**Regular Article**

**Effects of Astragalus Combined with Angelica on Bone Marrow Hematopoiesis Suppression Induced by Cyclophosphamide in Mice**

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Danggui Buxue Tang (DBT), a combination of Astragalus and Angelica at a 5:1 ratio, mainly promotes hematopoiesis. However, in the clinic, the combination ratio of Astragalus and Angelica to treat low hematopoietic function is not an absolute 5:1 ratio, suggesting that the herbs may promote hematopoiesis better after being combined at a certain range of ratios. The objective of this study is to investigate the effect of different ratio combinations of Astragalus and Angelica on bone marrow hematopoiesis suppression induced by cyclophosphamide (CTX) and to probe the interaction and mechanism of Astragalus combined with Angelica in promoting hematopoiesis. Following establishment of the model, mice were administered with Astragalus (6.00 g·kg⁻¹), Angelica (3.00 g·kg⁻¹), and combinations of Astragalus and Angelica at different ratios, including 10:1 (Astragalus 9.81 g·kg⁻¹ + Angelica 0.98 g·kg⁻¹), 5:1 (Astragalus 9.00 g·kg⁻¹ + Angelica 1.80 g·kg⁻¹), 2:1 (Astragalus 7.71 g·kg⁻¹ + Angelica 3.08 g·kg⁻¹), 1:1 (Astragalus 5.40 g·kg⁻¹ + Angelica 5.40 g·kg⁻¹), 1:2.5 (Astragalus 3.08 g·kg⁻¹ + Angelica 7.71 g·kg⁻¹), 1:5 (Astragalus 1.80 g·kg⁻¹ + Angelica 9.00 g·kg⁻¹), and 1:10 (Astragalus 0.98 g·kg⁻¹ + Angelica 9.81 g·kg⁻¹). Our results suggested that Astragalus mixed with Angelica synergistically promoted hematopoiesis best when the combination ratio of Astragalus and Angelica was 1:1, 1:2.5 or 1:5; moreover, the effect of Angelica was greater than that of Astragalus. The potential mechanisms of the combinations of Astragalus and Angelica that promote hematopoiesis include the dissolution of the effective components, promoting the synthesis and secretion of hematopoietic growth factor (HGF) and the proliferation of hematopoietic progenitor cells (HPCs).

**Key words** Astragalus; Angelica; bone marrow hematopoiesis suppression; hematopoietic growth factor (HGF); hematopoietic progenitor cell (HPC); chemical component

Astragalus is often combined by Angelica to prevent and cure diseases in traditional Chinese medicine (TCM). The most popular formulation is Danggui Buxue Tang (DBT) composed of Astragalus and Angelica at a 5:1 ratio, which is mainly used to treat anemia due to various causes via promoting hematopoiesis. In recent years, studies have shown that DBT also has other pharmacological effects, such as enhancing immunity, antagonizing diabetic nephropathy, cardiovascular protection, anti-fibrosis, estrogen-like effect, among others.

Concerning the hematopoietic function of the Astragalus–Angelica combination, most studies have revealed that Astragalus mixed with Angelica at 5:1 can strongly promote hematopoiesis. For example, one study demonstrated that DBT boosts the formation of red blood cells (RBC) in an inhibition model of bone marrow hematopoiesis function, both the decocation and granule of DBT can promote the recovery of peripheral blood cells and bone marrow nucleated cell (BMNC) and accelerate the proliferation of hematopoietic stem cells (HSCs) or hematopoietic progenitor cells (HPCs), thus stimulating the hematopoietic function. However, another study confirmed that combinations of Astragalus and Angelica displayed synergism in nourishing and tonifying blood in a model induced by N-acetylpenhydroxycine and cyclophosphamide (CTX) within the ratio ranges of 1:5–5:1; of note, the effect was more remarkable when Astragalus 90–180g was coupled with Angelica 10–40g, and Astragalus 20–100g was coupled with Angelica 50–100 g. These findings indicate that the ratio of Astragalus combined with Angelica at 5:1 is most likely not absolute for promoting hematopoiesis. Additionally, in the clinic, the combination ratio of Astragalus and Angelica for treating hematopoietic function deficiency is inconsistent. To further clarify a reasonable combination of Astragalus and Angelica for improving hematopoietic dysfunction, we probed the hematopoietic effect of different combinations of Astragalus and Angelica using a mouse model of bone marrow hematopoiesis suppression mediated by CTX.

