Toxicity Profile of Pyrrolizidine Alkaloid-Containing Medicinal Plants: Emphasis on Senecio Species

Manuela G. Neuman* • Vanessa Steenkamp**

ABSTRACT

Pyrrolizidine alkaloids (PAs) are found in various plant genera worldwide. Poisoning by PA-containing plants is usually accidental, by the ingestion of grain inadvertently contaminated with seeds of pyrrolizidine-containing weeds, or the consumption of herbal or bush tea, or when taken as herbal infusions for medicinal purposes. In this paper the toxicity of PA-containing plants, with emphasis on Senecio spp. is reviewed. Although the toxicity of Senecio has been documented in numerous case reports, the mechanism of toxicity is not fully known. Elucidating the factors involved in herbal remedies-induced toxicity has medical significance. Currently, there is no antidote for natural substances that induce liver damage. It is important to understand the need for monitoring the use of herbal medicine in order to optimize herbal/traditional medicine use and maximize the clinical and economical benefits. It is also necessary to enhance communication between scientists and physicians of all disciplines involved in complementary alternative medicine and clinical toxicology.

Keywords: case reports, herbal remedies, in vitro, liver, veno-occlusive disease

CONTENTS

BACKGROUND ..................................................................................................................... 104
METABOLISM .................................................................................................................. 104
EFFECTS OF PYRROLIZIDINE ALKALOIDS ................................................................. 105
Clinical and pathological ............................................................................................... 105
Experimental .................................................................................................................... 106
REPORTS OF POISONINGS ........................................................................................... 106
Precautions with herbal remedy use .............................................................................. 106
REFERENCES ................................................................................................................ 107

BACKGROUND

Pyrrolizidine alkaloids (PAs) are a class of secondary metabolites (Hartmann et al. 2004) present in over 450 different plant species that grow worldwide (Neuman et al. 2007). Presently there are over 300 PAs characterized, with the principal medicinal genera containing PAs being Senecio. Poisonings by plants containing PAs are usually accidental: by the ingestion of grain contaminated with pyrrolizidine-containing weeds, as has been reported in Tadjikistan with Heliotrope (Mayer and Luthy 1993) and South Africa with Senecio (Selzer and Parker 1951), consumption of herbal or bush teas made from Crotalaria or Comfrey (Huxtual 1990), or when Senecio was taken as herbal infusions for medicinal purposes (Steenkamp et al. 2000). The toxic effects are usually not detected until irreversible liver damage has occurred (Neame and Pillay 1964).

Various Senecio species are used medicinally. In South Africa, the leaves of S. latifolius are prepared as a paste and applied to treat wounds and burns, whereas decoctions are used to speed up childbirth or to induce abortion (Watt and Breyer-Brandwijk 1962; Hutchings 1989). S. aureus is used to treat injuries and serves as a diaphoretic and diuretic (Varga and Veale 1997), whereas S. bicolor is used as eye drops to treat cataracts and conjunctivitis (Dharmananda 2001). S. longilobus is widely used by an Indian tribe in Arizona as medicine (Stillman et al. 1977a) and S. monoenensis by the Seri Indians as a remedy for flu (Felger and Moser 1974). In traditional Chinese folk medicine, S. scandens (qianliang) is a medication for bacterial diarrhoea, enteritis, conjunctivitis and respiratory tract infections (Tang 1995). Two other species, S. argunensis and S. integrifolis, are also employed in Chinese medicine, both for the treatment for febrile disease, inflammation, diarrhoea and cataracts (Zhao et al. 1998). In Peru, a decoction of the leaves of S. calcitoides, S. tephraxoides, and S. canescens is drunk to treat coughs, bronchitis and asthma (Fernandez-Zuniga et al. 1996) whereas, S. rhizomatosus is used to treat wounds and pneumonia as well as to increase biliary secretion (De Feo 1992).

