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A case of chemotherapy-induced congestive heart failure successfully treated with Chinese herbal medicine

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Available online 12 January 2015

KEYWORDS

Traditional Chinese medicine;
Chemotherapy;
Heart failure;
Zhi Gan Cao Tang

Summary

Objective: A case is presented to illustrate a potential effect of Chinese herbal medicine (CHM) formulas in treating chemotherapy-induced cardiotoxicity.

Clinical presentation: An 18-year-old adolescent male with refractory acute lymphoblastic leukemia (ALL) had experienced anthracycline-induced congestive heart failure (CHF) for 3 weeks. Under intensive care with conventional therapy, the patient still had exercise intolerance and depended on supplemental oxygen all day. Therefore, he consented to treatment with traditional Chinese medicine (TCM) for alternative therapy.

Interventions and outcomes: This patient was treated with modified Zhi Gan Cao Tang (ZGCT), three times a day for 2 months. After 6 days of CHM treatment, the patient could tolerate daily activity without supplemental oxygen. After 2 months of CHM treatment, the follow-up chest X-ray showed great improvements in pulmonary edema and cardiomegaly.

Conclusions: In this case, anthracycline-induced cardiotoxicity resolved slowly following the administration of modified ZGCT. It is suggested that the CHM formula has a protective effect on the progression of CHF secondary to the use of anthracyclines in pediatric cancer. Further studies to determine the mechanism and clinical trials are warranted.

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Introduction

Several antineoplastic drugs in clinical use are known to induce cardiac side effects. These effects encompass a wide variety of clinic-pathologic syndromes with minor to severe clinical consequences.¹ Anthracycline is emerging as an active treatment against acute lymphoblastic leukemia (ALL). The antitumor activity of this agent is based on

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interactions with nuclear components, especially DNA and type II topoisomerases; however, the drug may also inhibit DNA and RNA synthesis.² Clinical studies have demonstrated that high doses of anthracyclines can prolong the 5-year event-free survival rate in patients with high-risk ALL,³ but the dose-limiting cardiotoxic effects make the disease prognosis poor and can be fatal.

Each one of these anthracyclines has been implicated in the development of cardiac toxicity leading to cardiomyopathy and congestive heart failure. The side effects may be caused by the activation of apoptotic mechanisms of cardiomyocytes during therapy, resulting in reduced LV systolic performance.⁴ Doxorubicin, a daunorubicin analog, has been reported to produce acute and self-limiting cardiomyopathy within 2–3 days of its administration. In a few cases, it has been connected to life-threatening cardiogenic complications at cumulative doses of 500–550 mg/m². Other risk factors for anthracycline-induced cardiotoxicity include high dose intensity, younger and older age at diagnosis, radiotherapy, combination therapy with other cardiotoxic chemotherapy, and longer length of follow-up.^{5,6} Concurrent therapies that protect against anthracycline-induced cardiomyopathy, such as probucol, carvedilol, dextrazoxane, and antioxidant nutrients, have been proposed.⁷ However, no effective and clinically applicable preventive treatment has yet been widely adopted.

Herein we report a case of severe cardiac toxicity in conjunction with the administration of doxorubicin plus FLAG regimes (fludarabine, cytarabine, and granulocyte colony-stimulating factor (G-CSF) support), successfully treated with Chinese herbal medicine (CHM) over a 2-month follow-up period.

Case report

An 18-year-old adolescent with relapsed ALL was admitted to the pediatric intensive care unit (PICU) with a 5-day history of severe dyspnea during 21 days of administration of the last dose with doxorubicin plus FLAG regimes. He had been diagnosed 4 years earlier as T-cell ALL-L1 morphology in the very high risk (VHR) classification, according to the protocol of the Taiwan Pediatric Oncology Group (TPOG)-ALL-2002, and was given complete chemotherapy.⁸ One year later, he relapsed with pancytopenia and increased blast cells in the peripheral blood smear examination, and received induction, consolidation, first maintenance, and subsequent maintenance therapy according to the Memorial Sloan-Kettering-New York 2 (MSK-NY 2) protocol,⁹ which resulted in partial response of the disease. He had previous progression 4 months prior to this admission, at which time he was offered one cycle of chemotherapy with doxorubicin plus FLAG regimens for refractory ALL from August 25 to 30, 2012.¹⁰ Treatment consisted of 50 mg/m² i.v. doxorubicin on days 1, 3, and 5, plus fludarabine 30 mg/m² and cytarabine 2000 mg/m² on days 1–5. Antiallergic medication was given with each cytarabine administration, which consisted of methylprednisolone 40 mg administered once a day from day –1 to day 5. During the interim cycle, he developed a grade-4 granulocytopenia. Treatment continued with a

20% dose reduction of fludarabine on day 2, and addition of G-CSF from day 2 to day 5. The patient received one complete cycle of treatment, but complained of delicate symptoms such as cough, slight chest discomfort, and a low grade fever the third week after this chemotherapy course.