**MATERIALS AND METHODS**

**Experimental Animals** ICR mice of specific pathogen free (SPF), 18–22 g, were provided by the Experimental Animal Center in Hunan University of Chinese Medicine. The animal protocols were approved by the Animal Ethics Committee of Hunan University of Chinese Medicine (approval number: 43004700005819, date: April 25, 2014), and the disposal of animals during the experiment was consistent with “Guidance Suggestions for the Care and Use of Laboratory Animals” from the Ministry of Science and Technology of the People's Republic of China.

**Plants and Identification** Astragalus membranaceus Bunge var. mongholicus (Bunge) P. K. Hsiao was derived from Inner Mongolia, and the voucher specimen number was A0060. Angelica sinensis (Oliv.) Diels [A. polymorpha...
Maxim. Var. *sinensis* Oliw.] originated from Gansu, and the voucher specimen number was A0042. All of the herbs were provided and identified by the Pharmacy Department of Hunan University of Chinese Medicine (Herbs Appraiser: Prof. Zuo Yajie), and the voucher specimens were stored in the Affiliated Hospital Herbarium, Hunan University of Chinese Medicine.

**Preparation of Chinese Herbal Decoction**  
*Astragalus*, *Angelica* and different ratio combinations of *Astragalus–Angelica* (10:1, 5:1, 2.5:1, 1:1, 1:2.5, 1:5, and 1:10) were prepared. First, the crude herbs of *Astragalus* and *Angelica* were weighed according to the ratios, chopped and put into the leacher for extraction three times using the water reflux method. Next, 8 times water was added to boil for 2 h and the water extract was obtained after being cooled; then, 6 times water was applied to the extract, boiled for 1 h and extracted, and this was repeated twice. Then, all of the extracts were mixed together, filtered and condensed with a 60°C vacuum, and this was repeated twice. Then, all of the extracts were weighed according to the ratios, chopped and put into the leacher for extraction three times using the water reflux method. Next, 8 times water was added to boil for 2 h and the water extract was obtained after being cooled; then, 6 times water was applied to the extract, boiled for 1 h and extracted, and this was repeated twice. Then, all of the extracts were mixed together, filtered and condensed with a 60°C vacuum, the final concentration of *Astragalus*, *Angelica* and the combinations was 0.6 g·mL\(^{-1}\), 0.3 g·mL\(^{-1}\), and 1.08 g·mL\(^{-1}\), respectively. Following 0.1% of sodium benzoate addition, the concentrate was sub-packaged and stored at \(-4^\circ\)C.

**Determination of Effective Components**  
Calycosin glycoside, calycosin, formononetin and astragaloside IV are the characteristic components of *Astragalus*, and ferulic acid is the characteristic component of *Angelica*. Therefore, the components were measured to identify *Astragalus* and *Angelica* by ultra performance liquid chromatography tandem mass-spectrometry (UPLC-MS/MS). The chemical structures of the five components are shown in Fig. 1.

**Key Instrument**  
Waters-Xevo-G2-S QToF UPLC-MS/MS (Acquity system, V4.1 Masslynx chromatography workstation, Waters Co., U.S.A.).

**Chromatographic Conditions**  
ACQUITY UPLC BEH C\(_{18}\) chromatographic column (2.1×50 mm, 1.7 µm). The mobile phase: 0.1% methane acid (A)→acetonitrile (B); gradient elution (0–1 min, 95→90% A; 1–4 min, 90→80% A); 4–7 min, 80→65% A; 7–10 min, 65→50% A; 10–11 min, 50→5% A; 11–12 min, 5→5% A; 12–13 min, 5→95% A; 13–15 min, 95→95% A). The column temperature was 35°C, the flow rate was 0.4 mL·min\(^{-1}\), and the injection volume was 1 µL.

**Preparation of Reference Substance Solution**  
Reference substances of ferulic acid, calycosin glycoside, calycosin, formononetin, and astragaloside IV were weighed precisely and dissolved with methanol, and the concentrations were 89.6, 83.2, 82.0, 69.6, and 84.0 µg·mL\(^{-1}\), respectively.

**Preparation of Test Solution**  
Using 0.5 mL of Chinese herbal decoction in a 10 mL centrifuge tube, 8 mL of methanol was added, mixed and centrifuged for 15 min at 4000 r·min\(^{-1}\). The supernatant was supplemented with 30% methanol solution to 50 mL and filtered with a 0.22 µm microporous membrane to acquire the test solution. Based on the above conditions, UPLC fingerprints of *Astragalus* and *Angelica* were measured (Fig. 2). In Fig. 2, ferulic acid, the specific component in *Angelica* could be detected, and calycosin glycoside, calycosin, formononetin and astragaloside IV in *Astragalus* were also observed.

