PHYTOTHERAPY RESEARCH
Phytother. Res. **27**: 1745–1755 (2013)
Published online 19 February 2013 in Wiley Online Library
(wileyonlinelibrary.com) **DOI**: 10.1002/ptr.4942

REVIEW

Potential Genotoxicity of Traditional Chinese Medicinal Plants and Phytochemicals: An Overview

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In the last decades, cases of poisoning due to herbal medicines have occurred in many countries; Chinese herbal medicines (CHMs) are occasionally involved. The experience gained from traditional use is efficient to detect immediate or near-immediate relationship between administration and toxic effects but is quite unlikely to detect medium- to long-term toxicities; thorough investigations of herbal medicines (toxicity assessments, active pharmacovigilance) appear then essential for their safe use. Genotoxicity is an especially insidious toxicity that may result in carcinoma development years after exposure; it can arise from multiple compounds, with or without metabolic activation. The present work reviews traditional CHMs and phytochemicals that have been shown to present a genotoxic hazard. Copyright © 2013 John Wiley & Sons, Ltd.

Keywords: Chinese herbal medicine; phytochemicals; genotoxicity; aristolochic acids; pyrrolizidine alkaloids.

INTRODUCTION

Herbal medicines are worldwide used and have attracted renewed interest in developed countries over the last decades. The main reasons are some disappointment of patients towards conventional allopathic medicines in terms of effectiveness and/or safety, alleged satisfaction with therapeutic outcomes (Abbot and Ernst, 1997; Huxtable, 1990), a rewarding feeling of participating in the choice of therapeutic means and the (fallacious) perception that herbal medicines are inherently 'natural', hence safe. Some patients prefer herbal medicines for cultural and personal beliefs, philosophical views on life and health (Ernst and White, 2000). In China, the use of herbal medicines represents approximately 40% of all healthcare services delivered. The percentage of the population which has used herbal medicines at least once is estimated at 48% in Australia, 70% in Canada, 42% in USA, 38% in Belgium and 75% in France (Foster *et al.*, 2000; WHO, 2002). Despite the positive perception of herbal medicines, poisoning cases have been reported in the literature (Vanherweghem and Degaute, 1998; Cosyns *et al.*, 1999; Ernst, 2002). Chinese herbal medicines (CHMs) have been involved, and some medicines have been shown to contain toxic compounds. Generally, these compounds react with cellular macromolecules, including DNA, causing cellular toxicity and/or genotoxicity (Rietjens *et al.*, 2005a). A 'genotoxin' is a chemical or agent that can cause DNA or chromosomal damage (Phillips and Arlt, 2009), and 'genotoxicity' refers to potentially harmful effects on genetic material which are related to the induction of permanent transmissible changes in the amount or structure of the genetic material (Cavalcanti *et al.*, 2010; Ogura *et al.*, 2008; Sanchez-Lamar *et al.*, 2002). Genotoxicity, broadly defined as 'damage to the genome', is a distinct and important type of toxicity as specific genotoxic events are considered hallmarks of cancer (Ellinger-Ziegelbauer *et al.*, 2009). In theory, a single hit to DNA may be sufficient to start genomic instability; it is a heavy matter of debate whether a threshold does exist and whether an acceptable exposure level could be defined for genotoxic compounds. Unfortunately, in most published works on herbals, the identities and/or amounts of genotoxicants have not been determined, papers dealing either with structural identification or with genotoxicity testing often without quantitative information.

Although the use of CHMs is rapidly spreading around the world, reviews on their potential genotoxicity are not only scarce, but also quite outdated. The present paper aims at compiling data on major genotoxicities of traditional CHMs and their phytochemicals and at developing suggestions for the safe use of these medicines.

TRADITIONAL CHMs AND ARISTOLOCHIC ACIDS

After the nephrotoxic and carcinogenic activity of the nitroaromatic compounds aristolochic acids (AAs) (Fig. 1) was discovered (Vanherweghem *et al.*, 1993), several studies investigated their genotoxic activity (Fang *et al.*, 2011; Hwang *et al.*, 2012), proving that

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Received 12 October 2012
Revised 02 January 2013
Accepted 09 January 2013

