Safety Issues of Chinese Medicine: A Review of Intoxication Cases in Hong Kong

NG Kit-ying, CHENG Chuen-lung, XU Hong-xi∗

Hong Kong Jockey Club Institute of Chinese Medicine, Hong Kong, China

Abstract: Chinese medicine (CM) has been widely used in Hong Kong for centuries. In fact, CM practitioners currently provide over 20% of the medical consultations in Hong Kong and about 60% of the population has previously used CM. In 1999, the Legislative Council passed the Chinese Medicine Ordinance with aims to govern practice, use, trading, and manufacturing of CM in Hong Kong. While it is usually safe to use CM under proper application and guidance, there have been frequent reports on intoxication and fatalities. The misuse of potent/toxic CM, because of misidentification or overdose, can cause severe aconite, aristolochic acid, anticholinergic, podophyllin, grayanotoxin, pyrrolizidine alkaloids, matrine, gelsemine, teucvin, and strychnine poisoning. In this review, we summarized CM intoxication cases in Hong Kong, concentrating on the discussion of toxic and adverse effects as well as the quality control of CM. By increasing the awareness of CM toxicity, we hope to enhance the regulatory process and stimulate further research on their toxic dose, toxic ingredients, intoxication mechanisms, and quality control.

Introduction

Chinese medicine (CM), with its thousands of years of history, is deeply rooted in the Chinese culture and is therefore widely accepted and used by the general public in Hong Kong. Over the years, it has become more popular as an alternative treatment for chronic diseases and medical problems that cannot be adequately dealt with by modern Western medicine. According to the thematic household survey conducted by the Census and Statistics Department, nearly 20% of medication in Hong Kong was prescribed by CM practitioners (Census and Statistics Department, 2002). Another study conducted by the Chinese University of Hong Kong indicated that about 60% of people in Hong Kong have previously used CM (The Chinese Medicinal Material Research Centre, 1991).

Hong Kong continues to be mainland's largest trading partner for CM herbs and enjoys unique geographical location, well-established regulation and logistic, concessionary tax rates, good trading reputation as well as extensive international business network. With Closer Economic Partnership Arrangement (CEPA), both mainland and international CM manufacturers are enticed to set up production facilities and headquarters in Hong Kong. Products are then sold to the mainland market as tariff-free premium goods and to the global market. In Hong Kong, the practice, use, trading, and manufacturing of CM are governed by the Chinese Medicine Ordinance (CMO), which supposedly should ensure their safety and quality.

However, over the past few decades, CM intoxication episodes concerning intrinsic toxicity, misidentification or erroneous substitutions with other herbs, incorrect preparation and/or dosage, adulteration with orthodox western medicine or their modified analogues, lack of quality control and standardization,

herb-drug interaction and adverse or life-threatening effects have been frequently reported. With the advocate for the modernization of CM, modifications to the ancient formulae and drug preparation, extraction and processing methods have resulted in even more frequent reports on adverse reactions. In this review, published reports of CM intoxication cases in Hong Kong are analyzed and the safety issues concerning CM-provoked toxic and adverse effects as well as the quality control of CM are discussed. Other common CM-related intoxication cases like those due to contamination with heavy metals/pesticides/microbes, or inappropriate labeling and/or advertising will not be discussed in detail; more information on these topics is available on the Department of Health website.

Factors affecting safety of CM

There are several factors affecting the safety of CM consumption. In general, these adverse effects can be attributed to both intrinsic and extrinsic factors. As in those of orthodox medicine, intrinsic factors can be subdivided into type A, B, C, and D reactions (De Smet, 1995). Type A reaction includes predictable toxicity, overdose, and pharmaceutical interactions; Type B reaction includes idiosyncratic reactions; Type C reaction includes adverse effects developed during long-term therapy; and Type D reaction includes delayed effects like carcinogenicity and teratogenicity. Extrinsic adverse effects can be attributed to non-compliance of good agricultural practice (GAP), good extraction practice (GEP), good manufacturing practice (GMP), and good sales or supply practice (GSP) resulting in contamination (heavy metals/pesticides/microbes), lack of standardization, erroneous substitution with toxic herbs, and adulteration with Western medicine or their analogues, incorrect preparation and/or dosage and inappropriate labeling. The extent to which the intrinsic and extrinsic factors lead to overall adverse effects varies considerably with the nature of the medicinal herbs. In general, intrinsic factors impose a greater impact on the overall adverse effects than extrinsic factors, which could be prevented with Standard Operational Practice (SOP).

There are more than 5000 medicinal species in China. Several CM are known to be toxic including *Flos Daturae*, *Flos Rhododendri Mollis*, *Rhizoma Podophylli Emodis*, *Rhizoma Dysosmatis*, *Radix Sophorae Tonkinensis*, *Radix Aconiti*, *Radix Aconiti Kusnezoffii*, *Radix Aconiti Lateralis*, *Semen Strychni*, and *Venenum Bufonis*. According to the Pharmacopoeia of the People's Republic of China (PPRC), toxic herbs can be classified as very toxic (9 entries), toxic (38 entries), and slightly toxic (22 entries) (Pharmacopoeia Commission of the P. R. China, 2005). However, these data must be interpreted with great caution because the concept of toxicity of medicinal materials may vary between medical doctors and traditional Chinese medicines (TCM) practitioners and the PPRC only accounts for the toxic herbs with intrinsic adverse effects.