**Main Reagents**  
Recombinant human granulocyte-macrophage colony-stimulating factor (rhG-CSF, approval-number: 13041105) was produced by Beijing Shuanglu (SL) Pharmaceutical Co., Ltd. CTX was purchased from Jiangsu Hengrui Medicine Co., Ltd. (batch number: 13110725).

Enzyme-linked immunosorbent assay (ELISA) kit for the detection of erythropoietin (EPO), thrombopoietin (TPO) and granulocyte-macrophage colony-stimulating factor (GM-CSF) was provided by Wuhan Beinglay Biotech Co., Ltd. (batch number: 201405). Recombinant human erythropoietin (rHEPO, batch number: 311PEPO28), recombinant murine thrombopoietin (rmTPO, batch number: 1212PMPT0), recombinant murine interleukin-3 (rmIL-3, batch number: 107PMIL3) were purchased from PROSPEC Company (Israel). Recombinant murine granulocyte-macrophage colony stimulating factor (rmGM-CSF, batch number: 107MGMC01) was purchased from PeproTech Company (U.S.A.). ISCOVE’s modified Dulbecco’s medium (IMDM, batch number: NAGI440) was purchased from Hyclone Company (U.S.A.). Two methoxy benzidine staining solution (batch number: SLBD1984AS-D) was provided by Sinopharm group (China). Naphthol-AS-D chloroacetate esterase (NAS-DCE, batch number: 615051) staining solution, acetylcholinesterase staining solution (batch number: HL0111A16) were bought from Shanghai Harling Biotechnology Co., Ltd. (China).

![Fig. 1. Chemical Structures of Five Components](image-url)
The reference substances of ferulic acid (batch number: 110773-201313), calycosin glycoside (batch number: 111920-201304), formononetin (batch number: 111703-200603) and astragaloside IV (batch number: 110781-201314) were purchased from the National Institutes for Food and Drug Control, and the reference substance of calycosin (batch number: YY90514) was purchased from Shanghai Yuanye Biotechnology Co., Ltd. The purity of each reference substance was greater than or equal to 98%.

**Animal Model** To establish the model of bone marrow hematopoietic function suppression, intraperitoneal injection of CTX (CTX in normal saline, 40 mg·kg⁻¹·d⁻¹) was performed for 3 d. The normal control group received an intraperitoneal injection of normal saline at the same dose. Blood

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Fig. 2. UPLC Fingerprints of Five Components of Astragalus and Angelica

A. UPLC fingerprints of five components in a mixed reference substance. B. UPLC fingerprints of Astragalus. The result showed that the main components in Astragalus, including calycosin glycoside, calycosin, formononetin and astragaloside IV, could be observed. C. UPLC fingerprints of Angelica. Ferulic acid, the specific component in Angelica, could also be detected.
samples were collected from the fundus venous plexus before CTX injection and on the 1st, 3rd, 5th, 7th, 9th, and 11th day after CTX injection. White blood cells (WBC), RBC, hemoglobin (HGB) and platelets (PLT) in the peripheral blood were counted with an automatic blood cell analyzer. In the experiment, we found that the peripheral blood displayed a gradual decline and then a self recovery after CTX injection for a period of time. In detail, after CTX was injected, the numbers of WBC, RBC, and PLT decreased to a minimum on the 5th day, and HGB decreased to a minimum on the 7th day; on the 9th day, the peripheral hemogram gradually returned to normal (Fig. 3). Therefore, based on these results, we established the bone marrow hematopoietic suppression model through a continuous CTX injection for 3 d and used the 5th day following CTX injection as the time point for observations.

Animal Experimental Method  ICR mice were randomly divided into 12 groups: normal group, model group, rhG-CSF positive group, Astragalus group, Angelica group, and Astragalus–Angelica combination groups with different ratios (10:1, 5:1, 2.5:1, 1:1, 1:2.5, 1:5, and 1:10). After being adaptively fed for 5 d, the animals were administered as follows: the mice in the normal group and model group were given normal saline (10 mL·kg\(^{-1}\)) intragastrically each day for 8 d. On Day 4, the mice in the model group also received an intraperitoneal injection of CTX (40 mg·kg\(^{-1}\)) for 3 d. The mice in the herb treatment groups were gavaged with different plant extracts for 8 d along with the establishment of the model on Day 4. The mice in the rhG-CSF control group were given normal saline every day for 8 d as above; the model was established on Day 4 and rhG-CSF (45 µg·kg\(^{-1}\)) was given a subcutaneous injection on Day 6 for 3 d. The animals in each group were decapitated and detected on Day 9.

