US Department of Health and Human Services, Food and Drug Administration, Northeast Regional Laboratory, 158-15 Liberty Avenue, Jamaica, New York, 11433-1034, U.S.A.

NMR regulatory analysis: determination and characterization of chinese-herb aristolochic acids

G. M. HANNA

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George M. Hanna, Food and Drug Administration, Northeast Regional Laboratory 158-15 Liberty Avenue, Jamaica, New York, 11433-1034, U.S.A.
ghanna@ora.fda.gov

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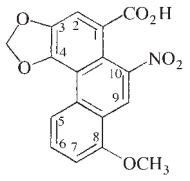
^{1}H NMR methodology for the simultaneous determination and characterization of the nephrotoxic components of *Aristolochia* plants aristolochic acid I (AA-I) and aristolochic acid II (AA-II) was developed utilizing a 400 MHz spectrometer without the need of reference standards. The developed methodology is able to differentiate and assess chemical structures of these toxic injurious compounds. The quantity of each was calculated on the basis of the integrals for the signals of the H-7 and H-8 of the phenanthrene ring of AA-I and AA-II at δ7.38 and δ8.31, respectively, and the vinylic protons of the internal standard maleic acid at δ6.06. The accuracy of the method was established through the analysis of synthetic mixtures containing the internal standard maleic acid, with purified AA-I or combined AA-I and AA-II sodium salts. Excellent agreements were verified between the assay results and the quantities in the mixtures. The mean $\pm$ SD recovery values for purified AA-I and combined AA-I and AA-II from two sets of 10 synthetic mixtures were $99.8 \pm 0.6\%$ and $99.6 \pm 0.8\%$, respectively. The assay of 4 lots of commercial aristolochic acid by ^{1}H NMR spectroscopy indicated AA-I and AA-II contents in the ranges 45.3–97.1% and 0–15.4%, respectively.

1. Introduction

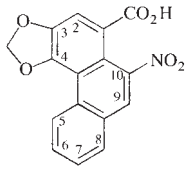
A number of *Aristolochia* species from the family Aristolochiaceae have been used in herbal medicines throughout the world as anti-inflammatory agents for gout, arthritis, rheumatism and chronic inflammatory skin diseases. Herbal teas containing *Aristolochia* herbal and radix were used for centuries for wound-healing, activation of the immune system and therapy of neoplasm. *Aristolochia clematitis L.* is common in Mediterranean countries and Central Europe. From the root and upper parts of the plant aristolochic acids were isolated. Pure aristolochic acids with limited amounts were used to stimulate phagocystosis (Kluthe et al. 1982). Aristolochic acids consist of about 14 compounds found as constituents in plants from the family Aristolochiae. There are over 600 species in the family, found both in Asia and in the United States.

Aristolochic acids were found in capsules of slimming regimen (Schmeiser et al. 1996; Vanherweghe 1998). Many cases of extensive interstitial fibrosis of the kidneys were observed in Belgian women after using the herbal slimming pills. Similar cases were also reported in France (Stengel and Jones 1998), in Japan (Tanaka et al. 2000), and in Great Britain (Lord et al. 1999). Although several *Aristolochia* species are officially described in the Chinese Pharmacopoeia and therefore are used in China, including *Aristolochia contorta* (fruit), *Aristolochia debilis* (radix, herb and fruit), *Aristolochia manshuriensis* (stem) and *Aristolochia fangchi* (root). Epidemiological observations related to those in Belgium have not been reported from China, except for some cases reported in Taiwan (Yang et al. 2000). Aristolochic acids are known as antineoplastic agents with acute nephrotoxicity (De Broe 1999). Aristolochic acids nephropathy is a progressive form of renal fibrosis that develops in many individuals who took weight-reducing pills containing Chinese herbs. It was stated that one of the herbs in these pills was replaced by *Aristolochia fangchi* Y. C. Wu ex L. D. Chou et S. M. Hwang, which is nephrotoxic and carcinogenic (De Broe 1999; Martinez et al. 2000; Nortier et al. 2003). The reason was a close similarity in Chinese terminology to another herb called Fang Ji or *Stephania tetrana* S. Moore (Menispermaceae), which was the actual intended ingredient in the weight loss formulas (Nortier et al. 2000). Because of the similarity of Chinese names for several herbs and Chinese tradition of interchangeability of similarly named herbs, the possibility always exists for accidental substitution of harmless herbs with *Aristolochia* species. *Aristolochia* species include Birthwort, Dutchman's pipe and Virginia snakeroot. Among plants that are considered to have a possibility of adulteration are *Asarum canadense*, *Bragantia wallichi*, and species of *Akebia*, *Clematis*, *Cocculus*, *Diploclisia*, *Menispermum*, *Sinomenium* and *Stephania*.