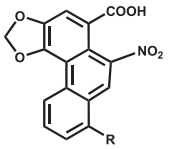
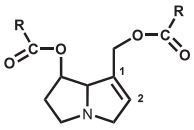
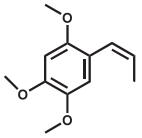
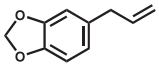
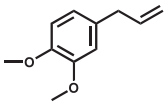
Chemical structures	Some concerned herbs
 Aristolochic acid I: R=H Aristolochic acid II: R= OCH_3	*Aristolochia contorta* Bunge; *A. crispulatum* C.Y.Cheng & C.S.Yang; *A. debilis* Sieb. et Zucc.; *A. fangchi* Y.C. Wu ex L.D. Chow & S.M. Hwang; *A. forbesii* Maxim.; *A. fukienense* C.Y.Cheng & C.S.Yang; *A. himalaicum* Hook.f. & Thomson ex Klotzsch; *A. ichangense* C.Y.Cheng & C.S.Yang; *A. manshuriensis* Kom; *A. maximum* Hemsl.; *A. sieboldii* Miq.; *A. sinarum* Lindl.; *A. splendens* C.Y.Cheng & C.S.Yang; *Asarum heterotropoides* F.Schmidt Herbs shown to be adulterated or contaminated by *Aristolochia sp* (non-exhaustive list): *Akebia quinata* Decne.; *Akebia trifoliata* (Thunb.) Koidz.; *Aucklandia costus* Falc. (= *Aucklandia lappa* Decne); *Clematis armandii* Franch.; *Clematis chinensis* Osbeck.; *Clematis hexapetala* L.f.; *Clematis hexapetala Pall.* (*Clematis flammula* L.); *Clematis hexasepala* DC.; *Clematis mandshurica* Rupr.; *Clematis montana* Bach.-Ham.ex DC.; *Clematis recta* L.; *Cocculus trilobus* (Thunb.) DC. (= *Cocculus orbiculatus* (L.) DC.); *Inula britannica* L.; *Inula helenium* L.; *Inula racemosa* Hook.f.; *Saussurea lappa* (Decne.) Sch.Bip.; *Saussurea costus* (Falc.) Lipsch.; *Stephania tetrandra* S.Moore; *Trichosanthes kirilowii* Maxim.; *Vladimiria souliei* (Franch.) Y.Ling (see also: American Herbal Pharmacopoeia, 2006)
 Pyrrolizidine alkaloids	*Borago officinalis* L.; *Crotalaria assamica* Benth; *Crotalaria sessiliflora L.*; *Emilia sonchifolia* DC; *Eupatorium cannabinum* L.; *Heliotropium indicum* L.; *Jacobaea vulgaris Gaertn.* (= *Senecio jacobaea* L.); *Petasites officinalis* Moench.; *Senecio vulgaris* L.; *Symphytum officinale* L.; *Tussilago farfara* L.
Components of various essential oils	
 β-asarone	*Acorus calamus* L.; *Acorus tatarinowii* S.; *Asarum forbesii* Maxim; *Orthodon asaroniferum* Fujita; *Orthodon isomyristicineferum* Fujita; *Piper lolot* DC
 Safrole	*Sassafras sp*; *Ocotea pretiosa* (Nees) Mez.; *O. cymbarum* Poepp. ex Nees; *Cinnamomum camphora* Nees; *Piper sp* (part of betel quid)
 Methyleugenol	> 450 plant species from 80 families (Tan and Nishida, 2012) including medicinal and alimentary species, such as basil, tarragon, lemon grass, bay leaf, nutmeg, allspice, cloves or mace

Figure 1. Some chemical structures of importance to genotoxicity assessment.

AAs are genotoxic in both bacterial and mammalian cells (Furihata *et al*., 1984; Kohara *et al*., 2002; Pezzuto *et al*., 1988). AA-related DNA adducts were notably shown in renal tissues of patients (Schmeiser *et al*., 1996); these mutagenic adducts are poorly repaired (Sidorenko *et al*., 2012) and can persist for years in DNA (Bieler *et al*., 1997; Nortier *et al*., 2000). AAs were detected in plants that originate from the Aristolochiaceae family, notably in the genus *Aristolochia* and *Asarum*. Whereas other species are commonly adulterated with AA-containing species [e.g. *Akebia trifoliata* (Thunb.) Koidz., *Clematis armandii* Franch., *Stephania tetrandra*, *S. Moore*], some species are potentially but not commonly adulterated [e.g. *Clematis chinensis* Osbeck, *Saussurea costus* (Falc.) Lipsch.] (Table 1 and American Herbal Pharmacopoeia, 2006). The presence of AAs I and II has already been reported in different Asian medicinal plants as well as in slimming products. These products have been banned for example in Belgium, UK, Canada, Australia and Germany (Hashimoto *et al*., 1999; Lee *et al*., 2002; Lee *et al*., 2001).

Aristolochia species (Aristolochiaceae)

Labeling of herbal products may not accurately reflect their content; adverse events or interactions attributed to specific herbs may actually be due to misidentified plants, pharmaceutical drugs or heavy metals. It is therefore important to determine the presence of toxic components and adulterants in herbal medicines to ensure safety of the patients. In many countries including Belgium, France (Stengel and Jones, 1998), Spain (Cosyns *et al*., 1994), UK (Lord *et al*., 1999) and Japan (Tanaka *et al*., 1997a; Tanaka *et al*., 1997b) *Aristolochia fangchi* was the cause of the so-called 'Chinese herbs nephropathy', an original type of subacute interstitial fibrosis of the kidney. Poisonings attributed to Fang-Ji (*Stephania tetrandra* S. Moore) in a weight-loss preparation (Cosyns *et al*., 1994; Depierreux *et al*., 1994; Kessler, 2000; Reginster *et al*., 1997; van Ypersele de Strihou, 1995; Vanherweghem *et al*., 1993) were actually caused by Guang-Fang-Ji (*Aristolocholia fangchi* Y.C. Wu ex L.D. Chow & S.M. Hwang) (Vanhaelen *et al*., 1994). The latter contains AAs, and the adulteration was dramatic, leading to nephrotoxic and carcinogenic events in more than 100 women (Zhou *et al*., 2004). The confusion in this case has arisen from the similarity of the names in Chinese.