Intrinsic potent/toxic herbal ingredients causing poisoning

Some Chinese medicinal materials, as with Western pharmaceuticals, are inherently toxic with a narrow safety margin and must be used with great caution. In some cases, erroneous substitutions of herbs with toxic ones were reported primarily due to similarities in name and physical appearance (But, 1993). Thirty-one types of potent/toxic Chinese herbal medicine namely arsenic trioxide, arsenolite, *Calomelas*, *Cinnabaris*, *Flos Daturae Metelis*, *Flos Rhododendri Mollis*, *Huechys*, *Hydrargyri Oxydum Rubrum*, *Lytta*, mercurous chloride and mercuric chloride, mercury, *Mylabris*, *Radix Aconiti Brachypodi* or *Radix Aconiti Szechenyiani*, *Radix* or *Rhizoma Podophylli Emodis* or *Radix* or *Rhizoma Dysosmatis*, *Radix Sophorae Tonkinensis*, *Realgar*, unprocessed *Fructus Crotonis*, unprocessed *Radix Aconiti*, unprocessed *Radix Aconiti Kusnezoffii*, unprocessed *Radix Aconiti Lateralis*, unprocessed *Radix Euphorbiae Fischerianae*, *Radix Euphorbiae Ebracteolatae* or *Radix Stellariae*, unprocessed *Radix Kansui*, unprocessed *Resina*

Garciniae Morellae, unprocessed *Rhizoma Arisaematis*, unprocessed *Rhizoma Pinelliae*, unprocessed *Rhizoma Typhonii* or *Radix Aconiti Coreani*, unprocessed *Semen Euphorbiae*, unprocessed *Semen Hyoscyami*, unprocessed *Semen Strychni*, and *Venenum Bufonis* are listed under Schedule One of CMO with restricted uses in Hong Kong.

Common symptoms of herbal poisoning include nausea, vomiting, and dizziness. Poisoning cases can further be confirmed by morphological identification (pharmacognosy) if the herb is available or more specifically by chemical analysis for toxin identification. The current practice in hospital is to collect herbal leftover and patients' urine samples for identification of chemical markers or intoxication-related biomarkers by chromatography and mass spectrometry (MS).

Aconite poisoning   Aconite-containing CM including *Radix Aconiti* and *Radix Aconiti Kusnezoffii*, which possess analgesic and anti-inflammatory activities and have been used for the treatment of various muscoskeletal disorders, are the most common cause of herbal poisoning. The aconitum alkaloids are the active ingredients of these herbs and the source of toxicity. However, the amount and type of aconitum alkaloids present can vary considerably depending on the species, geographical source, time of harvest, and method of processing. In standard practice, herbal practitioners recommend preparing *Aconitum* L. in decoction so as to reduce toxicity by boiling in water where aconitine alkaloids will be hydrolyzed into less toxic benzoylaconine and other aconite derivatives. This processing method may reduce the alkaloids contents by as much as 90% (Cao, 1993; Chan *et al*, 1994b).

Despite this, aconite poisoning still commonly occur. Neurological and cardiac toxicity may be triggered through the activation of sodium channels by C_{19}-diterpenoid esters, aconitine and other derivatives. Initial symptoms of intoxication include paresthesia, numbness in mouth and limbs, nausea, vomiting, muscle weakness, hypotension, bradycardia, and dizziness. In more serious cases, ventricular dysrhythmias and refractory cardiovascular collapse can occur (Dickens *et al*, 1994; Tai *et al*, 1992; Tomlinson *et al*, 1993). According to Chan (Chan, 2002a), 31 patients were admitted to public hospitals because of aconite poisoning between 1989 and 1991 including two deaths from ventricular arrhythmias. As reported by the Toxicology Reference Laboratory, ten cases of aconitine poisoning in Hong Kong were reported from March 2004 to May 2006; four of the ten cases were inadvertent contamination where aconite herbs were not listed in the written prescription (Poon *et al*, 2006). Erroneous substitution with an aconite herb was suspected.

In these hidden aconite poisoning cases, timely and appropriate sample collections for laboratory confirmation are necessary. Leftover herbal broth and urine samples were analyzed to provide solid evidence for aconite poisoning and yunaconitine, a highly toxic aconitum alkaloid in *Radix Aconiti* from Yunnan province of China, was commonly detected. Other aconitum alkaloids like aconitine, hypaconitine, and mesaconitine were detected in other aconite poisoning cases (Ito *et al*, 1997).   These cases highlight the importance of quality assurance/control in herbs with low safety margin.

Aristolochic acid poisoning   Aristolochic acid (AA) is a nephrotoxic and urothelial carcinogen. Poisoned patients develop transient proteinuria, renal failure and bladder carcinoma (Chen *et al*, 2001; Nortier *et al*, 2000). Due to their highly toxic effects, sale and importation of AA-containing herbs and related products have been prohibited in Hong Kong since June 1, 2004. However, several poisoning cases have still occurred due to erroneous substitution of herbs at wholesale level, in which *Herba Solani Lyrati* was accidentally replaced by toxic *Herba Aristolochiae Mollissimae* and *Radix Stephaniae Tetrandrae* was replaced by toxic *Radix Aristolochiae Fangchi*.

A case of AA-induced nephropathy complication has been reported in 2004 (Lo *et al*, 2004). Another

patient developed nephropathy complication after ingestion of *Herba Aristolochiae Mollissimae* in 2005 where AA was detected in the herbal leftover by high performance liquid chromatography-diode array detection (HPLC-DAD) and electrospray ionization (ESI)-tandem MS (Lo *et al.*, 2005). Prolonged uses of AA-containing herbs like *Caulis Aristolochiae Manshuriensis*, *Radix Aristolochiae Fangchi*, *Fructus Aristolochiae*, *Radix Aristolochiae*, and *Herba Aristolochiae* have been reported to be associated with kidney failure and cancer of urinary tract.

Anticholinergic poisoning　*Flos Daturae* and *Flos Rhododendri Mollis* have long been used for treating asthma, chronic bronchitis, epigastric pain, and toothache. Their constituents, namely scopolamine, hyoscyamine, and atropine, are acetylcholinergic receptor antagonists or muscarinic receptor agonists and so may cause anticholinergic poisoning. Intoxication symptoms include confusion or coma, flushed dry skin and mouth, dilated pupils, tachycardia, fever, and urinary retention (Tse *et al*, 1989).

Between 1985 and 1987, several cases with features of Atropine poisoning were suspected. In some cases, Atropine was detected, while in other cases, Atropine was not detected, but was believed to be contaminated with Atropine-like (structurally and toxicologically) substances (Tse *et al*, 1989).