The causal compounds of nephropathy, nephrotoxicity (Mengs and Stotzem 1993; Vanherweghem et al. 1993; Vanhaelen et al. 1994), mutagenicity and carcinogenicity (Schmeiser et al. 1986; Götzl and Schimmer 1993) which are contained mainly in the genus *Aristolochia* of Aristolochiaceae are aristolochic acids I (AA-I) and II (AA-II)



AA-I



AA-II

(Rücker and Chung 1975; Mengs 1987 Pfau et al. 1990). They are derived from the phenanthrene ring system and bearing a nitro and a carboxyl substituent. Reduction of the nitro group to an amino group yields to the corresponding aristolactams. Nitro reduction is the crucial pathway in the metabolic activation of AA (Stiborova et al. 2001). The mutagenic activities of AA-I and AA-II were reported to be primarily due to base substitution mutations (Pezzuto et al. 1988). Frameshift mutations were found to play a marginal role in mutagenesis of both AA-I and AA-II (Abel and Schimmer 1983; Pool et al. 1986). In human lymphocytes, AA-I and AA-II induce chromosome aberrations and sister chromatid exchange (SCE) (Routledge et al. 1990). The fact that both AA-I and AA-II are strong fore stomach carcinogens by oral administration to rats led to the use in animal experiments as a model for human stomach cancer (Burlinson 1989; Schmeiser and Wiessler 1996). It was found that the reduction of the nitro group in AA-I and AA-II might be the activation mechanism to form the cyclic *N*-acylnitrinium ion with delocalized positive charge as the ultimate carcinogenic intermediates which were able to bind to DNA (Krumbiegel et al. 1986). The four-electron reduction of the nitro group was the critical step in activation of the AA-I and AA-II (Hashimoto et al. 1999). The use of *Aristolochia* species in herbal medicines is no longer permitted in the US, EU and many other countries due to the toxicity of aristolochic acids which have shown to be nephrotoxic, carcinogenic and mutagenic.

This work was therefore carried out to develop a sensitive ^{1}H NMR method for the characterization and the specific determination of injurious nephrotoxic AA-I and AA-II which are commonly found in herbal supplements and were reported to cause acute hepatitis and endstage renal failure.

2. Investigations, results and discussion

Only mixtures of aristolochic acid I, 8-methoxy-3, 4-methylenedioxy-10-nitrophenanthrene-1-calboxylic acid (AA-I), and aristolochic acid II, 8-methoxy-3, 4-methylenedioxy-10-nitrophenanthrene-1-calboxylic acid (AA-II) are marketed. The identification without pure standard by Rf values of TLC spots or retention time of HPLC may be misleading. Actually, there are two reports (Zhu and Phillipson 1996; Hashimoto et al. 1999) in which AA-I and AA-II peaks of HPLC were reversed on the assignments. It is desirable to quickly confirm their presence in mixed standard materials by their NMR spectra.

Table 1: ^{1}H NMR spectral assignments of AA-I in DMSO-d_6

Chemical shifts[a] (δ ppm)	Multiplicity	Coupling constant (Hz)	Number of protons	Assignments
8.66	*dd*	J = 8.0, 1.8	1	H-5
8.59	*s*		1	H-9
7.86	*td*	J = 8.0, 1.8	1	H-6
7.82	*s*		1	H-2
7.38	*dd*	J = 8.0, 1.8	1	H-7
6.50	*s*		2	OCH_2O
4.07	*s*		3	OCH_3

[a] Chemical shifts were measured Vs. TSP, sodium 3 (trimethylsilyl) tetradeuterio propionate

In order to establish the accuracy of the ^{1}H NMR developed method, AA-I and AA-II were purified from commercial mixtures by preparative TLC. The TLC conditions were as follows: plate, silica gel $60F_{254}$ plate; solvent system, $CHCl_3$-MeOH-H_2O (60 : 40 : 10); detection UV irradiation. AA-I and AA-II showed Rf values of 0.52 and 0.47 respectively, and their separation was excellent. Under UV 254 nm irradiation both showed up as spots of the same dark color. AA-I and AA-II spots were individually scraped and extracted. An alternative purification method was also used, the mixture of AA-I and AA-II underwent CC on Sephadex LH-20 and eluted with H_2O and MeOH to give AA-I and AA-II successively. ^{1}H NMR spectra of dry residues of AA-I and AA-II indicated that both methods were effective in preparation of purified samples of AA-I and AA-II.