In addition to *Aristolocholia fangchi*, other medicinal *Aristolochia* species, such as *Aristolochia debilis* Sieb. et Zucc., *Aristolochia manshuriensis* Kom. *Aristolochia sinarum* Lindl. or *Aristolochia contorta* Bunge contain AAs (Chen *et al*., 2011; Hashimoto *et al*., 1999) and were also found to be genotoxic (Gotzl and Schimmer, 1993; Schmeiser *et al*., 1986). These plants are components of

Table 1. List of some genotoxic pyrrolizidine alkaloid-containing Chinese medicinal herbs

Botanical name	Chinese name	Medicinal purpose	Major genotoxic pyrrolizidine alkaloids	Daily administration (Pharmacopoeia of the People's Republic of China, 2005)	References
Crotalaria mucronata (Fabaceae)	Zhu shi dou	Folk medicine	Retrorsine	Adulterant of Astragali complanati semen (9 – 18 g)	(Fu *et al.*, 2007; Bensky *et al.*, 2004)
Emilia sonchifolia DC (Asteraceae)	Yang ti cao, Yi dian hong	Antipyretic, diarrhea, hemoptysis	Senkirkine	Adulterant of Taraxaci herba (9 – 30 g)	(Roeder, 2000; Bensky *et al.*, 2004)
Farfugium japonicum Kitam (Asteraceae)	Lian peng cao	Colds and flu	Senkirkine	not available	(Roeder, 2000)
Gynura bicolor DC (Asteraceae)	Guan yin xian	Dysmenorrhea, tuberculous hymoptysis	Retrorsine	not available	(Buckmaster *et al.*, 1977)
Gynura segetum Merr. (Asteraceae)	Ju shan qi, Tu san chii	Hemoptysis, peripheral blood circulation disorder	Seneciphylline	Adulterant of Atractylodis macrocephalae rhizoma (6 – 15 g)	(Roeder, 2000; Bensky *et al.*, 2004)
Heliotropium indicum L. (Boraginaceae)	Da wei yao	Ulcer, wounds and local inflammations	Heliotrine, lasiocarpine	not available	(Roeder, 2000)
Lappula intermedia M. Popov (Boraginaceae)	He shi	Ascariasis, oxyuriasis, infantile malnutition	Lasiocarpine	1 - 1.2 g	(Roeder, 2000)
Ligularia hodgsonnii Hook (Asteraceae)	Dian zi yuan	Antitussive	Clivorine	Adulterant of Asteris radix (5 - 9 g)	(Lin *et al.*, 2000)
Senecio chrysanthemoides DC (Asteraceae)	Chien li kuang, Tsang tu san chi	Traumatic injury, breast abscesses	Seneciphylline	not available	(Roeder, 2000)
Symphytum officinale L. (Boraginaceae)	Ju he cao	wound, stomach ulcers, other diseases of the digestive tract	Riddelliine	not available	(Guo *et al.*, 2007; Oberlies *et al.*, 2004)

traditional Sino-Japanese prescriptions (e.g. 'Kampo') used as a diuretic and analgesic (Hashimoto *et al.*, 1999).

Asarum species (Aristolochiaceae)

Asarum spp contain essential oils and AAs have been shown in many species (Jong *et al.*, 2003; Yang *et al.*, 1997). A few *Asarum* species are used to prepare 'Xixin' (Asari radix, 'Saishin' in Japanese), which is one of the important crude drugs in Chinese medicine exported all over the world. It has been used as an analgesic, antitussive and anti-allergic medicine (Hashimoto *et al.*, 1994). The following *Asarum* species contain AAs and are components of 'Xixin': *A. heterotropoides* F.Schmidt, *A. crispulatum* C.Y. Cheng & C.S. Yang, *A. forbesii* Maxim., *A. himalaicum* Hook.f. & Thomson ex Klotzsch, *A. sieboldii* Miq., *A. debile* Franch., *A. maximum* Hemsl., *A. ichangense* C.Y. Cheng & C.S. Yang, *A. fukienense* C.Y. Cheng & C.S. Yang (Jong *et al.*, 2003), *A. splendens* C.Y. Cheng & C.S. Yang (Chen *et al.*, 2011; Hashimoto *et al.*, 1999).

TRADITIONAL CHMs AND PYRROLIZIDINE ALKALOIDS

Pyrrolizidine alkaloids are esters of hydroxylated 1-methylpyrrolizidines (Fig. 1) (Fang *et al.*, 2011; Prakash *et al.*, 1999). Many studies showed that 1-2 unsaturated pyrrolizidine alkaloids are hepatotoxic and exhibit a large variety of genotoxicities, inducing DNA binding, DNA cross-linking, DNA-protein cross-linking, sister chromatid exchange and chromosomal aberrations (Fu *et al.*, 2001; Fu *et al.*, 2002a; Fu *et al.*, 2004; Fu *et al.*, 2002b; Roeder, 2000). Culvenor estimates that about 3% of the world flowering plants contain toxic pyrrolizidine alkaloids (Culvenor, 1980) and three families are particularly concerned, *Boraginaceae*, *Compositae* (*Asteraceae*, mainly in the Senecioneae and Eupatorieae tribes) and *Leguminosae* (*Fabaceae*, essentially the *Crotalaria* genus) (Hartmann and Witte, 1995).