Poisoning can be attributed to erroneous substitution between *Flos Campasis* and toxic *Flos Daturae* which are similar in dried appearance. Anticholinergic poisoning occurred in a seven-year-old girl who presented symptoms of delirium and confusion. Herbal leftover examination proved erroneous substitution (But *et al*, 1999). Other cases of anticholinergic poisoning from Chinese herbal medicine have also been reported (Chan *et al*, 1994a).

Podophyllin poisoning　*Guijiu* is the dried rhizomes and roots of *Dysoma versipellis* (Hance) M. Cheng. The rhizomes and roots of *Podophyllum hexandrum* Royle and *Podophyllum emodi* Wall. ex

Royle are also used as *Guijiu*, which has therapeutic application in rheumatism, trauma, snakebite, cough, and warts. Podophyllin is the main toxic constituent and can result in poisoning if it is not destroyed by heating or boiling in water. The common cause of poisoning is the erroneous substitution of *P. hexandrum* for *Gentiana scabra* Bunge or *Clematis chinensis* Osbeck. The initial symptoms of *Podophyllum* L. poisoning include vomiting and diarrhea, numbness, poor sensory responses, and motor coordination. High doses of *Podophyllum* L. (> 20 g) can lead to encephalopathy and neuropathy (Chan, 1991; Ng *et al*, 1991). In 1989, misidentification between *P. hexandrum* and *G. scabrta* led to two cases of neuropathy and encephalopathy (But *et al*, 1996). *Podophyllum* L. may induce brain damage by inducing edematous change of anterior horn cells as well as damaging dorsal root ganglia and axons. Between 1995 and 1996, twelve cases of misidentification between *C. chinensis* and *P. hexandrum* had caused neuropathy and one fatality. During the same year, the Department of Health also detected *Podophyllum* L. in 22 of 234 samples analyzed and then requested all shops to stop selling *C. chinensis* (But *et al*, 1996).

Grayanotoxin poisoning　A 57-day-old infant presented with vomiting, convulsion, and shock after consuming milk mixed with decoction of *Rhododendron simsii* Planch. Grayanotoxin was detected in both urine and decoction samples. Grayanotoxin I, the principle toxin found in *Rhododendron* L. and other plants of the Ericaceae family (Lai and Chan, 1999), binds to sodium channels in the cell membrane and in turn prevent their activation. Thus, excitable tissues are kept in a depolarized state, triggering the influx of calcium into cells (Kimura *et al.*, 2000). Common symptoms of grayanotoxin poisoning include nausea, vomiting, swearing, salivation, dizziness, weakness, blurred vision, convulsions, and loss of consciousness. Grayanotoxin also causes adverse effects in cardiovascular system

leading to systemic hypotension, bradycardia, and atrioventricular block.

Pyrrolizidine alkaloids poisoning Retronecine-type and otonecine-type pyrrolizidine alkaloids (PAs) are hepatotoxic. PAs poisoning can lead to veno occlusive diseases with symptoms like arterial hypertension, right ventricular hypertrophy, abdominal pain, ascites, hepatomegaly, and elevated serum transaminase level (Bah *et al*, 1994). It has been reported in Hong Kong that PAs poisoning from Comfrey tea consumption has led to one death due to veno occlusive disease (Kumana *et al*, 1983).

Matrine poisoning *Radix Sophorae Tonkinensis*, also called *Shandougen*, is often used for treating flu symptoms, sore throat, gum pain, and swelling. Two alkaloids, d-matrine and d-oxymatrine are the principle active compounds in *Shandougen*. Symptoms of poisoning like diaphragmatic paralysis and respiratory muscle paralysis can develop if more than 10 g of the herb is taken. Two poisoning cases were confirmed as revealed by matrine detection in patients' urine samples (Clinical Toxicology Task Force for Chinese Medicine, 2002).

Gelsemine poisoning Gelsemine is a natural toxin found in some Chinese medicinal plants, for example, *Gelsemii elegans* (Gardn. et Champ.) Benth. Accidental consumption of *G. elegans* can lead to a range of toxicity reactions, from severe dizziness to respiratory failure. Nine cases of gelsemine poisoning were confirmed upon diagnostic urine alkaloids profiling by liquid chromatography (LC)-tandem MS (Lai and Chan, 2009). As *Radix Gelsemii Elegantis* resembles *Radix Fici Simplicissimae* morphologically, erroneous substitution of the toxic CM for the non-toxic one may have led to poisoning.

Teucvin poisoning *Teucrium viscidum* Bl., used for the treatment of rheumatoid arthritis, hematemesis, and dysmenorrheal, was reported to cause hepatotoxicity. Other *Teucrium* species under Teucrium L. genus like *T. chamaedrys* L., commonly known as *Germander*, *T.*

capitatum L. and *T. polium* L. are also known to be hepatotoxic. A woman consumed herbal remedy containing *T. viscidum* Bl. to treat lower back pain, but developed acute hepatitis and other symptoms like jaundice, nausea, and vomiting (Poon *et al*, 2008). Gas chromatography-MS analysis (GC-MS) detected the presence of teucvin which is a furano neoclerodane diterpenoid of *Germander* and shares great similarities with teucrin A; activation of its furano ring by cytochrome P450 3A results in toxic reactive epoxides that provoke direct toxicity and secondary immune response.

Strychnine poisoning *Semen Strychni*, the dried ripe seed of *Strychnos nux-vomica* L., has been used for the treatment of rheumatism, musculoskeletal injuries, and limb paralysis. A woman consulted a CM practitioner for her neck pain and was prescribed 15 g of *Semen Strychni* which greatly exceeded the recommended dose of 0.3–0.6 g daily. She developed poisoning symptoms with tonic contractions of all her limb muscles and carpopedal spasm. Strychnine and brucine in *Semen Strychni* were found to be responsible for the poisoning (Chan, 2002b).