On September 21, he started complaining of a dry debilitating cough and dyspnea that gradually got worse. He was markedly hypoxic, with resting arterial blood gases at room air, pO₂ 39.8 mm Hg, pCO₂ 66.1 mm Hg, pH 7.36, bicarbonate 36.5 mmol/L, and oxygen saturation 64%. A chest radiograph compared to pre-chemotherapy therapy revealed diffuse alveolar opacities with perihilar consolidations, pleural fluid, and an enlarged cardiac silhouette. An electrocardiogram revealed sinus tachycardia with rare ventricular premature depolarizations, and portable echocardiography detected left ventricular dysfunction with ejection fraction (EF) <40%. He was briefly treated on supplemental oxygen via bi-level positive airway pressure (Bi-PAP), diuretic infusion, vasodilators, and inotropes with an initial clinical diagnosis of congestive heart failure (CHF). Empiric antibiotics were also prescribed for possible common or opportunistic infections. Due to the symptoms and chest plain film persisting, he consented and began to treatment with traditional Chinese medicine (TCM) on October 12, 2012.

On TCM observation, he was very fragile, perspiring, and dyspneic at rest. His temperature was 36.1 °C, arterial blood pressure 128/69 mm Hg, respiratory rate 40/min, and pulse rate 110/min. He had widespread inspiratory crackles on auscultation and signs of heart failure such as raised venous pressure or peripheral edema. The oxygen saturation was 96% under supplemental oxygen 30% via a Venturi face-mask. His hemoglobin was 8.5 g/dL, WBC 4 × 10³/μL (88% neutrophils), and platelets 4 × 10³/μL. Biochemical analyses showed AST and ALT were twice the upper limits and revealed an increase in total bilirubin. The patient had burnt black and chapped skin and lips. Tongue diagnosis revealed a rag texture, purple-redness, dryness, and peeled-like coating. A TCM diagnosis of a deficiency of Heart-yin and -yang, accompanied by Heat entering nutrient-blood, were concluded. The prescription of TCM herbal formulas included ingredients (Table 1) administered daily in three separate doses. The herbal formulas used were all prepared and extracted using standard procedures of the Taiwan Good Manufacturing Practice (GMP) by the certified company Sun Ten Pharmaceutical Co., Ltd. (Taiwan). A rapid response to the TCM treatment allowed the removal of supplemental oxygen for hours. Clinical improvement was evident after 6 days of treatment; after 12 days of treatment, he needed no oxygen at rest and diuretics could be tapered off. His repeated chest plain film study on October 20, 2012, disclosed a slowly resolving pulmonary venous congestion. Upon discharge, after 18 days of TCM treatment, he was in good clinical condition with oxygen saturation 98% via pulse oximetry.

The patient returned to TCM clinics every two weeks for follow-up and received continuous CHM treatment until December 2012. His subsequent chest plain film studies indicated no sign of recurrence of pulmonary edema and cardiomegaly. The EF has risen to 55% that indicated previous cardiac damage. The series of chest X-ray, clinical

Table 1 Ingredient and function of traditional Chinese herbal formulas.

