aminoglutethimide, Bexarotene, Bosentan, Carbamazepine, Dexamethasone, Efavirenz, Fosphenytoin, Griseofulvin, Modafinil, Nafcillin, Nevirapine, Oxcarbazepine, Phenobarbital, Phenytoin, Primidone, Rifabutin, Rifampin, and Rifapentine (Liu et al., 2007). Co-administration of these agents with medicinal herbs containing unsaturated PAs would thus not be recommended.

Conversely, agents capable of inhibiting hepatic CYP3A function could be expected to reduce the formation of highly reactive PA biotransformation products. Such agents could include *Piper nigrum* (Bhardwaj et al., 2002; Rezaee et al., 2014) and medications including Amiodarone, Amprenavir, Aprepitant, Atazanavir, Chloramphenicol, Clarithromycin, Conivaptan, Cyclosporine, Darunavir, Dasatinib, Delavirdine, Diltiazem, Erythromycin, Fluconazole, Fluoxetine, Fluvoxamine, Fosamprenavir, Imatinib, Indinavir, Isoniazid, Itraconazole, Ketoconazole, Lapatinib, Miconazole, Nefazodone, Nelfinavir, Posaconazole, Quinupristin, Ritonavir, Saquinavir, Tamoxifen, Telithromycin, Troleandomycin, Verapamil, and Voriconazole (Liu et al., 2007). However, such a strategy has not been assessed in clinical models to date.

In vitro models have clearly demonstrated that lowered glutathione status is associated with greater hepatotoxicity from PA ingestion (Yan and Huxtable, 1995; Yang et al., 2016). The concurrent or pre-ingestion of agents capable of enhancing hepatic glutathione stores would also be expected to attenuate the production of the highly reactive PA metabolites. Agents capable of enhancing hepatic glutathione stores include SAMe (Vendemiale et al., 1989) and n-acetyl cysteine (El-Serafi et al., 2018), as well as orally administered glutathione itself (Sacco et al., 2016). However, utilisation of these tools as a way to attenuate the hepatotoxic nature of unsaturated PAs has not yet been adequately evaluated.

2.3.3 Worldwide Recommendations on PA Ingestion

This section will provide an overview of how PA ingestion has been handled by a number of different authorities:

- The German Federal Ministry of Health has developed recommendations around the use of PA-containing medicinal herbs. A maximum dose has been set at 1 µg/day of unsaturated PAs. If the duration of administration is more than six weeks, the recommended total dose of unsaturated PAs is reduced to 0.1 µg/day. Use of PA-containing herbal products by pregnant and lactating women is strictly prohibited, as it is believed that foetuses and infants are particularly susceptible to PA-induced poisoning (CONTAM, 2011; Bundesinstitut für Risikobewertung, 2013).
- The World Health Organization International Programme on Chemical Safety (WHO-IPCS) has concluded that a dose equivalent to 10 µg heliotrine/kg body weight (b.w.) per day may lead to acute or sub-acute liver disease in humans. The WHO-IPCS stated it was not possible to evaluate the human cancer risk due to PA ingestion because of a lack of information on the long-term follow-up of populations that have been exposed to unsaturated PAs over time in low doses or acutely in a high doses. They did caution, however, that PAs are potentially carcinogenic in humans based on available animal and *in vitro* data (WHO-IPCS, 1988; WHO-IPCS, 1989).
- The UK Committee on Toxicity (COT) determined that the potent PA riddelliine at a dose of 0.1 µg/kg b.w. per day would not be expected to result in non-cancer (e.g., acute) adverse effects based on animal data. In regard to cancer risk, the COT defined the benchmark lower dose confidence limit for a 10% excess cancer risk (BMDL10) figure of 73 µg/kg b.w. per day. This was derived from a 2-year carcinogenicity study utilising the potent PA lasiocarpine in rats. Allowing a margin of exposure (MOE) of at least 10,000 (to compensate for the knowledge gap in translating the animal data to the human situation) indicated that a daily dose of 0.007 µg/kg b.w. per day is unlikely to increase cancer risk. Nor would ingestion of such doses be expected to result in acute (e.g., non-cancer) adverse effects. (Committee on Toxicity of Chemicals in Food 2008)
- The Australian New Zealand Food Authority (ANZFA) utilised human data from case reports to estimate a no-observed-effect level for veno-occlusive disease of 10 µg/kg b.w. for unsaturated PAs. This value was then divided by an uncertainty factor of 10 to take into account human variability, resulting in a provisional tolerable daily intake of 1 µg/kg b.w. per day. The ANZFA report concluded that there is no evidence that PAs are carcinogenic in humans (Australian and New Zealand Food Authority 2001).
- The European Medicines Agency has set a permitted daily intake of 0.007 µg PA/kg b.w. based on the same data used by the UK COT (discussed above) (Committee on Herbal Medicinal Products 2014).

The unsaturated PA dose recommendations made by the majority of these organisations do not, however, take into account relative potencies of the different PAs. With the exception of the ANZFA recommendations, dose recommendations were calculated on the most potent class of PAs. *Tussilago farfara* does contain this class of PAs in small amounts, whereas the other two species (*Symphytum officinale* and *Borago officinalis*) do not. It is worth noting that the PAs found in these latter species are several orders of magnitude less potent than lasiocarpine and riddelliine, which are the PAs used to calculate safe ingestion levels in the critical animal experiments. However, it should be kept in mind that comfrey contains substantially higher amounts of PAs than the other two herbs.

2.4 Animal studies

A comprehensive review of animal studies which address the PAs in these three plants is beyond the scope of this report. However, the following observations are relevant:

The animal studies which initiated the concern about PAs particularly of comfrey (Culvenor et al., 1980; Hirono et al., 1978) were based on animals being fed extremely high doses of comfrey or its isolated PAs, and were widely criticised in herbal circles (Pembery, 1982; Whitelegg, 1996). However, some of the more recent studies include more realistic doses of the whole plant. Recommendations regarding safe dosage of PAs are based on extrapolation from long-term animal studies involving the single PAs riddelliine and lasiocarpine. These are used as model substances as it is understood that there is a common mode of action for all 1,2-unsaturated PAs. Because human data is insufficient for making decisions about dosage, data from studies on these two model substances (NTP, 1978; "Toxicology and carcinogenesis studies of riddelliine (CAS No. 23246-96-0) in F344/N rats and B6C3F1 mice (gavage studies)," 2003) have been used to calculate the recommended daily intake of PAs (European Medicinces Agency, 2014; Committee on Toxicity of chemicals in food, 2008).

Critics of the early studies questioned the reliability of evidence based on high doses of a single PA rather than the whole plant, especially a PA that is not found in comfrey. A more recent study by Guo et al. (2007) compared patterns of carcinogenesis-related gene expression in rats fed riddelliine and those fed comfrey root, and found these patterns to be very similar to the original extrapolation of results and conclusion that the PAs in comfrey are linked to its carcinogenicity.

However other studies found conflicting results, for example Gomes et al. (2010) finding that comfrey root extract reduced cell proliferation in rats where tumours had been stimulated. Benedek et al. (2010) used an extract of comfrey root in a bacterial reverse mutation assay (Ames Test) and found no evidence of mutagenicity.

Experiments on rodents investigating pharmacokinetics of unsaturated PAs have used lasiocarpine (which is found in *S. caucasicum* but not *S. officinale*) and senecionine and seneciphyline (both found in *Tussilago farfara*). These studies reported that the PAs concentrated in the liver and lung tissue and were excreted in both urine and milk (Cupp, 2000; DeSmet et al., 1992).

THE PLANTS

However there is significant inter-species variation in relation to sensitivity to PAs. Questions remain as to the transferability of evidence of their toxicity between different animal species, and the applicability of this evidence to humans (Moreira et al., 2018). Recent findings of significant differences in PA metabolism between human, pig, cow, horse, rat, rabbit, goat and sheep liver indicate the importance of further investigations into this area (Kolrep et al., 2018). Kolrep et al., (2018) went further to suggest that the species–specific balance between activation and inactivating pathways or toxification and detoxification of PAs determines the degree of toxicity of the PAs and is a crucial element in risk assessment. This concept is explored further in the pathogenesis of PA-induced toxicity section 2.3.2.

Despite these limitations, animal studies provide a basis for caution regarding the consumption of comfrey. The results are mixed, and herbalists will argue that the consumption of riddelliine and lasiocarpine should not be extrapolated to the consumption of plants which do not contain these PAs. However the work of Guo et al. (2007) indicates that riddelliine and comfrey in one instance at least, have provided similar results. Finally, excretion of PAs in milk is noted, with obvious implications for nursing mothers.

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ADVERSE EVENT REPORTING

In this section, data is obtained from the major international pharmacovigilance repository, the World Health Organisation-Uppsala Monitoring Centre's VigiBase. As herbal medicines are not subject to the same regulatory requirements regarding suspected Adverse Drug Reaction (ADR) reporting as pharmaceuticals, spontaneous reporting systems are the principal source of data for adverse events of herbal products.

3.1 Background

Accurate attribution of causality when a pharmaceutical or herbal medicine is suspected of precipitating an adverse reaction requires information about what was ingested, the dose and duration of use, the temporal association of consumption with the onset of symptoms, together with observations following withdrawal of the suspected medicine. An understanding of the patient's medical history, the pharmacological plausibility of the symptoms, and other possible causes, including adulteration, contamination or overdose are also important considerations (WHO, 2004;Teschke, 2013). A rechallenge, where the patient is monitored while resuming consumption of the suspected intervention, would usually provide the clearest evidence, but is often unethical.

In addition, decisions about causality where adverse reactions may be associated with plants require the authentication of the species and the part of the plant ingested, and the plant must be referred to by botanical name. If a herbal product is involved, then details of the manufacturer, batch number and expiration date are also relevant. Species identification can be a complex task as herbal medicines are often consumed in combination. Where this occurs, each species should ideally be identified. When it is not possible to obtain enough of the plant and part to allow botanical identification via morphological features, an analysis of constituents may occur in isolation.

The patient's signs and symptoms may direct investigations where these are characteristic of a specific toxicity, for example the bradycardia caused by the cardiac glycosides found in foxglove species (such as *Digitalis purpurea* and *Digitalis lanata*) and the pharmaceutical derived from these plants (Janssen, 2016). In some cases, this has enabled the diagnosis of foxglove poisoning to be made in individuals who have mistakenly ingested it, thinking that it was comfrey (see 2.2).

The global repository of data on suspected ADRs has been held since 1978 by the Uppsala Monitoring Centre (UMC) in collaboration with the World Health Organization. Its purpose is to establish an international signalling system to facilitate early detection of possible adverse reactions to drugs, including herbal medicines. It is intended to generate hypotheses and does not determine causality.

Post-marketing data describing suspected harm to patients is collected on an ongoing basis from health professionals, pharmaceutical companies and consumers via Individual Case Safety Reports (ICSRs) since 1978. Today these number over 16 million individual reports and are drawn from more than 110 countries. These include the UK's 'yellow card' with which UK herbalists will be familiar. The data, stored in an online database, VigiBase, provides indicators of potential concern (WHO, 2018; Meincke, 2017; Pokladnikova, 2016).

There is considerable variation in the level of detail recorded via ICSRs and in terms of those who provide this information. In some countries reporting is limited to health professionals, in others consumers are also able to report suspected reactions. In some countries herbs are regulated as foods, in others they are regulated as drugs.

Limitations include the frequency of missing data – many of the ICSRs are incomplete – and the problem of under-reporting, which is common in all spontaneous reporting systems. This may be more of a problem in relation to herbal products than to pharmaceuticals for the following reasons (Shaw, 2012):

- There may be a failure to associate the herb with the adverse event. This is particularly problematic in relation to PA-containing plants, where problems may occur months or years after ingestion. This makes it difficult for the patient to remember, and likely impossible for researchers to positively identify, the causative agents.
- The patient may stop taking the herb when they feel unwell.
- The physician/patient may be unaware that herbal ADRs should be reported.
- The patient may not consider the herb a 'medicine' and therefore fail to disclose its use to their physician.

Spontaneous reports are an important part of any assessment of ADRs. They are more likely to pick up immediate and especially idiosyncratic reactions rather than those associated with long-term consumption or chronic disease. However concern has been raised about the possibility of acute as well as chronic effects from PAs in herbs and herbal products (Knutsen, 2017).

3.2 ADRs related to Comfrey, Coltsfoot and Borage recorded in VigiBase

METHODS

The ICSRs for *Tussilago farfara*, *Borago officinalis*, *Symphytum officinale* and *Symphytum x uplandicum* were accessed from the VigiBase database.

RESULTS

There were no adverse events associated with *Borago officinalis*, one for *Tussilago farfara*, nine for *Symphytum x uplandicum* and sixty-three for *Symphytum officinale*. The route of application was not reported for the *Tussilago farfara* event. All nine events associated with *Symphytum x uplandicum* were associated with topical applications. Of the sixty-three associated with *Symphytum officinale*, fifty-two were for topical applications, and for one case the route of administration was not recorded. Details of ADRs associated with topical application were not analysed.

Of the ten remaining cases, nine were listed as associated with the product Mediflor. In the remaining case the product name was not provided. All cases were reported in Europe, and only three of the reported cases involved liver-associated symptoms.

These results are summarised in Figure 3.1

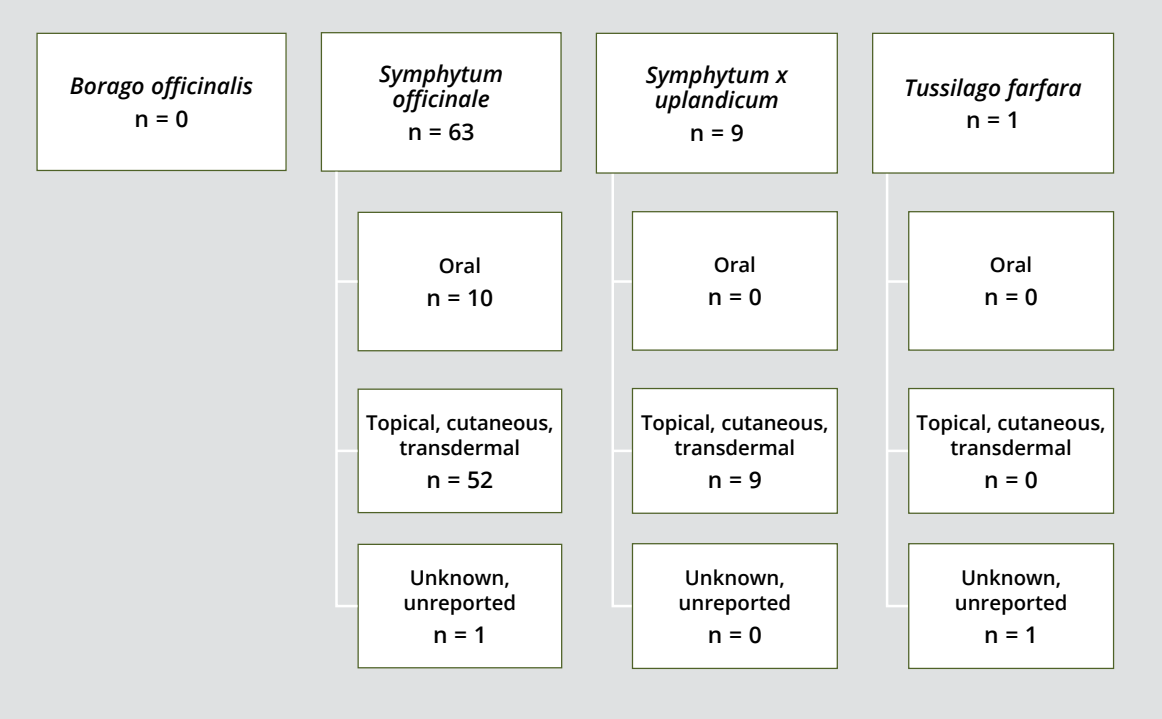

Figure 3.1 Summary of Individual Case Safety Reports from VigiBase

DISCUSSION

Further details of the product Mediflor were unable to be found via internet searching. Mediflor is a brand which supplies a range of herbal products, but there was no reference to a unique product 'Mediflor'. Clarification from UMC revealed that the name 'Mediflor' was entered into the WHODrug (the VigiBase drug dictionary which connects drug names with a set of ingredients) in 2006, and it was listed as containing *Symphytum officinale*.