PAs have a widespread toxic potential of which liver toxicity is so far the most extensively investigated. In this paper, we review the toxicity of PA-containing plants, with emphasis on Senecio spp., a plant widely used medicinally.

METABOLISM

PAs share a common pyrroline structure, consisting of two fused five-membered rings joined by a single nitrogen atom at position 4 to form a heterocyclic nucleus. Chemically at
A least three conditions are essential for hepatotoxicity of PAs: (I) a 1-2 double bond in the necine base, (ii) esterification of the hydroxyl group in one or more positions and (iii) a branched carbon chain in at least one of the ester side chains (Fig. 1).

PAs occur in the plant both as the free base and the N-oxide. Both forms are relatively non-toxic, but the free base is dehydrogenated by hepatic cytochrome P-450 to ester pyrrole intermediates, dehydro-alkaloids (DHA), which are potent electrophiles (Culvenor et al. 1970). CYP3A4 is the major enzyme involved in bioactivation and detoxification of senecionine in human liver (Miranda et al. 1991). The electrophiles can: i) react with water or glutathione (GSH) to form detoxified products (glutathionyl-6,7-dihydro-1-hydroxymethyl-5H-pyrrolizine), ii) alkylate liver macromolecules, or iii) be released into the circulation (Yan et al. 1995) (Fig. 2). The latter is responsible for the pathological changes observed. Up to 80% of the pyrrolizidine ring is excreted in the urine unchanged.

The toxicity of different PAs is proportional to the i) fraction of alkaloid that is converted to pyrrole, ii) rate of conversion and iii) chemical reactivity of the pyrrole produced (Mattocks, 1968). Furthermore, the metabolism and toxicity of PAs are markedly influenced by sulphur amino acid metabolites such as GSH or taurine, as PAs link to both to form non-toxic excretory products (Yan and Huxtable 1995, 1996). Taurine has many protective effects on the liver (Dokshina et al. 1974) and its supplementation has been shown to lower the toxicity and lethality of the PA, monocrotaline, as has supplementation with cysteine or methionine (Yan and Huxtable 1998). Selective induction or inhibition of P450s by drugs or food may lead to changes in toxicity (Eisenstein et al. 1979).

The toxicity of PAs also depends on the exposure time, dosage amount, and susceptibility of the organism (Wainwright and Schonland 1977). A dose of 10 mg/kg per day produces acute toxicity within 1-6 days. On the contrary ingestion of 0.1 mg/kg/day, poses chronic toxicity that presents clinically within months. In humans, the dosage appears to fall within the range of 0.1–10 mg/kg per day (Culvenor 1983).

Additionally, the extent of PA toxicity depends on the nutritional status of the subject. Rats fed a low protein diet were shown to exhibit higher mortality rates than those fed a normal diet (Schoental and Magee 1957). Young animals are more susceptible to the toxic actions of PAs (Schoental 1959; Fowler 1968). Newborn rats are more susceptible to the necrogenic effects of senecionine and monocrotaline, since the liver microsomal hydroxylating activity is low (McLean 1970).

**EFFECTS OF PYRROLIZIDINE ALKALOIDS**

**Clinical and pathological**

PAs are hepatotoxins, which have both acute and chronic effects in man and animals (Wainwright and Schonland, 1977). The manifestation of toxicity is as veno-occlusive liver disease (VOD) (Willmot and Robertson 1920) where
centrilobular haemorrhage (congestion) and centrilobular necrosis of the liver occurs. There is a growing concern over the use of herbal remedies containing PAs since pyrrolizidine-induced liver damage can be cumulative. In the characteristic clinical and biochemical case of poisoning, ascites occurs in 96% of patients, hepatomegaly in 85% and elevated liver enzymes in 92% (Neame and Pillery 1964).

