Alerts, Notices, and Case Reports

Lethal Ingestion of Chinese Herbal Tea Containing *Ch'an Su*

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TRADITIONAL CHINESE HERBAL REMEDIES are readily available from herb and grocery stores in most Chinese communities. Some of these products contain possibly toxic ingredients, are often imported into the United States illegally, and are associated with unproven claims of therapeutic benefit. This report describes the multifaceted investigation following the death of a young Chinese woman after she consumed a herbal remedy mixture prepared as a tea. A highly toxic ingredient in the tea was identified as secretions of the parotid and sebaceous glands of a toad (*Bufo bufo gargarizans* Cantor or *Bufo melanosticus* Schneider) that when used as a Chinese remedy is known as *ch'an su*. Although cases of toad-related poisonings have been described,[1-5] this is the first report of a death following the ingestion of a herbal remedy containing *ch'an su*. This investigation highlights the difficulties in identifying toxic components of herbal mixtures, the need for the cooperation of specialists of many fields to identify the causative agent(s) in these deaths, and the need for better controls over the use and availability of certain potent Chinese herbal remedies.

Report of a Case

The patient, a 25-year-old woman, presented to an emergency department because of a sudden onset of epigastric tenderness, several episodes of vomiting, and difficulty breathing that began immediately after she consumed tea brewed from a prescribed Chinese herbal remedy mixture. Abdominal pain was reported to have been present for a few days. The patient had a miscarriage about four months previously, and a few days before admission, she had been informed by her physician that she was again pregnant. She sought out a Chinese herbalist who prescribed a herbal remedy mixture to help her "sustain" her pregnancy. An in-store employee hired by the herbalist dispensed the remedy in two equal portions. After making one of the portions into a tea and drinking it for the first time that day, she immediately complained of perioral numbness. A few minutes after consuming slightly less than one "bowl" (about 100 ml) of the tea, she complained to her husband of nausea, then vomited and had increasing abdominal pain. She was immediately taken to the emergency department by her husband. She had no other relevant medical history.

On physical examination, the patient appeared listless but in no apparent distress; she had nausea but no diaphoresis. She was not cyanotic and had no signs of labored breathing. Her blood pressure was 120/80 mm of mercury, pulse rate 88 beats per minute, respirations 20 per minute, and temperature 36°C (96.8°F). The heartbeat was regular, the lungs were congested on expiration, and the abdomen was soft and nontender. The patient was drowsy but able to follow simple commands. The hemoglobin level was 125 grams per liter (12.5 grams per dl), the leukocyte count was 19.5 $\times$ 10^9 per liter (19,500 per mm^3), with 0.68 (68%) neutrophils, 0.28 (28%) lymphocytes, 0.03 (3%) monocytes, and 0.01 (1%) band forms. The serum sodium level was 133 mmol per liter; potassium, 3.9 mmol per liter; carbon dioxide content, 19 mmol per liter; and glucose, 8.4 mmol per liter (151 mg per dl). The blood urea nitrogen concentration and serum creatinine level were within normal limits. A portable x-ray film showed no evidence of cardiac enlargement or lung disease.

About 30 minutes after admission to the emergency department, the patient became pale, bradycardic, and progressively hypotensive. After the administration of atropine sulfate, sinus tachycardia developed and then cardiopulmonary arrest. During an hour-long resuscitative effort, the patient had a grand mal-type seizure. Despite all efforts, ventricular tachycardia developed, then intractable fibrillation. The patient died 2½ hours after she initially ingested the tea. The electrocardiogram was limited to a resuscitation rhythm strip.

A full autopsy was done less than nine hours after death. Gross findings were limited to a small amount of green-brown aspirated fluid in the proximal bronchi and a slightly enlarged uterus containing normal placental tissue consistent with a gestation of about one month. On microscopic examination, there was pronounced congestion of the viscera and minute foci of hepatic necrosis, all deemed consistent with vigorous resuscitation. Multiple

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cardiac sections, including of the conduction system, showed no inflammation or fibrosis.

Notably, investigating pathologists had abrupt, transient, severe rhinorrhea, coughing, and perioral numbness from handling the unused second portion of the herbal remedy mixture obtained from the family.