Toxic pyrrolizidine alkaloid-containing plants are found in South Africa, Central Africa, India, Jamaica, Canada, Europe, New Zealand, Australia, the United States and China (Steenkamp *et al.*, 2000; Stegelmeier *et al.*, 1999). More than 90 pyrrolizidine alkaloids were identified in 49 pyrrolizidine alkaloid-containing Chinese medicinal plants. Among these plants, one plant belongs to the Orchidaceae family, 5 to the Fabaceae (Leguminosae) family, 9 to the Boraginaceae family and 37 to the Asteraceae (Compositae) family (Fu *et al.*, 2002a). Some pyrrolizidine alkaloids found in Chinese medicinal plants (Table 1) exert mutagenic effects in *Salmonella typhimurium* TA100 in the presence of hepatic S9 activation enzyme system. These pyrrolizidine alkaloids, naturally occurring carcinogens, include clivorine, heliotrine, lasiocarpine, retrorsine, senkirkine, seneciphylline and riddelliine (Fu *et al.*, 2007).

TRADITIONAL CHMs AND OTHER GENOTOXIC PHYTOCHEMICALS

Essential oil components

Essential oils are volatile and very complex natural mixtures extracted from various aromatic plants. They are

often characterized by a few major components at fairly high concentrations (20–70%) compared to others components present in trace amounts. Essential oils may contain monoterpenoids, sesquiterpenoids, C6–C3 and C6–C1 units, coumarins, a few alkaloids, sulfured compounds and degradation products of terpenoids and lipids; menthol, geraniol, thymol, mentone, cerpineol, cinnamaldehyde, carvacrol, thymol, carvone safrole, eugenol, methyleugenol, estragole, β-asarone and anethole are examples of major components present in some essential oils (Bakkali *et al*., 2008).

Potential genotoxic volatile phytochemicals such as safrole, eugenol, methyleugenol, estragole and β-asarone (Fig. 1) are found in some Chinese medicinal plants (Liu *et al*., 2004; Munerato *et al*., 2005; Smith *et al*., 2010; Zhang *et al*., 2005). Safrole is the major component of the oil of sassafras (*Sassafras albidum* (Nutt.) Nees) (Poppenga, 2002), of *Ocotea pretiosa* (Nees) Mez., *O. cymbarum* Poepp. ex Nees, *Cinnamomum camphora* Nees and of betel quid (betel leave, *Piper betle* L. and areca nut, *Areca catechu* L.), a chewing preparation widely used in Taiwan (Chung *et al*., 2009); it is also a minor constituent of other essential oils and spices such as anise, sweet basil, cinnamon, nutmeg, mace and black pepper (Johnson *et al*., 2001). Sassafras oil has had a traditional and widespread use in herbal medicine; however, in 1960, the FDA banned its use as a food and flavoring additive because of the high content of safrole and its proven carcinogenic effects (Heikes, 1994). Safrole displays a significant genotoxic effect in the wing spot test of *Drosophila melanogaster* (Liu *et al*., 2004; Munerato *et al*., 2005). Persistent DNA adducts derived from epoxide metabolites (epoxidation of the lateral chain) have been identified both *in vitro* and *in vivo* (Daimon *et al*., 1997, 1998; Dietz and Bolton, 2007; Gupta *et al*., 1993; Luo and Guenthner, 1996; Randerath *et al*., 1993).

Related allylbenzene derivatives (eugenol, methyleugenol and estragole), extracted from clove oil and present in several spices including basil, cinnamon and nutmeg, are similarly genotoxic (Jeurissen *et al*., 2008; Munerato *et al*., 2005; Smith *et al*., 2010).

β-asarone has a strong genotoxic potency. Safrole, methyleugenol, β-asarone and α-asarone are found in the essential oil of *Asarum forbesii* Maxim (Aristolochiaceae), a wild aromatic plant growing in damp soil in Hunan Province, China (major component, α-asarone, 58.8%). It is used in folk medicine as an analgesic, diuretic and for the treatment of bronchial asthma and cough (Zhang *et al*., 2005). The quite comparable essential oil of *Acorus gramineus* Soland (Acoraceae) (major component, β-asarone, 80.4%) is also commonly used as analgesic. *Acorus calamus* L. and *Acorus tatarinowii* S. (Parab and Mengi, 2002; Zhang *et al*., 2005) are also medicinal plants containing β-asarone, present at 91.7% and 80.1% of the oil, respectively (Zhang *et al*., 2005). The former is used in cough, bronchitis, gout, inflammation, convulsions, depression and other mental disorders, tumors, hemorrhoids, skin diseases, numbness and general debility (Parab and Mengi, 2002). The rhizome of *Acorus tatarinowii* Schott has been widely used as a traditional Chinese medicine (TCM) for treating central nervous system disorders (Tong *et al*., 2010).

Pulegone and menthone (*Mentha pulegium* L.), camphor (*Salvia fruticosa* Mill.) and anethole (various Apiaceae) were found positive in some genotoxicity tests, but their genotoxic potential remains to be confirmed or infirmed (Franzios *et al*., 1997; Hasheminejad and Caldwell, 1994; Nestman and Lee, 1983; Kim *et al*., 1999). In the Ames test, terpineol was found active (Gomes-Carneiro *et al*., 1998), and cinnamaldehyde, carvacrol, thymol and carvone exerted weak mutagenic effects (Stammati *et al*., 1999).