Detected Indexes  Peripheral Hemogram  At 24 h after the last administration, the venous blood was taken from the fundus venous plexus. Then, WBC, RBC, HGB and PLT were counted with an automatic blood cell analyzer. Heparin was used as an anticoagulant.

Contents of EPO, TPO, and GM-CSF in Serum  Venous blood was centrifuged (3000 r·min\(^{-1}\)) for 10 min to obtain the serum, and the contents of EPO, TPO, and GM-CSF were detected using ELISA kits.

Spleen Index (SI)  The spleen also has hematopoietic function in addition to being an immune organ. In mice, the spleen is a lifelong hematopoietic organ, compensating for extramedullary hemopoiesis when bone marrow function is impaired. Thus, SI was measured in our study. Following decapitation of the mice, the connective tissues on the surface of the spleen were removed, the blood was removed with filter paper, and the spleen was weighed quickly. SI was calculated by the following formula: SI = spleen weight (mg)/mouse weight (g).

Bone Marrow Nucleated Cell (BMNC) Count  The right femur of the mice was obtained, and the marrow cavity of the femur was flushed with 1 mL phosphate buffered saline (PBS).
and prepared into a cell suspension. Subsequently, the cell suspension was diluted 20 times with 2% glacial acetic acid to count the number of BMNC.

**Morphology Analysis of Bone Marrow** Bone marrow tissues were dyed with hematoxylin and eosin (H&E) staining, and the percentage of the bone marrow hematopoietic tissue area in the bone marrow cavity area was calculated using Image-Pro Plus software.

**Cell Culture and Colony Assay of Bone Marrow HPC**

As was found in the above research, *Astragalus* combined with *Angelica* at 1 : 1, 1 : 2.5, and 1 : 5 promoted hematopoiesis the strongest while the effect of *Astragalus* combined with *Angelica* at 10 : 1 was weaker. Therefore, we chose the combination ratios of 10 : 1, 5 : 1, 1 : 1, and 1 : 5 for the following study.

First, the femur was removed to collect the bone marrow after the mice were sacrificed, a single cell suspension of bone marrow was made via filtration using a syringe needle No. 4 and the BMNC were counted. The concentration of BMNC was adjusted to \(1 \times 10^6\) and the BMNC suspension were added into 6-well plates, adjusted to 1 mL with IMDM and cultured at 37°C, 5% CO₂ and saturated humidity. Third, colony forming unit-erythrocyte (CFU-E) was observed and counted on the 3rd day of culture. Burst forming unit-erythroid (BFU-E), colony-forming unit-granulocyte macrophage (CFU-GM), and megakaryocyte colony forming unit (CFU-MK) were observed and counted on the 7th day. BFU-E and CFU-E were identified by 2-methoxy benzidine staining as red positive cells. For BFU-E, >50 cells or for CFU-E, >8 cells was identified as a colony. CFU-GM was dyed red with NAS-DCE as a positive cell, and greater than 50 cells was considered a colony. CFU-MK was stained by acetylcholinesterase and identified as a colony if the cell was brown and there were greater than 3 cells.

**Content Determination of Five Components in Different Ratio Combinations of *Astragalus* and *Angelica***

UPLC-MS/MS was used to determine the contents of five components.14) Key Instrument

<table>
<thead>
<tr>
<th>Component</th>
<th>Retention time (min)</th>
<th>Relative molecular mass</th>
<th>Enhanced production (m/z)</th>
<th>Cone voltage</th>
<th>Collision energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferulic acid</td>
<td>3.59</td>
<td>194.18</td>
<td>145.03</td>
<td>20</td>
<td>14</td>
</tr>
<tr>
<td>Calycosin glycoside</td>
<td>4.04</td>
<td>446.40</td>
<td>285.09</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>Calycosin</td>
<td>6.31</td>
<td>284.26</td>
<td>270.06</td>
<td>30</td>
<td>25</td>
</tr>
<tr>
<td>Formononetin</td>
<td>7.09</td>
<td>268.27</td>
<td>254.06</td>
<td>30</td>
<td>25</td>
</tr>
<tr>
<td>Astragaloside IV</td>
<td>8.74</td>
<td>784.97</td>
<td>143.11</td>
<td>30</td>
<td>25</td>
</tr>
</tbody>
</table>