**Experimental**

After a single dose of PAs the sequence of events in time appears to be: failure of DNA-mediated RNA synthesis concurrent with failure of cytoplasmic protein synthesis and disaggregation of polysomes; failure of pyruvate oxidation; loss of glycogen; structural damage to mitochondria; increased lysosomal activity; failure of mitochondrial nicotinamide adenine dinucleotide dependent enzyme synthesis; failure of nuclear nicotine adenine dinucleotide dependent enzyme synthesis and necrosis (McLean, 1970). Most of the toxic effects of pyrrolizidine alkaloids are produced by alkylation of DNA and proteins. The toxicity of PAs can be increased by co-medication with Phenobarbital, a potent inducer of cytochromes (Popat et al. 2001). S. latifolius was found to have a concentration- and time-dependent toxic effect in human hepatoblastoma cells, HepG2 (Neuman et al. 2007). Similarly, the same species showed dose-dependent gross morphological changes in the human hepatoma cell line, HuH-7 (Steenkamp et al. 2001). Necrosis was evident when the cells were treated with high concentrations of *S. latifolius* extract whereas at lower *S. latifolius* extract concentrations, nuclear fragmentation, destruction of the cytoskeleton and apoptosis was observed (Steenkamp et al. 2001). Treatment of HuH-7 cells with the PA, retrorsine, led to multinucleation, failure of spindle formation and clumping of the nuclear chromatin (Steenkamp et al. 2001). GSH depletion was found to be an early and critical event in the mechanism of Senecio-induced cytotoxicity in HepG2 cells (Neuman et al. 2007). Treatment of cells with N-acetyl-cysteine was found to prevent Senecio-induced GSH depletion and result in decreased cytotoxicity (Neuman et al. 2007). The PA, lasiocarpine, was shown to be genotoxic in a primary hepatocyte culture/DNA repair test (Williams et al. 1980). Seneconine has been shown to have a dose-response effect in cultures of rat hepatocytes (Green et al. 1981). Cytotoxicity was evident from the presence of lactate dehydrogenase in culture medium and loss of cells from the substratum. Furthermore, genotoxicity was noted from stimulation of DNA repair and evidence of covalent binding (Green et al. 1981). These findings of genotoxicity and cytotoxicity in vitro experiments on rat primary hepatocytes with seneconine, retrorsine, senechinyllyne, 19-OH-seneconine, trans-4-OH-2-hexenal and trans-4-OH-2-nonenal, also predicting their toxic potential for animals (Green et al. 1981). Senecio toxicity was shown to be present in the presence of lactate dehydrogenase in culture medium and loss of cells from the substratum. Furthermore, genotoxicity was noted from stimulation of DNA repair and evidence of covalent binding (Green et al. 1981). These findings of genotoxicity and cytotoxicity in vitro experiments on rat primary hepatocytes with seneconine, retrorsine, senechinyllyne, 19-OH-seneconine, trans-4-OH-2-hexenal and trans-4-OH-2-nonenal, also predicting their potential carcinogenic role (Griffin and Segall 1986). Mutagenicity (Yamanaka et al. 1979) as well as chromosomal aberrations and inhibition of RNA synthesis (Reddy et al. 1968) has been reported for PAs. Genotoxicity of 16 PAs was indicated using in vitro tests for induction of somatic mutation and mitotic recombination in cells of the developing wing primordial of *Drosophila melanogaster* (Frei et al. 1992). The latter authors concluded that the genotoxic potential of PAs in the wing spot test of *Drosophila* and their carcinogenic potential in mammals seem to be correlated. Fu et al. (2004) reviewed the mechanisms by which PAs exert genotoxicity and tumorigenicity. Senecio species may cause other extrahaematological manifestations such as teratogenesis (Cooper and Huxtable 1999).

The liver is a major component of the reticulo-endothelial system involved in the immune response. The immune system in C57Bl/6 is a sensitive target of monocrotaline toxicity (Deyo and Kerkvliet 1990). The PA metabolite, dehydroheliotridine has significant immunosuppressant activity in mice when given at half the LD50 dose (Percy and Pierce 1971).