Other genotoxic components

Many traditional Chinese medicinal plants were positive in genotoxicity assays (Ames, Micronucleus or Unscheduled DNA synthesis assays) (Table 2). However, the genotoxic compounds of most of them are not identified, the genotoxicity tests being often performed on crude extracts of plants; the levels of exposure to genotoxins can then hardly been determined. When available, the typical daily exposures to the herbs have been indicated. This gives only a rough idea of risk but may point out to high-intake herbs that warrant urgent safety assessment and/or regulation. Results from *in vitro* genotoxicity assays (Ames assay, *Saccharomyces cerevisiae* assay) and *in vivo* genotoxicity assays (sister chromatid exchanges, micronuclei and sperm-shape abnormality assays in mice) showed that the hydroalcoholic extract of *Punica granatum* L. whole fruit is genotoxic. However, this fruit is used by traditional medicine in America, Asia, Africa and Europe for the treatment of different types of diseases (Gracious-Ross *et al*., 2001; Kim *et al*., 2002; Murthy *et al*., 2004). Artesunate, a semisynthetic antimalarial derivative from artemisinin (a sesquiterpene lactone endoperoxide derived from the Chinese herb *Artemisia annua L.*), induces DNA breakage in a dose-dependent manner as shown using single-cell gel electrophoresis (Comet assay) (Covello *et al*., 2007; Efferth *et al*., 2008). This genotoxic effect was confirmed by measuring the level of γ-H2AX, which is considered to be an indication of DNA double-strand breaks (Li *et al*., 2008). Berberine, the main alkaloid of *Coptis chinensis* Franch., which is used in TCM for the treatment of gastrointestinal complaints (dysentery, diarrhea) and inflammatory symptoms, also exhibited genotoxic activity (Li-Weber, in press; Zhang *et al*., 2011).

The situation appears complex as some compounds appear positive in classical genotoxicity tests but without toxicological incidence. For example, quercetin induces positive response in the comet assay (Anderson *et al*., 1998; Charles *et al*., 2012), Cu(II)-dependent strand breakages (Rawle *et al*., 2008) and is strongly clastogenic (Snyder and Gillies, 2002). *In vivo* studies, however, show no evidence of quercetin genotoxicity or carcinogenicity (Utesch *et al*., 2008; Rietjens *et al*., 2005b).

Table 2 indicates that scarce data are available for only a few herbs; generally, only limited genotoxicity tests are performed, with sometimes questionable value of the studies as the identity and quality of tested herbs or Good Laboratory Practices quality assurance are often missing. This yields a truly incomplete picture of the question; conflicting data are generated, the same herb being described as genotoxic, antigenotoxic or safe depending on the author and the performed tests. Authentication and quality control of tested herbals as well as standardization and quality assurance of genotoxicity assays are certainly needed.

Table 2. Some traditional Chinese medicinal herbs with genotoxicity in the Ames, Unscheduled DNA synthesis (UDS) or Micronucleus (MN) tests. Adapted from Ueng *et al.* (1997), Scarpato *et al.* (1998) and Kevekordes *et al.* (1999)