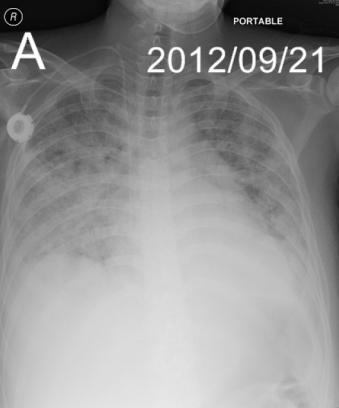
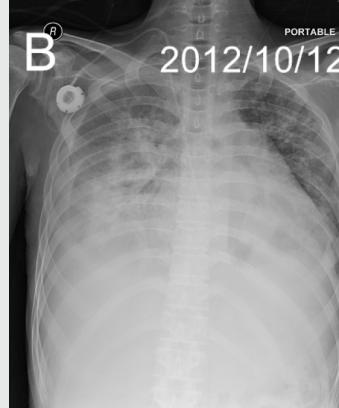
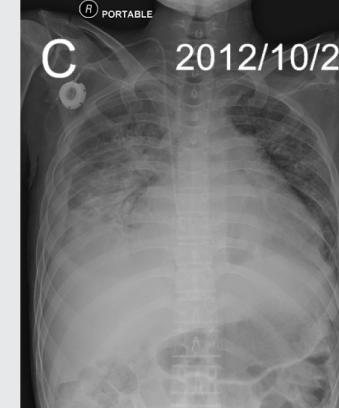
Chinese name (daily dosage)	Composition or Latin name	Function	Indication
Zhi Gan Cao Tang (7.5 g)	Zhi Gan Cao (<i>Radix Glycyrrhizae preparata</i>), Ren Shen (<i>Radix Ginseng</i>), Gui Zhi (<i>Ramulus Cinnamomi</i>), E Jiao (<i>Colla Corii Asini</i>), Sheng Di Huang (<i>Radix Rehmanniae</i>), Mai Men Dong (<i>Radix Ophiopogonis</i>), Huo Ma Ren (<i>Fructus Cannabis</i>), Sheng Jiang (<i>Rhizoma Zingiberis recens</i>), Da Zao (<i>Fructus Jujubae</i>)	<ul style="list-style-type: none"> • Nourish heart yin and yang • Tonify heart qi and blood • Strengthen the heart <p>Normalize the pulse</p>	Qi and blood deficiency, lung and heart deficiency, shortness of breath, dry cough, insomnia, restlessness, wasting, spontaneous sweating, palpitations, dry skin, lingering fever in palms and soles, fatigue, dry mouth, thirst, dry stools, knotted/irregular or rapid pulse.
Bie Jia (1.8 g)	<i>Carapax Trionycis</i>	<ul style="list-style-type: none"> • Nourish yin • Clear heat • Disperse blood • Soften hard lumps <p>Disperse accumulations</p>	Yin deficiency heat, fever, low-grade fever from yin deficiency, bone-steaming fever, chronic malaria, sub-costal hardness, lumbago, amenorrhea, dysmenorrhea, mass in the abdomen, internal liver wind, tremors, convulsions in children.
Gui Ban (1.8 g)	<i>Plastrum Testudinis</i>	<ul style="list-style-type: none"> • Nourish yin • Settle yang • Strengthen tendons and muscles • Cool blood <p>Supplement blood</p>	Deficiency of kidney yin, bone-steaming fever, night sweating, chronic cough, dry throat and mouth, involuntary emission, uterine bleeding, morbid leukorrhea, flaccidity and debility of loins and knees, chronic dysentery, chronic malaria.
Mu Li (1.8 g)	<i>Concha Ostreae</i>	<ul style="list-style-type: none"> • Tranquilize spirit • Subdue exuberance of liver yang • Astringe fluids • Nourish yin • Soften hardness • Disperse accumulations • Antacid action <p>Control pain</p>	Internal heat due to yin deficiency, deficiency fatigue, steaming bone syndrome, fever with irritability and fidgets, insomnia, palpitation, anxiety, abnormal daytime/nighttime sweating, epilepsy, involuntary emission, functional bleeding, morbid leukorrhea, scrofula, subcutaneous nodule.
Bai Shao (0.9 g)	<i>Radix Paeoniae alba</i>	<ul style="list-style-type: none"> • Tonify blood • Astringe yin • Control pain <p>Alleviate sudden onset of disease</p>	Blood deficiency, chest, abdominal, flank and costal pain, muscle pain and tightness, spasm of the limbs, diarrhea, dysentery, fever due to yin-deficiency, menstrual disorders, painful menstruation, functional bleeding, leukorrhea, spermatorrhea, dry skin.

presentation and relevant data before and after TCM treatment are summarized on Table 2. He remained in good condition during this 2-month treatment period, but he died unexpectedly 4 months after the reported event due to *Klebsiella pneumoniae* septicemia.

