International Journal of Biomedical and Pharmaceutical Sciences ©2009 Global Science Books

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

Manuela G. Neuman[1*] • Vanessa Steenkamp[2]**

[1] *In Vitro* Drug Safety and Biotechnology, Department of Pharmacology, Faculty of Medicine, University of Toronto, South Tower of the MaRS Discovery Centre, 101 College Street, South Tower, suite 300, lab.351, Toronto, Ontario, M5G 1L5, Canada

[2] Department of Pharmacology, University of Pretoria, Faculty of Health Sciences, PO Box 2034, Pretoria, 0001, Pretoria, South Africa

Corresponding author: * **Manuela.neuman@utoronto.ca** ** **vanessa.steenkamp@up.ac.za**

* **Web-page: http://www.marsdd.com/MaRS-Centre/Our-Tenants/In-Vitro-Drug-Safety-and-BioTechnology.html**

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

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* (Huxtable 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. monoensis* by the Seri Indians as a remedy for flu (Felger and Moser 1974). In traditional Chinese folk medicine, *S. scandens* (qianliguang) is a medication for bacterial diarrhoea, enteritis, conjunctivitis and respiratory tract infections (Tang 1995). Two other species, *S. argunensis* and *S. integrifolius*, 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. culcitioides*, *S. tephrosioides*, 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

Received: 11 March, 2009. Accepted: 9 June, 2009.

Invited Mini-Review

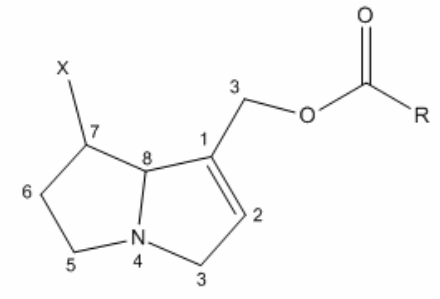

Fig. 1 Structure of a hepatotoxic pyrrolizidine alkaloid. X = RCO=O, HO or O.

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

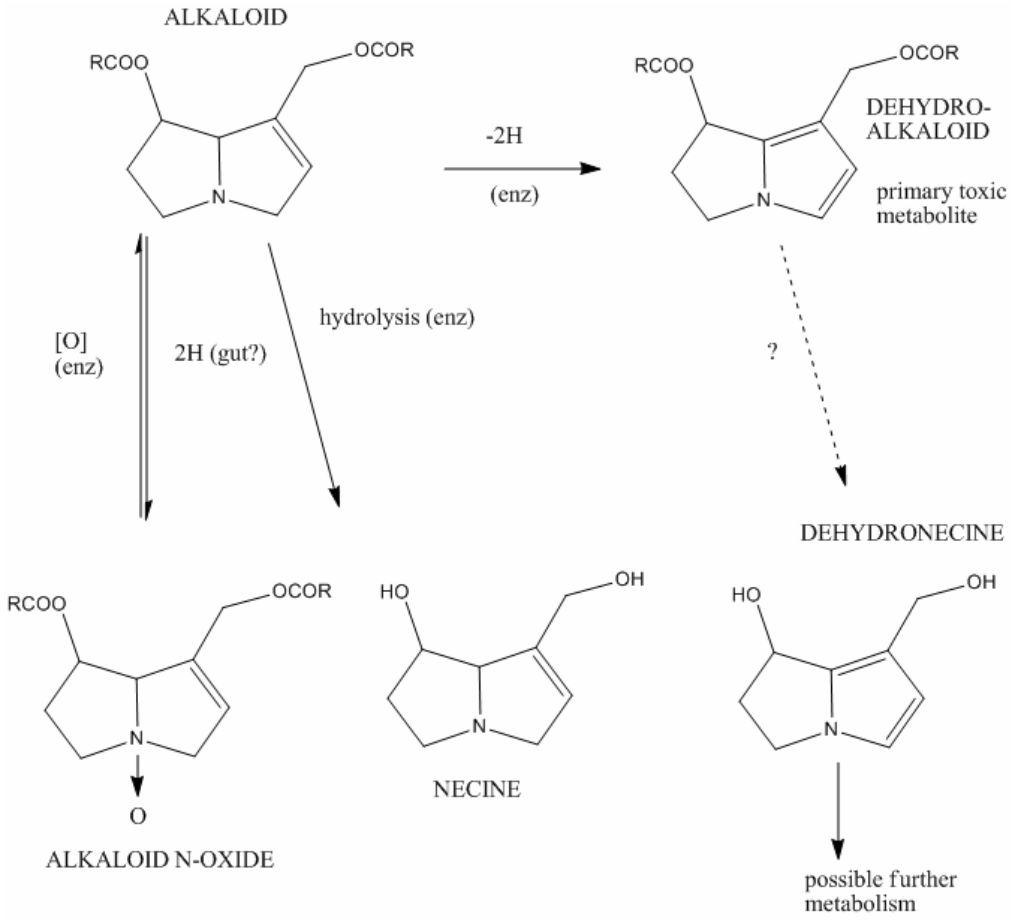

Fig. 2 Metabolism of pyrrolizidine alkaloids.