Adulteration with prescribed medication and/or analogues

Though a lot of proprietary CM claim to contain only herbal medicine, reports have revealed that these products were illegally adulterated by manufacturers with orthodox western medicine, their untested structurally modified analogues and/or banned drug ingredients. Serious side effects may occur in patients who consumed these so-called "CM products" and fatalities have been reported. In addition, the presence of undeclared drugs could cause potential overdose when consumers take these "CM products" concomitantly with orthodox Western medicine. According to the *Pharmacy and Poisons Ordinance of Hong Kong*, products containing Western drug ingredients should be registered before import and on sale in Hong Kong. These products can only be sold with doctor's prescription and taken

under pharmacist's supervision. However, cases of Western drug adulteration in proprietary CM have continuously been reported by the Department of Health. The number of cases reported has surged since 2004 in parallel with a registration system for proprietary CM and a licensing system for wholesalers and retailers of CM as well as wholesalers and manufacturers of proprietary CM. These cases include adulteration with, for example, weight-reducing drugs like Amfepramone, Diethyl propion, Ephedrine, Fenfluramine, and *N*-nitro-sofenfluramine (Kwan *et al*, 2006; Yuen *et al*, 2006; Yuen *et al*, 2007), male erectile dysfunction aiding drugs like Sildenafil, Tadolafil, Vardenafil, and their structurally modified analogues (Lam *et al*, 2008; Poon *et al*, 2007), anti-convulsants like Phenytoin, Carbamazepine, and Valproate (Lau *et al*, 2000), as well as pain-killers, antipsychotics, antihistamines, and anti-infective agents or a mixture of them.

Herb-drug interactions

Herb-drug interactions can be significant especially for Western drugs with narrow therapeutic indices, for instance, Warfarin. Since CM prescription frequently consist of decoctions of a number of different herbs, potential herb-drug interactions could occur if patients use both Western medicine and CM concomitantly, which is a common practice in Hong Kong. Warfarin was reported to interact with (1) *Lycium barbarum* L. resulting in reduced anticoagulant response to Warfarin (Lam *et al*, 2001), (2) *Salvia miltiorrhiza* Bge. leading to over-anticoagulation and bleeding (Chan, 2001; Tam *et al*, 1995), and (3) herbal product "*Guilinggao*" causing anti-platelet and/or anti-thrombotic effects (Wong and Chan, 2003).

Herbs causing adverse effects or increased surgical risks

A survey concerning Chinese herbal medicine intake among preoperative patients in Hong Kong revealed that 90% of the interviewee used CM regularly in the form of traditional soup or tea. Prior to admission, 25% of the patients consulted traditional CM practi-

tioners for their current illness and 13% were taking CM (Critchley *et al*, 2005). Seven herbs including *Eucommia ulmoides* Oliv, *Coptis chinensis* Franch., *Forsythia suspensa* (Thunb.) Vahl, *Aucklandia lappa* Decne., *Atractylodes macrocephala* Koidz., *Dendrobium nobile* Lindl., and *Corydalis turtschaninovii* Bess. have been shown to have vasodilatory and blood pressure lowering effects (Wong *et al*, 2003). Seven other CM including *Duchesnea indica* (Andr.) Focke, *Auricularia polytricha* (Mont.) Sacc., *Dichroa febrifuga* Lour., *Melia toosendan* Sieb. et Zucc., *Herbal Leonuri Japonici*, *Dianthus superbus* L., and *S. miltiorrhiza* have been associated with liver dysfunction and bleeding (Critchley *et al*, 2003). Several other herbs, namely *Polygonum multiflorum* Thunb., *Cassia obtusifolia* L., *Melia toosendan* Sieb. et Zucc., *Rheum palmatum* L., *Scolopendra subspinipes mutilans* L. Koch, *Alisma orientalis* (Sam.) Juzep., *Glycyrrhiza uralensis* Fisch., and *Mentha haplocalyx* Briq. have been linked to high mortality in chronic hepatitis B patients (Yuen *et al*, 2006).

Authentication and quality assurance/ control of CM

Authentication of herbs based on morphological characteristics can be achieved by experts in pharma-cognosy. However, in some cases, herbs may share very similar features making difficult to differentiate them with the naked eye and other traditional methods. This can result in misidentification and cause toxicity problems as exemplified in the sections above. Proper authentication of CM is therefore crucial for ensuring safety and appropriate use, and achieving maximum therapeutic potency. As such, DNA and chemical methods have been developed for more reliable and accurate authentication and quality assurance/control of CM.

DNA methods are able to achieve such high accuracy because genetic materials are unique to each individual herb irrespective of physical morphology, age, time of harvest, storage and method of processing, physiological conditions, and environmental factors.

These methods can be broadly categorized into polymerase chain reaction (PCR)-based, hybridization-based and sequencing-based methods (Yip *et al*, 2007). The PCR-based method is used to amplify the regions of interests in the genome and the amplified products can be distinguished by gel electrophoresis. Authentication of *Pinellia ternata* (Thunb.) Breit. and its adulterants, *Pinellia pedatisecta* Schott and *Arisaema heterophyllum* Bl. were carried out by PCR method (Lin *et al*, 2006). One pair of gene-specific primers was designed for the amplification of the 5' and 3' flanking region of mannose-binding lectins of *P. ternata* and another pair of primers for the amplification of the open reading frame region of mannose-binding lectins of *P. ternata*, *P. pedatisecta*, and *A. heterophyllum*. The PCR products, digested with restriction enzymes, differed in molecular size and could be distinguished by gel electrophoresis. *Gentiana macrophylla* Pall. has been reported to be differentiated from the other three species of the *Gentiana* L. genus by real-time PCR (Xue *et al*, 2008). A pair of primers flanking the ribosomal DNA internal transcribed spacer 2 (ITS2) sequence, which is highly unique for *G. macrophylla* and another pair of primers flanking the 5.8S gene region, which is highly conserved among angiosperm, were designed for species identification. The hybridization-based method, in which two single-stranded nucleic acids are annealed to form a double-stranded nucleic acid, has been used for the species identification in *Dendrobium* Sw. (Li *et al*, 2005), *Fritillaria* L. (Tsoi *et al*, 2003), *Datura* L. (Trau *et al*, 2002) and *Pinellia* Ten (Carles *et al*, 2001). A silicon-based DNA microarray, as a rapid tool for quality control and safety monitoring of herbal pharmaceuticals and nutraceuticals, was developed for the identification of toxic traditional Chinese medicinal plants (Carles *et al*, 2005). Species-specific oligonucleotide probes derived from the 5S ribosomal RNA (rRNA) gene of *Aconitum carmichaeli* Debeaux, *Aconitum kusnezoffi* Reichb., *Alocasia macrorrhiza* (L.) Schott, *Croton tiglium* L., *Datura inoxia* Mill., *Datura metel* L., *Datura tatula* L.,