Discussion

In Taiwan, combination of conventional treatment with TCM for treating chemotherapy-induced cardiotoxicity is rare. We present here the case of a patient who was hospitalized

Table 2 The series of chest plain films, clinical manifestations, laboratory data, and related western treatment before and after TCM treatment.

Date	Sep 21. 2012	Oct 12. 2012	Oct 20. 2012	Dec 03. 2012
Chest X-ray				
The weeks after chemotherapy	3	6	7	12
TCM intervention (days)	—	+ (0)	+ (9)	+ (53)
Clinical manifestation	Dyspnea, orthopnea, oliguria, anxiety, pink and frothy sputum	Dyspnea, orthopnea, peripheral edema, fatigue, cold limbs, tremulousness	Mild dyspnea, tachypnea, dizziness, abdominal distension	Orthostatic intolerance, gum bleeding
NYHA	IV	IV	IV	III
Laboratory data	•WBC (seg/lym/blast) 1.6 K/ μ L (89/4.0/0%) •HB 8.9 g/dL •Plt 9 K/ μ L •Cr 0.22 mg/dL	•WBC (seg/lym/blast) 4.0 K/ μ L (88/1.0/0%) •HB 8.5 g/dL •Plt 4K/ μ L •Cr 0.14 mg/dL	•WBC (seg/lym/blast) 3.6 K/ μ L (89/6.0/0%) •HB 9.3 g/dL •Plt 32 K/ μ L •Cr 0.12 mg/dL	•WBC (seg/lym/blast) 20.2 K/ μ L (9.0/17/66%) •HB 10.4 g/dL •Plt 4 K/ μ L •Cr 0.71 mg/dL
Cardiac echo	EF < 40%	—	—	EF = 55%
Oxygen delivery method	BiPAP (FiO ₂ 100%)	O ₂ V-M (FiO ₂ 30%)	O ₂ nasal cannula (3 L/min)	Room air
Pediatric medication	•Inotropic agent (dobutamine IVF run 10 mcg/kg/min, and digoxin 0.125 mg PO qd) •Diuretics (bumetanide 0.5 mg IV q4 h) •Rh-g-csf 300 mcg IVF qd •Steroid (methylprednisolone 55 mg IV q6 h) •Antibiotics (meropenem, teicoplanin, and voriconazole)	•Inotropic agent (dobutamine IVF run 6.4 mcg/kg/min) •Diuretics (furosemide 20 mg IV qd) •Steroid (methylprednisolone 10 mg IV q12 h) •Antibiotics (cefuroxime)	•Steroid (presnisolone) 10 mg PO qd) •Antibiotics (cotrimoxazole)	•Antibiotics (cotrimoxazole)

BiPAP, Bi-level positive airway pressure; EF, ejection fraction; IV, intravenous; IVF, intravenous fusion; NYHA, The New York Heart Association; PO, by mouth; TCM, traditional Chinese medicine; V-M, venturi mask.

in PICU for cardiotoxic adverse effects while on anthracycline for refractory ALL. From September 21 to October 20 of 2012, he was treated conservatively with diuretics and developed long-term dependence on supplemental oxygen. Because there was no spontaneous improvement of clinical symptoms or chest radiography, he chose complementary CHM treatment.

When a diagnosis of cardiotoxicity is strongly suspected, confirming the etiology as anthracycline-induced in nature may be challenging, short of exclusion, given the rarity of the disease and its clinical presentation. Regarding the agents used in this case, doxorubicin is known to cause cardiotoxicity related to myocardial damage in as many as 10–26% of cancer patients.⁵ Studies have shown that after completion of anthracycline therapy, 65% of survivors of childhood leukemia develop progressive cardiotoxicity after 6 years.^{11,12} The pathophysiology of cardiotoxicity in long-term survivors is characterized by reduced left ventricular wall thickness and mass, which is indicative of decreased cardiac muscle and depressed left ventricular contractility, itself indicative of unhealthy heart muscle. The occurrence of anthracycline-induced cardiotoxicity is progressive from the first dose, but in some individuals rapid deterioration in cardiac function can be found even at low doses.¹³ The adverse effect, which is directly related to the cumulative (total) dose of anthracyclines received, is irreversible. Once CHF has occurred after anthracycline administration, the mortality rate can exceed 50% within 2 years of treatment cessation.¹⁴ In our patient, chemotherapy-induced CHF was characterized by a cumulative anthracycline dose of 563 mg/m² over the previous 4 years in the absence of any known cause of cardiomyopathy and with evidence of multi-organ involvement directly attributable to the ALL or otherwise unexplained in the clinical setting.