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 Pillay 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 nicotine 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). Senecionine 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 were supported by *in vitro* experiments on rat primary hepatocytes with senecionine, retrorsine, seneciphylline, 19-OH-senecionine, *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 the *in vivo* tests for induction of somatic mutation and miototic 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 extrahepatic 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 C57BI/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 (Weston *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 drunk herbal tea throughout pregnancy (Roulet *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. jacobea* (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 after wheat was contaminated with *Senecio* seeds (Altaee and Mahmood 1998).

Populations in some countries are exposed to low levels of alkaloids in commonly available foodstuffs, such as honey in Australia and the USA (Deinzer *et al.* 1977), milk (Panter and James 1990) and comfrey in Europe (Weston *et al.* 1987). S. *jacobaea* has been reported to contribute to honey production in Albania, Brazil, Italy, Switzerland, United Kingdom, USA, and Zimbabwe (Edgar *et al.* 2002). The latter authors estimated that 3.9 µg of PAs is present in one gram of honey from *S. jacobaea*. Although acute PA poisoning is still unlikely even when assuming maximum honey consumption of around 93 g per day for adults and 32 g per day for infants, the German Federal Health Bureau (1992) and the International Programme on Chemical Safety (1988) both concluded that the level of PAs present in honey may contribute to chronic liver disease and liver tumours.

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).

Elucidating 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 contents of herbal remedies are often poorly understood, making it difficult to discern the toxicity level and almost impossible to place regulatory standards on which remedies are safe. Secondly, many patients are reluctant to disclose practice of folk remedies. This complicates the diagnosis process, which is already compromised due to poor analytical techniques (Pessayre *et al.* 1999; Zimmerman 1999).

Currently, there is no antidote for natural-substances that induce liver damage including veno-occlusive disease. 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 social 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

Altaee MY, Mahmood MH (1998) An outbreak of veno-occlussive disease of the liver in northern Iraq. *Eastern Mediterranean Health Journal* **1**, 142-148

Bateman J, Chapman D, Simpson D (1998) Possible toxicity of herbal remedies. *Scottish Medical Journal* **43**, 7-15

Chan P, Mahler J, Bucher JR, Travlos GS, Reid JB (1994) Toxicity and carcinogenicity of riddelline following 13 weeks of treatment to rats and mice. *Toxicon* **32**, 891-908

Cooper RA, Huxtable RJ (1999) The relationship between reactivity of metabolites of pyrrolizidine alkaloids and extrahepatic toxicity. *Proceedings of the West Pharmacology Society* **42**, 13-16

Culvenor CCJ (1983) Estimated intakes of pyrrolizidine alkaloids by humans. *Journal of Toxicology and Environmental Health* **11 (4-6)**, 625-635

Culvenor CCJ, Edgar JA, Smith LW, Tweeddale HJ (1970) Dihydropyrrolizidines III. Preparation and reactions of derivatives related to pyrrolizidine alkaloids. *Australian Journal of Chemistry* **23**, 1853-1867

Culvenor CCJ, Clarke M, Edgar JA, Frahn JL, Jago MV, Peterson JE, Smith LW (1980) Structure and toxicity of the alkaloids of Russian comfrey (*Symphytum x uplandicum* Nyman), a medicinal herb and item of human diet. *Experientia* **36**, 377-379

De Feo V (1992) Medicinal and magical plants in the northern Peruvian Andes. *Fitoterapia* **63**, 417-440

Deinzer ML, Thomson PA, Burgett DM, Isaacson DL (1977) Pyrrolizidine alkaloids: their appearance in honey from tansy ragwort (*Senecio jacobaea* L.). *Science* **195**, 497-479

Deyo JA, Kerkvliet NI (1990) Immunotoxicity of the pyrrolizidine alkaloid monocrotaline following subchronic administration to C57BI/6 mice. *Toxicological Sciences* **14**, 842-849