Dysosma pleiantha (Hance) R. E. Woodson, *D. versipellis*, *Euphorbia kansui* S. B. Ho, *Hyoscyamus niger* L., *Pinella cordata* N. E. Br., *P. pedatisecta*, *P. ternata*, *Rhododendron molle* (Blume) G. Don, *S. nux-vomica*, *Typhonium divaricatum* (L.) Decne., and *Typhonium giganteum* Engl. and the leucine transfer RNA gene of *Aconitum pendulum* Busch and *Stellera chamaejasme* Linn. were immobilized *via* dithiol linkage on a silicon chip. Target gene sequences of interest were then amplified, which allows parallel genotyping of multiple toxic plant species. The sequencing method allows determination of the sequences of nucleotide bases in DNA or RNA. *Radix Aucklandia*, the dried root of *Saussurea lappa*, shares the same trade name "*Muxiang*" with *Vladimiria berardioidea* (Franch.) Ling, *Vladimiria souliei* (Franch.) Ling, *Inula helenium* L., *Inula racemosa* Hook. f. in the Asteraceae family and toxic *Aristolochia debilis* Sieb. et Zucc. in the Aristolochiaceae family. Authentication of *Radix Aucklandia* was conducted by ITS DNA and 5S rRNA sequencing (Chen *et al*, 2008a). In pyrosequencing analysis, *Akebia quinata* (Thunb.) Decne and *Aristolochia manshuriensis* Kom. displayed distinctive DNA sequence variations including single nucleotide polymorphisms, differences in the copy number of repeated sequences, and patterns of insertions and deletions that allow the differentiation of the two herbs (Han *et al*, 2005). Furthermore, phylogenetic relationships among different species can be determined (Mizukami *et al*, 1998) and adulterants can be identified by performing sequence searches from databases such as Genbank (Mihalov *et al*, 2000). The establishment and application of a DNA method for differentiating easily confused species could prevent herbal poisoning.

Many chemical methods can also be adopted for authentication and quality assurance/control purposes, such as chemical testing and thin layer chromatography (TLC). However, chemical group reactions may not be specific enough and TLC may not achieve the separation quality and reproducibility required for authentication

and quality assurance/control. On the other hand, chemical fingerprinting can be a powerful tool for authentication and quality assurance/control when a chemical fingerprint, characterized by distinct pattern of several chemical markers, marks the unique identity of the herb. By optimizing extraction, separation and measurement conditions, five principal alkaloids namely benzoylmesaconine, mesaconitine, aconitine, hypaconitine, and deoxyaconitine have been detected in four species of *Aconitum* L. using HPLC method (Wang *et al*, 2006). HPLC-DAD have also been deployed for simultaneous quantitative determination of six *Aconitum* L. alkaloids including aconitine, mesaconitine, hypaconitine, benzoyl-aconine, benzoylmesaconine, and benzoylhypaconine in processed and unprocessed aconite roots, as well as 12 proprietary CM containing processed aconite roots (Xie *et al*, 2005). Satisfactory chromatographic separations of the six *Aconitum* L. alkaloids have been achieved. The HPLC chromatograms of the six alkaloids in processed aconite roots reveal a distinguishable pattern that differs from the unprocessed roots. In processed aconite root, contents of aconitine, mesaconitine, and hypaconitine were decreased while that of benzoylmesaconine was increased. Similarly, the quality of crude and processed *S. nux-vomica* seeds was determined by examining the contents of two major toxic alkaloids, strychnine and brucine, and a major non-alkaloid constituent, loganic acid using HPLC-UV methods (Han *et al*, 2008). Contents of strychnine, brucine, and loganic acid were found to decrease in processed seeds where the relative peak area of strychnine-to-loganic acid was a reliable key quality control parameter for processed *Strychnos* Linn. seeds.

The LC-tandem MS method is another powerful tool for specific identification and quantitative analysis of toxic ingredients. A LC-ESI-MS method has been used for the determination of AA-I and AA-II in raw herbs medicinal preparation (Chan *et al*, 2003). The MS/MS product ion, [M + H − 44] (+), was used for the quantitative measurement of AA-I and AA-II. By employing LC/(+)ESI/MS(3) in combination with selected reaction monitoring, aconitine, and mesaconitine were found to decompose while the content of hypaconitine remained more or less the same in processed aconite (Chen *et al*, 2008b). By determining the ratio of aconitine and mesaconitine to hypaconitine, processed and unprocessed aconite roots can be readily distinguished with limits of detection between 0.03 and 0.08 ng/mL. This allow rapid, sensitive, and selective quality control for raw and proprietary CM. Chemical fingerprinting and LC-tandem MS methods facilitates the quality control of herbal medicine by mediating the authentication of genuine species, evaluation of post-harvesting handling, assessment of intermediates and finished products, and detection of harmful or toxic ingredients (Li *et al*, 2008).