Protons resonances were assigned based on chemical shift values, spin multiplicities, and coupling constants. The ^{1}H NMR spectrum of AA-I in DMSO-d_6 showed a signal for aromatic methoxyl group protons at δ4.07 ppm (3H, s). Three mutually coupled aromatic protons of the phenanthrene ring appeared at δ7.38 (1H, dd, J = 8.0, 1.8 Hz), δ7.86 (1H, td, J = 8.0, 1.8 Hz) and δ8.66 (1H, dd, J = 8.0, 1.8 Hz) could be assigned to H-7, H-6 and H-5, respectively. The lower field signal at δ8.66 is characteristic of the C-5 proton in AA-I. Two singlets at δ7.82 (1H, s) and δ8.59 (1H, s) could be assigned to H-2 and H-9, respectively. The signal appeared at δ6.50 (2H, s) could be assigned to the methylenedioxy protons.

^{1}H NMR spectrum of AA-II in DMSO-d_6 showed four mutually coupled aromatic protons of the phenanthrene ring appearing at δ7.90 (1H, dd, J = 8.0, 1.8 Hz), δ7.95 (1H, td, J = 8.0, 1.8 Hz), δ8.31 (1H, dd, J = 8.0, 1.8 Hz) and δ9.14 (1H, dd, J = 8.0, 1.8 Hz), and could be assigned to H-7, H-6, H-8, and H-5, respectively. The lower field signal at δ9.14 is characteristic of the C-5 proton in AA-II. Two singlets at δ7.85 (1H, s) and δ8.59

Table 2: ^{1}H NMR spectral assignments of AA-II in DMSO-d_6

Chemical shifts[a] (δ ppm)	Multiplicity	Coupling constant (Hz)	Number of protons	Assignments
9.14	*dd*	J = 8.0, 1.8	1	H-5
8.59	*s*		1	H-9
8.31	*dd*	J = 8.0, 1.8	1	H-8
7.95	*td*	J = 8.0, 1.8	1	H-6
7.90	*dd*	J = 8.0, 1.8	1	H-7
7.85	*s*		1	H-2
6.50	*s*		2	OCH_2O

[a] Chemical shifts were measured Vs. TSP, sodium 3-(trimethylsilyl) tetradeuterio propionate

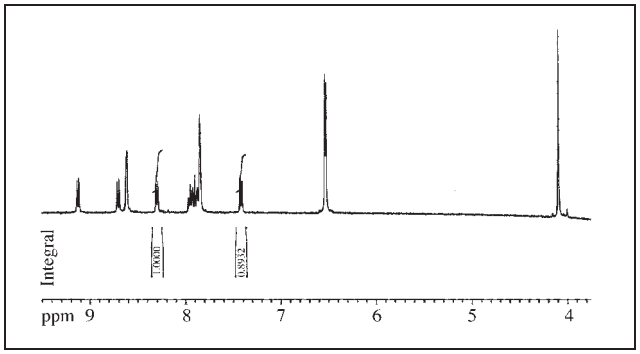


Fig. 1: 400.13 MHz ^{1}H NMR spectra of AA-I (45.3%), and AA-II (51.7%), 8.11 and 9.31 μM, respectively, in DMSO-d_6.

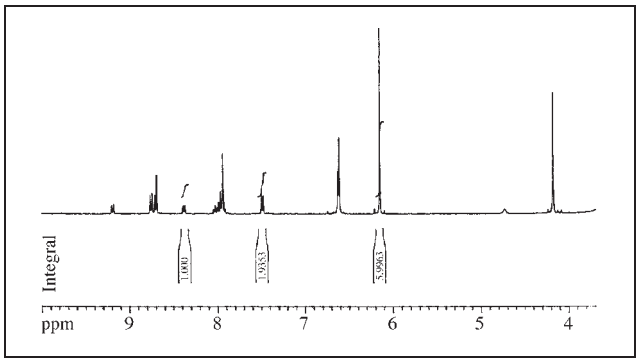


Fig. 2: 400.13 MHz ^{1}H NMR spectra of AA-I (64.6%), and AA-II (32.4%), and maleic acid, 10.67, 5.85, and 20.05 μM, respectively, in DMSO-d_6.

