OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2017.

Research Article
Effect of Danggui Buxue Tang on Erythropoiesis in Acute Chemotherapy Injured Mice

[1]Junhua He, [2]Zhaozhi Zeng, [3]Jun Chen, [1]Jinquan Wang, [1]Qizhu Chen and [1]Huaben Bo

[1]School of Bioscience and Biopharmaceutics, Guangdong Province Key Laboratory for Biotechnology Drug Candidates, Guangdong Pharmaceutical University, 510006 Guangzhou, Guangdong, China
[2]Medical Laboratory Animal Center, Guangdong Pharmaceutical University, 510006 Guangzhou, Guangdong, China
[3]College of Pharmacy, Guangdong Pharmaceutical University, 510006 Guangzhou, Guangdong, China

Abstract

Background and Objective: Hypocythemia is frequently developed in hematological and cancer patients. However its current therapies may induce the transmission of both viral and bacterial infection and lead to autoimmune pure red cell aplasia. The Chinese herbs exert their pharmacological effects through a multi-component and multi-target way coupled with its minimal side-effects. Danggui Buxue Tang (DBT) has been widely used for menopausal women as dietary supplements in China. This study aimed to evaluate the quality of Danggui Buxue Tang on the whole and the pharmacological effect from different levels. **Materials and Methods:** An HPLC method was established to develop chromatographic fingerprints of Danggui Buxue Tang from different areas. The fingerprints were analyzed with similarity evaluation system for chromatographic fingerprint of TCM. Comparison of peripheral blood count in different groups using HS-18 type fully automated hematology analyzer. Semi-solid medium was used to detect the number of erythroid progenitor cells. Erythroid cells numbers were detected by flow cytometry. The one-way analysis of variance (ANOVA) was used to determine statistical significance. **Results:** The experimental results showed that the fingerprints of the 6 samples of Danggui Buxue Tang had high similarity. So the mutual mode fingerprint for Danggui Buxue Tang was established and used for Danggui Buxue Tang quality control. However, only 17 common peaks in HPLC chromatogram were observed in 6 samples and the relative peak areas of common peaks were obviously different in different samples. Pharmacodynamic experiment showed that Danggui Buxue Tang could increase the number of peripheral reticulocyte, but had no effect on the number of peripheral red blood cells. Danggui Buxue Tang could increase the number of burst-forming unit-erythroid, colony forming unit-erythroid and erythroid cells in bone marrow. **Conclusion:** The fingerprint technique can be used for the quality evaluation and control of DBT. Danggui Buxue Tang can promote erythropoiesis in acute chemotherapy injured mice.

Key words: Fingerprint, chemotherapy, traditional Chinese medicine, erythropoiesis, erythroid cell, quality control

Received: Accepted: Published:

Citation: Junhua He, Zhaozhi Zeng, Jun Chen, Jinquan Wang, Qizhu Chen and Huaben Bo , 2017. Effect of danggui buxue tang on erythropoiesis in acute chemotherapy injured mice. Int. J. Pharmacol., CC: CC-CC.

Corresponding Author: Huaben Bo, School of Bioscience and Biopharmaceutics, Guangdong Province Key Laboratory for Biotechnology Drug Candidates, Guangdong Pharmaceutical University, 510006 Guangzhou, Guangdong, China Tel: 86-20-39352199 Fax: 86-20-39352201

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Erythrocytes are the most common type of blood cell. They can take up oxygen in the lungs or gills and release it into tissues, also have immune function[1]. So erythrocytes are often referred to as "red guard". Hypocythemia is frequently developed in hematological and cancer patients who undergo bone marrow suppression resulting from chemotherapy or radiotherapy[2]. In severe cases, erythrocyte transfusion may be required to increase the number of erythrocytes. However, erythrocyte transfusion may induce the transmission of both viral and bacterial infection[3]. In clinical practice, erythropoietin (EPO) can also stimulate production of erythrocytes. But EPO may potentially lead to autoimmune pure red cell aplasia[4]. In view of these clinical risks, extensive efforts have been devoted to develop new therapeutic strategies that would promote erythropoiesis but with minimal side-effects. Recently, traditional Chinese herbs have captured the world's attention due to its high effectiveness against many diseases coupled with its minimal side-effects[5-7].

In traditional Chinese herbs treatment, Chinese herbal prescriptions (comprise several crude drugs) play an important role in clinical applications. Danggui Buxue Tang (DBT) is one famous traditional Chinese prescription consisting of two crude herbs, including Radix Astragali (Huangqi; RA) and Radix Angelicae Sinensis (Danggui; RAS) in a ratio of 5:1. This prescription has been widely used for menopausal women as dietary supplements in China[8,9].

Pharmacological results indicated that DBT has the abilities to stimulate the immune system[10,11], to promote hematopoietic functions[12], to stimulate cardiovascular circulation, to prevent osteoporosis[13], to increase anti-oxidation activity[14,15], to improve chronic fatigue syndrome[16] and to prevent and treat Diabetic Nephropathy (DN)[17]. However, a direct study of the effect of DBT on erythropoiesis has not been performed. In current study, the effects of DBT on erythropoiesis will be evaluated using a mouse model.

The Chinese herbs exert their pharmacological effects through a multi-component and multi-target way[18-20]. So it is difficult to clarify the material basis of Chinese herbs. More important active compounds are missing in controlling the quality of the herbal decoction, which consequently hinders the development of DBT as disease and disorder remedies. Fingerprint of Chinese herbs can reflect comprehensively the category and content of chemical composition of Chinese herbs and then reflect the quality of Chinese herbs. On the premise that the material basis of Chinese herbs is not clear, the quality of Chinese herbs will be controlled more effectively and more completely by chromatographic fingerprint. The chromatographic fingerprint technique had already been extensively used for the quality evaluation and control on the whole[21,22]. A quality-assured drug is the prerequisite for accurate experiment. The objective of study was to control the quality of DBT using the fingerprint of DBT by HPLC.