Detection and quantification of toxic herbal ingredients and/or their metabolites in biological samples

Analytical methods for detection and quantification of the respective toxic alkaloids are of great importance for diagnosis and prognosis of herbal poisoning as well as other clinical and forensic toxicology (Maurer, 2007). The LC-tandem MS method is a powerful tool for specific identification and quantitative analysis of toxic ingredients. By LC-atmospheric pressure chemical ionization-MS and LC-ESI-MS/MS methods, toxic alkaloids including aconitine, atropine, colchicine, coniine, cytisine, nicotine and its metabolite cotinine, physostigmine, and scopolamine in plasma were detected and quantified (Beyer *et al*, 2007). Besides, rapid and simultaneous determination of five toxic alkaloids, namely brucine, strychnine, ephedrine, aconitine, and colchicine in human blood and urine samples have been reported using HPLC-ESI-MS operated in the multiple reaction monitoring mode (Qiu *et al*, 2008). The five alkaloids were well-separated within seven minutes in a single run with limits of quantification between 0.01 and 0.20 ng/mL. The LC-MS method used on a group of

aconitum alkaloids like aconitine, benzoylaconine, crassicauline A, deacetyl-crassicauline A, hypaconitine, benzoylhypaconine, mesaconitine, benzoylmesaconine, yunaconitine, and deacetylranaconitine in urine samples from intoxicated victims form an important diagnostic aid for acute aconite poisoning of unclear origin (Lai *et al*, 2006). Similarly, detection of aconitum alkaloids and their metabolites in the post mortem femoral blood and urine by HPLC-DAD (Elliott, 2002), in human urine by LC-ESI-MS (n) (Zhang *et al*, 2005), in human plasma samples by LC-ESI-time-of-flight-MS (Kaneko *et al*, 2006), in human urine, liver, and kidney by LC-MS-MS (Van Landeghem *et al*, 2007) have been reported. This LC-MS method has been applying in clinical investigation of suspected herbal poisoning in Hong Kong hospitals. With chemical markers, toxic components in species causing intoxication can further be confirmed by LC-MS analysis of blood and urine samples. Development of LC-MS based assay as diagnostic aids for clinical and forensic toxicological tasks would be promising owing to its soft ionization, high selectivity, and high sensitivity.

Conclusion

There is a common misconception among the general public that since traditional CM are of natural origins and have been used clinically for thousands of years, they are all safe without any side effects. Actually, some medicinal plants are inherently toxic with a narrow safety margin and even the improper use of non-toxic CM can result in various adverse effects. Special attention should be paid to possible herb-drug interactions since it is quite common for people in Hong Kong to use Chinese and Western medicine concomitantly.

Although intoxication cases have been frequently reported, only some of the biologically active ingredients and underlying intoxication mechanisms have been fully elucidated; further basic scientific research on these toxic ingredients and their intoxication mechanism is needed. Information on the toxic dose and toxicity profile of commonly used toxic herbs are also relatively limited. More research efforts should be devoted to these safety issues so that specific guidelines on the appropriate dosage of these herbs can be implemented.

In addition to understanding their toxicity, it is important to develop reliable analytical methods for the quality control of these potent/toxic herbs. A research organization for CM-related drug control should be setup by the government to strengthen and safeguard the quality of imported raw CM as well as proprietary CM. Regular trainings and seminars should be provided to CM practitioners/dispensaries/pharmacies and the public to strengthen their knowledge of the authentication, proper handling, processing, and usage of CM. It is expected that these measures taken at the community, scientific, and legal level would be effective at ensuring the overall safety and therapeutic potency of CM in Hong Kong.

References

Bah M, Bye R, Pereda-Miranda R, 1994. Hepatotoxic pyrrolizidine alkaloids in the Mexican medicinal plant Packera candidissima (Asteraceae: Senecioneae). *J Ethnopharmacol* 43: 19-30.

Beyer J, Peters FT, Kraemer T, Maurer HH, 2007. Detection and validated quantification of toxic alkaloids in human blood plasma-comparison of LC-APCI-MS with LC-ESI- MS/MS. *J Mass Spectrom* 42: 621-633.

But BW, Chan WH, Yau YS, Chan GL, 1999. Acute poisoning with Chinese medicinal herb-*Flos Daturae* (Nanyanghua). *HK J Paediatr (New series)* 4: 48-49.

But PP, 1993. Need for correct identification of herbs in herbal poisoning. *Lancet* 341: 637.

But PP, Tomlinson B, Cheung KO, Yong SP, Szeto ML, Lee CK, 1996. Adulterants of herbal products can cause poisoning. *BMJ* 313: 117.

Cao H, 1993. Determination of aconitine in wutou and related herbal drugs (roots of Aconitum) and their processed products in Hong Kong market. *China J Chin Mater Med* 18: 279-281, 318.

Carles M, Lee T, Moganti S, Lenigk R, Tsim KW, Ip NY, Hsing IM, Sucher NJ, 2001. Chips and Qi: microcomponent- based analysis in traditional Chinese medicine. *Fresenius J Anal Chem* 371: 190-194.

Carles M, Cheung MK, Moganti S, Dong TT, Tsim KW, Ip NY, Sucher NJ, 2005. A DNA microarray for the authentication of toxic traditional Chinese medicinal plants. *Planta Med* 71: 580-584.

Census and Statistics Department. 2002. Thematic household Survey Report No. 12. The Government of the Hong Kong Special Adminsitration Region, Hong Kong.

Chan JC, Chan TY, Chan KL, Leung NW, Tomlinson B, Critchley JA, 1994a. Anticholinergic poisoning from Chinese herbal medicines. *Aust N Z J Med* 24: 317-318.

Chan TY, Tomlinson B, Tse LK, Chan JC, Chan WW, Critchley JA, 1994b. Aconitine poisoning due to Chinese herbal medicines: a review. *Vet Hum Toxicol* 36: 452-455.

Chan TY, 2001. Interaction between warfarin and danshen (Salvia miltiorrhiza). *Ann Pharmacother* 35: 501-504.

Chan TY, 2002a. Incidence of herb-induced aconitine poisoning in Hong Kong: impact of publicity measures to promote awareness among the herbalists and the public. *Drug Saf* 25: 823-828.

Chan TY, 2002b. Herbal medicine causing likely strychnine poisoning. *Hum Exp Toxicol* 21: 467-468.