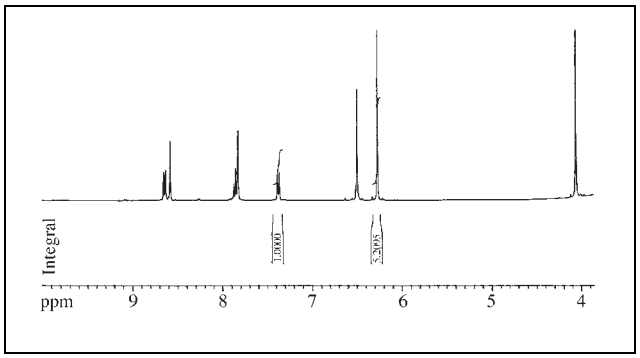


Fig. 3: 400.13 MHz ^{1}H NMR spectra of AA-I (97.1%), and maleic acid, 16.04, and 20.05 μM, in DMSO-d_6.

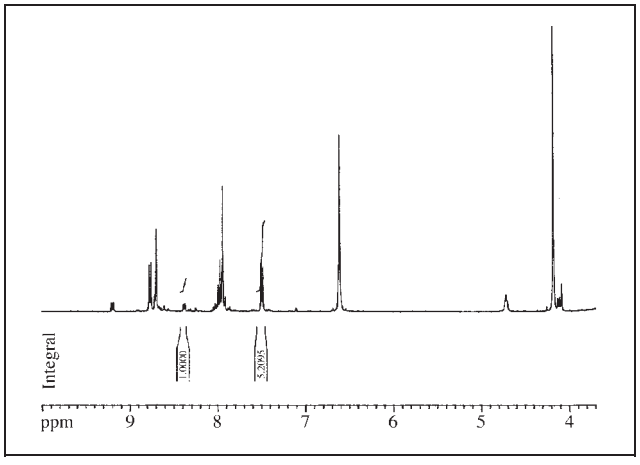


Fig. 4: 400.1 MHz ^{1}H NMR spectra of AA-I (84.1%), and AA-II (15.4%), 13.89 and 2.77 μM, respectively, in DMSO-d_6.

Table 3: Results of the assay of AA-I and AA-II sodium salts in synthetic mixtures by ^{1}H NMR spectroscopy[a]

No.	Internal standard maleic acid added[b] (mg)	Mixture of AA-I and -II sodium salts[a]				
		added (mg)	found (mg)			recovered[b] (%)
			AA-I	AA-II	Total	
1	1.15	4.46	3.00	1.47	4.47	100.2
2	1.15	7.04	4.71	2.35	7.06	100.3
3	1.15	3.57	2.33	1.19	3.52	98.6
4	1.15	4.56	3.01	1.50	4.51	98.9
5	1.15	7.51	5.04	2.52	7.56	100.6
6	1.15	8.51	5.61	2.81	8.42	98.9
7	1.15	9.02	5.96	2.98	8.94	99.1
8	1.15	6.07	4.01	2.01	6.02	99.2
9	1.15	6.47	4.31	2.15	6.46	99.2
10	1.15	8.03	5.39	2.70	8.09	100.7
Mean						99.6
SD						0.8

[a] Dried in vacuum for 24 h
[b] Added from a 49.53 μM solution of maleic acid in DMSO-d_6 (200 μL)
[b] Recoveries were calculated from (100 × amount found)/amount added

Table 4: Results of the assay of AA-I in synthetic mixtures by ^{1}H NMR spectroscopy

No.	Internal Standard, maleic acid added[b] (mg)	AA-I[a]		
		added (mg)	found (mg)	recovered[c] (%)
1	1.15	5.46	5.54	99.9
2	1.15	6.14	6.18	100.6
3	1.15	4.87	4.83	99.3
4	1.15	4.56	4.54	99.5
5	1.15	6.51	6.48	99.5
6	1.15	8.53	8.57	100.5
7	1.15	7.12	7.08	99.4
8	1.15	6.27	6.28	99.8
9	1.15	5.47	5.49	100.3
10	1.15	8.43	8.32	98.7
Mean				99.8
SD				0.63

[a] Purified and dried in vacuum for 24 h
[b] Added from a 49.53 μM solution of maleic acid in DMSO-d_6 (200 μL)
[c] Recoveries were calculated from (100 × amount found)/amount added

Table 5: Results of the assay of commercial mixtures AA-I and AA-II sodium salt by ^{1}H NMR spectroscopy

Lot	AA-I sodium salt (%)	AA-II sodium salt (%)
A	81.6	15.4
B	45.3	51.7
C	64.5	32.4
D	97.1	0

(1H, s) could be assigned to H-2 and H-9, respectively. The singlet appear at δ6.50 (2H, s) could be assigned to the methylenedioxy protons.