Further investigation by UMC revealed that in six of the nine case reports, there was no mention of *Symphytum* in the 'free text' section of the case reports, although other herbs were listed. This suggests a problem with the information recorded which has now been reported. However, it means that the products recorded in VigiBase as containing *Symphytum officinale* 'might not have contained *Symphytum*' (pers.comm. ADRinfo 6.9.2018).

CONCLUSION

The number of reports made in relation to these plants is negligible. Further, doubt has been cast on the accuracy of reporting in relation to the *Symphytum* products.

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SYSTEMATIC REVIEW OF CASE REPORTS

4.1 Introduction

According to the Oxford Centre of Evidence-Based Medicine (CEBM) the most reliable evidence to inform questions about the potential harm of a treatment are systematic reviews of randomised trials, followed by individual randomised controlled placebo trials (Howick, 2016). In the case of PA containing plants the only experimental studies about potential toxicity come from laboratories where *in vitro* and *in vivo* studies involving cell lines and various animal species provide insights into potential toxicity and mechanism of action. Given the limitations in translation to oral consumption by humans, these studies are considered as level 5 evidence; the lowest level. Observational studies based on methodologically sound non-randomised cohorts provide level 3 evidence, and as discussed in section 3, the only documented population-based evidence for the safety of comfrey, coltsfoot and borage is provided by the adverse drug reaction (ADR) records, a form of post market surveillance which is not based on rigorous methodology.

The literature on PA-containing plants does include another source of information about their human consumption; that is case reports. The CEBM considers case series, or case reports to provide level 4 evidence of harm due to an intervention and consider a systematic review of all the available case studies to deliver the most reliable evidence from this source (Howick, 2016).

Systematic reviews involve four replicable steps. First a rigorous search of the literature is undertaken to ensure the greatest number of studies meeting the general inclusion criteria are evaluated; secondly, these papers are read in detail and inclusion and exclusion criteria applied. In the next step the core papers whose content can contribute to answering the research question are independently and critically assessed by at least two authors. Only papers that are considered of methodological adequacy are retained. Finally, authors employ a systematic, replicable approach to the amalgamation of data to inform decision making. This rigorous process increases the reliability of the results.

The question this systematic review examines is whether the herbs comfrey (*Symphytum officinale*), coltsfoot (*Tussilago farfara*) and borage (*Borago officinalis*) are safe for oral consumption by humans, particularly in a clinical context. Each case study reports an adverse event considered to be due to the ingestion of one of these herbs. The question for the reviewers then, is to ascertain whether the case study provides sufficient and appropriate information to allow an attribution of causation to be made. The choice of instrument to guide this process is critical to the reliability of the systematic review.

This section opens with a description of contemporary instruments designed to assess causation in the case of adverse reactions to interventions. It explains the decisions to combine the Quality of Case Reports of Adverse Events instrument (QCRAEI), the Roussel Uclaf Causality Assessment Method (RUCAM), and the World Health Organisation-Uppsala Monitoring Centre system for standardised case causality assessment (WHO-UMC) in the critical appraisal of the case studies. The three assessment instruments can be found in Appendix 3. Next the systematic review is described, starting with the literature search, followed by the critical appraisal of included studies. Summaries of each included study can be found in Appendix 4. The results are then reported. This is followed by an expanded account of one case (Schroff et al., 2013), based on de-identified patient notes. This not only provides deeper insights into the causality in this case, but emphasises the need to better educate clinicians about how best to report adverse events involving plants. Appendix 5 reports a search for patient notes for each of the cases reviewed. Finally, in section 4, the potential role of *Symphytum officinale* and *Tussilago farfara* in each case study is considered with a focus on the adequacy of botanical identification.

4.2 Tools and instruments – how they were chosen and limitations

The extensive literature on pharmacovigilance discusses what information should be gathered and by whom in order to make an accurate attribution of causality when a pharmaceutical or herbal medicine is suspected of precipitating an adverse reaction (WHO, 2004; Edwards, 2012; Shaw et al., 2012) and this is discussed in 3.1.

The organ most susceptible to damage caused by PAs is the liver, where toxicity results in hepatic veno-occlusive disease (HVOD). This involves progressive injury to the wall of the hepatic sinusoid with ensuing loss of sinusoidal lining cells, sinusoidal haemorrhage and mild damage to the central vein endothelium (DeLeve et al., 1999). A liver specific causality assessment tool would be well suited to capture information about pharmacological plausibility when assessing the safety of comfrey, coltsfoot and borage. The ingestion of PA-containing plants could also be associated with other adverse events involving different symptoms and signs, as a reaction to any of the ingested constituents including PAs and a review of the safety of these plants would not be complete if this possibility was disregarded.

A number of instruments have been developed to aid collection of the appropriate data and assess the cause of adverse reactions in a range of contexts, from acute admission to a toxicology ward or following administration of a medication while in hospital care (Koh et al., 2010; Lindblad et al., 2017). An appropriate tool on which to base attributions of causality in the context of this review would be:

- Designed to assess adverse events involving plants,
- Validated in the literature for its specificity and reproducibility in determining causality,
- Appropriate for application to case reports,
- Able to capture hepatotoxicity while also
- Gathering data related to other symptoms and signs of toxicity.

A search of the literature was conducted and a number of instruments were considered for this review. Six assessment instruments were examined in detail. The table below summarises these tools and highlights their strengths and limitations for reporting adverse events related to herbal medicines based on previously published case reports.

Table 4.1 Causality Assessment Tools

Tool First author date	Validation		Assessment of causality includes:				
	Validated to Assess Causality	*Validated to assess causality resulting in hepatotoxicity*	*Herbal Material Assessed for Authentication*	*Herbal Dosage and duration*	*Botanical Name(s) of Herbal Medicine(s)*	*Laboratory investigation and imaging*	*Concomitant diseases and medications*
CIOMS[1] & updates Danan 1993 Tescke 2008	Yes	Yes Yes	No	Yes	Product name only	Yes	Yes
RUCAM[2] Danan 2015	Yes	Yes Yes	No	Yes	Product name only	Yes	Yes
WHO-UMC[3]	Yes	No	No	Yes	No	Yes	Yes
Naranjo scale Naranjo 1981	No	No	No	No	No	Yes	No
Maria and Victorino scale Maria 1997	No	Unclear	No	No	No	No	No
Ethnobotanical, ethnopharmacological surveys Rodrigues 2013	No	No	Yes	Yes	Yes	No	Yes

1 Council for International Organizations of Medical Sciences

2 Roussel Uclaf Causality Assessment Method

3 WHO-UMC system for standardised case causality assessment

Several of the tools were validated in the literature and could therefore be relied upon to inform causal attributions in the context of pharmaceuticals. Only one tool adequately covered the identification of herbs. Published in 2013 by Rodrigues and Barnes, this instrument is an adaptation of the approach used in ethnobotanical and ethnopharmacological surveys to the area of pharmacovigilance. The resulting tool collects details regarding the composition of the prescription/recipe, therapeutic use, preparation and storage, route of administration, adverse/undesirable/unexpected effects of the prescription and its ingredients, cautions and contraindications, foods and sexual taboos and other information relevant to an adverse reaction (Rodrigues & Barnes, 2013). Despite these strengths, the current discussion of the safety of comfrey, borage and coltsfoot originates in the Western herbal tradition and employs the language of medical science, so this instrument was not considered an appropriate choice.

Like Rodrigues and Barnes, Naranjo's assessment was developed for suspected ADRs in general, rather than for drug induced liver injury (DILI) or herb induced liver injury (HILI). Answers to the questions are scored and an algorithm is used to categorise the association of a herb or drug to an adverse effect. The instrument has the advantage of being simple and clear, however it is not recommended for hepatotoxicity assessments (García-Cortés et al., 2011).

In contrast, the WHO-UMC system for assessing causality based on case reports takes a qualitative approach, categorising the likelihood of a causal relationship between the material consumed and any adverse reaction into six levels of certainty (WHO-UMC). The categories are based on the quality of the documentation of observations as well as considerations such as temporal relationship, response to withdrawal, results of laboratory testing etc. A definition of each category is provided to guide assessors. The WHO-UMC Causality Categories are reproduced in Appendix 3. The lack of numerical data is considered a serious limitation by some authors, reducing objectivity and inter-rater reliability (Teschke et al., 2013). Nonetheless, the WHO-UMC method is internationally agreed upon and used globally to assess causality of adverse events from all causes.

The remaining three instruments focus on ADR causing hepatotoxicity. The Council for International Organizations of Medical Sciences (CIOMS) scale is designed for the assessment of suspected drug/herb induced liver injury and was originally published in 1993 (Danan & Benichou, 1993). It has been validated for hepatotoxicity and has good sensitivity, specificity and predictive validity, based on cases with a positive rechallenge test. It does not require an expert knowledge in hepatotoxicity to use accurately (Teschke et al., 2014). However this scale has limited exclusions of alternative causes and has qualitatively graded risk factors, so is less objective than the more recent version now known as the Roussel Uclaf Causality Assessment Method (RUCAM) (Teschke et al., 2014). Maria and Victoriana (1997) published an alternative instrument aiming to improve on the CIOMS by simplifying it and adding some additional elements. However it has been found to be less reliable, and in particular to deal poorly in situations where there was a long latency period and which involved chronic liver conditions (Lucena et al., 2001). As this is the case with PA toxicity, this instrument was excluded.

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The RUCAM was published in 2015 with further refinements published in 2016 and is an updated version of the COIMS method. The RUCAM uses an algorithm to quantitatively assess likelihood of causality in cases of suspected DILI and HILI (Danan & Teschke, 2015; Danan & Teschke, 2016). RUCAM is the most comprehensive tool for determining hepatotoxicity and it uses the initial ratio of the liver enzymes alanine aminotransferase (ALT) and alkaline phosphatase (ALP) to categorise the nature of the hepatotoxicity into hepatocellular (which includes hepatic veno-occlusive disease), cholestatic or mixed. This data is collected in routine liver function tests (LFT) and so is likely to be included in a published case report. The timing of the onset of symptoms in relation to the period of exposure to the herb or drug, and the follow-up once the intervention is withdrawn, captures the challenge and dechallenge. Other possible causes of compromised liver function are comprehensively collected, including concomitant medication, pre-existing illness and lifestyle risk factors like alcohol intake. The RUCAM has been extensively validated and has high sensitivity (86%), specificity (89%), positive predictive value (93%) and negative predictive value (78%) in relation to the causality of hepatotoxicity when prospectively assessed. The standardised and structured format of the RUCAM, as well as its comprehensive approach allows it to be adapted for use with case reports, although accuracy would be compromised by missing data. A limitation of both the CIOMS and the RUCAM is their focus on herbal medicine products, rather than whole fresh or dried herbs or extemporaneously prescribed combinations.

No single instruments met all prerequisites, so a combination of two internationally recognised assessments was employed. The RUCAM, with its quantitative algorithm for assessing causality in relation to hepatotoxicity was applied to all cases with liver involvement and the qualitative WHO-UMC scale covering all ADR was applied to all reports. Preliminary screening of the case reports was conducted using the Quality of Case Reports of Adverse Events instrument (QCRAEI), a tool designed specifically to assess the quality of case reports of adverse events (Agbabiaka et al., 2008). This tool was developed and tested for reliability by 16 experts (researchers and clinicians) using the Delphi method, and later slightly modified to better assess case reports of adverse events potentially associated with herbal medicines (Agbabiaka et al., 2008; Hung et al., 2011). The instrument consists of 20 items used in herbal medicine and covers four main aspects of pharmacovigilance. These are herbal medicine identification and authentication, drug information and therapeutic regimens, patient history, diagnosis and medications, and details of the adverse events (Hung et al., 2011). Each item is scored between 0 not reported, 1 unclear and 2 reported, for a maximum quality rating of 40. The total score is described in categories from lowest to highest quality. The QCRAEI, the RUCAM and the WHO-UMC are available in Appendix 3 and the categories employed by each are summarised in Table 4.2.

4.2.1 Steps in the critical appraisal of case reports

To reliably establish that consumption of one or more of the three species was the cause of an adverse reaction, a two-stage appraisal was applied. First the adequacy of the information provided to make a causality assessment was considered. Reports that were of found to be of low quality were discarded as unreliable. Those of adequate quality were then assessed using two different instruments.

4.3 Finding the case studies

Two of the authors (IB and JH) performed a computer-based search of MEDLINE, CINAHL Plus, AMED, GreenFILE, Health Source: Nursing/Academic Edition, PsycARTICLES, Psychology and Behavioral Sciences Collection, PsycINFO, and EMBASE databases (from inception to Nov 2017). The keyword search terms for these specific PA-containing herbs ["Borago", "borage", "pyrrolizidine", "lycopsamine", " supinidine", "amabiline", "cynaustine", "*Symphytum*", "comfrey", "symphytine", "7-acetyllycopsamine", "7-acetylintermedine", "intermedine", "lycopsamine", "symlandine", "myoscorpine", "echinatine", "echimidine", "heliosupine N-oxide", "heliotrine", "lasiocarpine", "viridiflorine", "uplandicine", "*Tussilago*", "coltsfoot", "senkirkine", "senecionine"] were combined with keyword search terms for hepatotoxicity ["liver", "hepat*", "megalocytosis", "venoocclusive disease", "cirrhosis", "carcinoma", "adenoma", "hyperplasia", "mutagenicity", "genotoxicity", "safety", "adverse events", "toxic*, "hepatotoxicity", "drug induced liver injury", "DILI", "herb induced liver injury", "HILI", "intrinsic liver toxicity"] using the combination term AND. The search term NOT ["*Senecio* OR *Heliotropium*"] was added to reduce the number of irrelevant papers. Bibliographies of case reports discussing the use of PA-containing medicinal plants and potential hepatotoxicity, as well as previous reviews, and the authors' personal libraries were hand-searched for additional references. There were no language restrictions.

Symphytum officinale

4.5 Method of data abstraction, inclusion and exclusion criteria

A wide search strategy was employed to identify literature to inform various sections of this review. Study selection criteria for this the systematic review of case studies were defined a priori and these are listed in Figure 4.1. Details of the search are described below.

Inclusion Criteria	Exclusion Criteria
Borago	Other species
Borage	Animal poisoning
Symphytum	Veterinary
Comfrey	Regulation
Tussilago	Food contamination (including honey)
Coltsfoot	Animal experiments
Peer reviewed papers	Mechanisms of toxicity
Human case report	Pharmacokinetics
Human case series	Plant occurrence or prevalence
* Note: only genus and common names were used rather than species, to ensure that cases on related species were captured.	Review articles
	Editorials
	Otherwise not relevant

Figure 4.1 Inclusion and exclusion criteria

The titles and abstracts of the articles found were screened by two authors (IB and JH), and those that appeared to meet the criteria were examined in further detail. Case reports that discussed the potential toxic effect of the consumption of *Borago officinalis*, *Symphytum officinale* or *Tussilago farfara* in humans were included. Study report outcomes, methods, and methodological quality were evaluated independently by two investigators. Reports were abstracted and assessed by SE, CA, SS and IB. Any discrepancies were resolved by discussion of the paper or, if necessary, a third reviewer was brought in to arbitrate. Key data abstracted for analysis included the herb reported to be used, its form and dosage, who the prescriber was, duration of treatment, patient details, patients' medical history, use of concomitant medications by the patient, presenting symptom picture, onset and course of symptoms, treatment and care received, investigatory findings, and authors causal attribution.

The quality of the case report was assessed using Quality of Case Reports of Adverse Events Assessment Instrument (QCRAEA), and each report was scored out of 40 and ranked into one of five categories from Low to High quality with a sixth category for reports which were unsuitable for the purpose. Details are provided in Table 4.2, which describes these categories and those of the other assessment tools used. Papers rated moderate or good quality were then assessed in terms of causality by both the RUCAM and the WHO-UMC. Authors of published case reports were contacted via email or mail in an effort to collect additional data on each case report. See Table 4.2.