Methods

The tea was strongly suspected of causing the death after the postmortem examination failed to identify any natural disease process, including myocarditis or other cardiac abnormalities, drugs of abuse, common poisons, or therapeutic drugs in toxic amounts. A multidisciplinary approach was used to isolate and identify the suspected agent and to evaluate the public health ramifications of this case. Chinese herbalists with more than 20 years of experience were consulted to verify and translate the original prescription and to identify its individual components. Clinical toxicologic screening by the medical examiner's office was done on postmortem blood and urine specimens. Mouse bioassay and advanced analytical assays were done by the California Department of Health Services (DHS) and the United States Department of Agriculture on specimens of the tea and the unused portion of the herbal mixture. Public health investigators from DHS reviewed the cases of other deaths in the community that, in hindsight, might be attributed to undiagnosed poisoning by herbal remedies.

TABLE 1.—*Preparation of Materials* and Results of Mouse Bioassay of a Chinese Herbal Remedy*

Material	Result: Time to Death, min:sec	Result: Dilution
Brewed tea (A)†	Death 3:45-4:45	No
Dilution of A	Death 37:50	1:9 H_2O
Tap water boiled in patient's teapot	No death	No
Unused herbal remedy mixture brewed by authors (B)	Death 2:30	No
Dilution of B	Death 19:59	1:10 H_2O
Component of B: unidentified brown powder	Death 2:53	No
Component of B: unidentified brown pellets	Death 4:01	No
Component of B: "glue?"‡ (*ch'an su?*)§	Death 3:53	No
Herbal remedy mixture obtained from same store using same prescription (C)	No death	No
Different components of C	No death	No
Ch'an su (D)	Death 6:30	1:1 H_2O

*See text for description of samples A through D.

†Prepared by the patient, instructions direct that the herbs boil in 4 bowls of water (approximately 425 ml) until the volume is reduced to 1 bowl (3 to 4 hours). After boiling, a separate package of "glue" is mixed with the tea.

‡"Glue" is "gelatinum asini" powder (ass-hide glue), a granular, powdery resinous material contained in a separate package. It is the same material used to make the gelatinum asini balls. This material is added immediately before drinking and after the other herbs have boiled for several hours.

§*Ch'an su* is a traditional Chinese herb consisting of dehydrated toad gland secretion formed into a resinous block.

But-Lau Method

Step 1	15 ml of the aqueous brewed herbal remedy are adjusted to pH = 9, using 1:1 (vol/vol) concentrated ammonium hydroxide:water
Step 2	Add 20 ml of diethyl ether and sonicate for 5 minutes
Step 3	Save the ether extract in a 125-ml separator funnel Repeat step 2 and pool the second ether extract
Step 4	Add 25 ml of 2% HCl to the separator funnel containing the pooled ether extracts and shake gently for 1 minute
Step 5	Repeat step 4 three times, saving the aqueous phase (total of 100 ml of 2% HCl)
Step 6	Adjust the aqueous phase to pH = 9 with concentrated ammonium hydroxide
Step 7	Extract the aqueous phase with 25 ml of diethyl ether
Step 8	Repeat step 7 three times, saving the combined ether extract in a 250-ml Erlenmeyer flask
Step 9	Dry with 10 grams of sodium sulfate and filter the ether extract
Step 10	Evaporate the ether extract to dryness under a stream of nitrogen using a water bath at room temperature
Step 11	Dissolve the residue in 1 ml of ether

Figure 1.—The But-Lau method of extraction is shown (Paul P. H. But, PhD, and F. W. Lau, Department of Biology and Chinese Medicinal Material Research Center, The Chinese University of Hong Kong, Hong Kong, written communication, June 1993). HCl = hydrochloride

The DHS carried out bioassays and analytical assays on the following specimens:

- The leftover brewed tea (sample A),
- Tea brewed from the second unused portion of the herbal remedy mixture (sample B),
- Tea brewed from a refill of the prescription obtained by DHS from the same store that dispensed the original herbal remedy mixture (sample C), and
- Tea brewed from a control specimen of known *ch'an su* obtained from a local herbalist (sample D).*

A mouse bioassay was done to evaluate the toxicity of these specimens. Three- to 4-week-old Swiss Webster mice of either sex weighing approximately 20 grams were administered intraperitoneally 1 ml of cooled brewed tea of samples A through D prepared as described in Table 1. The mice were kept in a facility accredited by the American Association for Accreditation of Laboratory Animal Care. After the samples were injected, adverse reactions were recorded for about 30 minutes or until death. Surviving mice were reexamined the next morning.

In the analytical assay to identify the specific toxic agents, aqueous leachates of samples A through D were extracted by the But-Lau method (written communication, Figure 1). Analysis was done using both high-resolution mass spectrometry with the direct introduction

*Sample D was obtained and tested after the initial analytical assays identified components of *ch'an su*.

of a probe and gas chromatography with mass spectrometry (Figure 2).