Zhi Gan Cao Tang (ZGCT) is an old CHM formula consisting of nine raw herbal components: Radix Glycyrrhizae, Radix Ginseng, Fructus Jujubae, Radix Rehmanniæ, Radix Ophiopogonis, Colla Corii Asini, Fructus Cannalis, Ramulus Cinnamomi, and Rhizoma Zingiberis Recens. This formula has long been used for treatment of the Heart-yin and -yang deficiency due to consumption of qi and yin by Heat-evil. It is especially prescribed for patients with cardiogenic arrhythmia, which caused by rheumatic heart disease, pericarditis, and ischemic heart disease.^{15,16} Glycyrrhizin and ginsenoside respectively extracted from Radix Glycyrrhizae and Radix Ginseng are the steroid-like compounds, structurally similar to cardiac glycoside such as digoxin and digitoxin.¹⁷ These steroid-like compounds have therapeutic effects on treating heart failure via the same molecular mechanism triggered by inhibition of Na⁺/K⁺-ATPase.¹⁷ Besides, Radix Rehmanniæ has a protective effect on adriamycin-induced cytotoxicity in cardiac muscle cells.¹⁸ In a meta-analysis review study, the author suggested that ZGCT plus biomedical treatment might be more beneficial for CHF than biomedical treatment alone [19].

In TCM theory, the clinic presentations of acute leukemia, such as fever, bleeding, anemia, hepatomegaly, splenomegaly, or lymphadenopathy, are related to the process of virtual essence with Heat-evil entering nutrient-blood. When it comes to the refractory stage of leukemia, the Heat-evil will burn out the yin-blood of the liver and kidney, further unbalancing the yin-yang.²⁰ We believe

repeated and external Toxin-evil (i.e., anthracycline) may increase the oxidative stress of cardiomyocytes; in other words, they may aggravate the burden of Heart-yang, giving purge retention of blood-stasis and fluid from the Lung. At that time, Bai Shao, Mu Li, Bie Jia, and Gui Ban can be sequentially added on the basis of ZGCT to help recuperate yang, clean heat and nourish yin, and purge retention of fluid from the Heart and Lung. Application of modified ZGCT in treating chemotherapy-induced CHF in patients with refractory ALL could elevate the clinical efficacy, which is of great value in clinical practice.

A limited amount of data has indicated that TCM herbs could relieve anthracycline-induced cardiotoxicity. Choi et al. showed that licorice extracts had a protective effect against doxorubicin-induced cardiac apoptosis in vitro.²¹ The therapeutic effects on anthracycline-induced CHF were likely related to the major ingredient of ZGCT, Radix Glycyrrhizae preparata. After 2 months of treatment with modified ZGCT formulas, the patient's exertional dyspnea and fragility subsided, consistent with the regression of pulmonary edema. This experience suggested that TCM could be used to treat the progression of congestive heart failure secondary to chemotherapy; in this case, strategies to prevent or reduce doxorubicin cardiotoxicity were not manipulated.

Conclusion

A patient diagnosed with CHF secondary to anthracycline-induced cardiotoxicity was successfully treated with CHM herbal formulas alone for 2 months. Although the mechanism of CHM has not yet been identified in this case, the authors believe that modified ZGCT exerted its function on Heart-yang tonification over heart failure, as well as on yin-blood supplement and Heat-evil cleaning from the course of ALL. CHM herbal formulas could play an alternative role in preventing the progression of anthracycline-induced cardiotoxicity in cases of poor response to conservative treatment. Further studies to determine the mechanism and clinical trials are warranted.

Conflict of interest statement

No financial conflicts exist.

References

1. Simbre VC, Duffy SA, Dadlani GH, Miller TL, Lipshultz SE. Cardiotoxicity of cancer chemotherapy: implications for children. *Paediatr Drugs* 2005;7:187–202.
2. Rabban A, Finn RM, Ausio J. The anthracycline antibiotics: antitumor drugs that alter chromatin structure. *Bioessays* 2005;27:50–6.
3. Silverman LB, Gelber RD, Dalton VK, Asselin BL, Barr RD, Clavell LA, et al. Improved outcome for children with acute lymphoblastic leukemia: results of Dana-Farber Consortium Protocol 91-01. *Blood* 2001;97:1211–8.
4. Lipshultz SE. Exposure to anthracyclines during childhood causes cardiac injury. *Semin Oncol* 2006;33:S8–14.
5. Swain SM, Whaley FS, Ewer MS. Congestive heart failure in patients treated with doxorubicin: a retrospective analysis of three trials. *Cancer* 2003;97:2869–79.