Table 4.2 Summary of the assessment instruments role, scoring systems and categories of the assessment instruments, the QCRAEI, the WHO-UMC and the RUCAM

Instrument	QCRAEI Scoring	WHO-UMC Methodology	RUCAM Scoring
Role to assess:	*The quality of reporting of case in which an adverse event occurred.*	*The association of herb with reported adverse event by a qualitative method.*	*The association of herb with adverse event employing a quantitative method using an algorithm.*
Ranks 1	High 29-40	Certain	Highly probable ≥9
2	Upper medium 22-28	Probable	Probable 6-8
3	Lower medium 15-21	Possible	Possible 3-5
4	Low quality 0-14	Unlikely	Unlikely 1-2
5	Excluded causality ≤ 0	Conditional/Unclassifiable	Un-assessable ≤ 0
6		Un-assessable/Unclassifiable	

QCRAEI Quality of Case Reports of Adverse Events Instrument (Agbabiaka et al., 2008)
WHO-UMC World Health Organisation Uppsala Monitoring Centre system for standardised case causality assessment (WHO-UMC)
RUCAM Roussel Uclaf Causality Assessment Method (Danan & Teschke 2015)

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4.6 Study selection

Our search of MEDLINE, CINAHL Plus, AMED, GreenFILE, Health Source: Nursing/Academic Edition, PsycARTICLES, Psychology and Behavioral Sciences Collection, and PsycINFO databases yielded a total of 1110 citations (Figure 1). EMBASE discovered 1691 articles. Hand-searching discovered an additional 3 articles. In total, 2804 reports were screened. Papers were excluded if they were duplicates (720), animal experiments (228), discussions on mechanisms of toxicity or pharmacokinetics (462), review articles and editorials (332), public literature (28), reports on analytic chemistry (85), therapeutic intervention trials (19), reports on other plant species (179), discussed accidental poisonings from PA-containing foods or honey (59), were agricultural or veterinary in nature (106), or were otherwise not relevant (508). Seventy-eight papers were chosen to be examined in greater detail. We excluded 67 of these papers, as they were not original case reports reporting on the ingestion of one of the three herbs in question – leaving 11 relevant case reports. A diagram illustrating the flow of citations through the screening process can be seen in Figure 4.2.

4.7 Results and discussion

Eleven case reports and letters published in the peer reviewed literature met the inclusion criteria (Bach et al., 1989; Freshour et al., 2012; Gyorik & Stricker, 2009; Jones & Taylor, 1989; Miskelly & Goodyer, 1992; Rasenack et al., 2003; Ridker et al., 1985; Roulet et al., 1988; Schroff et al., 2013; Weston et al., 1987; Yeong et al., 1990). These cases were published between 1985 and 2013 and reported adverse events considered to be associated with the ingestion of either comfrey or coltsfoot in five countries (three each from the UK and USA, two each from Switzerland and New Zealand and one from Germany). No published reports of adverse events associated with Borage were found. Eight of the cases concerned HVOD, two involve pulmonary pathology, one with associated hepatic dysfunction, and one describes a case of deep vein thrombosis and associated pulmonary embolism. The oldest patient affected was 77 years, and the youngest was still in utero when symptoms were detected. Three of the cases report a fatal adverse event, five patients recovered, two patients became asymptomatic, but were not fully recovered at the time of publication and no information about recovery was provided in one case. Table 4.3 provides a summary of these cases. More detailed summaries are provided in Appendix 4.

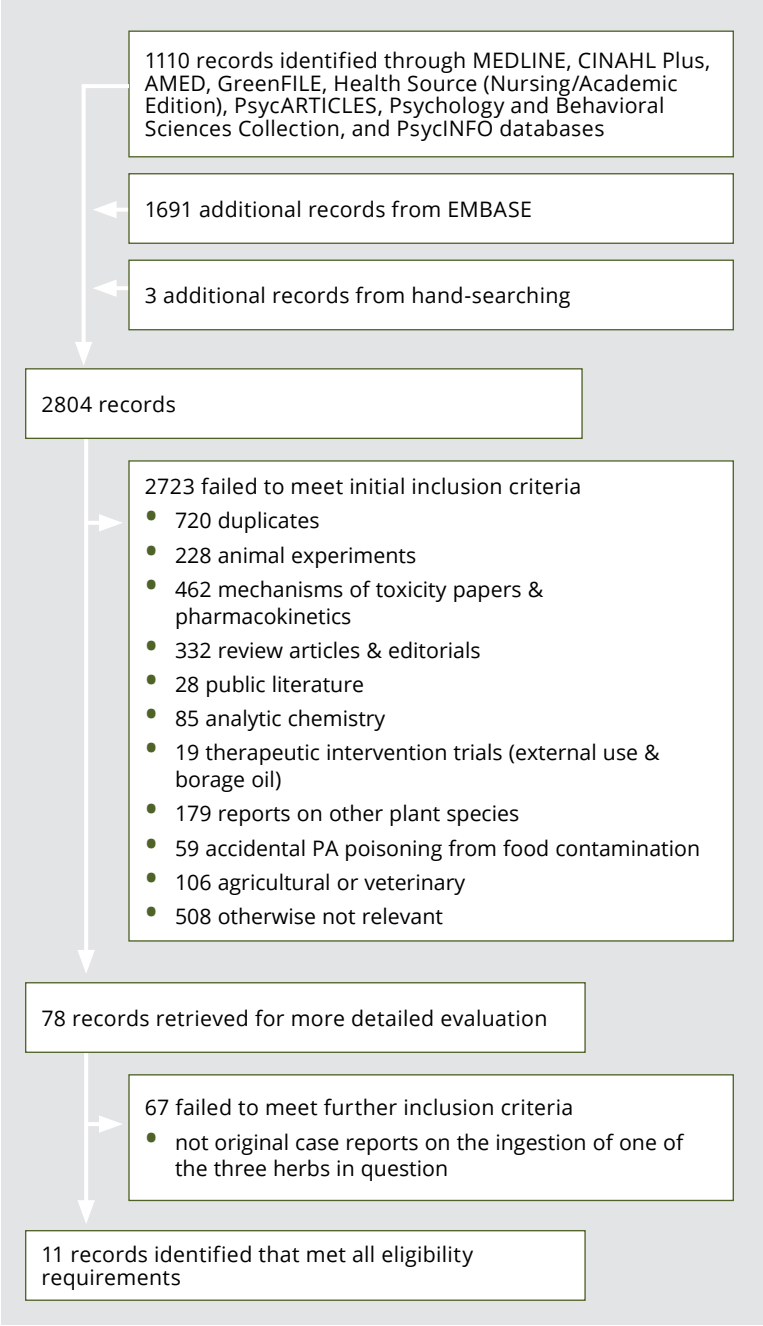

Figure 4.2: Flow of citations and articles through the phases of screening and eligibility evaluation

4.7.1 Quality assessment of case reports of adverse events

All case reports were screened for quality using the Quality of Case Reports of Adverse Events instrument QCRAEI (Agbabiaka et al., 2008). Just one of the case reports met the criteria for 'High' quality reporting, (Miskelly & Goodyer, 1992). Five were rated 'Upper Medium' quality (Gyorik & Stricker, 2009; Ridker et al., 1985; Roulet et al., 1988; Weston et al., 1987; Yeong et al., 1990), while four reports (Bach et al., 1989; Freshour et al., 2012; Rasenack et al., 2003; Schroff et al., 2013) were considered 'Medium low' quality. Jones and Taylor's (1989) short letter was excluded from further assessment due its 'Low' quality. Given the sparsity of the data reporting the consumption of the relevant herbs, it was decided only to exclude the report found to be of low quality from further critical appraisal.

Table 4.3 Summary case characteristics

Author date	Ridker 1985	Weston 1987	Roulet 1988	Jones 1989	Bach 1989	Yeong 1990	Miskelly 1992	Rasenack 2003	Gyorick 2012	Freshour 2012	Schroff 2013
Adverse event/ diagnosis	Hepatic VOD	Hepatic VOD	Hepatic VOD	Hepatic VOD	Hepatic VOD	Hepatic VOD	Pulmonary VOD with hepatic involvement	Hepatic VOD	Pulmonary hypertension	Deep vein thrombosis and pulmonary embolism	Hepatic VOD
Outcome at publication	Recovered	Recovered	Death	Not reported	Partially recovered	Death	Recovered	Death	Recovered	Recovered	Partially recovered
Country	USA	UK	Switzerland	NZ	USA	NZ	UK	UK	Switzerland	USA	Germany
Age/gender	49 years female	13 years male	5 days female	NR	47 years female	23 years male	77 years female	Foetus 27 weeks male	66 years female	27 years Male	63 years female
Herb of interest	Comfrey	Comfrey	Coltsfoot	Comfrey	Comfrey	Comfrey	Comfrey	Comfrey	Comfrey	Coltsfoot	*Tussilago* and *Petasites*
ID/ID constituents	PAs	NR	PAs	NR	NR	NR	NR	PAs	NR	NR	NR
Dose Duration	Estimated exposure to PAs 0.7-0.74 mg/kg b.w./ day for 6 months	No information About 3 years	Estimated exposure equiv. dry weight herb 0.6 mg/kg b.w./day for 9 months	NR	Up to 10 cups of comfrey tea a day and handfuls of comfrey-pepsin tablets Over 12 months	4-5 young comfrey leaves, steamed daily 7 – 14 days	1/2 tsp three times a day where comfrey 6 parts in 27, 6 days out of 7, for 6 months	2 gm of mixed herbs used daily in cooking during pregnancy Estimated quantity of PAs 0.014 – 0.021 µg/kg b.w./day.	Daily ingestion of 1-1.5L of a herbal tea blend for several months.	NR	10 leaves Acute poisoning
Concomitant herbs/ medications **Identified/ Not identified**	Yes Unidentified	Yes Prednisolone sulphasalazine	Yes Unidentified	Yes Unidentified	NR	No	Yes Unidentified	Yes Herb teas Unidentified herbs with no PAs.	Yes, eight other herbs. Unidentified.	Yes Twelve herbs Species named.	NR
Other		Pre-existing Crohn's disease	Mother pruritis from 4th month. Caesarean following vaginal bleeding at 36 weeks.	Multiple sclerosis	Hospitalised with ascites 6 years after ceasing comfrey.	Oedema noted prior to ingestion of comfrey.		Mother aged 27 years, 3rd pregnancy	History of arterial hypertension, T2DM moderate adiposity and mild renal insufficiency.		

VOD Veno-occlusive disease
NR Not reported
PA Pyrrolizidine alkaloids

4.7.2 Application of causality assessments to the case reports and case studies

The WHO-UMC instrument was applied to the ten cases with a rating of medium low quality and above. The likelihood of the ingested plants having a causative role in the adverse reaction was rated as 'Probable' in three case (Rasenack et al., 2003; Roulet et al., 1988; Weston et al., 1987) and 'Possible' in six (Bach et al., 1989; Gyorik & Stricker, 2009; Miskelly & Goodyer, 1992; Ridker et al., 1985; Schroff et al., 2013; Yeong et al., 1990), while the case reported by Freshour et al. (2012) was found 'Unassessable'. The RUCAM was applied to the eight cases of appropriate quality where hepatic involvement was reported. In five of the reports the ingested plant material was rated as 'Unlikely' to cause the liver injury (Bach et al., 1989; Roulet et al., 1988; Schroff et al., 2013; Weston et al., 1987; Yeong et al., 1990). In the cases reported by Miskelly and Goodyear (1992) and Rasenack et al. (2003), herb induced liver injury was rated as 'Possible'. One report was found 'Unassessable' (Ridker et al., 1985). The assessment was carried out by 4 of the authors, independently (IB, SS, SE and CA). Disagreements were resolved by discussion. Details of the assessments can be viewed in Table 4.4.

The causality assessments categorise the probability of association into five ranks in the RUCAM and six in the WHO-UMC system (as shown in Table 4.2). None of the cases met the criteria for the first rank in either rating method; 'Certain' (WHO-UMC) or 'Highly probable' (RUCAM) or the second category of the RUCAM ('Probable'). However eight reports were ranked as 'Probable/Likely', the second rank in the WHO-UMC method, or in the third rank of either the RUCAM ('Possible') or WHO-UMC ('Probable').

The report by Freshour and colleagues was rated of low medium quality on the QCRAEI and was considered unassessable/unclassifiable by the WHO-UMC method. There was no liver involvement in this case so it was not rated according to the RUCAM. Another case was considered 'unassesable' when rated on the RUCAM, although the reporting quality was rated 'upper medium' and it was assessable by the WHO-UMC method (Ridker et al., 1985). These findings highlight the pros and cons of using a dual assessment system; results were contradictory in some instances, although more of the human data was assessed for causality in total, than if just one method was relied upon.

Based on these results a cautionary position would advise against the ingestion of the plant materials consumed by these patients. However, to understand the possible role of *Symphytum officinale* or *Tussilago farfara* in these cases, it is necessary to review the source of the herbs and the data authenticating the plant material. If the plants were authenticated in the course of the treatment of the patient, this information was omitted in eight of the eleven case reports and it was incomplete in all of them. Most of the reports omitted a number of other details explaining why only one case (Miskelly et al., 1992) was found to be of 'High' quality based on the QCRAEI. Refer to Table 4.3 for details.

Table 4.4 Assessment of case reports reporting an adverse event potentially associated with *Symphytum officinalis* or *Tussilago farfara*, for quality and strength of association

QCRAEI Quality of reporting	Author date	WHO-UMC Causality	RUCAM Causality of HILI/DILI
High quality	Miskelly 1992	Possible	Possible
Upper medium quality	Yeong 1990	Possible	Unlikely
	Roulet 1988	Probably/likely	Unlikely
	Weston 1987	Probable	Unlikely
	Ridker 1985	Possible	Un-assessable
	Gyorik 2009	Possible	
Low medium quality	Bach 1989	Possible	Unlikely
	Schroff 2013	Possible	Unlikely
	Rasenack 2003	Probably/Likely	Possible
	Freshour 2012	Un-assessable/unclassifiable	
Low quality	Jones 1989		

QCRAEI Quality of Case Reports of Adverse Events Instrument (Agbabiaka et al., 2008)
WHO-UMC World Health Organisation Uppsala Monitoring Centre system for standardised case causality assessment (WHO-UMC)
RUCAM Roussel Uclaf Causality Assessment Method (Danan & Teschke 2015)
No liver involvement

4.8 Search for further information

These case reports are necessarily brief. It is neither possible nor ethical to publish complete case notes. However there was simply insufficient information to make a reliable assessment about the possible involvement of comfrey and coltsfoot in the published reports. Attempts were made to contact each of the authors to request further information about these patients, including case notes if available. The Human Research Ethics Committee (HREC) of Southern Cross University approved this research (Approval number ECN: 17-250). Full details of this aspect of the research are found in Appendix 5.

In most cases the authors were either uncontactable (5), or unable to provide further information (5) as case notes were no longer available. Note that some of these cases are over 30 years old. However in one case, Schroff et al., (2013) further information was obtained.

The original report of acute liver failure after accidental intake of PA-containing plants listed Dr Frieder Schroff as the lead author from the Department of Toxicology in Germany in 2012. Attempts to contact Dr Schroff were unsuccessful. However the senior author (last listed author) on this original case report, Professor Florian Eyer, was able to provide the de-identified medical and supporting documentation surrounding this case for our re-evaluation as per the HREC application.

This documentation provides highly relevant details which were not in the published case report. These details include concomitant medications, possible alternative causes of liver injury and a toxicological report on the plant material. In combination, this enables a significantly more robust assessment of the case than was available from the published study.

From this case involving accidental intake of PA-containing plants, the following are examined in the safety assessment of coltsfoot. A timeline of events, summaries of outcomes and biochemical and imaging data commencing with hospital admission, toxicological analysis of the plant material and subsequent follow-up of this patient are provided. The information gathered in this process is applied through the causality assessment tools and some concluding remarks about PA toxicity are offered.

This is the only case report where the causality assessment tools pre- and post-communication with the author are able to be applied.

A summary of the case is found in Table 4.5 and the timeline of events in Table 4.6.

Table 4.5 Summary of case reported by Schroff et al., 2013

Patient	63-year-old female
Outcome	Slow recovery
Location	Germany
Source	*Petasites* and *Tussilago*
Identification	The herbal material tested was the material picked and ingested by the patient as part of a Korean meal. The testing sample came from the patient's kitchen. Samples of *Petasites* and *Tussilago* were examined for PA content as identified in case notes but not in the original publication
Dose	About 10 leaves, including both of the herbs (about 100 grams)
Duration	Consumed in one dose on a single occasion.
Who prescribed?	Self
Prescribed in response to:	Ingredients for a Korean recipe.
Concomitant herbs and or medications	Not reported in the published case study, found in patient's case notes
Presenting symptoms, diagnostic details and course of adverse event	Symptom onset 3 hours after consumption of *Petasites* and *Tussilago* with nausea and vomiting. Hospitalised next day (19/06/2012) with abdominal pain and signs of hepatic failure with markedly elevated liver enzymes, low prothrombin and thrombocytopenia. Intense treatment for acute poisoning in ICU. Liver biopsy at day 14 revealed veno-occlusive disease with intraluminal fibrin clots in the sinusoids. Discharged from ICU at 24 days, still with elevated liver enzymes. Mild ascites and similarly elevated liver enzymes present 3 months later when a liver biopsy showed slight progression of the veno-occlusive liver disease with partly obliterated lumina of central veins, perivenular drop out hepatocytes and perisinusoidal fibrosis.
Medical and or social history	Recovery complicated by a fall which occurred in hospital and necessitated neurorehabilitation. Longstanding history of depression since 2004
Authors comment on causation	'Veno-occlusive liver-disease caused by pyrrolizidine alkaloid containing herbs may account for significant morbidity.'