In addition to the lower field characteristic signal of the C-5 proton which appeared at δ9.14 in AA-II and at δ8.66 in AA-I, there are more dissimilarities between the ^{1}H NMR spectrum of AA-II and that of AA-I. The four mutually coupled aromatic signals at δ7.90 (1H, dd, J = 8.0, 1.8 Hz), δ7.95 (1H, td, J = 8.0, 1.8 Hz), δ8.31 (1H, dd, J = 8.0, 1.8 Hz) and δ9.14 (1H, dd, J = 8.0, 1.8 Hz), instead of three mutually coupled aromatic signals at δ7.38 (1H, dd, J = 8.0, 1.8 Hz), δ7.86 (1H, td,

J = 8.0, 1.8 Hz) and δ8.66 (1H, dd, J = 8.0, 1.8 Hz) and the aromatic methoxyl group at δ4.07 (3H, s). The assignments of AA-I and AA-II are given in Table 1 and 2, respectively.

The simultaneous quantification of AA-I and AA II in commercial mixtures was achieved by measuring the intensity of the signals for the H-7 of AA-I at δ7.38 (1H, dd, J = 8.0, 1.8 Hz), H-8 of AA-II at δ8.31 (1H, dd, J = 8.0, 1.8 Hz) and the vinylic protons of the internal standard maleic acid at δ6.06 (2H, s). The selection of maleic acid was based on its solubility and convenient position. The specimen of maleic acid used had a declared *trans* form fumaric acid content of <0.5%. Applying a purity correction factor for maleic acid in the calculation was unnecessary since the intensity of the two signals corresponding to both forms can be used in the calculation without affecting the accuracy of the results.

The accuracy of the ^{1}H NMR spectroscopic method was tested by analyzing a set of 10 synthetic mixtures containing the internal standard, maleic acid and AA-I or AA-I and AA-II sodium salts in the amounts listed in Tables 3 and 4. These results indicated that the accuracy of the proposed method was not affected by the range of relative proportions of analytes to internal standard. Four aristolochic acid lots from different commercial sources were also analyzed by the proposed ^{1}H NMR spectroscopic method. The ^{1}H NMR spectra are shown in Fig. 1–4 and the results of analysis are summarized in Table 5.

In summary, the method described here was effective in distinguishing AA-I and AA-II in a mixture by their ^{1}H NMR spectra. Quantitative analyses of AA-I and AA-II were successfully carried out. The purified forms of AA-I and AA-II were used only to check the accuracy of the method. In conclusion, the ^{1}H NMR spectroscopic method greatly simplified the characterization and the simultaneous quantitative analysis of the injurious AA-I and AA-II in mixtures. Additional to its ability to concurrently measure both forms, it does not require the use of any of the pure analytes as reference standards.

3. Experimental

3.1. Apparatus

All ^{1}H NMR spectra were obtained on a model AMX-400 spectrometer operating at 400.13 MHz (Brüker Instruments, Inc., Billerica, MA) and 28 °C. The chemical shifts were referenced to TSP, sodium 3-(trimethylsilyl) tetradeuteriopropionate.

3.2. Materials

Dimethyl-d_6 sulfoxide, DMSO-d_6, 99.9 atom % D, sodium 3-(trimethylsilyl) tetradeuteriopropionate (TSP) and maleic acid, 99.5 + %, were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). Aristolochic acid (mixed reagents of AA-I and AA-II) 97% and aristolochic acid sodium salt (mixed reagents of AA-I and AA-II), 97%, were from ICN Biomedicals, Inc. (Irvine, CA, USA).