Results

The ingredients in the herbal prescription are listed in Table 2. Based on a literature review, all of the prescribed herbs were nontoxic at the dosages given. According to the Chinese herbalists who examined the unused portion of the herbal remedy, however, one component in the mixture actually dispensed (sample B) did not match the expected prescribed ingredient. The "gelatinum asini" balls were absent, and two unidentified components, small brown pellets and powder, were found that did not resemble any commonly known botanical products. The brown powder was presumably from the breakup of the pellets. The identity of the gelatinum asini powder (ass-hide glue) was questionable.

Postmortem routine toxicologic screening failed to detect either the parent compound or the metabolites of cocaine, amphetamines, opiates, phencyclidine, marijuana, benzodiazepines, ethanol, volatiles, cyanide, strychnine, barbiturates, tricyclic antidepressants, methadone, or other commonly abused drugs. Only lidocaine used in the resuscitation effort was detected in the blood during conventional testing. In retrospect, after the causative agent was identified, testing of the serum using the Abbott TDx assay[6] for digoxin showed a concentration of 6.3 nmol per liter (4.90 ng per ml; toxic, >2.6 nmol per liter [>2 ng per ml]).

Mouse bioassay results verified the herbal remedy mixture as being highly toxic (see Table 1). The administration of decoctions of samples A, B, and D, as well as those prepared from the three components of sample B (unidentified brown powder, unidentified brown pellets, and "glue") caused death in as little as 2½ minutes. Diluting the decoctions before administering resulted in a proportional delay in the time of death. The mice receiving samples A, B, or D exhibited similar symptoms that included piloerection, labored breathing, apparent hypoxia (poor color and bulging eyes), oral discharge, extension and twitching of fore and hind limbs, and in some cases, generalized convulsions followed by death. Decoctions prepared from sample C, although allegedly the same herbal remedy mixture as sample B, were not lethal to mice and produced no obvious adverse effects during the period observed.

Gas Chromatography-Mass Spectrometry

Each extract of herbal remedy was injected separately into the Hewlett Packard (HP) 5890 gas chromatograph (GC) with an HP 5971A mass selective detector. The conditions for the GC system are as follows: splitless injections of 2 µl were made into a 15-m, 0.25-µm internal diameter DB-1 column with 0.25-µm film thickness; the initial column temperature was 150ºC for 2 minutes, ramped at a rate of 20ºC per minute until it reached 300ºC and held for 12 minutes; linear velocity of the column was 35 cm per second; the transfer line temperature was 310ºC. The mass spectroscopy work was performed in the full-scan mode, with the scan range from 35 to 650 atomic mass units and the scan time 1 scan per second, with a solvent delay of 5 minutes and electron multiplier voltage of 1,976 volts.

Figure 2.—The method of gas chromatography with mass spectrometry is described.

TABLE 2.—*Prescription for a Chinese Herbal Remedy Provided to the Patient*

Herb	Quantity, grams
Astragalus root	15
Gelatinum asini ball (*Equus asinus*)*	18
Cacumen biotae (biota tops, *Biota orientalis*)	15
Alismatis rhizome	18
Peony root	15
Glycyrrhizae species root, raw	6
Rehmannia root, cooked	30
Cornus fruit	15
Phellodendron bark (*Phellodendron amurense*)	4.5
Poria species, with host wood attached	24
Gelatinum asini powder†	15

*Gelatinum asini powder (ass-hide glue) plus talcum powder baked for 5 minutes.
†In a separate package, to be added after the volume has been reduced.

Preliminary mass spectral data for components of sample B identified that bufodienolides were the principal toxic components. Based on molecular weights assigned from the ammonia chemical ionization spectra of components of sample B and comparison of the high-resolution spectra of components of sample B with a 120,000-spectra database, including those of the known constituents of *ch'an su*,[7,8] the four major constituents appeared to be bufotalin, cinobufotalin, γ-bufotalin (or its isomer, bufogenin B), and bufalin. Subsequently, samples A through D were extracted under identical conditions, and their total ion current chromatograms from gas chromatography with mass spectrometry are shown in Figure 3. Samples A, B, and D had patterns identical to the major fragments from the bufotalin and bufotenin library mass spectra. None of these compounds were found in sample C.