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On admission to hospital, standard liver tests were carried out, which were positive for Hepatitis A (patient had been exposed to it, or vaccinated against it), and negative for Hepatitis B and C, and HIV. She was on significant amounts of concomitant medication, some of which may have been affecting the liver and confirmatory metabolites of these drugs were identified through toxicology testing.

Table 4.6 Timeline of events from admission to hospital with acute symptoms until the patient's follow-up liver biopsy.

Date	Event
18/6/12	Collected and ate approximately 100 grams of plant material most likely to be *Petasites albus* and *Tussilago farfara* mistaken for plants she was familiar with in Korea.
19/6/12	Hospitalisation due to vomiting, nausea, epigastric pain
20/6/12	Elevation in liver enzymes, thrombocytopaenia, increased D-dimer, leukopaenia, moved to toxicology ward at different hospital, intensive care
22/6/12	Moved to general ward at hospital
11/7/12	Thrombocytes normal. Sonography: hepatopathy, hepatomegaly, ascites
13/7/12	Discharged from hospital, requiring monitoring for ascites and weight by GP
21/8/12	Hospitalisation due to increasing ascites
23/8/12	Fall in hospital, headache
24/8/12	CCT – bleeding in cerebellum & oedema: suboccipital craniotomy performed. (External ventricular drain (EVD) inserted to relieve elevated intracranial pressure when normal flow of the cerebrospinal fluid inside the brain is obstructed).
29/8/12	Tracheotomy: px has been difficult to intubate, extubation attempt unsuccessful. Slow reaction time on waking. CCT & increase of swelling, so decompression of suboccipital craniotomy. New EVD inserted.
8/9/12	EVD removal
10/9/12	Moved to another clinic, intensive nursing
11/9/12	Moved to acute ward of this clinic
13/9/12	Ventriculitis, intensive nursing, enteral feeding Sonography of abdomen: ascites (little ascites in upper region, obvious ascites in lower region)
20/9/12 (continuing)	Rehabilitation (dysphagia & difficulty with balance) inc. physiotherapy, ergotherapy, neuropsychological diagnosis and therapy. Px had difficulties with memory & concentration. Exacerbation of 'delirantes syndrome' (hallucinatory psychosis). Improved with quetiapine, an antipsychotic.
25/9/12	Sonography of abdomen: slightly more ascites (little ascites in upper region, obvious ascites in lower region)
22/10/12	Sonography of abdomen: no ascites
23/10/12	Liver biopsy: veno-occlusive disease, slight progression of changes within the tissue. No cirrhosis, no malignancy. Due to the bleeding of the brain anticoagulant therapy not recommended.

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Table 4.7 Assessment of quality and causation from published report and patients case notes.

Data source	QCRAEI	WHO-UMC	RUCAM
Published case report Schroff et al 2013	Lower medium quality 18/38	Possible	Unlikely
Case notes provided by senior author in 2018	High quality 33/38	Probable	Possible

QCRAEI Quality of Case Reports of Adverse Events Instrument (Agbabiaka et al., 2008)
WHO-UMC World Health Organisation Uppsala Monitoring Centre system for standardised case causality assessment (WHO-UMC)
RUCAM Roussel Uclaf Causality Assessment Method (Danan & Teschke 2015)

4.8.1 Toxicological analysis

The patient's symptoms were understood to relate to the plants recently collected by the patient and consumed as part of her meal. The uneaten portion of the meal was taken from the patient's kitchen and sent for toxicological analysis (pers. com. Florian Eyer).

The Toxicology Department Laboratory received a sample of the herbs ingested in the patient's meal for analysis for toxic pyrrolizidine alkaloids on 20/07/2012. The analysis was carried out by Helmut Wiedenfeld (Toxicologist). This is a direct quotation from the translation of his report:

Methods:

The sample contained 52.58 g dried leaf material *Petasites albus (P. albus)* and18 g dried leaf material *Tussilago farfara* (*T. farfara*). Analysis for toxic pyrrolizidine alkaloids (PAs) was completed as described in the journal Deutsche Apotheken Zeitung 137: 4070-4075 (1997). PAs were determined by gas chromatography, and structures were identified by GC-MS and also compared with authentic reference material.

Results:

Petasites albus: 52.58 g: 1489 mg seneciphyllin; 207.9 mg senecionin; *Tussilago farfara*: 18g: 0.194 mg senkirkin

Assessment and evaluation:

Pyrrolizidine alkaloids (PAs) can cause liver damage, typically veno-occlusive disease. Depending on their chemical structure, their toxicity can be understood on a spectrum from non-toxic to highly toxic. The PAs found in these two species (seneciphyllin, senecionin, senkirkin) are highly toxic.

In addition to the chemical structure, it is important to assess the amount of plant ingested over what period of time as PAs are metabolised to toxic agents in the liver.

In this case it would be helpful to know the amount of plant material that was ingested.

Conclusion

Our analysis allows the conclusion that oral consumption of *P. albus* carries with it a very high risk of toxicity. This contrasts with *T. farfara* where sub-chronic to chronic damage could be expected. In this case I would not say that there is acute danger from a one-off/singular intake of *Tussilago*.

Note:

There is an extremely high level of toxic PAs identified in this sample of *P. albus*, and normally the levels identified in this plant are decidedly below this amount. However external factors including stress, drought and UV radiation are known to influence alkaloid development positively.

4.8.2 Discussion

In this case, the generous provision of the case notes by the paper's senior author have enabled a much fuller picture of this patient's experience to be presented. Highly relevant details, including that the plant material was tested for PAs, the patient's concurrent medications and a full relevant medical history, were not in the published case report.

The toxicology report not only authenticated the plant material but provided details of the content of the PAs. Further, the toxicologist's assessment of the relative toxicity of butterbur (*Petasites albus*) compared to coltsfoot (*Tussilago farfara*) was that it was unlikely that the one dose of coltsfoot would have caused VOD and acute liver failure.

Data in the published case report (Schroff et al., 2013) was compared with the case notes and subsequent communication with the case study author across the two causality assessment tools (Danan & Teschke, 2015, WHO-UMC). The results can be seen in Table 4.6. The information provided was scored on the QCRAEI too, to indicate what a publication including the relevant additional material would have scored, even though this is not the application it was designed for (Agbabiaka et al., 2008). The value of having better quality data to inform the question of causation is seen in relation to the RUCAM. When this method was applied to the published case report the association of the ingested herbs to the adverse event was rated as 'Unlikely'. With all the details available, the probability of an association was ranked as 'Possible'. The ranking in the WHO-UMC system also changed from 'Possible' to 'Probable'. This confirms that when all relevant data is available in a case report more accurate estimates of causality can be made (Agbabiaka et al., 2008).

4.8.3 Acknowledgements

Florian Eyer has been enormously generous with time and answering questions and this is greatly appreciated. The further documentation has been instrumental to our discussion around the safety of the oral consumption of coltsfoot. Thanks are also extended to Martina Pattison who translated the numerous German documents into English.

4.9 The role of comfrey and coltsfoot

Definitive botanical identification is not reported in the eight cases where a causal association is considered a possibility when rated according to RUCAM or the WHO-UMC (Bach et al., 1989; Gyorik & Stricker, 2009; Miskelly & Goodyer, 1992; Rasenack et al., 2003; Ridker et al., 1985; Roulet et al., 1988; Schroff et al., 2013; Weston et al., 1987; Yeong et al., 1990). In three cases evidence of plants containing PAs found in the plant material, or hepatic metabolites from biopsy or autopsy specimens was published and the patient case notes in the Schroff case also provide this information (Rasenack et al., 2003; Ridker et al., 1985; Roulet et al., 1988; Schroff et al., 2013).

4.9.1 Plant material containing PAs reported

Ridker and colleagues (1985) report a case of a 49-year-old female with hepatic VOD who ingested MU-16 tea (manufactured in Japan) and Comfrey-pepsin tablets (manufactured in the USA). This report was of 'Upper middle' quality rated on QCRAEI (Agbabiaka et al., 2008). The plant material was analysed and the exposure to PAs was estimated at 0.7-0.74 mg/kg b.w./day for 6 months. Assuming a 65kg weight, this would equate to 48.1mg comfrey root/day. This would contain ~130 µg PAs per day. Given the "safe" recommendation is 0.1 µg/day (for the most potent PAs), this would equate to a long-term safe dose of 10µg/d due to the 100-fold weaker PAs in comfrey. So this level of ingestion is still more than 10 times the "safe" daily level for chronic exposure based on relative PA toxicity (Bundesinstitut für Risikobewertung, 2013). However no specific alkaloid was named, the content of the Comfrey-pepsin capsule was described as a white powder which doesn't seem consistent with a root extract and the reviewers were unable to access further information. This report was classified as 'Possible' by the WHO-UMC criteria, but 'Unlikely" on the RUCAM. While the characteristic pathology is strongly suggestive of exposure to unsaturated PAs, a direct relationship to comfrey has not been definitively established.

The fatal exposure to unsaturated PA in utero is reported by Roulet et al. (1988). The expectorant formula consumed daily by the mother was analysed, along with autopsy specimens. The tea was purchased from a pharmacy and contained ten ingredients, 9% w/w was *Tussilago farfara*. Thin layer chromatography (TLC) was used to analyse a sample of the tea and senecionine, one of the most potent PAs was identified (Merz and Schrenk 2016), along with its less toxic N-oxide. The author estimates maternal exposure as equiv. dry weight herb 0.6 mg/kg b.w./day for 9 months, however the reliability of this quantification is in doubt as the stated analytical method was TLC with the full procedure utilised not being described. Without the use of densitometry, TLC does not offer accurate quantitative insight into the samples being analysed (Reich & Schibli, 2007).

Senecionine is found in *Adenostyles alliariae*, *A. glabra*, *Petasites hybridus*, *Senecio spp.* as well as *Tussilago farfara*. That *Tussilago* was the only, or primary source of senecionine in the expectorant has since been questioned. In a letter to the editor published in Journal of Pediatrics in 1989 Spang reported that the tea had been further researched by the local cantonal office and was found to have 'contained not only leaves and flowers of *Tussilago farfara* but also roots of *Petasites officinalis* (*Radix petasitidis*)' (Spang, 1989). These two herbs were also noted in the acute poisoning and development of HVOD in the patient discussed in Schroff et al. (2013). Leaves from these two species were gathered in the wild by a Korean woman who thought she was picking a safe vegetable found in her homeland (Eyer, 2018 pers. comm). In the view of the toxicologist, the *Petasites* was much more likely to have been responsible for her acute symptoms than the *Tussilago*. Could the expectorant tea have been adulterated by *Petasites*?

In 2003 Rasenack et al. reported another fatal case, involving exposure to PAs in utero. This report was found to be of 'Low medium' quality and was categorised in the third rank ('Possible') in both RUCAM and WHO-UMC method. PAs were found in a combination of herbs used daily in cooking and reported to contain comfrey. Analysis of specimens taken from a biopsy of the foetal liver revealed dehydrolycopsamine and dehydrointegerrimine, metabolites of lycopsamine and integerrimine, respectively, while the PAs identified in the plant material were lycopsamine and interrimine and their O7-acetyl derivatives. Lycopsamine is found in *Borago officinalis*, *Symphytum officinale*, *S. asperum* and *S. x uplandicum* and rated of mildest toxicity (Roeder, 1995; Merz and Schrenk 2016). The reviewers were unable to identify a PA known as interrimine. The authors of the case report state the PAs were consistent with *heliotropium* and comfrey, possibly suggesting echinatine, the only unsaturated PA reported in both plants (El-Shazly & Wink, 2014). However, findings from the foetal biopsy suggests integerrimine may have been present in the cooking herbs. Integerrimine is considered by Merz and Shrenk (2016) to be the most potent class of PA and is found in *Petasites hybridus*, *Senecio spp.* and *Tussilago farfara*, but not in comfrey (Nedelcheva et al., 2015; Robins, 1993). Without access to the original toxicology report it is not possible to clear up these discrepancies. However while exposure to unsaturated PAs is undoubtedly the cause of the tragic death reported, the definitive evidence of a causal role for *Tussilago* is lacking.

4.9.2 Case reports where evidence of plant identification is absent

A further four reports describe hepatic or pulmonary VOD, including the fatal intoxication of a 23-year-old male after the ingestion of a 4-5 steamed leaves daily for 7 to 14 days (Yeong et al., 1989). The leaves are reported to have been *Symphytum*, however corroborative evidence is not available. In this case the patient is described as 'emaciated' with 'gross ascites' when hospitalised and to have eaten a vegetarian diet for 4 years characterised by 'binge-eating' single foods for days or weeks. This suggests that the patient's nutritional status is likely to have contributed to his vulnerability to PAs, possibly lowering his glutathione status, consequent of inadequate dietary intake of protein and selenium which are prerequisites for the synthesis of the tripeptide glutathione (Lamba et al., 2002). Clarity around the temporal association of ingestion of the steamed leaves and the onset of the hepatic VOD is also unclear. The patient had a 3-month

history of influenza-like symptoms followed by malaise and night sweats and both peripheral oedema and abdominal distension 3 weeks prior to hospital admission while the steamed leaves were apparently eaten for just two weeks prior to admission. This case report was categorised as 'Unlikely' using RUCAM, but 'Possible' when the WHO-UMC method was applied. It is possible that the patient may have been particularly vulnerable to PAs, however this report does not establish a causal relationship to *Symphytum*.

Weston et al. (1987) reports the case of a male aged 13 who consumed comfrey tea regularly for about 3 years as part of a therapeutic regime to manage Crohn's disease. The boy developed hepatic VOD. Withdrawal of the tea was followed by improvement in liver function. This report was classified as 'Probable' on the WHO-UMC scale and 'Possible' on the RUCAM. While the identity of comfrey was not confirmed, the association to PAs is clearly a strong possibility. The patient was previously and at times concurrently, treated with prednisolone for his inflammatory bowel disease. Since corticosteroids are known to be metabolized in liver, and to induce P450 3A (Matoulkova et al., 2014), this combination may have increased the production of the toxic dehydro-metabolites of whatever PAs were ingested.

The case reported by Miskelly and Goodyer (1992) (the only report rated as of 'High' quality by the QCRAEI criteria) involved a 77-year-old female with pulmonary VOD, who used several herbal tea blends for around 6 months, including comfrey. The case was ranked as 'Probable' using the WHO-UMC method and 'Possible" on RUCAM. Withdrawal of the herb tea resulted in improvement of pulmonary symptoms and a drop in liver enzyme values; the patient declined a liver biopsy. Miskelly and Goodyer name both skullcap (presumably *Scutellaria lateriflora*) and comfrey and writes: 'Both comfrey and skullcap may produce an acute hepatitis but only comfrey is known to produce pulmonary lesions, and then only in rats. This suggests that the pulmonary lesions were evidence of endothelial hyperplasia and due to comfrey' (Miskelly & Goodyer, 1992).

The hepatotoxicity attributed to skullcap is now known to be caused by germander (including Teucrium canadensis and T. chamaedrys) – common adulterants of skullcap. Germander is also known as pink skullcap, and has strong morphological similarities with skullcap (Engels, 2009; Chen et al., 2010). The herbal formula taken by this patient may well have included germander, not skullcap. Without identification of the herbs involved, it is not possible to establish the role comfrey, or any other herb, may have played in this case.