The PA, monocrotaline, has been shown to cause pulmonary vascular inflammation (WHO 1988). Rats fed the PA, riddelline (10 mg/kg), for 13 weeks; showed inflammatory cell infiltration, which included accumulations of macrophages in the lungs, liver and kidneys (Chan et al. 1994).

**REPORTS OF POISONINGS**

Veno-occlusive liver disease (VOD) has been associated with consumption of PA-containing dietary supplements (Ridker et al. 1985). VOD leads to cirrhosis and eventually death. There are many reported cases of poisonings by PAs which have resulted in death: Afghanistan (Tandon et al. 1978), Britian (Winston et al. 1987), Egypt (Safouh et al. 1965), Hong Kong (Kumana et al. 1985), India (Tandon et al. 1976), Israel (McLean, 1974), Jamaica (Hill 1952), Scotland (Bateman et al. 1998) and the United States (Huxtable 1990) and Peru (Ortiz et al. 1995).

Various reports describe cases where VOD developed due to PAs: a newborn infant developed VOD through breast milk from the mother who drank herbal tea throughout pregnancy (Rout et al. 1988), four adults who had drunk herbal tea (Kumana et al. 1985), a 18-month-old-boy given herbal tea (Sperl et al. 1995), a preterm neonate who developed VOD due to the mother using a herbal mixture for cooking (Rasenack et al. 2003) and in the UK by drinking tea prepared from comfrey (Culvenor et al. 1980; Weston et al. 1987). VOD epidemics due to cereal contamination have occurred in places like Afghanistan (Tandon et al. 1978) and Central India (Tandon et al. 1976), the former due to contamination with Heliotrope and the latter with Crotalaria species.

A number of cases of PA toxicity due to Senecio species have been published. In Europe the offending Senecio spp. is mainly *S. jacobeae* (Huxtable 1980). Tomioka et al. (1995) reported a case of a young woman developing VOD after cough remedies were prepared using *S. tephrosioides*. VOD has also been reported after a patient had taken commercial herbal preparations as an infusion for chronic constipation containing *S. vulgaris* (Vilar et al. 2000). In another case, *S. vulgaris* tea was drunk by an adult for 2 years and resulted in VOD (Ortiz et al. 1995). A herbal tea containing *S. longilobus* was given to a 6-month-old infant in Arizona (Stillman et al. 1977b) and a 2-month-old-boy as a cough mixture (Fox et al. 1978). In both cases *S. longilobus* was confused with *Gordolobo yerba*, a popular Mexican herb obtained from *Gnaphalium macounii*. A family of 16 from Iraq developed VOD due to cereal contaminated with *Senecio* seeds (Alaee and Mahmood 1998).

From 1974 onwards, cases of VOD have been reported by healthcare practitioners and patients are exposed to low levels of alkaloids in commonly available foodstuffs, such as honey in Australia and the USA (Deinzer et al. 1980), milk in Canada (Sibbald 1999), Europe (Weston et al. 1987), and in the USA (Deinzer et al. 1987). *S. jacobaea* has been reported to contribute to VOD in the USA (Deinzer et al. 1987). *S. jacobaea* and *S. vulgaris* are the most commonly found species in the USA and the UK (Weston et al. 1987).

Various reports of poisonings have occurred in places like Afghanistan (Tandon et al. 1978) and Central India (Tandon et al. 1976), the former due to contamination with Heliotrope and the latter with Crotalaria species.

A number of cases of PA toxicity due to Senecio species have been published. In Europe the offending Senecio spp. is mainly *S. jacobeae* (Huxtable 1980). Tomioka et al. (1995) reported a case of a young woman developing VOD after cough remedies were prepared using *S. tephrosioides*. VOD has also been reported after a patient had taken commercial herbal preparations as an infusion for chronic constipation containing *S. vulgaris* (Vilar et al. 2000). In another case, *S. vulgaris* tea was drunk by an adult for 2 years and resulted in VOD (Ortiz et al. 1995). A herbal tea containing *S. longilobus* was given to a 6-month-old infant in Arizona (Stillman et al. 1977b) and a 2-month-old-boy as a cough mixture (Fox et al. 1978). In both cases *S. longilobus* was confused with *Gordolobo yerba*, a popular Mexican herb obtained from *Gnaphalium macounii*. A family of 16 from Iraq developed VOD due to cereal contaminated with *Senecio* seeds (Alaee and Mahmood 1998).