MATERIALS AND METHODS

Plant materials and chemical reagents: Radix Astragali (RA) was obtained from Gansu Province and Inner Mongolia Province of China; Radix Angelicae Sinensis (RAS) was obtained from Gansu Province, Shanxi Province and Yunnan Province of China. The authentication of plant materials was performed morphological by one of the authors Dr. Chen during the field collection. Parts of the samples are currently deposited in the School of Bioscience and Biopharmaceutics, Guandong pharmaceutical University, Guangzhou, China. AR and HPLC grade reagents were from Thermo Fisher (Geel, Belgium). Iscoves modified Dulbecco medium (IMDM) was from Life. Fetal Bovine Serum (FBS) was form BI (Kibbutz BeitHaemek, Israel). The IL3, SCF, EPO and IL6 were from prospec (Ness Ziona, Israel). Anti-mouse CD16/CD32 and anti-mouse Ter119 APC-eFluor 780 were from eBioscience (San Diego, USA).

Other reagents used were of analytical-reagent grade or with equivalent purity.

Preparation of DBT: The preparation of DBT was based on those reported previously with minor modifications[15]. Briefly, plant materials were ground into a powder and then RA and RAS were accurately weighed according to a ratio of 5:1 and mixed well in a vortex. The mixture was extracted in 8 volume of water by boiling for 2 h. The extraction was filtered and the residue was re-extracted under the same conditions. The pooled extraction was condensed to 1 g mL^{-1} (w/v, crude drugs/water) by rotavapor (Shanghai, China) and stored at -80°C.

HPLC fingerprint of DBT: Preparation of HPLC test sample was based on those reported previously with minor modifications[23]. Briefly, ethanol was added to the extract to a final concentration of 70%. The mixture was centrifuged at 12,000×g for 10 min. The supernatant was collected and dried by rotavapor (yamato RE311, China). The residues were dissolved in 1 mL of methanol and filtered through a Pall 25 mm syringe filter (0.2 μm) for HPLC analysis.

A waters HPLC system consisting of a waters 2695 pump, a 717 auto-sampler, a UV/VIS Photodiode Array 2996 Detector, an Empower program and an Agilent TC-C18 (4.6×150 mm,

5 μm) was used for all analysis. Acetic acid water (0.1%, solvent A) and methanol (Solvent B) were used as mobile phases at a flow rate of 0.5 mL min^{-1} at room temperature. The gradient profile was 5-22% B at 0-7.5 min, 22-26% B at 7.5-17.5 min, 26-45% B at 17.5-42.5 min, 45-65% B at 42.5-62.5 min, 65-95% B at 62.5-67.5 min. A sample of 20 μL was injected for HPLC analysis. The detection wavelength was set at 254 nm.

Animals: A total of 36 adult male KM mice, aged 7-8 weeks, were obtained from the Guangdong Medical Laboratory Animal Center (Guangdong, China). All animals were kept under standard lighting conditions (12 h with alternate day and night cycles) and were given free access to food and water. The animals were treated according to the Guidelines of Animal Care and Use Committee of Guangdong Pharmaceutical University and the study was approved by the local Animal Research Welfare Committee.

After 1 week of adaptation, the mice were randomly divided into six groups: Three DBT-treated groups, EPO-treated group, model group and normal control group. Adriamycin (4 mg kg^{-1} day^{-1}) was given by injection (i.p.) daily for 3 days to establish the hypocythemia model. Normal saline was given by injection (i.p.) in normal control group. DBT (5 g kg^{-1} day^{-1}), DBT (15 g kg^{-1} day^{-1}) and DBT (25 g kg^{-1} day^{-1}) were given by injection (i.g.) for 5 days starting from the day after chemotherapy in three DBT-treated groups. The EPO (200 U kg^{-1} day^{-1}) was given by injection (i.p.) in the EPO-treated group. Normal saline was injected (i.g.) in model group and normal control group.

Determination of peripheral blood cells: Twenty four hours after the last administration, blood from each group was collected from plexus venosus behind the eyeball. White Blood Cells (WBC), Red Blood Cells (RBC) and reticulocyte were analyzed by HS-18 type fully automated hematology analyzer (Italy).

Preparation of bone marrow single cell suspension: Twenty four hours after the last administration, mice were sacrificed with cervical dislocation. Femurs were removed from mouse in a sterile environment and trimmed the ends of the long bones to expose the interior marrow shaft. Bone marrow cells were rinsed with IMDM supplemented with 10% FBS. To make a single cell suspension, gently draw medium and cells up and down with a 3 cc syringe and 21 g needle.

Erythroid progenitor assays: Burst-forming unit-erythroid (BFU-E) was cultured in methylcellulose (1%) supplemented with 15% FBS, 1% BSA, 10 μg mL^{-1} insulin, 200 μg mL^{-1} transferrin, 50 ng mL^{-1} SCF, 10 ng mL^{-1} IL3, 10 ng mL^{-1} IL6 and 3 U mL^{-1} EPO. Colony Forming Unit-Erythroid (CFU-E) were cultured in methylcellulose (1%) supplemented with 15% FBS, 1% BSA, 10 μg mL^{-1} insulin, 200 μg mL^{-1} transferrin and 3 U mL^{-1} EPO. Bone marrow single cells (2×10^5 cells mL^{-1}) were seeded in triplicated and cultured at 37 in 5% CO_2 atmosphere. The CFU-E was counted under inverted microscope (Olympus IX71) after 3 days of culture. The BFU-E was counted after 7 days of culture.

Flow cytometric analysis of erythroid cells: One hundred microliters of bone marrow single cell suspension (1×10^7 cells mL^{-1}) was used for flow cytometric analysis. Bone marrow cells were pre-incubated with 0.5 μg of anti-mouse CD16/CD32 for 20 min on ice prior to staining. Then bone marrow cells were stained with 0.25 μg of anti-mouse TER119 APC-eFluor 780 for 30 min in the dark on ice. As a control, additional bone marrow cell suspensions were stained with mouse isotope control APC-eFluor 780. After washing twice with PBS, the cells were resuspended with flow cytometry staining buffer (pH 7.0). Then the cells were analyzed with flow cytometry (Beckman gallios) and the data were processed using EXPO32 software.