3.3. Preparation of purified samples

TLC Separation was used for purification of individual AA-1 or AA-II. The TLC conditions were as follows: plate, silica gel 60F_{254} plate; solvent system, $CHCl_3$-MeOH-H_2O (60 : 40 : 10); detection UV irradiation. AA-I and AA-II showed Rf values of 0.52 and 0.47. Under UV 254 nm irradiation both showed up as spots of the same dark color. AA-I and AA-II spots were individually scraped and extracted with 50% MeOH, then underwent ultra sonication for 30 min and next filtered (45 μm pore size) and evaporated under reduced pressure.

Column chromatography as an alternative purification method was also used, the mixture of AA-I and AA-II underwent on Sephadex LH-20 and eluted with H_2O and MeOH to give AA-I and AA-II successively. The purities of AA-I and AA-II were evaluated by their individual ^{1}H NMR spectrum.

3.4. Standard solutions

Stock solutions of maleic acid (5.74 mg/mL) were prepared in DMSO-d6, transferred to glass vials, and immediately crimped-sealed with Teflon-coated rubber septa and aluminum seals. When needed, these solutions were withdrawn through the septa by means of a liquid-tight micro-liter syringe.

3.5. Procedure

Synthetic formulations containing aristolochic acid and maleic acid, were prepared by first adding an accurately weighed quantity of aristolochic acid or aristolochic acid sodium salt to a 5 mm NMR tube, added 0.200 mL maleic acid stock solution, dissolved in DMSO-d6, adjusted the volume to approximately 1.0 mL, and immediately capped, mixed, placed in the spectrometer, and the spectrum was obtained. The amounts of AA-I and AA-II were calculated by measuring integrals for the signals of the H-7 and H-8 of the phenanthrene ring of AA-I and AA-II at δ7.38 and δ8.31, respectively and the vinylic protons of the internal standard maleic acid at δ6.06 and using the following equations:

$$\text{AA-I, mg} = [\text{AI/Am}] \times [\text{EWI/EWm}] \times \text{Mm} \quad (1)$$

where AI is the integral value of aristolochic acid-I, Am is the integral value of the internal standard, EWI is the formula weight of aristolochic acid-I sodium salt divided by the number of protons (i.e., 363.2/1 = 363.2), EWm is the formula weight of the internal standard divided by the number of absorbing protons (i.e., 116.1/2 = 58.05), and Mm is the amount of internal standard added, mg.

$$\text{AA-II, mg} = [\text{AII/Am}] \times [\text{EWII/EWm}] \times \text{Mm} \quad (2)$$

where AII is the integral value of aristolochic acid-II, Am is the integral value of the internal standard, EWII is the formula weight of aristolochic acid-II sodium salt divided by the number of protons (i.e., 333.2/1 = 333.2), EWm is the formula weight of the internal standard divided by the number of absorbing protons (i.e., 116.1/2 = 58.05), and Mm is the amount of internal standard added, mg.

References

Abel G, Schimmer O (1983) Induction of structural chromosome aberrations and sister chromatid exchanges in human lymphocytes *in vitro* by aristolochic acid. Hum Genet 64: 131–133.

Burlinson B (1989) An in vivo unscheduled DNA synthesis (UDS) assay in the rat gastric mucosa: preliminary development. Carcinogenesis 10: 1425–1428.

De Broe ME (1999) On a nephrotoxic and carcinogenic slimming regimen. Am J Kidney Dis 33: 1171–1173.

Gotzl E, Schimmer O (1993) Mutagenicity of aristolochic acids (I, II) and aristolic acid I in new YG strains in *Salmonella typhimurium* highly sensitive to certain mutagenic nitroarenes. Mutagenesis 8: 17–22.

Hashimoto H, Higichi M, Makono B, Sakakibara I, Kubo M, Komatsu Y, Maruno M, Okada M (1999) Quantitative analysis of aristolochic acids, toxic compounds, contained in some medicinal plants. J Ethnopharmacol 64: 185–189.

Kluthe R, Vogt A, Batsford S (1982) Double blind study of the influence of aristolochic acid on granulocyte phagocytic activity. Arzneimittelforsch 32: 443–445.

Krumbiegel G, Hallensleben J, Mennicke WH, Rittmann N, Roth HJ (1987) Studies on the metabolism of aristolochic acids I and II. Xenobiotica 17: 981–991

Lord GM, Tagore R, Cook T, Gower P, Pusey CD (1999) Nephropathy caused by Chinese herbs in the UK. Lancet. 354: 481–482.

Martinez MC, Nortier J, Vereerstraeten P, Vanherweghem JL (2000) Progression rate of Chinese herb nephropathy: impact of Aristolochia fangchi ingested dose. Nephrol Dial Transplant 17: 408–412.