Discussion

Ch'an su was identified as the toxic ingredient after mass spectral analysis indicated the presence of known constituents of this ingredient in the specimens. Testing the victim's postmortem serum revealed unexpected, lethal amounts of cardiac glycosides. Based on this information, the expert herbalists' evaluation, the deceased's symptoms, and the results of the bioassay, we concluded that *ch'an su* was the causative toxic agent and had been placed in the herbal remedy mixture instead of the prescribed gelatinum asini (ingredient used to make the gelatinum asini balls and gelatinum asini powder). The final unanswered question was, "How did this substitution occur?" Public health investigation, though limited, did not suggest that a contaminated batch of toxic herbs had been imported into the community. Investigation of the circumstances by the medical examiner's office and the DHS did not uncover any facts to indicate that a deliber-

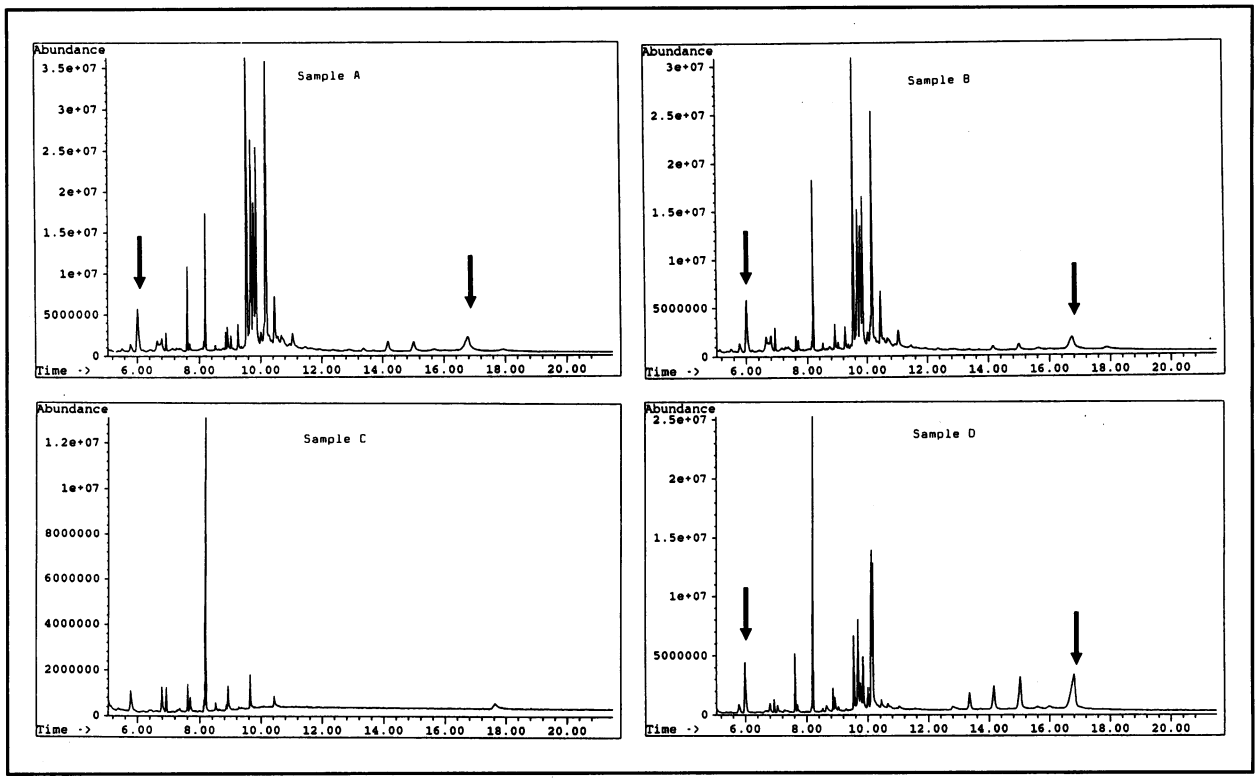

Figure 3.—The four total ion current chromatograms corresponding to samples A, B, C, and D, respectively, are shown. A compound having a retention time of 16.7 minutes has similar mass spectral peaks in samples A, B, and D and the bufotalin library mass spectrum. Samples A, B, and D also contain another compound that has a retention time of 6.0 minutes and major fragments that are identical to the library reference mass spectra of 3-[2-(dimethylamino)ethyl]-1H-indol-5-ol (bufotenin). Sample C has neither of these compounds in the extract.

ate substitution had occurred. Because *ch'an su* and gelatinum asini are both imported as dark-brown resinous blocks that become virtually indistinguishable when ground into powder or pellets, we conclude that the most likely scenario was an inadvertent substitution of *ch'an su* when the herbal prescription was dispensed.