6. Wouters KA, Kremer LC, Miller TL, Herman EH, Lipshultz SE, et al. Protecting against anthracycline-induced myocardial damage: a review of the most promising strategies. *Br J Haematol* 2005;131:561–78.
7. Simpson C, Herr H, Courville KA. Concurrent therapies that protect against doxorubicin-induced cardiomyopathy. *Clin J Oncol Nurs* 2004;8:497–501.
8. Liang DC, Yang CP, Lin DT, Hung IJ, Lin KH, Chen JS, et al. Long-term results of Taiwan Pediatric Oncology Group studies 1997 and 2002 for childhood acute lymphoblastic leukemia. *Leukemia* 2010;24:397–405.
9. Steinherz PG, Redner A, Steinherz L, Meyers P, Tan C, Heller G. Development of a new intensive therapy for acute lymphoblastic leukemia in children at increased risk of early relapse. The Memorial Sloan-Kettering-New York-II protocol. *Cancer* 1993;72:3120–30.
10. Quarello P, Berger M, Rivetti E, Galletto C, Masetti R, Manicone R, et al. FLAG-liposomal doxorubicin (Myocet) regimen for refractory or relapsed acute leukemia pediatric patients. *J Pediatr Hematol Oncol* 2012;34:208–16.
11. Lipshultz SE, Colan SD, Gelber RD, Perez-Atayde AR, Sallan SE, Sanders SP. Late cardiac effects of doxorubicin therapy for acute lymphoblastic leukemia in childhood. *N Engl J Med* 1991;324:808–15.
12. Lipshultz SE, Lipsitz SR, Sallan SE, Dalton VM, Mone SM, Gelber RD, et al. Chronic progressive cardiac dysfunction years after doxorubicin therapy for childhood acute lymphoblastic leukemia. *J Clin Oncol* 2005;23:2629–36.
13. Chatterjee K, Zhang J, Honbo N, Karliner JS. Doxorubicin cardiomyopathy. *Cardiology* 2010;115:155–62.
14. Jensen BV, Skovsgaard T, Nielsen SL. Functional monitoring of anthracycline cardiotoxicity: a prospective, blinded, long-term observational study of outcome in 120 patients. *Ann Oncol* 2002;13:699–709.
15. Chen WG, Ba ZM. Prof. ZHANG Yi's experience in treating severe arrhythmia. *J Tradit Chin Med* 2010;30:47–50.
16. Tang XL, Bao LF. Clinical application and research situation of Zhi Gan Cao Decoction (Decoction consists of *Glycyrrhiza uralensis* stirring frying with liquids). *J Tradit Chin Med Lit* 2004;2:47–51 (in Chinese).
17. Chen RJ, Jinn TR, Chen YC, Chung TY, Yang WH, Tzen JT. Active ingredients in Chinese medicines promoting blood circulation as Na+/K⁺-ATPase inhibitors. *Acta Pharmacol Sin* 2011;32:141–51.
18. Chae HJ, Kim HR, Kim DS, Woo ER, Cho YG, Chae SW. Saeng-Ji-Hwang has a protective effect on adriamycin-induced cytotoxicity in cardiac muscle cells. *Life Sci* 2005;76:2027–42.
19. Bai H, Li Y, Han K, Gong M, Ma A. Effectiveness of Chinese herbal medicine as an adjunctive treatment for dilated cardiomyopathy in patients with heart failure. *J Altern Complement Med* 2013;19:811–9.
20. Huang LM, Qiu HM. Thoughts on the treatment of acute leukemia fever by Chinese medicine. *Shanghai J Tradit Chin Med* 2003;37:11–3 (in Chinese).
21. Choi HJ, Seon MR, Lim SS, Kim JS, Chun HS, Park JH. Hexane/ethanol extract of *Glycyrrhiza uralensis* licorice suppresses doxorubicin-induced apoptosis in H9c2 rat cardiac myoblasts. *Exp Biol Med (Maywood)* 2008;233:1554–60.

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