Dharmananda S (2001) Safety issues affecting herbs: pyrrolizidine alkaloids. Institute for Traditional Medicine. Available online: http://www.itmonline.org/arts/pas.htm

Dokshina GA, Yartsev EI, Kolesnikov YA, Kostesha NY (1974) Influence of taurine on the recovery of tissue respiration and oxidative phosphorylation in liver mitochondria in irradiated rats. *Radiology* **14**, 44-48

Edgar JA, Roeder E, Molyneux, J (2002) Honey from plants containing pyrrolizidine alkaloids: a potential threat to health. *Journal of Agricultural and Food Chemistry* **50**, 2719-2730

Eisenstein D, Azari J, Huxtable RJ (1979) Attenuation of the toxicity of a pyrrolizidine alkaloid (monocrotaline) by metabolic inhibition. *Proceedings of the Western Pharmacology Society* **22**, 193-198

Felger RS, Moser MB (1974) Seri Indian pharmacopoeia. *Economic Botany* **4**, 414-436

Fernandez-Zuñiga G, Fernandez-Valderrama I, Hammond GB (1996) Investigation of the essential oils of *Senecio tephrosioides* and *Salvia oppositeflora*. *Revista Latinoamericana de Quimica* **25**, 14-16

Fowler ME (1968) Pyrrolizidine alkaloid poisoning in calves. *Journal of the American Veterinary Medical Association* **152**, 1131-1137

Fox DW, Hart MC, Bergeson PS, Jarrett PB, Stillman AE, Huxtable RJ (1978) Pyrrolizidine (Senecio) intoxication mimicking Reye's syndrome. *The Journal of Pediatrics* **93**, 980-982

Frei H, Luthy J, Brauchli J, Zweifel U, Wurler FE, Schlatter C (1992) Structure/activity relationships of the genotoxic potencies of sixteen pyrrolizidine alkaloids assayed for the induction of somatic mutation and recombination in wing cells of *Drosophila melanogaster*. *Chemico-biological Interactions* **1**, 1-22

Fu PP, Xia Q, Lin G, Chou MW (2004) Pyrrolizidine alkaloids – Genotoxicity, metabolism enzymes, metabolic activation, and mechanisms. *Drug Metabolism Reviews* **36**, 1-55

German Federal Health Bureau (1992) Bundesanzeiger, June 17, 4805. *Deutsche Apotheker-Zeitung* **132**, 1406-1408

Green CE, Segall HJ, Byard JL (1981) Metabolism, cytotoxicity, and genotoxicity of the pyrrolizidine alkaloid senecionine in primary cultures of rat hepatocytes. *Toxicology and Applied Pharmacology* **60**, 176-185

Griffin DS, Segall HJ (1986) Genotoxicity and cytotoxicity of selected pyrrolizidine alkaloids, a possible alkenal metabolite of the alkaloids, and related alkenals. *Toxicology and Applied Pharmacology* **86**, 227-234

Hartmann T, Theuring C, Beuerle T, Ernst L, Singer MS, Bernays EA (2004) Acquired and partially de novo synthesized pyrrolizidine alkaloids in two polyphagous arctiids and the alkaloid profiles of their larval food-plants. *Journal of Chemical Ecology* **30**, 229-254

Hill KR (1952) The vomiting sickness of Jamaica: a review. *The West Indian Medical Journal* **1**, 243-264

Hutchings A (1989) A survey and analysis of traditional medicinal plants as used by the Zulu, Zhosa and Sotho. *Bothalia* **19 (1)**, 111-123

Huxtable RJ (1980) Herbal teas and toxins: Novel aspects of pyrrolizidine poisoning in the United States. *Perspectives in Biology and Medicine* **24**, 1-14

International Programme on Chemical Safety (IPCS) (1988) Pyrrolizidine alkaloids environmental health criteria 80. WHO: Geneva. Available online: http://www.inchem.org/documents/ehc/ehc/ehc080.htm

Kumana C, Ng M, Lin HJ, Ko W, Wu PC, Todd D (1985) Herbal tea induced hepatic veno-occlusive disease: quantification of toxic alkaloid exposure in adults. *Gut* **26**, 101-104

MacLennan AH, Wilson DH, Taylor AW (1996) Prevalence and cost of alternative medicine in Australia. *Lancet* **347**, 569-573

Mattocks AR (1968) Toxicity of pyrrolizidine alkaloids. *Nature* **217**, 723-728

Mayer F, Luthy J (1993) Heliotrope poisoning in Tadjikistan. *Lancet* **342**, 246-247