Ch'an su, also known as toad venom, *senso*, or "bufonis venenum," is a concentrated secretion of parotid and sebaceous glands from either *B b gargarizans* Cantor or *B melanosticus* Schneider.[8] These preparations contain two groups of toxic compounds: the steroid derivatives that resemble cardiac glycosides, consisting of bufagenins (bufagins, bufadienolides) and bufotoxins, and the basic components that include epinephrine (adrenaline), norepinephrine, serotonin, and bufotenin (a hallucinogenic compound).[9-11] Traditional uses for *ch'an su* given in minute doses include the stimulation of myocardial contraction, diuresis, pain relief, anti-inflammatory effects, and topical use for skin eruptions. Toad venom has been reported to be abused either by "licking or smoking" to obtain the hallucinogenic effects.[12]

The signs and symptoms of *ch'an su* poisoning may include local irritation with profuse salivation; perioral numbness; nausea; vomiting; pain; arrhythmias such as ventricular fibrillation, tachycardia, bradycardia, and second-degree atrioventricular block; vasoconstriction; hypertension; hallucination; and seizures.[2-4,10,12,13] Many of these symptoms were displayed by the patient during the short clinical course. Although *ch'an su* toxicity in humans has been treated with supportive therapy, there is no known antidote for such poisoning. The use of a pacemaker for severe arrhythmias and charcoal lavage to absorb the toxic components has been suggested.[1,12] In dogs, the administration of propranolol has successfully reversed ventricular fibrillation caused by bufotoxin poisoning.[14]

Structural similarity between the cardiac glycosides of the toad venom and digoxin explains many of the observed symptoms and the cross-reaction in both the digoxin fluorescent immunoassay (Abbott TDx assay) and in radioimmunoassays. Several authors have referred to this finding as the presence of a digoxin-like immunoreactive substance.[2,15,16] In this case, we used the patient's elevated "digoxin" levels to confirm *ch'an su* as the toxic substance. The treatment of severe digoxin and digitoxin overdose with digoxin-specific immune Fab antibodies has been described.[17] Unfortunately, there is no research on the use of digoxin-specific Fab fragments with the cardiac glycosides found in *ch'an su*. Studies of polyclonal digoxin immune Fab with digoxin, digitoxin, and two common digoxin-derived glycosides, however, showed no important difference in the binding

capacities and the ability of the antibodies to abolish the arrhythmogenic effects of these glycosides.[18] This indicates a potential for the use of digoxin-specific Fab fragments in future cases of severe poisoning by *ch'an su* or other naturally occurring glycosides.

This case illustrates several possible problems with the evaluation and use of Chinese herbs. First, the generally accepted definition of a "herbal remedy" needs to be expanded. Chinese herbal remedies may contain not only botanical but also mineral and animal-derived ingredients. Therefore, in evaluating and identifying herbal remedies, consideration must be given to the likelihood of ingredients from a nonbotanical source.

Second, we must remember that many of the most effective pharmaceutical agents used today are based on products initially prescribed as herbal remedies. Many Chinese herbs continue to be commonly prescribed that exhibit potent pharmacologic activities and that may become deadly if not processed, prescribed, dispensed, or used properly.[19,20] Access to these products needs to be limited to professionals who possess the knowledge required to properly identify and use them. Although many western-trained herbalists have a broad-based knowledge of traditional Chinese medicine, dispensers in local herb stores often have not received formal training or apprenticeship and may have insufficient knowledge to dispense such potent pharmacologically active herbal remedies. Both China and Taiwan have laws regulating the use of the most toxic herbs, and proposed regulations on potent herbs used in Chinese medicine are being discussed in Hong Kong.[21] In the United States, not only should herbs be subject to the US standards of approval, but there is a growing need for uniform standards of practice in the herbal industry, to ensure that the herbal products being dispensed are of the highest quality and properly processed and to guard against errors during their dispensing.