The case study reported by Bach et al. (1989) is the only study that reports hepatic VOD years after the consumption of the source of the PA had stopped. The female involved was 47 years old when hospitalised with ascites and diagnosed with hepatic VOD. The authors suggest the pathology was caused by her daily ingestion of '10 cups of comfrey tea' and comfrey-pepsin tablets 'by the handful' for roughly two years. Ridker et al. also report a case in which a patient took comfrey-pepsin tablets. In the case Bach reported, four years after starting the comfrey, a LFT revealed the patient had elevated liver enzymes. By this time the patient had stopped taking comfrey for about two years. Four years after the LFT, the patient had developed palmar erythema and hepatomegaly with a liver biopsy the following year confirming hepatic VOD. This patient had no history of hepatitis or alcohol problems. The comfrey tea and tablets were sourced from a health food shop. Previous cases suggest that an analysis of the plant material is the only way to rule out other sources of PAs. However, it is likely that the tablets did contain comfrey. Although the PAs in comfrey are considered to be of the mildest potency, the exposure may have been both prolonged and 'handfuls' suggests a relatively high dose. When assessed using the WHO-UMC method this case was categorised as 'Possible' and the further review of the details confirms this assessment. When the RUCAM was applied to the case, the ranking was 'Unlikely' reflecting a lack of detail about concomitant medication and most health history.

4.10 Conclusion

An extensive search of the literature revealed eleven case reports where either comfrey or coltsfoot was considered by the authors to have a possible role in the patients' adverse reaction. Reporting in one case was of 'Low' quality (QCRAEI) (Jones & Taylor, 1989) and another report, which had no hepatic involvement, was found unassessable (WHO-UMC) (Freshour et al., 2012). These cases were not considered reliable enough to inform questions about safety.

A characteristic of the remaining case reports share is a lack of adequate information about the botanical identification of the plants consumed by the patient, although three of the reports did refer to PAs found in ingested plants or biopsy material. Lack of this information is the major limitation to accurate attribution of causality and the reliable assessment of the safety of comfrey and coltsfoot.

Mistaken identity or substitution may have played a role in three cases and was definitely involved in a fourth. The expectorant tea containing *Tussilago* reported by Roulet et al., 1988, may also have contained *Petasites* (Spang, 1989). It is plausible that germander, rather than skullcap may have been found in the herb blends containing comfrey taken by the patient in the case reported by Miskelly et al. (Chen et al., 2010). Rasenack et al. (2003) reported on the analysis of plant material and foetal biopsy and autopsy samples in a case where comfrey was suspected. However some of the PAs named are not found in comfrey, but in *Petasites, Senecio spp* and in *Tussilago* (Nedelcheva et al., 2015). Personal communication and the full case notes, including the toxicology report make it clear that the patient whose case was reported by Schroff et al. (2013) picked and cooked *Tussilago* and *Petasites* mistaking them for a plant native to Korea. These examples emphasise the importance of avoiding assumptions about the identity of herbs potentially associated with an adverse event.

SYSTEMATIC REVIEW OF CASE REPORTS

In three of the cases, including one reported by Gyorik et al. (2009) where the adverse event involved pulmonary hypertension, patients had a significant health history that may have made them more vulnerable to unsaturated PAs. One case involved a boy with Crohn's disease taking comfrey tea, at times with CYP3A inducing steroid medications (Weston et al., 1987). Another woman had several chronic conditions including hypertension and type 2 diabetes mellitus and minor renal insufficiency, (Gyorik & Stricker, 2009), while a third patient is reported to have eaten an inadequate diet for an extended period of time and was described as emaciated (Yeong et al., 1990). In these cases, the lack of definitive identification or confirmation of the PAs present means an opportunity to gain insight into two of the biologically plausible ways in which PAs may be more damaging in some populations than others, in particular where concomitant medications or lowered glutathione levels promote the synthesis or slow the removal of the toxic metabolites from the liver.

In two cases the patients took comfrey-pepsin tablets, and in both cases the dose may have been relatively high (Bach et al., 1989; Ridker et al., 1985). The history of the patient reported by Bach and colleagues follows the pattern of slowly deteriorating liver function eventually diagnosed as HVOD, several years after taking *Symphytum* for an extended period (about two years). This is the pattern observed in animal studies (Dusemund et al., 2018), not so clearly elucidated in any of the other cases. Both these patients had HVOD which is characteristic of PA poisoning, but without evidence that the comfrey-pepsin tablets did contain PAs only from *Symphytum* (along with the herbal tea, in the case reported by Ridker et al., 1985.).

Two of the cases reviewed involved in utero exposure to plants containing PAs, both with fatal consequences (Rasenack et al., 2003; Roulet et al., 1988). With insufficient evidence to differentiate the relative risk of one PA containing plant from another, the avoidance of sources of unsaturated PAs, including comfrey, coltsfoot and borage is strongly recommended in pregnancy and lactation.

The best available evidence in the literature concerning the oral consumption of *Symphytum officinale* and *Tussilago farfara* has been critically assessed in terms of quality of reporting and for causality. Without adequate reporting of the botanical identity of the plants involved it is not possible to reliably determine the degree to which these herbs are associated with HVOD or other symptoms or to make definitive statements about the safety of these herbs.

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Tussilago farfara

CONCLUSIONS AND RECOMMENDATIONS

This report assesses the evidence on the safety of comfrey, coltsfoot and borage to enable the NIMH to make recommendations to its members about safe herbal practice, and to inform the Institute's position in the context of regulation.

5.1 Findings

From the review conducted of the phytochemistry of the PAs, evidence suggests that there are significant concerns regarding the ingestion of both comfrey and coltsfoot. In relation to comfrey, the quantity of PAs is cause for concern. As outlined in Section 2.2, the plant is high in PAs although these particular PAs are low in potency – (~ 1000 µg/g in the leaves and ~ 2700 µg/g in the roots). In contrast, coltsfoot contains significantly lower amounts of PAs (55 µg/g) but these PAs are among the most potent and therefore potentially the most damaging. In contrast, the PAs contained in borage are low in amount (< 10 µg/g) and of the mildest potency.

Extrapolation from animal studies to humans is not straightforward, and in the case of PAs there is evidence that there is significant variation in PA metabolism between individual animal species, including humans. In most relevant animal studies which concern comfrey, the results are mixed. However caution is indicated regarding its ingestion in pregnancy and lactation. No pattern of suspected adverse events was found in the data available from WHO-UMC's VigiBase.

Human evidence of harm caused by these plants is weak, with reported cases being up to thirty years old. Insufficient detail and inconsistent information makes it difficult for clear assessments of causality to be made. With insufficient information about concomitant medication and without full relevant medical history it was impossible to make judgements about causality. While many of the cases were possibly associated with the ingestion of unsaturated PAs, they did not form a reliable body of evidence on which to draw conslusions about the oral consumption of the herbs at the centre of this review. There was one instance where further information was available, and this enabled a robust assessment of the case to be made.

The absence of definitive botanical authentication of the botanical material in many of the cases is of particular concern given the ongoing problems of adulteration or misidentification with these species.

5.2 Limitations of the evidence

As stated, there is little human evidence of harm. Arguments in the literature and by regulators concerning the toxicity of these plants are, with few exceptions, extrapolated from two sources:

- evidence from human exposure to other plant species (not comfrey, coltsfoot or borage) which contain PAs
- evidence from animal studies, often utilising isolated PAs, including those which are not found in comfrey, coltsfoot or borage

The extrapolation of evidence from animals to humans, and the extrapolation of evidence of harm from isolated PAs to those found in the context of the whole plant, raises questions about the toxicity of the whole plant within an individual. The paucity of data on the effects in humans of ingestion of whole plant extracts equates to a lack of research into the real-world application of the plants as they are used in clinical practice. There is an absence of reliable information on the action of the whole plant within an individual patient.

Only eleven case reports were retrieved from an extensive search of the literature. None of the cases concerned borage. The number of ADRs retrieved from the WHO database was also extremely small. While this does not diminish the seriousness of adverse events that arise from plant materials that may contain comfrey or coltsfoot, it does suggest this is an example of a rare harm. This partly explains the paucity of evidence, but also gives weight to the recommendation that a patient-specific risk:benefit analysis be conducted before these plants are recommended.

5.3 Consideration of the therapeutic benefits of the plants

While this report has focussed on the safety of these plants, any considerations of restricting access to them must balance potential harms with their therapeutic benefits. These benefits are recorded in contemporary and traditional texts and are borne out of the experience of those who use them in clinical practice or in everyday life.

The importance of comfrey in tissue healing has been widely acknowledged and for that reason non-PA containing variants have been developed to ensure that it can continue to be used. Borage leaf has captured recent attention with suggestions that it may have a role as a protective agent against gastric cancer. The suggestion that the phenolic content of the leaf may provide protection against its already low PA content is of particular interest to herbalists.

Well documented clinical data in the published literature is required if practitioners' experience is to be validated and taken into account by regulators.

5.4 At risk populations

These plants should not be used with pregnant and lactating women. They should also be avoided in individuals with low hepatic glutathione stores or selenium deficiency or those with pre-existing liver conditions. Caution should also be exercised in those taking herbs or medicines that induce the cytochrome P45O 3A enzyme system.

5.5 Strategies to mitigate potential harm

In terms of actions that may be taken to mitigate possible dangers of the ingestion of these herbs, wereiterate the advice that a healthy, plant-rich, diverse diet rich in foods that support the glutathionation heptic detoxification pathway is advisable – i.e., appropriate amounts of protein and selenium.

In addition, therapeutic interventions that are both liver-protective and increase bile flow could be recommended. It may be possible to identify populations who are more or less likely to be at risk by examining single nucleotide polymorphisms (SNPs) related to CYP3A activity. Patients could be genotyped for the CYP3A4*22 allele to determine whether they are poor metabolisers, intermediate-metabolisers, or entensive metabolisers (Elens, Gelder, Hesselink, Haufroid, & Schaik, 2013). Poor metabolisers would be at the least risk of PA-induced toxicity, as decreased CYP3A activity would result in attenuated production of the highly-reactive PA biotransformation products; whereas extensive metabolisers would be at increased risk of PA-induced toxicity due to increased production of these compounds. Concomitant use of CYP3A inducing herbs or medications with these plants should be avoided.

Care needs to be taken with botanical identification. This is an issue not only when individuals harvest the herb themselves, but also within the supply chain, given the frequency of reported adulteration. Plants should be sourced from identified stock or from a supply chain where plants are authenticated throughout.

5.6 Implications of this research

It has long been central to a herbalist's understanding of medicinal plants that the herb is more than the sum of its parts, and that the action of the whole plant cannot be fully explained by focussing only on individual constituents. The variability of plants due to environmental factors, and the range of extracting mediums and modes of preparation mean that they present a complex array of constituents. Further, the constituent profiles may, as in the case of PAs, alter in a quite dynamic fashion within the plant during its life cycle. Herbalists have long been comfortable with this complexityand have understood it as a strength rather than a weakness of their craft.

It is therefore of concern that these plants are being regulated on the basis of individual constituents without appropriate investigation of the activity of the whole plant, and without appropriate investigation of the interaction between the whole plant and the individual patient. This is counter to the understanding that herbalists have of their use of these particular plants, and of the way they undertake clinical practice. It also provides a worrying precedent should other herbs be found to contain constituents which in other circumstances, in other plants, in other concentrations, have been found to be problematic. The increasing sensitivity and specificity of analytical techniques means that such concerns are more than theoretical.

The issue with PAs continues to evolve. Interestingly, the same research that recently raised concerns about the PA content of *Eupatorium perfoliatum* (boneset) reported that no PAs were found the coltsfoot and borage samples analysed (Colegate, Upton, Gardner, Panter, & Betz, 2018). In a similar vein, Semeret et al. did not find senicionine in *Tussilago* (Seremet et al., 2013). This suggests that further research is required to confirm in which situations these compounds are present and in which they are absent or undetectable.

Further, PAs have been found in herbal teas prepared from plants not known to contain PAs, as a consequence of cross contamination and accidental co-harvesting with PA-containing plants. Effected plants have included *Valeriana officinalis*, *Hypericum perforatum*, *Mentha x piperita* and *Matricaria chamomilla* (Moreira, Pereira, Valentao, & Andrade, 2018). Thus for herbalists, safety assessments related to the ingestion of PAs have broader consequences than the ongoing availability of the three plants under review here.

Practitioners of herbal medicine may feel they are in the firing line with the potential loss of some of their valued tools of trade. However given the extent of their individual and collective clinical experience of the therapeutic effects of these plants, they are uniquely placed to contribute to debates about the risk:benefit analyses of their materia medica. The herbalist will continue to be the advocate for these plants when the evidence of harm is unreliable or inadequate.

CONCLUSIONS AND RECOMMENDATIONS

5.7 Recommendations

5.7.1 Regarding the continued practitioner use of comfrey, coltsfoot and borage

Given the uncertainties revealed by the research to date, it is difficult to draw firm conclusions. In each circumstance where consideration is given to the use of these plants, a risk:benefit analysis relative to that particular patient should be made, and a decision made as to whether the potential benefits outweigh the potential risks.

In relation to the use of these three plants, and particularly in relation to the use of comfrey and coltsfoot, practitioners should ensure that they:

- are well informed about the potential toxicity of the plant and the populations for which it should not be used
- are well informed about the weaknesses of the evidence on which recommendations for the limitations to the uses of the plants are made
- observe time-limits in relation to the use of comfrey and coltsfoot
- include dietary and supplementary recommendations to support protective liver function during treatment.

5.7.2 For future research

While caution is advised on the basis of current research, there are many unanswered questions in relation to the safety of these herbs, and many gaps in the current knowledge. A targeted program of research is recommended. The following are suggestions for further research:

5.7.2.1 N-of-1 trials may offer an appropriate methodological approach. This design is similar to placebo-controlled randomised double-blind clinical trial, but tests an intervention on just one patient. The Oxford Centre of Evidence–based Medicine considers this to be the level one evidence for an intervention in an individual, the same level as a systematic review with meta-analysis (Howick J, 2016). This research design may be appropriate for patients for whom comfrey is well indicated, and would contribute to building a body of first-rate evidence to inform clinical and regulatory decisions.

5.7.2.2 Retrospective epidemiological investigations should be conducted. This might include, for example, inviting participants who have drunk comfrey tea regularly in the past for an extended period of time, and checking their liver enzymes. Consideration of liver biopsies should also be given. This would build on the work of Anderson and McLean, which provided evidence of safe consumption over an extended period. While this sample was small (N=29) no evidence of liver damage was observed in liver function tests, even for individuals who had taken comfrey regularly for over twenty years. (Anderson & McLean, 1989).

5.7.3.3 All adverse events reporting forms and regulatory documentation should document herbal material assessed for authentication. These requirements should be added to assessment tools and included in their scoring profile

5.7.3.4 The opinion of a toxicologist with expertise in this area would provide a current assessment of the relative toxicities of the relevant PAs. This is an ever-changing area of research and regular reviews are warranted. Unfortunately this was beyond the scope of the current report.

5.7.3.5 Given that differences in PA metabolism is to be expected between individuals due to the genetic polymorphisms that determine, for example, differences in CYP3A metabolism, identification and testing of these markers may be a way to ascertain if an individual patient is more at risk through using these plants. Further, consumption of specific foods and medications may well increase the production of toxic secondary compounds through induction of CYP3A, while others may have the opposite effect. Further research is required in these areas.

5.7.3.6 The considerable variation in PA production over the plant's life cycle, and their variation in response to environmental conditions and environmental stressors, suggests there is potential for further understanding in this area to contribute to decision-making about harvest times which minimise Pas, and times when their harvest should be avoided.

5.7.3.7 Animal experimentation, while an ethical barrier for many herbalists, could test the biological plausibility of a number of hypotheses, and enable these ideas to be rejected or taken to the next level of evidence. Questions may include:

- What is the toxicity of whole plant extracts as opposed to the toxicity of individual PAs?
- What is the toxicity of these extracts when administered orally rather than via parenteral administration?
- Does increased glutathione status (achieved via nutritional or herbal supplementation) significantly attenuate possible harms from PA ingestion?
- What is the difference in toxicity when plants from older leaves as opposed to younger leaves are consumed? This is an issue both with comfrey and coltsfoot.

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APPENDICES

A1

HISTORY OF THE *SYMPHYTUM* CONTROVERSY

From the late 1970s, a longstanding perception of comfrey as a safe herb has been challenged, and this needs to be seen against the backdrop of historical events.