McLean EK (1970) The toxic actions of pyrrolizine (*Senecio*) alkaloids. *Pharmacology Reviews* **22**, 430-463

McLean E (1974) *Senecio* and other plants as liver poisons. *Israel Journal of Medical Sciences* **10**, 436-440

Mercier M (1984) Information on the International Programme on Chemical Safety, UNEP/ILO/WHO. *Geographia Medica* **14**, 344-359

Miranda CL, Reed RL, Guengerich FP, Buhler DR (1991) Role of cytochrome P450 IIIA4 in the metabolism of the pyrrolizidine alkaloid senecionine 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* **38**, 729-732

Neuman MG, Jia A, Steenkamp V (2007) *Senecio*-induced apoptosis *in vitro*. *Canadian Journal of Physiology and Pharmacology* **85**, 1063-1075

Ortiz Cansado A, Crespo Valadés E, Morales Blanco P, Sáde Santamaria J, González Campillejo JM, Ruiz Téllez T (1995) Veno-occlusive liver disease due to intake of *Senecio vulgaris* tea. *Gastroenterology and Hepatology* **18**, 413-416

Panter KE, James LF (1990) Natural plant toxicants in milk: a review. *Journal of Animal Science* **68**, 892-904

Percy JJ, Pierce AE (1971) Immunosuppressive activity of the pyrrolizidine alkaloid metabolite dehydroheliotridine. *Immunology* **21**, 273-280

Pessayre D, Feldman G, Haouzi D, Fau A, Moreau A, Neuman MG (1999) Hepatocyte apoptosis triggered by natural substances (cytokines, other endogenous molecules and foreign toxins). In: Cameron RG, Fauer G (Eds) *Handbook of Experimental Pharmacology: Apoptosis Modulation by Drugs* (Vol 142), Springer Verlag, Heidelberg, pp 69-109

Popat A, Shear NH, Stewart M, Thomson S, Malkiewicz I, Neuman MG

(2001) The hepatotoxicity of *Callilepis Laureola*, a South African herbal medicine, in HepG2 cells *in vitro*. *Clinical Biochemistry* **34**, 219-227

Rasenack R, Muller C, Kleinschmidt M, Rasenack J, Wiedenfeld H (2003) Veno-occlusive disease in a fetus caused by pyrrolizidine alkaloids of food origin. *Fetal Diagnosis and Therapy* **18**, 223-225

Reddy J, Harris C, Svoboda D (1968) Inhibition by lasiocarpine of RNA synthesis, RNA polymerase and induction of tryptophan pyrrolase activity. *Nature* **217**, 659-661

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

Roulet M, Laurini R, River L, Calame A (1988) Hepatic veno-occlusive disease in a newborn infant of a women drinking herbal tea. *The Journal of Pediatrics* **112**, 433-436

Safouh M, Shehata AH, Elwi A (1965) Hepatic vein occlusion disease in Egyptian children. *Archives of Pathology* **79**, 505-511

Schoental R, Magee P (1957) Chronic liver changes in rats after a single dose of lasiocarpine, a pyrrolizidine (*Senecio*) alkaloid. *The Journal of Pathology and Bacteriology* **74**, 305-319

Schoental R (1959) Liver lesions in young rats suckled by mothers treated with the pyrrolizidine alkaloids lasiocarpine and retrorsine. *The Journal of Pathology and Bacteriology* **77**, 485-495

Selzer G, Parker RGF (1951) Senecio poisoning exhibiting as Chiari's syndrome. A report on twelve cases. *American Journal of Pathology* **27**, 165-185

Sibbald B (1999) New federal office will spend millions to regulate herbal medicines, vitamins. *Canadian Medical Association Journal* **160**, 1355-1357

Sperl W, Stuppner H, Gassner I, Judmaier W, Dietze O, Vogel W (1995) Reversible hepatic veno-occlusive disease in an infant after consumption of pyrrolizidine-containing herbal tea. *European Journal of Pediatrics* **154**, 112-116

Steenkamp V, Stewart MJ, Zuckerman M (2000) Clinical and analytical aspects of pyrrolizidine poisoning caused by South African traditional medicines. *Therapeutic Drug Monitoring* **22**, 302-306

Steenkamp V, Stewart MMJ, van der Merwe S, Zuckerman M, Crowther NJ (2001) The effect of *Senecio latifolius* a plant used as a South African traditional medicine, on a human hepatoma cell line. *Journal of Ethnopharmacology* **78**, 51-58