**Statistical Analysis**

Statistical package SPSS17.0 (SPSS Inc., Chicago, IL, U.S.A.) was supplied to all analyses, data were expressed as mean ± standard deviation. One-way ANOVA was used for multi-group comparison, least significant difference (LSD) test or Dounnett’s T3 test was used for two-group comparison. Values of \(p<0.05\) were considered statistically significant. The total effect of the combination was analyzed using the multi index comprehensive index method.16) First, each index was standardized: standardized value=(treatment group−model group)/(control group−model group). Then, the weight coefficient was determined based

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**Table 1. The Constituents of CFU-GM, CFU-MK, CFU-E, and BFU-E Culture Systems**

<table>
<thead>
<tr>
<th>Constituent</th>
<th>CFU-GM</th>
<th>CFU-MK</th>
<th>CFU-E</th>
<th>BFU-E</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Mercapt ethanol (mL)</td>
<td>0.1 ((10^{-4})</td>
<td>0.1 ((10^{-4})</td>
<td>0.1 ((10^{-3})</td>
<td>0.1 ((10^{-3})</td>
</tr>
<tr>
<td>L-Glutamine (3%, mL)</td>
<td>0.015</td>
<td>0.015</td>
<td>0.015</td>
<td>0.015</td>
</tr>
<tr>
<td>Horse sera (mL)</td>
<td>0.25</td>
<td>0.4</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>rmGM-CSF (50ng·mL⁻¹, mL)</td>
<td>0.15</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>rhEPO (20U·mL⁻¹, mL)</td>
<td>—</td>
<td>0.05</td>
<td>0.05</td>
<td>0.1</td>
</tr>
<tr>
<td>rmIL-3 (20ng·mL⁻¹, mL)</td>
<td>—</td>
<td>0.05</td>
<td>—</td>
<td>0.05</td>
</tr>
<tr>
<td>rmTPO (5ng·mL⁻¹, mL)</td>
<td>—</td>
<td>0.1</td>
<td>—</td>
<td>0.1</td>
</tr>
<tr>
<td>BMNC (1×10⁶ mL⁻¹, mL)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>IMDM media (mL)</td>
<td>To 0.9</td>
<td>To 0.9</td>
<td>To 0.9</td>
<td>To 0.9</td>
</tr>
<tr>
<td>Agar (3%, mL)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

**Table 2. Parameters of Mass Spectrometry among Each Component**
on the importance of the index reflecting hematopoietic function and the opinions of experts, that WBC, RBC, HGB, and EPO was 1.7, GM-CSF, PLT, and BMNC was 1.1, and TPO and SI was 0.5 in our study. Weight score=standardized value×weight coefficient, and the total effect of the drug was equal to the sum of the weight score of all of the indexes. The greater the total effect obtained, the stronger the drugs promoted hematopoiesis. The interaction of Angelica and Astragalus was analyzed by multiple linear regression.17)

RESULTS
Effects of the Different Combinations of Astragalus and Angelica on Peripheral Hemogram (Fig. 4) Compared with the normal group, CTX significantly decreased the number of WBC, RBC, HGB, and PLT in peripheral blood (p<0.05 or p<0.01). In the rhG-CSF positive group, WBC was markedly increased (p<0.01) while RBC, HGB, and PLT did not significantly change (p>0.05) compared to the model group. Astragalus alone had no effect on the decreased peripheral hemogram (p>0.05), Astragalus–Angelica mixed at 10:1 only notably increased RBC (p<0.05) and had no effect on WBC, HGB, or PLT (p>0.05). Angelica alone and the combinations of Astragalus and Angelica at 5:1, 2.5:1, 1:1, 1:2.5, 1:5, and 1:10 significantly elevated WBC and PLT (p<0.05 or p<0.01). The increase of WBC in the combination of Astragalus and Angelica at the 1:2.5 ratio was higher than that of Astragalus, Angelica alone and the other combinations (p<0.05); the effect of Astragalus–Angelica combinations at 2.5:1 and 1:10 on PLT increase was the most obvious. Angelica alone and Astragalus–Angelica mixtures, including the ratios of 10:1, 5:1, 2.5:1, 1:1, 1:2.5, 1:5, and 1:10, prompted an increase of RBC (p<0.05 or p<0.01), and the effect was greatest with Astragalus–Angelica combined at 2.5:1. Astragalus combined with Angelica at the ratios of 5:1, 2.5:1, 1:2.5, 1:5, and 1:10 showed a significant increase of decreased HGB (p<0.05 or p<0.01).