Statistical analysis: The chromatographic fingerprints were analyzed with similarity evaluation system for chromatographic fingerprint of TCM (2004A edition).

Statistical chart was drawn by statistical software Graphpad Prism 5.0. Data was analyzed by statistical software SPSS 18.0 (Chicago, USA). All values were expressed as Mean±SD. The one-way ANOVA was used to determine statistical significance[24]. A value of $p<0.05$ was considered significant.

RESULTS

Fingerprint of DBT: The chromatographic fingerprints of DBT from different areas were analyzed with similarity evaluation system for chromatographic fingerprint of TCM (2004A edition). The result of experiments showed that the fingerprints of the 6 samples of DBT had high similarity (Fig. 1) and the similarity was over 0.933 compared with reference fingerprint. The fingerprint of DBT from Gansu (RA) and Shanxi (RAS) had the lowest similarity (0.933), the fingerprint from Inner Mongolia (RA) and Shanxi (RAS) had the highest similarity (0.996). The mutual mode fingerprint for DBT was established with similarity evaluation system for chromatographic fingerprint of TCM (2004A edition) and used for DBT quality control (Fig. 2).

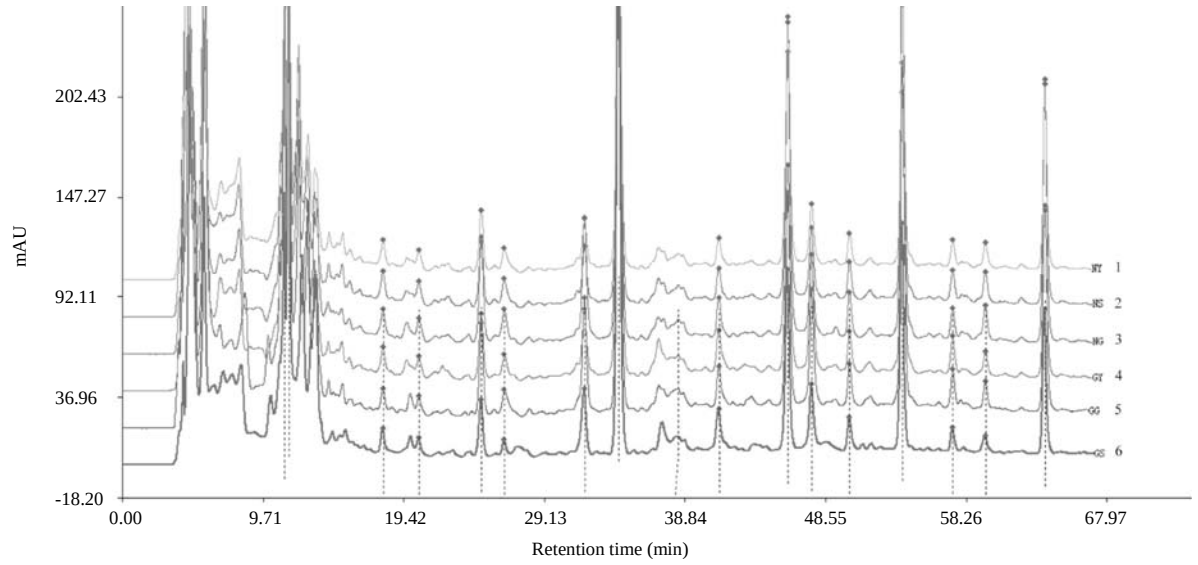

Fig. 1: Fingerprints of DBT from different areas

1: RA was from Inner Mongolia province; RAS was from Yunnan province, 2: RA was from Inner Mongolia province; RAS was from Shanxi province, 3: RA was from Inner Mongolia province; RAS was from Gansu province, 4: RA was from Gansu province; RAS was from Yunnan province, 5: RA was from Gansu province; RAS was from Gansu province and 6: RA was from Gansu province; RAS was from Shanxi province

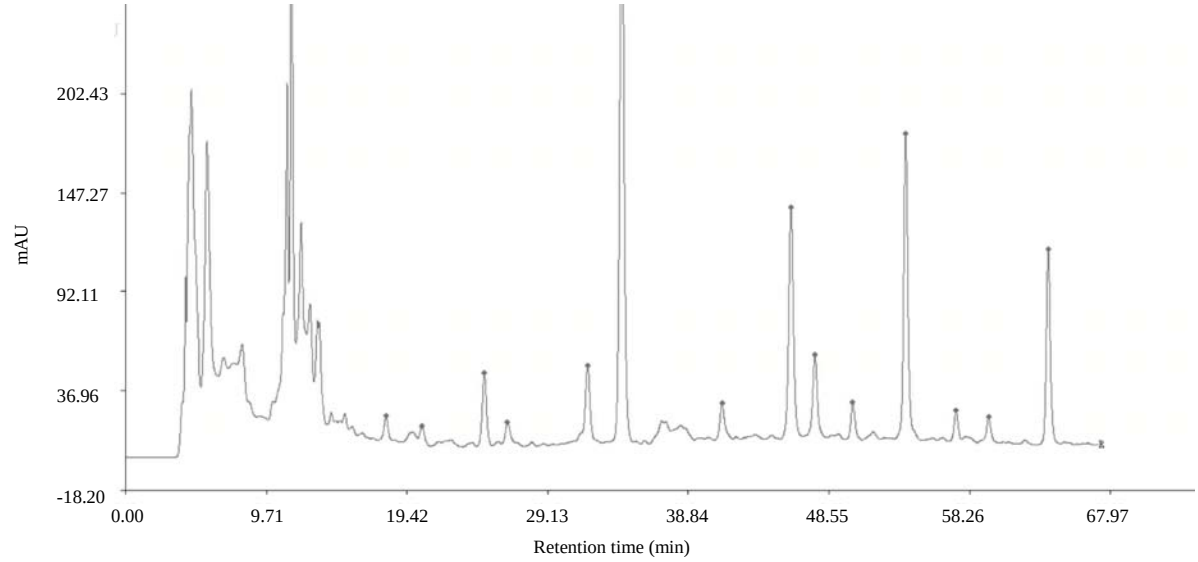

Fig. 2: Mutual mode fingerprints of DBT from different areas

In this study, major common peaks were identified by comparing the retention times of various components in chromatographic fingerprints of DBT from different areas. The result of experiments showed that 17 common peaks in HPLC chromatogram were observed in 6 samples. The relative peak areas of common peaks were obviously different in different samples. The relative peak areas of common peak 7 had the highest relative standard deviation (37.38%), the relative peak areas of common peak 8 had the lowest relative standard deviation (12.63%) (Table 1). These results showed that the fingerprints of the 6 samples of DBT have high similarity on the whole, but most individual chromatographic peaks have great differences.