Chan SA, Chen MJ, Liu TY, Fuh MR, Deng JF, Wu ML, Hsieh SJ, 2003. Determination of aristolochic acids in medicinal plant and herbal product by liquid chromatography-electrospray-ion trap mass spectrometry. *Talanta* 60: 679-685.

Chan YW, 1991. Magnetic resonance imaging in toxic encephalopathy due to podophyllin poisoning. *Neuroradiology* 33: 372-373.

Chen F, Chan HY, Wong KL, Wang J, Yu MT, But PP, Shaw PC, 2008a. Authentication of Saussurea lappa, an endangered medicinal material, by ITS DNA and 5S rRNA sequencing. *Planta Med* 74: 889-892.

Chen JH, Lee CY, Liau BC, Lee MR, Jong TT, Chiang ST, 2008b. Determination of aconitine-type alkaloids as markers in fuzi (Aconitum carmichaeli) by LC/(+)ESI/MS(3). *J Pharm Biomed Anal* 48: 1105-1111.

Chen W, Chen Y, Li A, 2001. The clinical and pathological manifestations of aristolochic acid nephropathy-the report of 58 cases. *Chin Med J* 81: 1101-1105.

Clinical Toxicology Task Force for Chinese Medicine. 2002. Handbook on Toxicological Aspect of Chinese Medicines. Hospital Authority, Hong Kong.

Critchley LA, Chen DQ, Chu TT, Fok BS, Yeung C, 2003. Pre-operative hepatitis in a woman treated with Chinese medicines. *Anaesthesia* 58: 1096-1100.

Critchley LA, Chen DQ, Lee A, Thomas GN, Tomlinson B, 2005. A survey of Chinese herbal medicine intake amongst preoperative patients in Hong Kong. *Anaesth Intensive Care* 33: 506-513.

De Smet PA, 1995. Health risks of herbal remedies. *Drug Saf* 13: 81-93.

Dickens P, Tai YT, But PP, Tomlinson B, Ng HK, Yan KW, 1994. Fatal accidental aconitine poisoning following ingestion of Chinese herbal medicine: a report of two cases. *Forensic Sci Int* 67: 55-58.

Elliott SP, 2002. A case of fatal poisoning with the aconite plant: quantitative analysis in biological fluid. *Sci Justice* 42: 111-115.

Han S, Kim S, Yang CH, Seo J, 2005. Genetical identification of Akebia and Aristolochia species by using Pyrosequencing. *J Biotechnol* 120: 360-363.

Han QB, Li SL, Qiao CF, Song JZ, Cai ZW, Pui-Hay BP, Shaw PC, Xu HX, 2008. A simple method to identify the unprocessed Strychnos seeds used in herbal medicinal products. *Planta Med* 74: 458-463.

Ito K, Ohyama Y, Konishi Y, Tanaka S, Mizugaki M, 1997. Method for the simultaneous determination of Aconitum alkaloids and their hydrolysis products by gas chromatography-mass spectrometry in human serum. *Planta Med* 63: 75-79.

Kaneko R, Hattori S, Furuta S, Hamajima M, Hirata Y, Watanabe K, Seno H, Ishii A, 2006. Sensitive analysis of aconitine, hypaconitine, mesaconitine and jesaconitine in human body fluids and Aconitum tubers by LC/ESI-TOF-MS. *J Mass Spectrom* 41: 810-814.

Kimura T, Kinoshita E, Yamaoka K, Yuki T, Yakehiro M, Seyama I, 2000. One site of action of grayanotoxin in domain 4 segment 6 of rat skeletal muscle sodium channel. *FEBS Lett* 465: 18-22.

Kumana CR, Ng M, Lin HJ, Ko W, Wu PC, Todd D, 1983. Hepatic veno-occlusive disease due to toxic alkaloid herbal tea. *Lancet* 2: 1360-1361.

Kwan TH, Tong MK, Leung KT, Lai CK, Poon WT, Chan YW, Lo WH, Au TC, 2006. Acute renal failure associated with prolonged intake of slimming pills containing anthraquinones. *Hong Kong Med J* 12: 394-397.

Lai CK, Chan AY, 1999. Tetrahydropalmatine poisoning: diagnoses of nine adult overdoses based on toxicology screens by HPLC with diode-array detection and gas chromatography-mass spectrometry. *Clin Chem* 45: 229-236.

Lai CK, Chan YW, 2009. Confirmation of gelsemium poisoning by targeted analysis of toxic gelsemium alkaloids in urine. *J Anal Toxicol* 33: 56-61.

Lai CK, Poon WT, Chan YW, 2006. Hidden aconite poisoning: identification of yunaconitine and related aconitum alkaloids in urine by liquid chromatography-tandem mass spectrometry. *J Anal Toxicol* 30: 426-433.

Lam AY, Elmer GW, Mohutsky MA, 2001. Possible interaction between warfarin and Lycium barbarum L. *Ann Pharmacother* 35: 1199-1201.

Lam YH, Poon WT, Lai CK, Chan AY, Mak TW, 2008. Identification of a novel vardenafil analogue in herbal product. *J Pharm Biomed Anal* 46: 804-807.

Lau KK, Lai CK, Chan AW, 2000. Phenytoin poisoning after using Chinese proprietary medicines. *Hum Exp Toxicol* 19: 385-386.

Li T, Wang J, Lu Z, 2005. Accurate identification of closely related Dendrobium species with multiple species-specific gDNA probes. *J Biochem Biophys Methods* 62: 111-123.

Li SL, Han QB, Qiao CF, Song JZ, Cheng CL, Xu HX, 2008. Chemical markers for the quality control of herbal medicine: an overview. *Chin Med* 28: 3-7.

Lin J, Zhou X, Gao S, Wu W, Liu X, Sun X, Tang K, 2006. Authentication of Pinellia ternata and its adulterants based on PCR with specific primers. *Planta Med* 72: 844-847.

Lo SH, Mo KL, Wong KS, Poon SP, Chan CK, Lai CK, Chan A, 2004. Aristolochic acid nephropathy complicating a patient with focal segmental glomerulosclerosis. *Nephrol Dial Transplant* 19: 1913-1915.