Stegelmeier BL, Edgar JA, Colegate SM, Gardner DR, Schoch TK, Coulombe RA, Molyneux RJ (1999) Pyrrolizidine alkaloid plants, metabolism and toxicity. *Journal of Natural Toxins* **8**, 95-116

Stillman AE, Huxtable RJ, Fox D, Hart M, Bergeson P (1977a) Pyrrolizidine (Senecio) poisoning in Arizona: severe liver damage due to herbal teas. *Arizona Medicine* **34**, 545-546

Stillman AS, Huxtable R, Consroe P, Kohnen P, Smith S (1977b) Hepatic veno-occlusive disease due to pyrrolizidine (*Senecio*) poisoning in Arizona. *Gastroenterology* **73**, 349-352

Tandon BN, Tandon HD, Tandon RK, Narndranathan M, Joshi YK (1976) An epidemic of veno-occlusive disease of liver in central India. *Lancet* **2**, 271-272

Tandon HD, Tandon BN, Mattocks AR (1978) An epidemic of veno-occlusive disease of the liver in Afghanistan: Pathologic features. *American Journal of Gastroenterology* **70**, 607-613

Tang W (1995) Mutagenic and carcinogenic constituents of Chinese herbal medicines. *Abstracts in Chinese Medicine* **6 (2)**, 227-271

Tomioka M, Calvo F, Siguas A, Sanchez L, Nava E, Garcia U, Valdivia M, Reatequi E (1995) Hepatic veno-occlusive disease associated with ingestion of *Senecio tephrosioides*. *Revista de Gastroenterologia del Peru* **15 (3)**, 299-302

Varga CA, Veale DJH (1997) Isihlambezo Utilization patterns and potential health effects of pregnancy-related traditional herbal medicine. *Social Science and Medicine* **44**, 911-924

Vilar JH, Garcia M, Cabrera P (2000) Veno-occlusive liver disease induced by *Senecio vulgaris* toxicity. *Gastroenterologia y Hepatologia* **23**, 285-286

Wainwright J, Schonland MM (1977) Toxic hepatitis in black patients in Natal. *South African Medical Journal* **51**, 571-573

Waring N (2000) Evaluating herbal remedies. What can the US learn from Germany? *Hippocrates* **14**, 25-27

Weston CF, Cooper BT, Davies JD, Levine DF (1987) Veno-occlusive disease of the liver secondary to the ingestion of comfrey. *British Medical Journal* **295**, 183

WHO (1988) Pyrrolizidine alkaloids. In: *International Programme on Chemical Safety, Environmental Health Criteria* 80. World Health Organization, Geneva, 337 pp

Williams G, Mori H, Hirono I, Nagao M (1980) Genotoxicity of pyrrolizidine alkaloids in the hepatocyte primary culture/DNA repair test. *Mutation Research* **79**, 1-5

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

Yamanaka H, Nagao M, Sugimura T, Furuya T, Shirai A, Matsushima T (1979) Mutagenicity of pyrrolizidine alkaloids in the salmonella/mammalian microsome test. *Mutation Research* **68**, 211-216

Yan CC, Cooper RA, Huxtable RJ (1995) The comparative metabolism of the four pyrrolizidine alkaloids, seneciphylline, retrorsine, monocrotaline and trichodesmine in the isolated perfused rat liver. *Toxicology and Applied Pharmacology* **133**, 277-284

Yan CC, Huxtable RJ (1995) Relationship between glutathione concentration and metabolism of the pyrrolizidine alkaloid, monocrotaline, in the isolated, perfused liver. *Toxicology and Applied Pharmacology* **130**, 132-139

Yan CC, Huxtable RJ (1996) Effects of taurine and guanidinoethane sulfonate on toxicity of the pyrrolizidine alkaloid monocrotaline. *Biochemical Pharmacology* **51**, 321-329

Yan CC, **Huxtable RJ** (1998) Effect of taurine on biliary metabolites of glutathione in liver perfused with the pyrrolizidine alkaloid, monocrotaline. *Advances in Experimental Medicine and Biology* **442**, 85-89

Zhao XZ, Wang ZT, Lin G, Cui YY, Zhang M, Xu LS (1998) Hepatotoxic pyrrolizidine alkaloids and traditional Chinese medicines. *Chinese Traditional Herbal Drugs* **29**, 343-346

Zimmerman HJ (1999) Hepatic metabolism of foreign compounds. In Hepatotoxicity. The adverse effects of drugs and other chemicals on the Liver. Lippincott Williams & Wilkins: Philadelphia, pp 11-40