From 1974 onwards, cases of VOD have been reported by healthcare practitioners and patients are exposed to low levels of alkaloids in commonly available foodstuffs, such as honey in Australia and the USA (Deinzer et al. 1980), milk in Canada (Sibbald 1999), Europe (Weston et al. 1987), and in the USA (Deinzer et al. 1987). *S. jacobaea* has been reported to contribute to VOD in the USA (Deinzer et al. 1987). *S. jacobaea* and *S. vulgaris* are the most commonly found species in the USA and the UK (Weston et al. 1987).

Various reports of poisonings have occurred in places like Afghanistan (Tandon et al. 1978) and Central India (Tandon et al. 1976), the former due to contamination with Heliotrope and the latter with Crotalaria species.

**Precautions with herbal remedy use**

Herbal products are gaining widespread use across the globe. In the United States, it is estimated that up to 40% of the adult population use herbal remedies (Waring 2000), with similar trends occurring in Canada (Sibbald 1999), Europe (Bateman et al. 1998) and Australia (MacLennan et al. 1996). In developing countries, medicinal plants undoubtedly play a valuable role in the treatment of disease, as
they form part of primary healthcare. The common view that all “natural” compounds are safe is a myth. As with most therapeutic drugs, however, there is also a potential to cause toxicity. Of all the plant species used in traditional medicine only ~1% has been scientifically shown to possess therapeutic value. Although medicinal plants offer significant therapeutic benefits, it is imperative that their potential risks are also recognized (Mercier 1984; Stegelmeier et al. 1999).

Eliciting the factors involved in herbal remedies-induced toxicity has medical significance. Fatal poisoning cases that result from the use (or misuse) of traditional herbal medicines continues to be a serious problem. This is especially true for PA containing plants. Standardization of the content of active compounds in remedies is required. This requirement, however poses a different problem as the content of active compounds in remedies is required. Treatment consists of stopping the intake and managing symptoms, but has been largely unsuccessful and has often resulted in death. A rational approach to limit or prevent hepatotoxicity due to herbal remedies is required. From a societal point of view, the mechanism of herbal remedies-induced liver toxicities may assist in gaining a larger recognition of the problem, which will be required for the development of educational strategies aimed at informing physicians and the public about the potential dangers of these commonly used remedies.

It is thus evident that it is important to understand the need for monitoring the use of herbal medicine in order to optimize herbal/traditional medicine use and maximize the clinical and economical benefits. It is also necessary to enhance communication between scientists and physicians of all disciplines involved in complementary alternative medicine and clinical toxicology. More importantly is to educate the public in understanding the limits and the possible danger of using uncontrolled “natural remedies”.

REFERENCES

Hutchings A (1989) A survey and analysis of traditional medicinal plants as used by the Zulu, Zovusa and Sofoto. Bothalia 19 (1), 111-123
Miranda CL, Reid RL, Guengerich FP, Buhrer DR (1991) Role of cytochrome P450 IIIA4 in the metabolism of the pyrrolizidine alkaloid senecioine in human liver. Carcinogenesis 12, 515-519
Neame PB, Pillay VKG (1964) Spontaneous hypoglycaemia, hepatic and renal necrosis following the intake of herbal medicines. South African Medical Journal 10, 729-732
Popp A, Shear NH, Stewart M, Thomson S, Malkiewicz I, Neuman MG...


Willmot FC, Robertson GW (1920) Senecio disease or cirrhosis of the liver due to Senecio poisoning. *Lancet* 2, 848-849