Lo SH, Wong KS, Arlt VM, Phillips DH, Lai CK, Poon WT, Chan CK, Mo KL, Chan KW, Chan A, 2005. Detection of Herba Aristolochia Mollissemae in a patient with unexplained nephropathy. *Am J Kidney Dis* 45: 407-410.

Maurer HH, 2007. Demands on scientific studies in clinical toxicology. *Forensic Sci Int* 165: 194-198.

Mihalov JJ, Marderosian AD, Pierce JC, 2000. DNA identification of commercial ginseng samples. *J Agric Food Chem* 48: 3744-3752.

Mizukami H, Shimizu R, Kohjyouma M, Kohda H, Kawanishi F, Hiraoka N, 1998. Phylogenetic analysis of Atractylodes plants based on chloroplast trnK sequence. *Biol Pharm Bull* 21: 474-478.

Ng TH, Chan YW, Yu YL, Chang CM, Ho HC. Leung SY, But PP,

1991. Encephalopathy and neuropathy following ingestion of a Chinese herbal broth containing podophyllin. *J Neurol Sci* 101: 107-113.

Nortier JL, Martinez MC, Schmeiser HH, Arlt VM, Bieler CA, Petein M, Depierreux MF, De Pauw L, Abramowicz D, Vereerstraeten P, Vanherweghem JL, 2000. Urothelial carcinoma associated with the use of a Chinese herb (Aristolochia fangchi). *N Engl J Med* 342: 1686-1692.

Pharmacopoeia Committee of the P. R. China. 2005. Pharmacopoeia of the People's Republic of China (vol.1). People's Medical Publishing House, Beijing.

Poon WT, Chau TL, Lai CK, Tse KY, Chan YC, Leung KS, Chan YW, 2008. Hepatitis induced by *Teucrium viscidum*. *Clin Toxicol (Phila)* 46: 819-822.

Poon WT, Lai CK, Ching CK, Tse KY, So YC, Chan YC, Hau LM, Mak TW, Chan AY, 2006. Aconite poisoning in camouflage. *Hong Kong Med J* 12: 456-459.

Poon WT, Lam YH, Lai CK, Chan AY, Mak TW, 2007. Analogues of erectile dysfunction drugs: an under- recognised threat. *Hong Kong Med J* 13: 359-363.

Qiu P, Chen X, Lin L, Ai, C, 2008. Simultaneous determination of five toxic alkaloids in body fluids by high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 875: 471-477.

Tai YT, But PP, Young K, Lau CP, 1992. Cardiotoxicity after accidental herb-induced aconite poisoning. *Lancet* 340: 1254-1256.

Tam LS, Chan TY, Leung WK, Critchley JA, 1995. Warfarin interactions with Chinese traditional medicines: danshen and methyl salicylate medicated oil. *Aust N Z J Med* 25: 258.

The Chinese Medicinal Material Research Centre. 1991. Report on the Utilization of Traditional Chinese Medicine in Hong Kong. The Chinese University of Hong Kong, Hong Kong.

Tomlinson B, Chan TY, Chan JC, Critchley JA, 1993. Herb-induced aconitine poisoning. *Lancet* 341: 370-371.

Trau D, Lee TM, Lao AI, Lenigk R, Hsing IM, Ip NY, Carles MC, Sucher NJ, 2002. Genotyping on a complementary metal oxide semiconductor silicon polymerase chain reaction chip with integrated DNA microarray. *Anal Chem* 74: 3168-3173.

Tse CY, Chin DK, Teoh R, 1989. Poisoning by Chinese herbs. *J Hong Kong Med Assoc* 41: 177-178.

Tsoi PY, Woo HS, Wong MS, Chen SL, Fong WF, Xiao PG, Yang MS, 2003. Genotyping and species identification of Fritillaria by DNA chips. *Acta Pharm Sin* 38: 185-190.

Van Landeghem AA, De Letter EA, Lambert WE, Van Peteghem CH, Piette MH, 2007. Aconitine involvement in an unusual homicide case. *Int J Legal Med* 121: 214-219.

Wang Z, Wen J, Xing J, He Y, 2006. Quantitative determination of diterpenoid alkaloids in four species of Aconitum by HPLC. *J Pharm Biomed Anal* 40: 1031-1034.

Wong AL, Chan JT, Chan TY, 2003. Adverse herbal interactions causing hypotension. *Ther Drug Monit* 25: 297-298.

Wong AL, Chan TY, 2003. Interaction between warfarin and the herbal product quilinggao. *Ann Pharmacother* 37: 836-838.

Xie Y, Jiang ZH, Zhou H, Xu HX, Liu L, 2005. Simultaneous determination of six Aconitum alkaloids in proprietary Chinese medicines by high-performance liquid chromatography. *J Chromatogr A* 1093: 195-203.

Xue CY, Li DZ, Wang QZ, 2008. Identification and quantification of the traditional Chinese medicinal plant Gentiana macrophylla using Taqman real-time PCR. *Planta Med* 74: 1842-1845.

Yip PY, Chau CF, Mak CY, Kwan HS, 2007. DNA methods for identification of Chinese medicinal materials. *Chin Med* 2: 9.

Yuen MF, Tam S, Fung J, Wong DK, Wong BC, Lai CL, 2006. Traditional Chinese medicine causing hepatotoxicity in patients with chronic hepatitis B infection: a 1-year prospective study. *Aliment Pharmacol Ther* 24: 1179-1186.

Yuen, YP, Lai CK, Poon WT, Ng SW, Chan AY, Mak TW, 2007. Adulteration of over-the-counter slimming products with pharmaceutical analogue--an emerging threat. *Hong Kong Med J* 13: 216-220.

Zhang HG, Sun Y, Duan MY, Chen YJ, Zhong DF, Zhang HQ, 2005. Separation and identification of Aconitum alkaloids and their metabolites in human urine. *Toxicon* 46: 500-506.