Symphytum officinale, common comfrey, is native to the UK and Europe and has a long history as a highly-valued medicinal plant. Of other *Symphytum* species, the most well known is *Symphytum x uplandicum*, Russian comfrey, a hybrid of *S. officinale* and *S. asperum* (prickly comfrey). This was brought from St. Petersburg to the UK by Henry Doubleday, a Quaker horticulturalist in the 1870s, only a few short decades after the Irish Potato Famine. While it was not his original intention for the plant's use, with food security as an issue of the time, Doubleday was soon promoting it as a vegetable for humans and a fodder for animals (Christy, 1877; Hills, 1953, 1976).

S. x uplandicum is a high-yield, non-invasive plant. The popularity it gained in the late 19th century waned until it was revived in the 1940s to counter wartime stock-feed shortages. Hills (1953) describes 'the haste and violence of its growth' in spring and early summer, a 'thick-necked muscular fury about Comfrey that reminds one of the sort of bull that needs watching' (p. 143). This combination of vigorous growth and high nutritional content made it suitable as animal feed, and its palatability led to its being promoted as a human food.

In the post-war era, it attracted the attention of the small group of UK organic farmers who were concerned at the increased use of pesticides and chemicals in agriculture. This group included Frank Newman-Turner, a herbalist, naturopath, author and editor of The Farmer, an organic farming periodical, and Lawrence D. Hills, journalist and founder of the Henry Doubleday Institute (now the charity Garden Organic) (Hills, 1976).

By the 1970s, *S. x uplandicum* had been exported to be grown for food and fodder in parts of Africa and India. Suggested comfrey recipes at this time included not only soups and green drinks, but the ground, dried leaves to be added to flour in breadmaking (Hills, 1976).

In 1976, concerns were raised about PA toxicity in humans after incidents in north-east Afghanistan central India (Mohabbat, 1976; Tandon et al., 1976). In late 1970s and early 1980s the possibility that some species of comfrey may cause problems for human health were raised in Japan by Hirono et al. (1978) and in Australia by Culvenor et al, (1980) on the basis of animal trials. Evidence that Culvenor's concerns are linked to the promotion of Russian comfrey as a food and his awareness of this movement can be found in the 1980 article in Experentia where he quotes Hills among the 'herbalists and organic gardeners [promoting it] as a medicinal herb, salad plant and "green drink"' and his suggestion that 'several hundred thousand people' were consuming comfrey regularly (Culvenor et al., 1980).

It appears that Hills and Newman Turner understood *S. x uplandicum* as a valuable crop for food as well as useful for a myriad of other uses, while Culvenor saw it as a 'potential dietary hazard' due to its pyrrolizidine alkaloid content.

References

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DISTRIBUTION OF PYRROLIZIDINE ALKALOIDS ACROSS BOTANICAL FAMILIES

A2

This table describes the plant genera in which pyrrolizidine alkaloids are found.

Family	Genera
Apocynaceae	• *Alafia* • *Anodendron* • *Fernaldia* • *Parsonsia* • *Urechites*
Asteraceae	• *Adenostemma* • *Adenostyles* • *Ageratum* • *Arnica* • *Brachyglottis* • *Cacalia* • *Chersodoma* • *Chromolaena* • *Cineraria* • *Conoclinum* • *Crassocephalum* • *Critonia* • *Doronicum* • *Emilia* • *Erechtites* • *Eupatorium* • *Farfugium* • *Gynura* • *Homogyne* • *Jacmaia* • *Kleinia* • *Ligularia* • *Llatris* • *Notonia* • *Odontocline* • *Petasites* • *Senecio* • *Syneilesis* • *Trichogonia* • *Tussilago* • *Werneria*

Family	Genera
Boraginaceae	• *Alkanna* • *Anchusa* • *Amsinckia* • *Arnebia* • *Asperugo* • *Borago* • *Buglossoides* • *Caccinia* • *Cerinthe* • *Cryptantha* • *Cynoglossum* • *Echium* • *Gastrocotyle* • *Hackelia* • *Heliotropium* • *Lappula* • *Lindelofia* • *Lithospermum* • *Macrotomia* • *Mertensia* • *Messerschmiedia* • *Molkiopsis* • *Myosotis* • *Neatostema* • *Nonea* • *Paracaryum* • *Paracynoglossum* • *Rindera* • *Solenanthus* • *Symphytum* • *Tournefortia* • *Trachelanthus* • *Trichodesma* • *Ulugbekia*
Ehretiaceae	• *Ehretia*
Celastraceae	• *Bhesa*
Convolvulaceae	• *Ipomoea*

Family	Genera
Fabaceae	• *Adenocarpus* • *Alexa* • *Buchenroedera* • *Castanospermum* • *Crotalaria* • *Laburnum* • *Lotononis*
Orchidaceae	• *Catasetum* • *Chysis* • *Doritis* • *Hammarbya* • *Kingiella* • *Liparis* • *Malaxis* • *Phalaenopsis* • *Trichoglottis* • *Vanda* • *Vandopsis*
Poaceae	• *Festuca* • *Lolium* • *Schismus* • *Thelepogon*
Ranunculaceae	• *Caltha*
Rhizophoraceae	• *Cassipourea*
Santalaceae	• *Amphorogyne* • *Thesium*
Sapotaceae	• *Mimusops* • *Planchonella*

Adapted from Roeder E (1999). Analysis of pyrrolizidine alkaloids. Current Organic Chemistry 3(6):557-576

A3

ASSESSMENT INSTRUMENTS

1. Quality Assessment of Case Reports of Adverse Events Instrument (QCRAEI)

Scoring instructions

Give a score of 2 points for each "yes" or 1 point for each "unclear" and 0 point for each "no".
Some questions have additional scoring instructions.

Low quality = 0-14; lower medium quality 15-21; upper medium quality 22-28; high quality 29-38

Category	Question	Rating *(please tick one box)* Yes	Unclear	No	Item Score
I Information about the medicinal product and treatment	***A – Drug information***				
	Are the following details reported?				
	1. Drug name (i.e. International Nonproprietary Name INN or generic name)	☐	☐	☐	
	2. Brand name and/or manufacturer	☐	☐	☐	
	B – Therapeutic regimen				
	Are the following details reported?				
	3. Daily dose and dosing regimen	☐	☐	☐	
	4. Route of administration *(i.e. oral, subcutaneous)*	☐	☐	☐	
	5. Length of treatment period *(dates and duration of treatment)*	☐	☐	☐	
II Patient history, diagnosis, medical condition(s) and medications	***A – Patient history***				
	Are the following details reported?				
	6. Age	☐	☐	☐	
	7. Sex	☐	☐	☐	
	B – Concurrent diseases and/or medical conditions:				
	8. Are concurrent diseases and/or medical conditions reported (including and allergies)? *(If explicitly stated that there were none, give 2 points)*	☐	☐	☐	
	C – Quality Assessment of Case Reports of Adverse Events Instrument				
	9. Are concurrent diseases analysed/considered with regards to their relevance to the AE? *(Give 2 points if considered in respect to AE or explicitly stated there are no concurrent diseases, give 1 point if mentioned but not adequately discussed and 0 point if not reported at all)*	☐	☐	☐	
	D – Concomitant medications				
	10. Are concomitant medications documented? *(if it is explicitly stated that there were none, give 2 points. 1 point if inadequately reported or unclear and 0 point if no mention of concomitant medications)*	☐	☐	☐	
	11. Are doses, therapeutic regimes and treatment duration reported for concomitant medication? *(all the information is required to score 2 points or if explicitly stated there were no concomitant medications, give 1 point if one or two of the information is reported otherwise give 0 point)*	☐	☐	☐	
	12. Is the involvement of concomitant medications with AE considered/discussed in the report? *(give 2 points if reported and discussed or stated there were no concomitant medications, 1 point if mentioned but not adequately discussed, 0 point if not reported)*	☐	☐	☐	

ASSESSMENT INSTRUMENTS

Category	Question	Rating (please tick one box) Yes	Unclear	No	Item Score
III – Adverse event/ drug interaction information	13. Is the AE/interaction described?				
	14. Is it reported how the AE (or drug interaction) was diagnosed using appropriate diagnostic tests (including lab tests)? Does the report state that the procedures listed below were carried out or explicitly state that they were not?				
	15. Dechallenge or dose manipulation *(give 2 points if either is reported and patients response discussed or explicitly stated that there was no dechallenge and 0 point if not reported at all)*				
	16. Rechallenge *(give 2 points if dose, name of drug or class of drug for rechallenge is reported and adequately discussed or if stated that rechallenge was unacceptable, dangerous or unethical. 0 point if not reported at all)*				
	17. Is there a description of the Algorithm/instrument/scale used for causality assessment *(i.e. WHO classification, any other published scale, or authors own clinical judgment)*				
	Herbal Preparation Information				
	Are the following details reported?				
	1. Full taxonomic name of the plant				
	2. Plant source (i.e. folium, radix, rhizome, . . .)				
	3. Extract name and/or manufacturer or brand name *(all the information is required to score 2 points, give 1 point if one or two of the information is reported otherwise give 0 point)*				
Total Score /40					

Adapted from:

Agbabiaka, T. B., Savović, J., Harris, R., & Ernst, E. (2008). The development of a tool to assess the quality of case reports of adverse events. International Journal of Risk and Safety in Medicine, 20(3), 123-133. doi:10.3233/JRS-2008-0435

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A3

2. WHO-UMC system for standardised case causality assessment

Case		
Causality term	**Assessment Criteria**	
Certain	Event or laboratory test abnormality, with plausible time relationship to drug intake Cannot be explained by disease or other drugs Response to withdrawal plausible (pharmacologically, pathologically) Event definitive pharmacologically or phenomenolgically (ie an objective and specific medical disorder or a recognised pharmacological phenomenon Rechallenge satisfactory, if necessary	
Probable/Likely	Event or laboratory test abnormality, with reasonable time relationship to drug intake Unlikely to be attributed to disease or other drugs Response to withdrawal clinically reasonable Rechallenge not required	
Possible	Event or laboratory test abnormality, with reasonable time relationship to drug intake Could also be explained by disease or other drugs Information on drub withdrawal may be lacking or unclear	
Unlikely	Event or laboratory test abnormality with a time to drug intake that makes a relationship improbable (but not impossible) Disease or other drugs provide plausible explanations	
Conditional/Unclassified	Event or laboratory test abnormality More data for proper assessment needed, or Additional data under examination	
Unassessable/Unclassifiable	Report suggesting an adverse reaction Cannot be judged because information is insufficient of contradictory Data cannot be supplemented or verified	

Available from **http://www.who.int/medicines/areas/quality_safety/safety_efficacy/WHOcausality_assessment.pdf** (accessed 21/9/2018)

3. Roussel Uclaf Causality Assessment Method (RUCAM) in Drug Induced Liver Injury

Worksheet available from https://livertox.nih.gov/rucam.html (accessed 19/9/2018)
Abbreviations used: ALT, alanine aminotransferase; Alk P, alkaline phosphatase; ULN, upper limit of the normal range of values Modified from: Danan G and Benichou C. J Clin Epidemiol 1993; 46: 1323-30.

RUCAM Causality Assessment

Drug: _____ Initial ALT: _____ Initial Alk P: _____ R ratio = [ALT/ULN] ÷ [Alk P/ULN] = _____ ÷ _____ = _____

The R ratio determines whether the injury is hepatocellular (R > 5.0), cholestatic (R < 2.0), or mixed (R = 2.0 – 5.0)

	Hepatocellular Type		Cholestatic or Mixed Type		Assessment
1. Time to onset					
	Initial Treatment	**Subsequent Treatment**	**Initial Treatment**	**Subsequent Treatment**	**Score** (check one only)
○ From the beginning of the drug:					
• Suggestive	5 – 90 days	1 – 15 days	5 – 90 days	1 – 90 days	☐ +2
• Compatible	< 5 or > 90 days	> 15 days	< 5 or > 90 days	> 90 days	☐ +1
○ From cessation of the drug:					
• Compatible	≤ 15 days	≤ 15 days	≤ 30 days	≤ 30 days	☐ +1
Note: If reaction begins before starting the medication or >15 days after stopping (hepatocellular), or >30 days after stopping (cholestatic), the injury should be considered unrelated and the RUCAM cannot be calculated.					
2. Course	**Change in ALT between peak value and ULN**		**Change in Alk P (or total bilirubin) between peak value and ULN**		**Score** (check one only)
After stopping the drug:					
• Highly suggestive	Decrease ≥ 50% within 8 days		Not applicable		☐ +3
• Suggestive	Decrease ≥ 50% within 30 days		Decrease ≥ 50% within 180 days		☐ +2
• Compatible	Not applicable		Decrease < 50% within 180 days		☐ +1
• Inconclusive	No information or decrease ≥ 50% after 30 days		Persistence or increase or no information		☐ 0
• Against the role of the drug	Decrease < 50% after 30 days **OR** Recurrent increase		Not applicable		☐ -2
○ **If the drug is continued:**					
• Inconclusive	All situations		All situations		☐ 0
3. Risk Factors:	**Ethanol**		**Ethanol or Pregnancy (either)**		**Score** (check one for each)
○ Alcohol or Pregnancy	Presence Absence		Presence Absence		☐ +1 ☐ 0
○ Age	Age of the patient ≥ 55 years Age of the patient < 55 years		Age of the patient ≥ 55 years Age of the patient < 55 years		☐ +1 ☐ 0

4. Concomitant drug(s):	**Score** (check one only)
○ None or no information or concomitant drug with incompatible time to onset	☐ 0
○ Concomitant drug with suggestive or compatible time to onset	☐ -1
○ Concomitant drug known to be hepatoxic with a suggestive time to onset	☐ -2
○ Concomitant drug with clear evidence for its role (positive rechallenge or clear link to injury and typical signature)	☐ -3

5. Exclusion of other causes of liver injury:		**Score** (check one only)
Group I (6 causes): ○ **Acute viral hepatitis** due to **HAV** (IgM anti-HAV), or ○ **HBV** (HBsAg and/or IgM anti-HBc), or ○ **HCV** (anti HCV and/or HCV RNA with appropriate clinical history) ○ **Biliary obstruction** (By imaging) ○ **Alcoholism** (History of excessive intake and AST/ALT ≥ 2) ○ **Recent history of hypotension, shock or ischemia** (within 2 weeks of onset) **Group II (2 categories of causes):** ○ Complications of underlying disease(s) such as autoimmune hepatitis, sepsis, chronic hepatitis B or C, primary biliary cirrhosis or sclerosing cholangitis; or ○ Clinical features or serologic and virologic tests indicating acute CMV, EBV, or HSV.	○ All causes in Group I and II ruled out	☐ +2
	○ The 6 causes of Group I ruled out	☐ +1
	○ Five or 4 causes of Group I ruled out	☐ 0
	○ Less than 4 causes of Group 1 ruled out	☐ -2
	○ Non drug cause highly probable	☐ -3

6. Previous information on hepatotoxicity of the drug:	**Score** (check one only)
○ Reaction labeled in the product characteristics	☐ +2
○ Reaction published but unlabeled	☐ +1
○ Reaction unknown	☐ 0

7. Response to readministration:			**Score** (check one only)
○ Positive	Doubling of ALT with drug alone	Doubling of Alk P (or bilirubin) with drug alone	☐ +3
○ Compatible	Doubling of the ALT with the suspect drug combined with another drug which had been given at the time of onset of the initial injury	Doubling of the Alk P (or bilirubin) with the suspect drug combined with another drug which had been given at the time of onset of the initial injury	☐ +1
○ Negative	Increase of ALT but less than ULN with drug alone	Increase of Alk P (or bilirubin) but less than ULN with drug alone	☐ -2
○ Not done or not interpretable	Other situations	Other situations	☐ 0
		TOTAL (add the checked figures)	

Abbreviations used: ALT, alanine aminotransferase; Alk P, alkaline phosphatase; ULN, upper limit of the normal range of values
Modified from: Danan G and Benichou C. J Clin Epidemiol 1993; 46: 1323-30.

CHARACTERISTICS OF CASE STUDIES

The following tables summarise the case studies which are discussed in detail in Section 4 of the report.