Botanical name[a]	Chinese name	Daily administration[b]	Genotoxicity assays		Comments on known compounds positive for genotoxicity[c]
Aconitum carmichaili Debx.	Fu zi or Wu tou	Roots, 1 – 1.2 g	Ames		n.a.
Agrimonia pilosa Ledeb. Var. *japonica* (Miq) Nakai	Xian he cao	Herb, 6 – 15 g	Ames		n.a. No genetic toxicity was detected in male mouse reproductive cells (Pang *et al.*, 2006) Mutagenicity inhibitor (Horikawa *et al.*, 1994)
Akebia trifoliata (Thunb.) Koidz.	Mu tong	Stems, 3 – 6 g	Ames		# Could be a confusion; longtime adulterated with *Aristolochia manshuriensis* Kom. (similar Chinese names) Aristolochic acids #
Alpinia oxyphylla Miq.	Yi zhi ren	Fruits, 3 – 9 g	Ames		Mutagenicity (Morimoto *et al.*, 1982)
Angelica dahurica Benth. et Hook Var. *pai-chi*	Bai zhi	Roots, 3 – 9 g	Ames		Furocoumarins # (photosensibilization #[d])
Arctium lappa L.	Niu bang zi	Fruits, 6 – 12 g	Ames		Mutagenicity inhibitor (Barnes *et al.*, 2007) HMPC concluded that the relevance of available studies for the assessment of preparations of the root is unclear (EMA, 2011).
Aristolochia debilis Sieb. et Zucc	Ma dou ling	Fruits, 3 – 9 g	Ames	MN	Aristolochic acids #
Aristolochia heterophylla Heml	Han fang ji	Roots, 4.5 – 9 g	Ames	MN	Aristolochic acids #
Aristolochia rigida Duch	Xi xin	Roots, 1 – 3 g			Positive in Escherichia coli (SOS chromotest) Aristolochic acids #
Asarum sieboldii F. Maekawa	Xi xien	Herb, 1 – 3 g	Ames		Aristolochic acids # Safrole #
Astragalus membranaceus (Fisch.) Bge. *var. mongholicus* (Bge.) Hsiao	Huang qi	Roots, 9 – 30 g	Ames	MN	Antigenotoxic (Liu *et al.*, 2009)
Benincasa cerifera Savi (synonym of *Benincasa hispida* (Thunb.) Cogn.)	Dong gua pi	Peels (exocarp), 3 – 9 g	Ames		n.a.
Bupleurum falcatum L.	Chai hu	Roots, 3 – 9 g	Ames	MN	n.a.
Carthamus tinctorius L.	Hong hua	Flowers, 3 – 9 g	Ames	MN	n.a.
Cassia angustifolia Vahl	Fan xie ye	Leaves, 2 – 6 g	Ames		# aloe-emodine (anthraquinone) (EMA, 2009)
Catalpa ovata G. Don	Xin shi	not available	Ames		# catalpin (iridoid) (Nozaka *et al.*, 1989)
Chrysanthemum morifolium Ramat.	Ju hua	Flowers, 5 – 9 g	Ames		n.a.
Cinnamomum mairei Levl.	Yin ye gui	Adulterant of Cinnamomi cortex (1.5 – 4.5 g)[c]		MN	# cinnamaldehyde
Coptis japonica Makino.	Huang lian alternate species	Rhizome, 2 – 5 g	Ames		# Berberine-type alkaloids Conflicting reports (WHO, 1999)
Corydalis bulbosa DC. or *Corydalis yanhusuo* W.T. Wang	Yan hu suo	Roots, 3 – 9 g	Ames		# Berberine-type alkaloids
Curcuma zedoaria (Berg.) Rosc.	E zhu	Roots, 6 – 9 g	Ames		Considered safe by HMPC (EMA, 2010)
Cuscuta chinensis Lam.	Tu si zi	Seeds, 6 – 12 g	Ames	MN	# Quercetin[e]

(Continues)

Table 2. (Continued)

Botanical name[a]	Chinese name	Daily administration[b]	Genotoxicity assays			Comments on known compounds positive for genotoxicity[c]
Datura metel L.	Yang jin hua	Flowers, 0.3 – 0.6 g			MN	n.a.
Dioscorea japonica Thunb.	Shan yao	Roots,15 – 30 g	Ames			n.a.
Elfvinga applanata Karst.	not available	not available	Ames			n.a.
Eriocaulon buergerianum Koern.	Gu jing cao	Flowers, 9 – 24 g	Ames		MN	n.a.
Eucommia ulmoides Oliv.	Du zhong	Barks, 6 – 9 g	Ames			Antigenotoxic
Evodia rutaecarpa (Juss) Benth.	Wu zhu yu	Fruits, 1.5 – 4.5 g	Ames			Aqueous and 70% ethanolic extract were not genotoxic (Yang, 2008)
Forsythia suspensa (Thunb.) Vahl	Lian qiao	Fruits, 6 – 15 g			MN	Conflicting reports (Ai, 2011)
Fritillaria cirrhosa D. Don	Chuan bei mu	Bulbs, 3 – 9 g		UDS		n.a.
Gentiana lutea L.	Long dan	Roots, 3 – 6 g	Ames			# gentiopicroside, hydroxyxanthones, gentisine and isogentisine HMPC concludes on the lack of preclinical safety studies (especially genotoxicity) (EMA, 2010)
Gentiana scabra Bunge	Long dan	Roots, 3 – 6 g	Ames			n.a. (# ?? similar to *G. lutea*)
Geranium thunbergii Sieb. et Zucc.	Lao he cao	Herb, 9 – 15 g	Ames			n.a.
Gleditsia sinensis Lam.	Zao jiao ci	Thorn, 3 – 9 g	Ames			n.a.
Hydnocarpus anthelmintica Pier. Ex Laness.	Da feng zi	Seeds, 0.3 – 1 g		UDS		n.a.
Inula britannica L. var. *chinensis* (Rupr.) Regel	Xuan fu hua	Flowers, 3 – 9 g	Ames			# Quercetin[e]
Lepidium apetalum Wild.	Ting li zi	Seeds, 3 – 9 g	Ames	UDS		n.a.
Lilium brownii F.E Brown var. *colchesteri* Wils	Bai he	Stems, 6 – 12 g		UDS		n.a.
Lonicera japonica Thunb.	Jin yin hua	Flowers, 6 – 15 g	Ames			n.a.
Lycium chinense Mill.	Di gu pi	Barks of roots, 9 – 15 g		UDS		n.a.
Mallotus japonicus Muell-Arg.	Ye wu tong	not available	Ames			n.a.
Milletia dielsiana Harms	Ji xue teng	Stems, 9 – 15 g		UDS		n.a.
Nelumbo nucifera Gaertn.	Lian zi	Seeds, 6 – 15 g	Ames	UDS	MN	Antigenotoxic (Sohn *et al*. 2003)
Notopterygium incisum Ting ex H.T. Chang	Qiang huo	Roots, 3 – 9 g			MN	# Furocoumarins (photosensibilization #[d])
Paeonia albiflora Pall. Var *trichocarpa* Bunge (alternate species to *Paeonia lactiflora* Pall.)	Bai shao	Roots, 6 – 15 g	Ames			Mutagenic (Morimoto *et al*., 1982)
Paeonia suffruticosa Andr.	Mu dan pi	Barks, 6 – 12 g			MN	n.a.
Phellodendron amurense Rupr.	Huang bai	Barks, 3 – 12 g	Ames		MN	Berberine-type alkaloids # Conflicting reports (Chung *et al*., 2004)
Platycodon grandiflorus Jacq. A. DC	Jie geng	Roots, 3 – 9 g			MN	n.a.
Prinsepia uniflora Batal.	Rui ren	not available	Ames	UDS	MN	n.a.
Prunella vulgaris L.	Xia ku cao	Fruit spikes, 9 – 15 g	Ames		MN	# Quercetin[e]