Effects of DBT on peripheral blood cell counts: Changes of peripheral red blood cells can directly reflect the capacity of

Table 1: Common peaks and peak area of fingerprints of DBT from different areas

No. of peak	Retention time (min)	Peak area (mAU) Origin GS	GG	GY	NG	NS	NY*	Reference fingerprint	RSD (%)
1	11.18	1445.80	3579.26	1947.41	2246.44	2221.16	2498.04	2323.02	30.66
2	11.50	3439.88	5353.03	4212.51	4645.32	4645.00	4106.56	4400.38	14.63
3	18.00	196.39	218.62	256.37	303.47	279.15	260.19	252.37	15.53
4	20.48	171.16	197.56	227.38	252.99	233.41	209.19	215.28	13.45
5	24.78	633.43	1024.15	797.29	1197.56	838.22	737.66	871.38	23.57
6	26.38	158.36	422.86	376.77	520.99	468.89	417.36	394.20	31.87
7	31.91	893.54	1554.21	904.93	1573.12	764.81	683.26	1062.31	37.38
8	34.27	6135.26	7273.29	7872.43	8574.15	7280.94	6351.61	7247.94	12.63
9	38.34	301.06	485.61	227.57	361.20	323.25	277.87	329.43	26.91
10	41.18	613.37	538.51	604.67	458.04	397.79	350.89	493.88	22.09
11	45.95	2174.03	2571.89	2658.99	3580.33	3493.40	3036.46	2919.18	18.91
12	47.59	837.87	1415.86	1487.44	1027.73	876.11	765.40	1068.40	29.00
13	50.21	342.59	424.11	442.01	475.92	415.28	350.63	408.42	12.79
14	53.85	2454.33	3834.60	3383.34	4706.23	3675.59	3156.39	3535.08	21.23
15	57.33	242.07	389.22	416.08	315.79	328.95	288.06	330.03	19.45
16	59.59	207.37	296.59	290.56	436.69	355.77	303.80	315.13	24.22
17	63.69	1504.24	2150.14	1868.84	2821.45	2384.88	2009.86	2123.24	21.25

GS: RA was obtained from Gansu province and RAS was obtained from Shanxi province, GG: RA and RAS were obtained from Gansu province, GY: RA was obtained from Gansu province and RAS was obtained from Yunnan province, NG: RA was obtained from Inner Mongolia province and RAS was obtained from Gansu province, NS: RA was obtained from Inner Mongolia province and RAS was obtained from Shanxi province, NY: RA was obtained from Inner Mongolia province and RAS was obtained from Yunnan province

Table 2: Changes of peripheral blood cells in different groups ($\bar{x}\pm s$)

Groups	Doses	n	RBC (10^{12})	Reticulocyte (10^{9})	WBC (10^{9})
Normal	-	5	10.44±1.05*	371.3±34.8	3.92±0.61*
Myelosuppression model	-	5	9.16±0.28	288.0±34	2.44±0.21
DBT	5 g kg^{-1} day^{-1}	5	8.58±0.51	293.2±35.8	3.80±0.5*
	15 g kg^{-1} day^{-1}	5	9.29±0.28	265.7±57.1	3.12±0.79
	25 g kg^{-1} day^{-1}	5	9.50±0.35	341.4±21.7*	3.06±0.76
EPO	200 U kg^{-1} day^{-1}	5	10.05±0.3*	355.4±33.4*	3.10±1.02

*p<0.05, significantly different compared with the model group

DBT on erythropoiesis. The result of experiments showed that the number of peripheral erythrocytes and reticulocyte of normal group were significantly higher than those in the model group ($p<0.05$), which suggested that the animal models with blood deficiency was successfully established. However, the number of peripheral red blood cells in three dosage group using DBT had no significant difference with model group ($p>0.05$). Further, we detected the peripheral reticulocyte counts. The result of experiments showed that the number of peripheral reticulocyte of the high dose group using DBT were significantly higher than those in the model group ($p<0.05$) (Table 2).

Meanwhile, the numbers of peripheral white blood cells were detected. The result of experiment showed that the number of peripheral white blood cells of the low dose group using DBT were significantly higher than those in the model group ($p<0.05$) (Table 2).

Effect of DBT on erythroid progenitor: Erythrocytes in peripheral blood are derived from erythroid progenitor. To further seek the possible potential target of DBT and demonstrate the effect of DBT on erythropoiesis, erythroid progenitors (CFU-E and BFU-E) in bone marrow were measured with Colony-Forming Cell (CFC) assay.

The result of experiments showed that erythroid progenitors could produce colonies of morphologically recognizable daughter cells in a semisolid matrix supplemented with appropriate growth factors (Fig. 3b, c). The number of BFU-E and CFU-E of the model group were significantly lower than those in the normal group ($p<0.05$) (Fig. 3a), which suggested that the animal model was successfully established. The number of CFU-E of the medium and high dose group using DBT were significantly higher than those in the model group ($p<0.05$), the number of BFU-E of the high dose group using DBT were significantly higher than those in the model group ($p<0.05$) (Fig. 3a). Results showed that BFU-E and CFU-E may be a therapeutic potential target for DBT.