Ridker et al., 1985

Patient	49 year old female
Outcome	Recovered
Location	USA
Source 1	MU-16 (tea from Japan) – exact components not reported
Identification	Tea bag from patient's supply analysed for PAs and pyrrolizidine N-oxide. Repeated 2 years later with specially purchased MU-16.
Dose and duration	480g of tea over 6 month period, estimated to provide total PA's 0.49-1.45 micrograms/kg of body weight per day.
Source 2	Comfrey-Pepsin capsules (from USA)
Identification	Analysed for PA and pyrrolizidine N-oxide. Note: dry weight equivalent content of comfrey not declared, and powder described as "white"
Dose Duration	6 capsules/day for 6 months estimated to provide total PA's 14.1 micrograms/kg body weight per day.
Combined dose and duration	Calculated minimum total PA's per day 700-740 micrograms per day over multiple months.
Who prescribed?	Not reported
Prescribed in response to:	Not reported
Concomitant herbs and or medications	Daily vitamin/mineral supplements (A, B C, E, K,Ca, Mg, K, Zn, Fe, lecithin); bovine adrenal extract; 3 cups chamomile tea per week No pharmaceutical medications.
Presenting symptoms, diagnostic details and course of adverse event	Progressive swelling of abdomen & extremities over last 4 months 'Several' hospital admissions without diagnosis. Budd-Chiari syndrome diagnosed on biopsy which revealed centrilobular necrosis and congestion. Hepatic venogram indicated moderate portal hypertension. Side to side portacaval shunt: successfully reduced preportal pressure and ascites; long term follow up after discharge revealed no serious problems except for transient bout of post-shunt encephalopathy attributed to ingestion of >200g protein in 12hr period
Medical and or social history	Two adult children.
Authors comment on causation	Hepatic veno occlusive disease due to chronic low dose PA and pyrrolizidine N-oxide ingestion from MU-16 tea and Comfrey-Pepsin capsules.

Weston et al., 1987

Patient	13 year old male
Outcome	Recovered from AE in context of pre-existing Crohn's disease
Location	UK
Material ingested	Comfrey tea
Identification	Not reported
Dose and duration	Not reported About 3 years
Who prescribed?	Naturopath
Prescribed in response to:	Management of Crohn's disease following parental decision to discontinue Pharmaceuticals reintroduced in response to symptom exacerbation.
Concomitant herbs and or medications	Prednisolone and sulphasalazine in acute exacerbations including at time of admission Acupuncture
Presenting symptoms, diagnostic details and course of adverse event	June 1986 presented with fatigue, diarrhoea and weight loss July 1986 hospital admission fatigue, diarrhoea, weight loss, fever, abdominal pain. Examination revealed ascites and tender hepatomegaly Abnormal findings: Hb 117 g/L, bilirubin 26 mmol/L, AST 87 IU/L, albumin 27g/l, Diagnosis: thrombotic variant hepatic veno-occlusive disease Bx intimal thickening, sinusoidal distension, loss of centrilobular hepatocytes Patient responded well to spironolactone, salt restriction and bed rest. Bowel disease remained relatively inactive with treatment with prednisolone and sulphsalazine, resumed school and 'tolerably well on his medication'
Medical and or social history	Diagnosis of Crohn's disease 1983
Authors comment on causation	Hepatic veno-occlusive disease caused by the ingestion of comfrey tea in child with possibly increased susceptibility due to sub optimal nutritional status secondary to Crohn's disease.

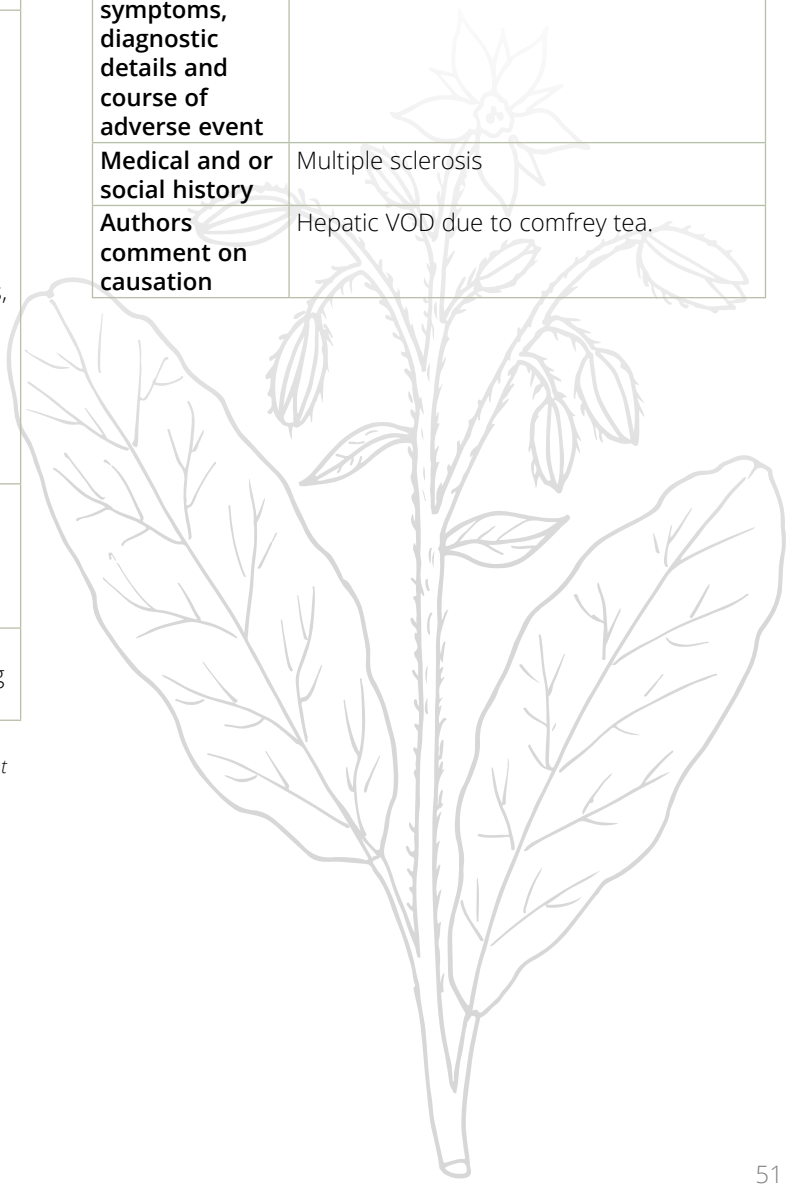
Roulet et al., 1988

Patient	Female neonate, onset of symptoms day 5, deceased day 28..
Outcome	Death
Location	Switzerland
Source	Herbal tea containing *Tussilago farfara* 9% w/w of tea (9 other unnamed ingredients)*
Identification	TLC: 0.6mg senecionine (measured as N-oxide) per kg dry weight
Dose and duration	A single cup daily through out pregnancy. Level of exposure to patient relatively low (0.60 mg/kg dry weight)
Who prescribed?	Not reported
Prescribed in response to:	Expectorant tea purchased at a pharmacy.
Concomitant herbs and or medications	Tea included 9 other unnamed herbs.
Presenting symptoms, diagnostic details and course of adverse event	Normal pregnancy except pruritis of unknown origin since 4th month. Vaginal bleeding led to caesarean at 36 weeks. Birth weight 2740g. Within 5 days of birth developed jaundice, hepatomegaly, ascites, apathy. Serum levels of Liver enzymes and bilirubin markedly elevated, level of some coagulation factors below normal. Liver biopsy day 27 of life: centrilobular fibrosis, neovascularisation, iron deposition, widespread circumferential connective tissue occlusion of small and medium hepatic veins. Patient died 38 days after birth. Autopsy confirmed hepatic veno occlusive disease
Medical and or social history	Occasional cannabis and hallucinogenic mushrooms months before pregnancy. Two siblings; normal births. Mother no abnormalities.
Authors comment on causation	Prolonged daily exposure to *Tussilago farfara* – led to cumulative toxicity causing veno-occlusive disease.

** Spang (1989) reported that the tea the roots of Petasites officinalis, another plant containing unsaturated PA's were found in samples of this tea adds a level of uncertainty to the connection between Tussilago and the death of the infant.*

Jones & Taylor, 1989 Letter

Patient	No details
Outcome	Not reported
Location	NZ
Source	Comfrey tea
Identification	Not reported
Dose and duration	Not reported
Who prescribed?	Self-prescribed
Prescribed in response to:	
Concomitant herbs and or medications	Other herbal teas No pharmaceutical medications
Presenting symptoms, diagnostic details and course of adverse event	Hepatic veno-occlusive disease
Medical and or social history	Multiple sclerosis
Authors comment on causation	Hepatic VOD due to comfrey tea.



CHARACTERISTICS OF CASE STUDIES

Bach et al., 1989 Case report

Patient	47 year old female
Outcome	Symptoms resolved, but liver damage still evident at time of report.
Location	USA
Source	Comfrey tea and comfrey-pepsin tablets
Identification	No identification reported.
Dose	10 cups of comfrey tea per day plus 'handfuls' of comfrey-pepsin tablets
Duration	Over a year.
Who prescribed?	Recommended by a homeopathic doctor and purchased in a health food store in 1978
Prescribed in response to:	Vague complaints of abdominal pain, fatigue and allergies.
Concomitant herbs and or medications	None reported.
History and symptoms of Adverse event	1982 LFT revealed aminotransferase levels twice upper limit of normal. Ingestion of comfrey had stopped for 2-3 years. 1986 hepatomegaly and palmar erythema investigated. Serum ATP levels twice normal levels detected. December 1986 hospitalised with 'massive' ascites, hyponatremia and confusion. Patient responded to low sodium diet, but ascites remained. A Denver shunt was inserted in January 1987 Fluid did not re-accumulate. First biopsy: January 1987: diseased histology including perivenular fibrosis consistent with hepatic venoocclusive disease. 1988 Shunt removed. Second biopsy: September 1989: pathological changes consistent with progressed veno-occlusive disease with further fibrosis and areas of collapse with fibrous septa surrounding parenchyma nodules.
Medical and or social history	No history of alcohol abuse or hepatitis
Authors comment on causation	VOD due to comfrey tea and tablets

Yeong et al., 1989

Patient	23 year old male
Outcome	Death
Location	New Zealand
Source	Not reported
Identification	Not reported
Dose	4-5 young comfrey leaves, steamed daily
Duration	7 – 14 days
Who prescribed?	Self-prescribed.
Prescribed in response to:	Not reported.
Concomitant herbs and or medications	No ethanol, marijuana, other medications or radiation exposure.
History and symptoms of Adverse event	When hospitalised patient was emaciated, febrile, with gross ascites, pleural effusions, peripheral oedema and hepatomegaly on percussion. LFT revealed multiple abnormalities. Two biopsies revealed multiple histological abnormalities; severe portal hypertension confirmed by liver angiography. Treatment with diuretics was initiated. Liver function continued to deteriorate. Patient developed a DVT in his left leg attributed to venous stasis secondary to tense ascites and deteriorating renal function. An operation to insert a meso-atrial shunt failed to prevent further deterioration and the patient died 7 days after the operation. Autopsy confirmed veno-occlusive disease.
Medical and or social history	Vegetarian diet for 4 years characterised by 'binge-eating' single foods for days or weeks. Three month history of influenza-like symptoms followed by malaise and night sweats. Peripheral oedema and abdominal distension 3 weeks prior to hospital admission. No other pathology found on autopsy.
Authors comment on causation	Temporal association indicative of causation due to ingestion of comfrey.

Miskelly 1992 letter

Patient	77 year old woman
Outcome	Recovered
Location	UK
Source	Comfrey tea
Identification	Not reported
Dose and duration	1/2 tsp three times a day where comfrey 6 parts in 27, 6 days out of 7, for 6 months
Who prescribed?	Self-prescribed
Prescribed in response to:	Unclear
Concomitant herbs and or medications	Three herbal formulations; a bowel tonic, a nervine and BFC (Dr Christopher's Bone Flesh Cartilage) which contained 9 species including comfrey and skullcap*, which was reported at I part in 27.
Presenting symptoms, diagnostic details and course of adverse event	Tiredness, anorexia and weight loss for 6 months, cough with green sputum for 3 months. Dark urine for a few weeks. On examination moderately jaundiced, no pyrexia, hepatosplenomegaly or ascites. Chest clear on auscultation. LFT revealed greatly elevated liver enzymes and bilirubin. Herbs continued. LFT unchanged 1 month later. Chest X-ray found reticulonodular shadowing in the right mid-zone and base. Stopped herbs. LFT repeated in 2 weeks, significant improvement revealed. Symptoms gradually improved over next 6 months. Liver function and chest x-ray were normal after 4 months; and patient began to gain weight.
Medical and or social history	Wasting disease
Authors comment on causation	Close temporal association of ingestion of herbal medicines, particularly the comfrey and skullcap, to changes to liver and lung, absence of other agents and cases reports and animal studies demonstrating veno-occlusive disease caused by PAs.

** More recent research has found no evidence that Skullcap (Scutellaria lateriflora) is hepatotoxic, but that it has frequently been substituted by the hepatotoxic germander (including Teucrium canadensis & T. chamaedrys). These species are hepatotoxic (Engels 2009).*

Rasenack et al., 2003

Patient	Female Foetus gestational age 27 weeks
Outcome	Death
Location	Germany
Source	Mixture of herbs imported from Turkey used in daily cooking.
Identification	No botanical identification. GC and GC-Mass spectrometry found pyrrolizidine alkaloids consistent with comfrey and *heliotropium*.
Dose and duration	Estimated the quantity of PAs used in cooking to be 20-30 times the recommended limit of PA or 0.014 – 0.021 µg/kg b.w./day.
Who prescribed?	Self-prescribed
Prescribed in response to:	Not reported
Concomitant herbs and or medications	Several unidentified herbs in the mixture.
Presenting symptoms, diagnostic details and course of adverse event	27 weeks gestation: Foetal skin oedema and polyhydramnios treated with corticosteroids; 30 weeks gestation: 2000mL amniocentesis; 31 weeks: Foetal ascites & skin oedema, intraperitoneal calcifications and hepatomegally. Mother cholestasis with pruritus. Mother treated with cholestyramine; 32 weeks: Mother dyspnoeic; bleeding from placental blood vessel, 2000mL amniocentesis, and subsequent emergency caesarean; upon birth infant ventilated, multiple blood transfusions. Death in 12 hours.
Medical and or social history	In first pregnancy caesarean section was performed postnatal development of the child was normal. 2nd pregnancy: L sided renal atrophy lead to nephrectomy and pregnancy spontaeously aborted in 8th week.
Authors comment on causation	VOD seems to be caused by a herbal mixture used for daily cooking which contained pyrrolizidine alkaloids.

CHARACTERISTICS OF CASE STUDIES

Gyorik & Stricker, 2009

Patient	66 year old female
Outcome	Resolved
Location	Switzerland
Source **Identification** **Dose** **Duration**	Mixture of herbs in a tea including Comfrey No One to one and a half litres a day of a tea containing nine herbs. Months
Who prescribed? **Prescribed in response to:**	Self Unclear
Concomitant herbs and or medications	Another 8 unidentified herbs mixed into tea.
Presenting symptoms, diagnostic details and course of adverse event	2006 hospitalised due to progressive dyspnoea. Blood gas analysis showed severe partial respiratory insufficiency. Liver enzymes were mildly elevated. Pulmonary hypertension confirmed. CT scan of chest showed a mild polyserositis with ground glass opacities in both lungs, not characteristic of pulmonary VOD according to two independent experts. At follow up, requested to stop herbal tea. In 2008 admitted with right heart failure. After diuretic therapy, a CT scan of the chest was repeated showing normal lung parenchyma with complete regression of previously reported opacities.
Medical and or social history	History of arterial hypertension, non insulin dependent diabetes, moderate adiposity and mild renal insufficiency.
Authors comment on causation	No other explanation being found, the authors conclude, the pulmonary hypertension to be 'possibly' caused by the prolonged use of herbal remedies containing comfrey.

Freshour et al., 2012

Patient	27 year old male
Outcome	Recovered (after treatment, should this be included when it has occurred?)
Location	USA
Source **Identification** **Dose** **Duration**	Coltsfoot No Not reported Not reported
Who prescribed? **Prescribed in response to:**	Self No reported.
Concomitant herbs and or medications	Nonsteroidal anti-inflammatories. Twelve other herbs consumed regularly are named.
Presenting symptoms, diagnostic details and course of adverse event	Hospitalised for DVT in right calf and pulmonary embolism. Discontinued herbs. Treated effectively with anti-coagulants which were to be continued on discharge.
Medical and or social history	Right leg pain secondary to back injury 2 weeks prior to admission. Self-treated with bed rest for 14 days. Regular alcohol consumption and 0.5 pack of cigarettes per day.
Authors comment on causation	No direct causal link was identified, but other causes of DVT in a young and otherwise healthy male were excluded, so the authors considered Coltsfoot as a possible causal agent.