					Mutagenicity inhibitor (Horikawa *et al.*, 1994, Feng *et al.*, 2010)
Rehmannia glutinosa f. *hueichingenesis*(fermented)	Shu di huang	Roots, 9 – 15 g		MN	Antimutagenic (Xu, 2010)
Rheum palmatum L.	Da huang	Roots, 3 – 30 g	Ames		anthranoids (aloe-emodin, emodin, chrysophanol and physcion) Mutagenicity inhibitor (Horikawa *et al.*, 1994) HMPC concludes that further investigations are needed to assess the carcinogenic risk definitely. The short-term use of *Rheum* can be regarded as safe (EMA, 2009)
Salvia miltiorrhiza Bunge	Dan shen	Roots, 9 - 15 g		UDS	Genotoxic studies of tanshinones are few and very Controversial (Cavalcanti *et al.*, 2008)
Schizandra chinensis (Turcz.) Baill.	Wu wei zi	Fruits, 3 - 6 g			tk gene mutation and chromosomal damage (Hu *et al.*, 2009)
Scutellaria baicalensis Georgi	Huang qin	Roots, 3 - 9 g	Ames		# Wogonin (Zhao *et al.*, 2011) Baicalein is antigenotoxic (Lee *et al.*, 2000)
Sinomenium acutum (Thunb.)	Fang ji	Roots, 4.5 - 9 g	Ames		# Could be a confusion with *Aristolochia spp*; similar Chinese names# Aristolochic acids
Smilax glabra Roxb.	Tu fu ling	Rhizome, 15 - 60 g	Ames		n.a.
Sophora flavescens Ait.	Ku shen	Roots, 4.5 - 9 g		MN	n.a.
Sophora japonica L.	Huai hua	Flowers, 5 - 9 g	Ames		Sophoricoside is not genotoxic (Kim *et al.*, 2001)
Sophora subprosarata Chun et Chen	Shan dou gen	Roots, 3 - 6 g	Ames		n.a.
Swertia japonica Makino	Dang yao	not available	Ames		Antimutagenic activity (Hiramatsu *et al.*, 2004)
Tetragonia tetragonoides O. Kuntze	Fan xing	not available	Ames		n.a.
Thuja orientalis L.(syn. of *Platycladus orientalis* (L.) Franco)	Bai zi ren	Seeds, 3 - 9 g	Ames	MN	# Possible contamination by aflatoxins (Bensky *et al.*, 2004)
Trapa bispinosa Roxb. Var *iinumai* Nakano	Ling jiao	not available	Ames		n.a.
Typhonium giganteum Engl	Bai fu zi	Rhizome, 9 - 30 g	Ames		n.a.
Zinziber officinale Rosc.	Sheng jiang	Roots, 3 - 9 g	Ames		# Gingerol and shogaol, particularly 6-gingerol. Antimutagenic effects also reported. HMPC considers toxicity studies of ginger inadequate at least regarding genotoxicity, carcinogenicity and, partially, reproductive and developmental toxicity (EMA, 2006)

[a]The reliability of botanical identifications has not been assessed by the authors of the present review.
[b]According to the Pharmacopoeia of the People's Republic of China, 2005.
[c]The # indicates only a putative link between genotoxicity and chemical composition; this link has not been inferred in the original genotoxicity study report.
[d]Depending on lightening conditions during the genotoxicity test, photosensitization is likely to occur.
[e]Quercetin is recognized to yield false positive data in the Ames test.