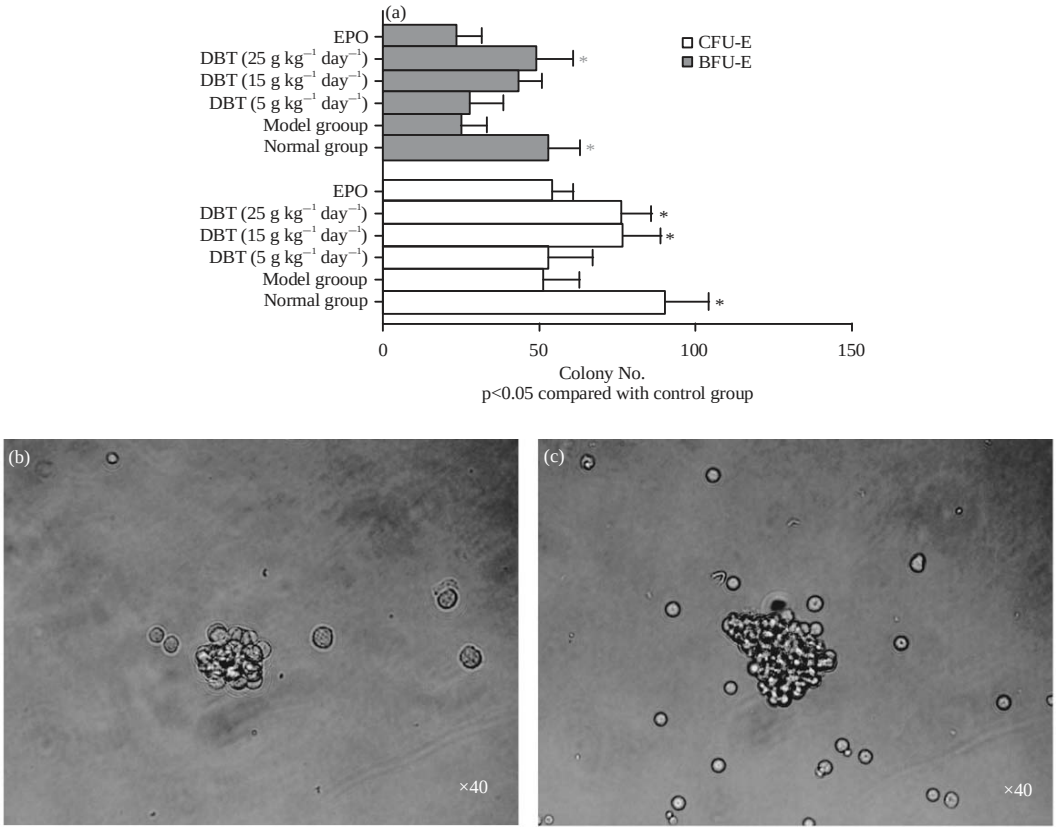

Fig. 3(a-c): Effect of DBT on erythroid progenitor (BFU-E and CFU-E), (a) Number of BFU-E and CFU-E, (b) Colony formed by CFU-E and (c) Colony formed by BFU-E
Values are Mean±SD

Effect of DBT on erythroid cell: The TER-119 monoclonal antibody reacts with mouse erythroid cells from pronormoblast to mature erythrocyte stages. In order to look for potential therapeutic targets for DBT, the number of erythroid cells was measured using TER-119 monoclonal antibody. The result of experiments showed that the percentage of erythroid cells in bone marrow of normal group had no significant difference compared with model group ($p>0.05$), however the percentage of erythroid cells in bone marrow of the medium and high dose group using DBT were significantly higher than those in the model group ($p<0.05$) (Fig. 4). Results showed further the capacity of DBT to promote erythropoiesis.

Effect of DBT on the total body weight and organ weight: The general effects of DBT on animals' well-being were also determined. Animal body weights in normal-, model-, DBT- and EPO-treated groups were determined on days 1, 2, 3, 4 and 5, respectively. The result of experiments showed that the weights showed a gradual increase in all groups (Fig. 5a). However, there was a body weight decrease in model group animals on day 2, but this decrease was not observed in DBT groups (Fig. 5a). It is possible that DBT protect the loss of body weight resulting from adriamycin. Then the effects of DBT on the ex-bone marrow, hematopoietic organs were investigated (Fig. 5b). The result of experiments showed that the weight of liver, spleen and kidney had no significant difference in all groups. In order to investigate the net effects of DBT on liver, spleen and kidney size, we normalized the weight of the organ to that of the body (Fig. 5c). The result of experiment showed that the weight of liver, spleen and kidney had no significant difference in all groups.

DISCUSSION

The Chinese herbs exert their pharmacological effects through a multi-component and multi-target way[18-20]. So it is difficult to elucidate pharmaceutical ingredients. On the premise of unknown pharmaceutical ingredients, quality of Chinese medicinal herbs may be evaluated on the whole. Fingerprint can reflect overall chemical features of Chinese medicinal herbs. High Performance Liquid Chromatography