Schroff 2013

Patient	63 year old female
Outcome	Slow recovery
Location	Germany
Source	*Petasites* and *Tussilago*
Identification	(Unclear if the material tested was the material ingested. But some samples of *Petasites* and *Tussilago* were examined for PA content found in case notes not publication)
Dose	About 10 leaves, including both of the herbs (about 100 gm)
Duration	Consumed in one dose on a single occasion.
Who prescribed?	Self
Prescribed in response to:	Ingredients for a Korean recipe.
Concomitant herbs and or medications	Not reported.
Presenting symptoms, diagnostic details and course of adverse event	Symptom on set 3 hours after consumption with nausea and vomiting. Hospitalised next day with abdominal pain and signs of hepatic failure with highly elevated liver enzymes, low prothrombin and thrombocytopenia. Intense treatment for acute poisoning in ICU. Liver biopsy at day 14 revealed veno-occlusive disease. Discharged from ICU at 24 days, still with elevated liver enzymes. Mild ascites and similarly elevated liver enzymes present 3 months later when a liver biopsy showed slight progression of the veno-occlusive liver disease.
Medical and or social history	Recovery complicated by a fall which occurred in hospital and necessitated neurorehabilitation.
Authors comment on causation	Veno-occlusive liver-disease caused by pyrrolizidine alkaloid containing herbs may account for significant morbidity.

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Weston, C. F. M., Cooper, B. T., Davies, J. D., & Levine, D. F. (1987). Veno-occlusive disease of the liver secondary to ingestion of comfrey. British Medical Journal (Clinical research ed.), 295(6591), 183. doi:10.1136/bmj.295.6591.183

Yeong, M. L., Swinburn, B., Kennedy, M., & Nicholson, G. (1990). Hepatic veno-occlusive disease associated with comfrey ingestion. Journal of Gastroenterology and Hepatology, 5(2), 211-214. doi:10.1111/j.1440-1746.1990.tb01827.

SECONDARY ANALYSIS OF CASE REPORTS

Published case reports are typically brief and do not include all the data collected about a patient and their medical history, but often concentrate on reporting the cause of a specific pathology. Some of the published studies date from several decades ago and protocols for the attribution of causation have become more rigorous and require specific identification of any plant material implicated. Therefore, it was considered important to seek additional information about published adverse events potentially involving the ingestion of borage, comfrey and coltsfoot.

Methods

Corresponding authors of case studies published in peer reviewed journals concerning these herbs were contacted by email or mail and invited to provide the de-identified medical records of the patient involved where appropriate patient permissions were in place. Authors were specifically asked for pathology, serology, imaging as well as any other medical history, all of which could facilitate detailed analysis of the case using the assessment tools previously described. If no response was received after a fortnight, follow up emails and letters were despatched. Where the corresponding author could not be found, or did not respond, contact details from other authors of the publication were sought and where possible, other authors were contacted. The Human Research Ethics Committee of Southern Cross University (SCU HREC) approved this research (Approval number ECN: 17-250).

Results

There was no response from any of the authors on five of the cases and it is unclear whether the correspondence reached and was viewed by the intended recipients (Freshour, Odle, Rikhye, & Stewart, 2012; Gyorik & Stricker, 2009; Roulet, Laurini, Rivier, & Calame, 1988; Weston, Cooper, Davies, & Levine, 1987; Yeong, Swinburn, Kennedy, & Nicholson, 1990). Authors of another five papers did respond, but were unable to provide case notes, because they were no longer working at the relevant institution or because the original notes were made over 20 years ago, and were thought to have been destroyed (Bach, Thung, & Schaffner, 1989; Jones & Taylor, 1989; Miskelly & Goodyer, 1992; Rasenack, Muller, Kleinschmidt, Rasenack, & Wiedenfeld, 2003; Ridker, Ohkuma, McDermott, Trey, & Huxtable, 1985). Table 1 describes the results of each request for access to original patient case notes.

One author did respond; Professor Eyer, the senior author on the paper published in 2013 (Schroff, Felgenhauer, Pfab, Stenzel, & Eyer, 2013), retrieved the original handwritten notes and converted them to pdfs. and sent them to one of the review authors (CA) to de-identify. This minor change to the original protocol was approved by the SCU HREC. The case notes were then translated and their contents is described in section 4.8

Table 1 Response to requests for access to the case notes with pathology serology and imaging relating to the published adverse event involving *Symphytum officinale or Tussilago farfara*

First Author Date	Response requests for access to patient case notes.
Ridker 1985	Responded. No longer has access to the case notes.
Weston 1987	Unable to contact authors.
Roulet 1988	Unable to contact authors.
Jones 1989	Institution has changed hands. New CEO unable to locate the authors.
Bach 1989	Responded. Patient notes are no longer available.
Yeong 1989	Unable to contact authors.
Miskelly 1992	No longer able to access the patient files. Patient files older than 20 years old are now destroyed.
Rasenack 2003	Response. Moved to another institution, but referred to a colleague Dr Helmut Wiedenfeld, who had retired and was unable to access the case.
Gyorick 2009	Unable to contact.
Freshour 2012	Unable to contact.
Schroff 2013	Response and case notes provided by senior author Prof Florian Eyer

Discussion

The additional information about the case reported by Schroff et al., 2013 confirms the value of referring to the original case notes when reviewing adverse drug reactions. Plant material may have been identified and thus may have informed, at least some of the case reports considered in this review. Without all the appropriate data, attributions of causality cannot be made.

With the increasing uptake of herbal medicines in the West (Casey, Adams, & Sibbritt, 2007; Posadzki, Watson, Alotaibi, & Ernst, 2012) adverse event reporting is becoming a public health issue (Howell et al., 2017; Walji et al., 2009). The promotion of a culture of pharmacovigilance among health professionals and consumers should result in increasingly safe practice. However, reports that lack botanical identification and toxicological analysis are likely to cause the spread disinformation.

Conclusion

Without access to original case notes and in particular details of how the plant material was identified along with associated toxicology reports, it is impossible to gain further insights into the causation of the adverse events described in the published case report.

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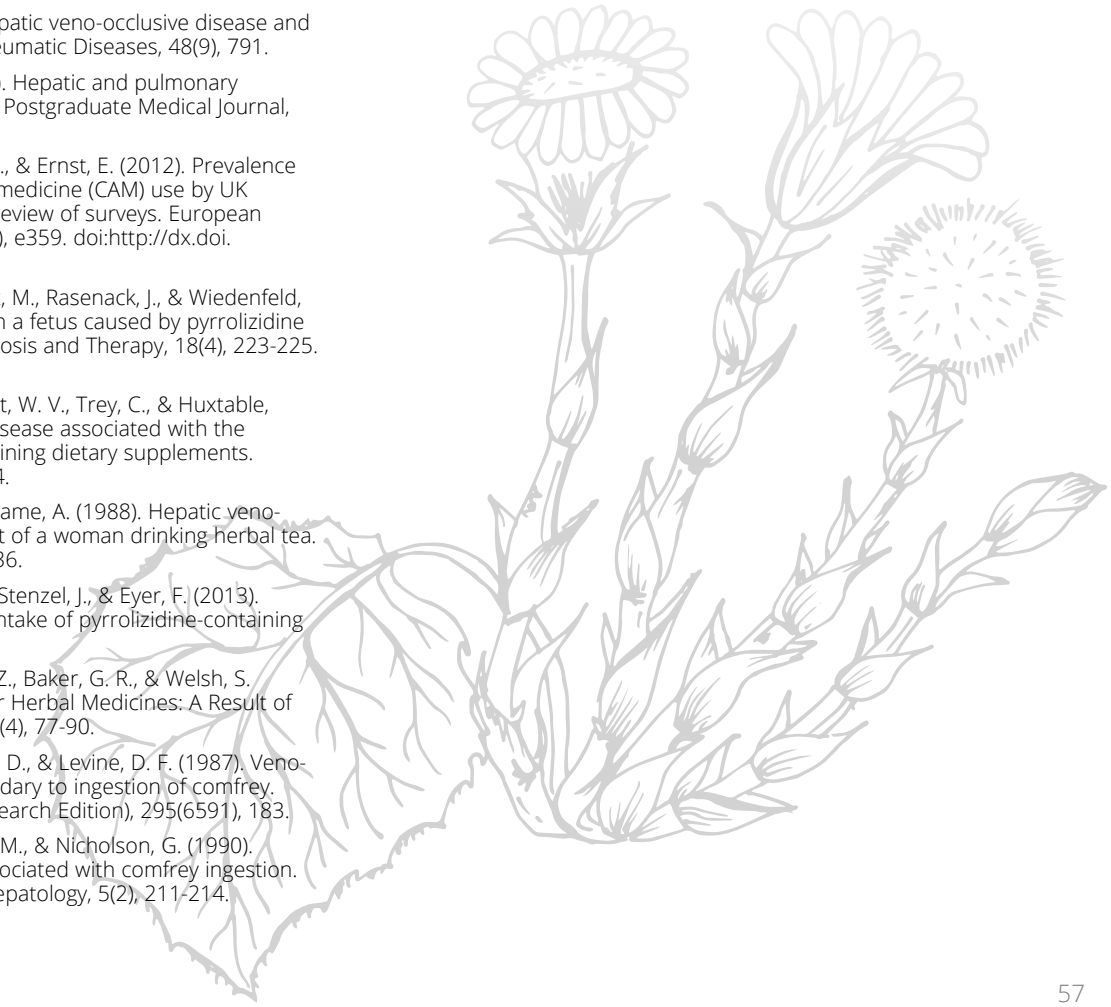

ABOUT THE AUTHORS

Catharine Avila PhD

Dr Catharine Avila is a public health researcher with a background in clinical naturopathy. For the last 17 years she has worked with Southern Cross University as a clinic supervisor, lecturer and researcher. She has 20 years of clinical experience in naturopathy which led to her doctoral research which examined the role of nutritional supplementation in premenstrual syndrome. Her research experience demonstrates a broad range interests from clinical trial management to projects focussing on natural plant pharmacology. Her recent projects include those addressing issues around the safety of medicinal plants in pregnancy and developing systematic review methodology for preclinical herbal research. Previous research includes mixed methods studies investigating rates of failure to attend general practice appointments in disadvantaged populations and an investigation of waiting times for appointments with GPs in the North Coast PHN.

Cathy project managed an Australian Primary Health Care Institute funded project exploring primary health care integration around a general practise superclinic. This led to research into the gap between the Public Health system definition of healthcare integration and the ways in which Australians actually manage their health, including consultations with CAM practitioners, CAM interventions and health promoting lifestyle practices. Cathy is currently engaged in practice-based research, building research capacity in a small group of naturopaths and supporting N-of-1 trials of CAM interventions. She is the lead researcher in a project funded by the Centre of Organics at Southern Cross University investigating herbal medicine products cultivated by organic and conventional methods.

Ian Breakspear MHerbMed (USyd) ND DBM CertPhyto FNHAA

Ian Breakspear is a Senior Lecturer (Naturopathy) at Endeavour College of Natural Health in Australia and has been extensively involved in herbal education and academic leadership for more than 20 years. In addition to his clinical qualifications in botanical medicine and naturopathy, Ian completed a Master of Herbal Medicines within the Faculty of Pharmacy, University of Sydney, specialising in phytopharmacology, toxicology, regulation, and phytochemical analysis.

From 2000-2009 Ian was a Board Member of the Naturopaths and Herbalists Association of Australia (NHAA), with four of those years in the position of Vice-President. His achievements in this role included a complete redevelopment of the NHAA's course accreditation and educational standards for herbal medicine, and in 2006 Ian was awarded with Fellowship of the NHAA for his contributions.

Ian has spoken at four international conferences on herbal medicine, and delivered numerous continuing education seminars to naturopaths, herbalists and pharmacists both nationally and internationally. He has contributed to various academic advisory panels, and was instrumental in work within the profession between 2013-2015 which led to the establishment of Bachelor's degree as the minimum educational standard in herbal medicine and naturopathy from 2015 onwards.

In addition to his educational activities, Ian continues to maintain a clinical practice in Sydney with a focus on assisting patients with cardiovascular disease and has contributed a chapter on the management of cardiovascular conditions in a forthcoming naturopathic medicine text. Ian is also a member of the Expert Scientific Steering Committee for Boundary Bend Olives and is currently conducting research comparing and contrasting the phytochemical profile of various commercially available olive leaf extracts.

Sue Evans PhD FNHAA

Dr Sue Evans has participated in shaping and documenting the changes in Western herbal medicine in Australia for over thirty years. Following a degree in social science she travelled to the UK to undertake herbal medicine training in the late 1970s and returned to Australia to practice, teach and research. Over the years, her understanding of medicinal plants has been enriched by exploring the interface between herbal medicine and history, philosophy, public health, politics and international development.

Through teaching and involvement with her profession she has been involved in a number of 'firsts', including: practicing in the first integrative medicine clinic in Melbourne; helping establish Melbourne's longstanding herbal support group, VicHerbs; being appointed foundation lecturer in herbal medicine at Southern Cross University, in the first state-funded university course in naturopathy in Australia; and being the inaugural chair of an independent advisory committee to the Naturopaths and Herbalists Association of Australia's Board.

She has published widely and lectured throughout Australia and internationally. She has observed the application and use of medicinal plants in other parts of the world, from Latin America (Brazil, Mexico, Chile and Peru) to Mongolia, as well as in the US and the UK, where she undertook her herbal medicine training. She is a Life Member of the NHAA and was awarded the Vis Mediatrix Naturae award at the 2016 Australian Naturopathic Summit. She is currently a senior lecturer in complementary medicines in the College of Health and Medicine at the University of Tasmania.

Jason Hawrelak
BNat (Hons), PhD, FNHAA, MASN, FACN

Dr Jason Hawrelak, is a research scientist, educator, nutritionist, and Western herbalist with more than 18 years' clinical experience. Dr Hawrelak did his Honours (First Class) and PhD degrees in the areas of the gastrointestinal microbiota, irritable bowel syndrome, the causes of dysbiosis, and the clinical applications of pre- and probiotics. He has written extensively in the medical literature on these topics – including 16 textbook chapters – and his research has been cited over 800 times. Jason regularly presents to other health professionals on the gut microbiome, probiotics, optimising gut health, and managing gastrointestinal conditions with natural medicines nationally and internationally, as well as complementary and alternative medicines more broadly.

He currently coordinates and teaches the Evidence-based Complementary Medicine programs in the College of Health & Medicine at the University of Tasmania (Hobart, Australia) and is the Gastrointestinal Imbalances lecturer in the Master of Science in Human Nutrition and Functional Medicine program at the University of Western States (Portland, Oregon). He is also a Visiting Research Fellow at the Australian Research Centre for Complementary and Integrative Medicine (ARCCIM) at the University of Technology Sydney (Sydney, Australia).

Jason is on the Medical Nutrition Council of the American Society for Nutrition and in July 2015, he was awarded a Fellowship from the American Society for Nutrition, for his significant contributions to the teaching and practice of nutrition in Australia and North America. Jason is also a Fellow of the Naturopaths and Herbalists Association of Australia. He is Chief Research Officer at ProbioticAdvisor.com, an on-line resource for clinicians and the public.

Ses Salmond PhD, BA, ND, DBM, DipHom, DipNut, DipRemMass, FNHAA

Dr Ses Salmond has been in practice as a herbalist, naturopath and homoeopath for 26 years in the not-for-profit community sector, at Leichhardt Women's Community Health Centre, Liverpool Women's Health Centre and the Arkana Therapy Centre. Her focus has been both in clinical practice and to provide quality grass roots herbal and naturopathic treatment to a diverse range of socio-economically disadvantaged women. Recently she has been active in coordinating outreach naturopathic clinics within Budyari Aboriginal Community Health Centre and residential retreats for First Nations peoples.

Ses completed her PhD on the Hep573 Study, a randomised, double-blind, placebo-controlled clinical trial of silymarin alone and silymarin combined with antioxidants to improve liver function and quality of life in people with chronic hepatitis C.

She has been a lecturer at two private colleges in Sydney for the past 17 years. She is a past Vice-President of the Naturopaths and Herbalists Association of Australia and is currently an advisor to the Board. Further, she has lectured nationally and internationally and was awarded the Douglas Piper Young Investigator Award in Clinical Sciences by the Gastroenterological Society of Australia in 2010 and the Australian Naturopathic Summit Naturopath of the Year in 2018. Dr Salmond has published widely in medical and naturopathic textbooks.