REGULATORY GUIDELINES FOR GENOTOXICITY TESTING OF HERBAL MEDICINAL PRODUCTS

As genotoxicity testing aims to yield information on all types of mutations, i.e. gene mutations, structural chromosome aberrations (clastogenicity) and numerical chromosome aberrations (aneugenicity), standard test batteries have been developed (Kirkland *et al*., 2005) which include the assessment of (i) mutagenicity in a bacterial reverse mutation test (Ames test) and (ii) genotoxicity in mammalian cells *in vitro* and/or *in vivo*; and this with and without metabolic activation (ICH, 2012). In Europe, the following tests are recommended by Organization for Economic Co-operation and Development (OECD) for assessing the genotoxicity of chemicals: *in vitro* Mammalian Chromosome Aberration Test 473, *in vitro* Mammalian Cell Micronucleus Test 487, *in vitro* Mammalian Cell Gene Mutation Test 476, Bacterial Reverse Mutation Test 471, *in vivo* Mammalian Bone Marrow Chromosome Aberration Test 475, *in vivo* Mammalian Erythrocyte Micronucleus Test 474 and *in vivo* Mammalian Spermatogonial Chromosome Aberration Test 483 (OECD, 2012). An overview of regulatory-accepted tests and recent advances in the field has been recently published (Ouedraogo *et al*., 2012). These tests are foreseen for chemicals, but the European Union devised guidelines for their application to herbs (HMPC, 2008a). This proved to be quite a challenge as some common flavonoids yield very positive Ames tests but are not carcinogen (cf the case of quercetin discussed in Section 4); this European Union regulation has been published after heavy debates on the topic (HMPC, 2008b). A recent paper apparently successfully tested and discussed its applicability but did not disclose actual data (Kelber *et al*., 2012).

In China, herbal products are covered by the regulatory guidelines for genotoxicity testing of medicinal products (SFDA, 2007) released by the Center for Drug Evaluation of China's State Food and Drug Administration in 2007. According to this regulation, *in vivo* and *in vitro* genetic endpoint detection methods can be divided into three categories: (i) gene mutations, (ii) chromosomal aberrations and (iii) DNA damage and repair. Bacterial reverse mutation assay, *in vitro* test of mammalian cells chromosomal damage or mouse lymphoma TK test, *in vivo* test of chromosomal damage of rodent hematopoietic cells and additional genotoxicity tests associated with carcinogenicity (liver UDS test, ^{32}P-marker test, transgenic mutation assay and tumor-associated genetic alterations and molecular study) could be used to evaluate the genotoxicity of compounds or herbals.

OUTLOOKS AND CONCLUSIONS

Traditional herbal medicines are perceived by the public as (relatively) safe, but, nowadays, the knowledge on the potential risks associated with this type of products increases. Potential harm can occur via inherent toxicity of herbs, as well as from contamination, adulteration, plant misidentification and interactions with other herbal products or pharmaceutical drugs (Jordan *et al*., 2010; Shaw, 2010; Zhang *et al*., 2012).

Genotoxicity can arise from multiple compounds, with or without metabolic activation. This is an especially insidious toxicity that may result in carcinoma development years after exposure; the experience gained from traditional use is efficient to detect immediate or near-immediate relationship between administration and toxic effects but is quite unlikely to detect this type of medium- to long-term toxicities.

The assessment of the safety and efficacy of Traditional CHMs is becoming an important issue for consumers, regulatory authorities and health care professionals. However, the research protocols, standards and methods for the evaluation of the safety and efficacy of CHMs are more complex than those for conventional medicines. Indeed, TCM presents a unique set of pharmaceutical theories that include particular methods for processing, combining and decocting CHMs, which probably contribute to reduce their eventual toxicities (for example, prolonged decoction may strongly reduce the content in genotoxic volatile compounds) and enhance their efficacies. There are however relatively few published papers on this aspect that certainly warrants attention. The evaluation of CHM safety appears complicated by multiple factors, such as the geographical origin of plant material, different processing techniques, dosage, route of administration and compatibility with other medicines (Zhang *et al*., 2012). Moreover, only a high-level quality control of both toxicologically investigated and marketed material will ensure the transferability of safety data to market realities.

It is important to establish standards, techniques and methods suitable for evaluating the safety of CHMs, by combining recent assessment techniques, such as bioinformatics, omics technologies and as a last resort the use of animals (Ouedraogo *et al*., 2012). This safety evaluation of CHMs ideally should include insidious toxicities such as genotoxicity, carcinogenicity, reproductive/developmental toxicity, hepatotoxicity, nephrotoxicity, neurotoxicity, cardiotoxicity and immunotoxicity.

Clearly, further investigations are necessary for the safe use of CHMs. Given the high number of marketed CHMs and TCMs for which no correct safety assessment has been performed, active pharmacovigilance is essential to rapidly detect eventual toxicological problems and to build up reliable information on the safe use of herbal medicines (Shaw *et al*., 2012; Zhang *et al*., 2012). To this end, cooperation between orthodox physicians and traditional practitioners is needed to bring together the full case details (Shaw *et al*., 2012). Independent scientific assistance on toxicological investigation and botanical verification is invaluable for full evaluation of any case report.

Consumers and regulatory agencies also need to be aware of the subtleties of the system of TCM (the naming systems, processing, substitutes, use of herbal combinations and types of extract used clinically); indeed, safety assessment of CHMs (botanicals) needs to be completed by safety data on the marketed preparations as processing and extraction may dramatically modulate the chemical composition and the toxicological profile.

Acknowledgements

This work has been performed in the frame of the FP7 '*Good Practice in Traditional Chinese Medicine Research in the Post-genomic Era*', Grant agreement No: 223154 (http://www.gp-tcm.org/), and International Science & Technology Cooperation Program of Zhejiang Province (Grant agreement No: 2012C24017). This work has also been partly funded by Grant of Education Department of Zhejiang Province (Grant agreement No: Y201223353) and Young Scientist Grant of Zhejiang Gongshang University (Grant agreement No: QY11-20).

Conflict of Interest

The authors have declared that there is no conflict of interest.

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