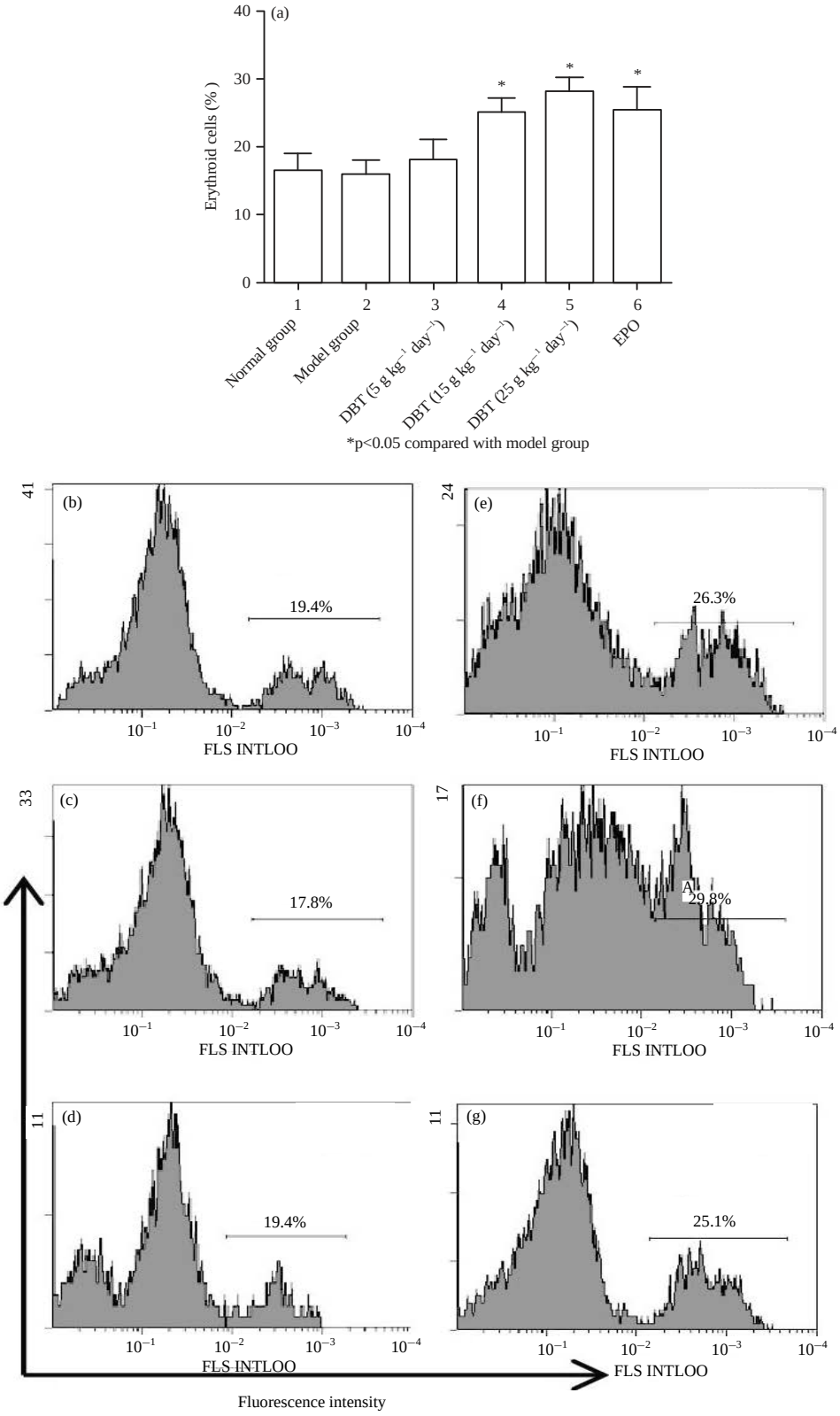

Fig. 4(a-g): Effect of DBT on erythroid cell, (a) Percentage of erythroid cells in bone marrow, (b) Normal group, (c) Model group, (d) DBT (5 g kg^{-1} day^{-1}), (e) DBT (15 g kg^{-1} day^{-1}), (f) DBT (25 g kg^{-1} day^{-1}) and (g) EPO
Values are Mean±SD

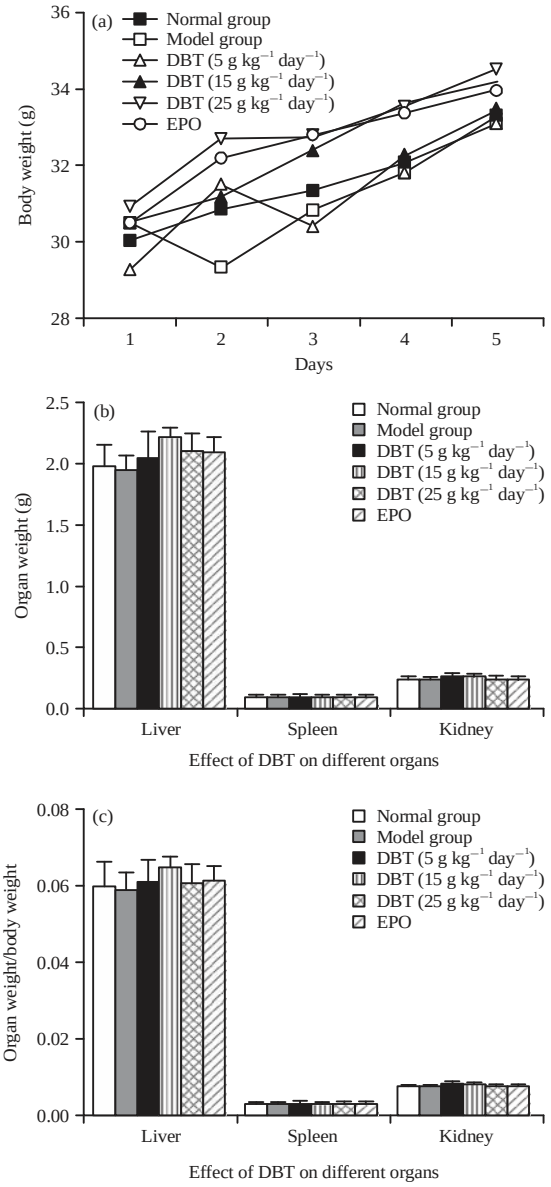

Fig. 5(a-c): Effect of DBT on animal, (a) Body weight, (b) Organ weight of liver, spleen and kindey and (c) Organ weight/body weight of liver, spleen and kindey
Values are Mean±SD

(HPLC) has the properties of high speed, sensitivity, repeatability and reliability. So fingerprint of DBT was established by HPLC in this study.

As a result of different growing environment, RA and RAS from different places have different chemical constituents. The effects of RA and RAS are controlled by chemical constituents. The result of experiments also showed that although the fingerprints of the 6 samples of DBT had high similarity on the whole and 17 major common peaks were found. However, the relative peak areas of common peaks were obviously different in different samples. So quality of DBT must be evaluated on the whole. Previous studies have also shown that four flavonoid compounds contents in RA from different places are different[25] and the quality control of RAS is not enough simply by ferulic acid content, other indicators should be chosen so that judgment is more scientific and accurate[26].