Third, the availability and use of Chinese herbs are increasing in the United States and are no longer limited to Asians. A recent study concerning the prevalence of unconventional therapies found that 1% of the general population in the United States has used herbal medicines.[22] Of those who also saw a physician, almost three quarters did not inform the physician of their additional alternative therapies. Even with knowledge of their patients' use of herbal remedies, most traditionally trained US physicians are unfamiliar with herbal properties and toxicities. Physicians and other health care professionals, especially those in geographic regions with ethnically diverse populations, need to become more sensitive to patients' use of alternative therapies. When those therapies involve herbal products, they need to familiarize themselves with their pharmacologic actions, interactions, and toxicities, just as physicians would with any other prescription medication. The use of the digoxin fluorescence immunoassay and digoxin-specific Fab fragments is an example of where knowledge may provide a rough toxicologic screen or suggest possible treatment modalities.

The rapidly growing herbal industry in the United States is substantially less regulated than its western medicine equivalent. Without uniform standards of practice and licensing of herbal practitioners and dispensers, consumers of herbal remedies have no assurance that products they receive are safe. The current public protest against government regulation needs to be examined critically in light of the serious public health ramifications, exemplified by this case, that may occur with the use of unregulated products or unlicensed practitioners.

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REFERENCES

1. Kwan T, Paiusco AD, Kohl L: Digitalis toxicity caused by toad venom. Chest 1992; 102:949-950

2. Lin CS, Lin MC, Chen KS, et al: A digoxin-like immunoreactive substance and atrioventricular block induced by a Chinese medicine 'kyushin.' Jpn Circ J 1989; 53:1077-1080

3. Chern MS, Ray CY, Wu D: Biologic intoxication due to digitalis-like substance after ingestion of cooked toad soup. Am J Cardiol 1991; 67:443-444

4. Hitt M, Ettinger DD: Toad toxicity. N Engl J Med 1986; 314:1517-1518

5. Licht LE: Death following possible ingestion of toad eggs. Toxicon 1967; 5:141-142

6. Cheung K, Hinds JA, Duffy P: Detection of poisoning by plant-origin cardiac glycoside with the Abbott TDx Analyzer. Clin Chem 1989; 35(pt 2):295-297

7. Brown P, Kamano Y, Pettit GR: High-resolution mass spectrometry—Bufadienolides I. Organic Mass Spectrom 1972; 6:47-74

8. Hsu HY, Chen YP, Shen SJ, Hsu CS, Chen CC, Chang HC: Oriental Materia Medica—A Concise Guide, 1st edition. Long Beach, Calif, Oriental Healing Arts Institute, 1986, pp 770-772

9. Meyer K: Cardiotoxic steroids from toads. Memorial Inst Butanian 1966; 33:433-440

10. Bedford PGC: Toad venom toxicity and its clinical occurrence in small animals in the United Kingdom. Vet Rec 1974; 94:613-614

11. Chen KK, Kovaříková A: Pharmacology and toxicology of toad venom. J Pharm Sci 1967; 56:1535-1541

12. Mack RB: Kiss me and the world is mine. N C Med J 1990; 51:375-377

13. Eliades D, Swindall B, Johnston J, Pamnani M, Haddy F: Hemodynamic effects of bufalin in the anesthetized dog. Hypertension 1989; 13(pt 2):690-695

14. Palumbo NE, Perri S, Read G: Experimental induction and treatment of toad poisoning in the dog. J Am Vet Med Assoc 1975; 167:1000-1005

15. Lin CS, Lin MC, Chen KS, Liu CB: Effect of a Chinese medicine 'kyushin' on serum digoxin concentration measurement in dogs. Jpn Circ J 1989; 53:108-112

16. Valdes R: Endogenous digoxin-like immunoreactive factors: Impact on digoxin measurements and potential physiological implications. Clin Chem 1985; 31:1525-1532

17. Smith TW, Butler VP, Haber E, et al: Treatment of life-threatening digitalis intoxication with digoxin-specific Fab antibody fragments. N Engl J Med 1982; 307:1357-1362

18. Cambridge D, Morgan CR, Allen G: Digoxin and digoxin derivative induced arrhythmias: In vitro binding and in vivo abolition of arrhythmias by digoxin immune Fab (DIGIBAND). Cardiovasc Res 1992; 26:906-911

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

20. Chan TYK, Chan JCN, Tomlinson B, Critchley JAJH: Chinese herbal medicines revisited: A Hong Kong perspective. Lancet 1993; 342:1532-1534

21. Working Party on Chinese Medicine—Interim Report. Hong Kong, Government Press, 1992

22. Eisenberg DM, Kessler RC, Foster C, Norlock FE, Calkins DR, Delbanco TL: Unconventional medicine in the United States—Prevalence, costs, and patterns of use. N Engl J Med 1993; 328:246-252