Mengs U (1983) On the histopathogenesis of rat forestomach carcinoma caused by aristolochic acid. Arch Toxicol 52: 209–220.

Mengs U (1987) Acute toxicity of aristolochic acid in rodents. Arch Toxicol 59: 328–331.

Mengs U, Stotzem CD (1993) Renal toxicity of aristolochic acid in rats as an example of nephrotoxicity testing in routine toxicology. Arch Toxicol 67: 307–311.

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

Nortier JL, Schmeiser HH, Muniz Martinez MC, Arlt VM, Vervaet C, Garbar CH, Daelemans P, Vanherweghem JL (2003) Invasive urothelial carcinoma after exposure to Chinese herbal medicine containing aristolochic acid may occur without severe renal failure. Nephrol Dial Transplant 18: 426–428.

Pezzuto JM, Swanson SM, Mar W, Che CT, Cordell GA, Fong HH (1988) Evaluation of the mutagenic and cytostatic potential of aristolochic acid (3,4-methylenedioxy-8-methoxy-10-nitrophenanthrene-1-carboxylic acid) and several of its derivatives. Mutat Res 206: 447–454.

Pfau W, Schmeiser HH, Wiessler M (1990) 32P-postlabelling analysis of the DNA adducts formed by aristolochic acid I and II. Carcinogenesis 11: 1627–1633.

Pool BL, Eisenbrand G, Preussmann R, Schlehofer JR, Schmezer P, Weber H, Wiessler M (1986) Detection of mutations in bacteria and of DNA damage and amplified DNA sequences in mammalian cells as a systematic test strategy for elucidating biological activities of chemical carcinogens. Food Chem Toxicol 24: 685–691.

Routledge MN, Orton TC, Lord PG, Garner RC (1990) Effect of butylated hydroxyanisole on the level of DNA adduction by aristolochic acid in the rat forestomach and liver. Jpn J Cancer Res 81: 220–224.

Rücker VG, Chung BS (1975) Aristolochic acids from Aristolochia manshuriensis. Planta Med. 27: 68–67.

Schmeiser HH, Bieler CA, Wiessler M, van Ypersele de Strihou C, Cosyns JP (1996) Detection of DNA adducts formed by aristolochic acid in renal tissue from patients with Chinese herbs nephropathy. Cancer Res 56: 2025–2028.

Schmeiser HH, Pool BL, Wiessler M (1986) Identification and mutagenicity of metabolites of aristolochic acid formed by rat liver. Carcinogen 7: 59–63.

Stengel B, Jones E (1998) End-stage renal insufficiency associated with Chinese herbal consumption in France. Nephrologie 19: 15–20.

Stiborova M, Frei E, Breuer A, Wiessler M, Schmeiser HH (2001) Evidence for reductive activation of carcinogenic aristolochic acids by prostaglandin H synthase – (32)P-post labeling analysis of DNA adduct formation. Mutat Res 493: 149–160.

Tanaka A, Nishida R, Maeda K, Sugawara A, Kuwahara T (2000) Chinese herb nephropathy in Japan presents adult-onset Fanconi syndrome: could different components of aristolochic acids cause a different type of Chinese herb nephropathy? Clin Nephrol 53: 301–306.

Vanherweghem LJ (1998) Misuse of herbal remedies: the case of an outbreak of terminal renal failure in Belgium (Chinese herbs nephropathy). J Altern Complement Med 4: 9–13.

Vanherweghem JL, Depierreux M, Tielemans C, Abramowicz D, Dratwa M, Jadoul M, Richard C, Vandervelde D, Verbeelen D, Vanhaelen-Fastre R, et al. (1993) Rapidly progressive interstitial renal fibrosis in young women: association with slimming regimen including Chinese herbs. Lancet. 341: 387–391.

Vanhaelen M, Vanhaelen-Fastre R, But P, Vanherweghem JL (1994) Identification of aristolochic acid in Chinese herbs. Lancet 343: 174.

Yang CS, Lin CH, Chang SH, Hsu HC (2000) Rapidly progressive fibrosing interstitial nephritis associated with Chinese herbal drugs. Am J Kidney Dis 35: 313–318.

Zhu M, Phillipson JD (1996) Hong Kong samples of the traditional Chinese medicine "Fang Ji" contain aristolochic acid toxins. Int J Pharmacognosy 34: 283–289.