In order to demonstrate the effect of DBT on erythropoiesis, adriamycin was first given by injection (i.p.) to establish the hypocytharemia model. Adriamycin can inhibit DNA replication by intercalating DNA[27]. Experimental results showed that the number of peripheral erythrocytes and reticulocyte, BFU-E and CFU-E of model group were significantly lower than those in the normal group ($p<0.05$), which suggested that adriamycin could inhibit erythropoiesis and the animal models with blood deficiency was successfully established. However the percentage of erythroid cells in bone marrow of normal group had no significant difference compared with model group ($p>0.05$). The most likely reason is that adriamycin not only inhibits erythropoiesis but also inhibit other bone marrow cells, which results in no significant change of the percentage of erythroid cells in bone marrow.

The number of peripheral red blood cells in three dosage group using DBT had no significant difference with model group ($p>0.05$). Considering that the lifespan of erythrocytes can reach 120 days in the peripheral blood and our experiment only last 8 days, the number of peripheral erythrocytes cannot sensitively reflect the effect of DBT. In order to guarantee precise and reliable results, the number of peripheral reticulocyte was detected to evaluate the effect of DBT on erythropoiesis. Reticulocytes are immature red blood cells. Reticulocytes develop and mature in the bone marrow and then circulate for about a day in the blood stream before developing into mature red blood cells. The number of reticulocytes is a good indicator of bone marrow activity because it represents recent production. Experimental results showed that the number of peripheral reticulocyte of the high dose group using DBT were significantly higher than those in the model group ($p<0.05$). So DBT can enhance bone marrow activity and promote erythropoiesis.

In the process of red blood carpasel maturation, a cell undergoes a series of differentiations[28]. First, pluripotent stem cells from bone marrow differentiate into erythroid progenitor (BFU-E and CFU-E) and then erythroid progenitor differentiate into erythrocyte through a multi-step process. In which many cytokines act as main effect molecules and many different developmental stages of erythroid cell (pronormoblast, basophilic normoblast, polychromatophilic, orthochromatophilic) are formed[29,30]. The DBT may exert its

pharmacological effects through a multi-target way and has the characteristic of whole regulating. In order to demonstrate the ability of DBT on erythropoiesis from different level and seek the potential target for DBT, erythroid progenitors (CFU-E and BFU-E) in bone marrow are measured with Colony Forming Cell (CFC) assay. Experimental results showed that DBT could increase the number of erythroid progenitor and the percentage of erythroid cells in bone marrow. Previous studies have shown that DBT can balance the expression of cytokine (EPO, TPO and GM-CSF) in bone marrow microenvironment[31], increase the recovery of the megakaryocytic series by antiapoptotic effects on M-07e cells[23], regulate the cell proliferation and differentiation of MG-63 cell[13] and activate extracellular signal-regulated kinase in cultured T-lymphocytes[10]. So DBT may exert its pharmacological effects through a multi-target way.

CONCLUSION

It is concluded that the fingerprints of the 6 samples of DBT have high similarity and the mutual mode fingerprint of DBT can be used for the quality evaluation and control of DBT. The DBT can increase the BFU-E and CFU-E cell count. The DBT can promote erythropoiesis in acute chemotherapy injured mice.

SIGNIFICANCE STATEMENTS

This study discovered the erythropoiesis of danggui buxue tang that can be beneficial for acute chemotherapy injured mice. This study will help the researcher to uncover the critical areas of quality control of DBT and erythropoiesis of DBT. This study revealed the new pharmacodynamics of DBT.

ACKNOWLEDGEMENTS

This study was supported by National Natural Science Foundation of China (Grant No. 81403317), Guangzhou Science and Technology Project (Grant No. 201707010441); Research projects for strong Chinese medicine province of Guangdong province (Grant No. 20131250), Guangdong Province Public Research and Capacity-Building of Special Foundation (Grant No. 2015A020211037) and Guangzhou Science and Technology Project (Grant No. 201607010087).

REFERENCES

1. Morera, D. and S.A. MacKenzie, 2011. Is there a direct role for erythrocytes in the immune response? Vet. Res., Vol. 42. 10.1186/1297-9716-42-89
2. Kamimura, K., Y. Matsumoto, Q. Zhou, M. Moriyama and Y. Saijo, 2016. Myelosuppression by chemotherapy in obese patients with gynecological cancers. Cancer Chemother. Pharmacol., 78: 633-641.
3. Kranenburg, F.J., M.S. Arbous, C. So-Osman and J.G. van der Bom, 2015. Blood is not for everyone: The usefulness of erythrocyte transfusion. Ned. Tijdschr. Geneeskd., Vol. 159.
4. Casadevall, N., K.U. Eckardt and J. Rossert, 2005. Epoetin-induced autoimmune pure red cell aplasia. J. Am. Soc. Nephrol., 16: S67-S69.
5. Seto, S.W., G.Y. Yang, H. Kiat, A. Bensoussan, Y.W. Kwan and D. Chang, 2015. Diabetes mellitus, cognitive impairment and traditional Chinese medicine. Int. J. Endocrinol., Vol. 2015.
6. Zhang, L.G., X.W. Ouyang, T.T. Wu, L.J. Ni and W.Z. Shi, 2014. Quantitative evaluation of *in vitro* effects and interactions of active fractions in a Chinese medicinal formula (Yaotongning capsule) on rat chondrocytes. J. Ethnopharmacol., 155: 1424-1432.
7. Shen, X., J. Xia and C.E. Adams, 2014. Acupuncture for schizophrenia. Cochrane Database Systematic Rev.
8. Calapai, G. and A.P. Caputi, 2007. Herbal medicines: Can we do without pharmacologist? Evidence-Based Compl. Altern. Med., 4: 41-43.
9. Meisler, J.G., 2003. Toward optimal health: The experts discuss the use of botanicals by women. J. Women's Health, 12: 847-852.
10. Gao, Q.T., J.K. Cheung, J. Li, Z.Y. Jiang and G.K. Chu *et al*., 2007. A Chinese herbal decoction, Danggui Buxue Tang, activates extracellular signal regulated kinase in cultured T lymphocytes. FEBS Lett., 581: 5087-5093.
11. Gao, Q., J. Li, J.K. Cheung, J. Duan and A. Ding *et al*., 2007. Verification of the formulation and efficacy of Danggui Buxue Tang (a decoction of Radix Astragali and Radix Angelicae Sinensis): An exemplifying systematic approach to revealing the complexity of Chinese herbal medicine formulae. Chin. Med., Vol. 2.
12. Gao, Q.T., J.K.H. Cheung, R.C.Y. Choi, A.W.H., Cheung and J. Li, *et al*., 2008. A Chinese herbal decoction prepared from Radix Astragali and Radix Angelicae Sinensis induces the expression of erythropoietin in cultured Hep3B cells. Planta. Med., 74: 392-395.
13. Choi, R.C.Y., Q.T. Gao, A.W.H. Cheung, J.T.T. Zhu and F.T.C. Lau *et al*., 2011. A Chinese herbal decoction, danggui buxue tang, stimulates proliferation, differentiation and gene expression of cultured osteosarcoma cells: Genomic approach to reveal specific gene activation. Evidence-Based Compl. Altern. Med., Vol. 2011. 10.1093/ecam/nen085
14. Gao, Q.T., J.K.H. Cheung, J. Li, G.K.Y. Chu and R. Duan *et al*., 2006. A Chinese herbal decoction, danggui buxe tang, prepared from radix astragali and radix angelicae sinensis stimulates the immune responses. Planta Med., 72: 1227-1231.

15. Dong, T.T., J.K. Zhao, G.T. Qiut, N.J. Zhao and D. Ran *et al*., 2006. Chemical and biological assessment of a Chinese herbal decoction containing radix astragali and radix angelicae sinensis: Determination of drug ratio in having optimized properties. J. Agric. Food Chem., 54: 2767-2774.
16. Liu, Y., H.G. Zhang and X.H. Li, 2011. A Chinese herbal decoction, Danggui Buxue Tang, improves chronic fatigue syndrome induced by food restriction and forced swimming in rats. Phytother. Res., 25: 1825-1832.
17. Ke, H.L., Y.W. Zhang, B.F. Zhou and R.T. Zhen, 2012. Effects of Danggui Buxue Tang, a traditional Chinese herbal decoction, on high glucose-induced proliferation and expression of extracellular matrix proteins in glomerular mesangial cells. Natural Prod. Res., 26: 1022-1026.
18. Liu, Z., F. Guo, Y. Wang, C. Li and X. Zhang *et al*., 2016. BATMAN-TCM: A bioinformatics analysis tool for molecular mechanism of traditional Chinese medicine. Scient. Rep., Vol. 6.
19. Zhang, J., H. Zou, Q. Zhang, L. Wang and J. Lei *et al*., 2016. Effects of Xiaoshuan enteric-coated capsule on neurovascular functions assessed by quantitative multiparametric MRI in a rat model of permanent cerebral ischemia. Altern. Med., Vol. 16.
20. Li, L., L. Zhang and C.C. Yang, 2016. Multi-target strategy and experimental studies of traditional Chinese medicine for Alzheimer's disease therapy. Curr. Top. Med. Chem., 16: 537-548.
21. Dai, W., L. Hu, L. Ji, J. Li, K. Bi and Q. Li, 2015. A comprehensive method for quality evaluation of houttuyniae herba by a single standard to determine multi-components, fingerprint and HPTLC method. Anal. Sci., 31: 535-541.
22. Cai, Y., X. Li, M. Li, X. Chen, H. Hu, J. Ni and Y. Wang, 2015. Traceability and quality control in traditional Chinese medicine: From chemical fingerprint to two-dimensional barcode. Evidence-Based Compl. Altern. Med., Vol. 2015.
23. Yang, M., G.C. Chan, R. Deng, M.H. Ng and S.W. Cheng *et al*., 2009. An herbal decoction of *Radix astragali* and *Radix angelicae sinensis* promotes hematopoiesis and thrombopoiesis. J. Ethnopharmacol., 124: 87-97.
24. Wittenborn, J.R., 1952. Critique of small sample statistical methods in clinical psychology. J. Clin. Psychol., 8: 34-37.
25. He, J.X., Q. Mq., J.Q. Zhang, Q. Tian, C. He, R.L. Yin and H. Li, 2017. Rapid identification of *Astragali radix* from different origins by UPLC combined with chemometrics methods. Chin. Trad. Herbal Drugs, 48: 179-184.
26. Xue-Rong, W., 2015. A correlation analysis of effective component content of Danggui in different origin. Clin. J. Chinese Med., 7: 15-16.
27. Mandziuk, S., R. Gieroba, A. Korga, W. Matysiak and B. Jodlowska-Jedrych *et al*., 2015. The differential effects of green tea on dose-dependent doxorubicin toxicity. Food Nutr. Res., Vol. 59.
28. Obeidi, N., A.A. Pourfathollah, M. Soleimani, M.N. Zarif and F. Kouhkan, 2016. The effect of Mir-451 upregulation on erythroid lineage differentiation of murine embryonic stem cells. Cell J., 18: 165-178.
29. Gothwal, M., J. Wehrle, K. Aumann, V. Zimmermann, A. Grunder and H.L. Pahl, 2016. A novel role for NF-E2 in erythroid maturation by modulation of mitochondrial autophagy. Haematologica.
30. Fang, S., H. Nurmi, K. Heinolainen, S. Chen and E. Salminen *et al*., 2016. Critical requirement of VEGF-C in transition to fetal erythropoiesis. Blood, 128: 710-720.
31. Yan, S., Y. Xie, B. Zhu, Y. Han and W. Guo, 2011. Effect comparison of different formulation of Dang–Gui–Bu–Xu-Tang on myelosuppression mouse. Asian Pacific J. Trop. Med., 4: 556-559.