Effects of Angelica–Astragalus Combinations on Hematopoietic Growth Factor (HGF), BMNC and SI (Fig. 5) Compared with the normal group, CTX reduced the contents of GM-CSF, TPO, and BMNC in serum and increased SI (p<0.05 or p<0.01). Compared with the model group, the contents of GM-CSF and BMNC in the rhG-CSF positive group were remarkably increased (p<0.05 or p<0.01), but EPO, TPO and SI did not change. Astragalus could elevate EPO (p<0.05) but had no effect on GM-CSF, TPO, BMNC or SI (p>0.05). Angelica was ineffective on all of the parameters (p>0.05). Astragalus–Angelica mixtures (1:1, 1:2.5, 1:5, 1:10) led to a significant accumulation of GM-CSF (p<0.05 or p<0.01). All of the ratio combinations of Astragalus and Angelica, except

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Fig. 4. Comparison of Peripheral Hemogram among the Groups (±s, n=14)
1. normal group; 2. model group; 3. rhG-CSF (45 µg·kg⁻¹); 4. Astragalus (6.00 g·kg⁻¹); 5. Angelica (3.00 g·kg⁻¹); 6. Astragalus–Angelica 10:1 (Astragalus 9.81+Angelica 0.98 g·kg⁻¹); 7. Astragalus–Angelica 5:1 (Astragalus 9.90+Angelica 1.80 g·kg⁻¹); 8. Astragalus–Angelica 2.5:1 (Astragalus 7.71+Angelica 3.08 g·kg⁻¹); 9. Astragalus–Angelica 1:1 (Astragalus 5.40+Angelica 5.40 g·kg⁻¹); 10. Astragalus–Angelica 1:2.5 (Astragalus 3.08+Angelica 7.71 g·kg⁻¹); 11. Astragalus–Angelica 1:5 (Astragalus 1.80+Angelica 9.00 g·kg⁻¹); 12. Astragalus–Angelica 1:10 (Astragalus 0.98+Angelica 9.81 g·kg⁻¹). Same as below. *p<0.05, **p<0.01, vs. normal group; ***p<0.05, ****p<0.01, vs. model group.
for 1:10, increased the effect on the decrease of EPO ($p<0.05$ or $p<0.01$). The combinations between *Astragalus* and *Angelica* at 2.5:1, 1:2.5, and 1:10 could markedly increase TPO ($p<0.05$). *Astragalus–Angelica* (5:1, 2.5:1, 1:1, 1:2.5, 1:5, and 1:10) notably increased BMNC while attenuating SI ($p<0.05$ or $p<0.01$).

**Effects of Bone Marrow Hematopoietic Tissue Area with Different Combinations of *Astragalus* and *Angelica* (Fig. 6)** In the normal group, the morphology and structure of the bone marrow were normal, and a large number of nucleated cells were observed. In the model group, the bone marrow was filled with fat cells, and the hematopoietic tissues were markedly diminished ($p<0.01$). Compared with the model group, fat cells were barely detected in the rhG-CSF group, and the hematopoietic tissues were notably increased ($p<0.01$). In groups receiving *Angelica* alone and combinations of *Astragalus–Angelica* at 5:1, 2.5:1, 1:1, 1:2.5, 1:5, and 1:10, fat cells were reduced to various degrees, nucleated cells were increased, and the area of hematopoietic tissues was enlarged compared to the model group ($p<0.01$). There was no statistical significance among each group ($p>0.05$). *Astragalus* alone and *Astragalus* combined with *Angelica* at a 10:1 ratio had no role in enhancing the bone marrow hematopoietic tissue area ($p>0.05$).

**Analysis of the Total Effect for Different Combinations of *Astragalus* and *Angelica* (Table 3)** The total effect of *Astragalus* combined with *Angelica* at the ratios of 1:1, 1:2.5, and 1:5 was maximal while the effects of the ratios 5:1, 2.5:1, and 1:10 were lower, but the effect of the 10:1 ratio was the lowest. Therefore, we inferred that the effect of promoting hematopoiesis in *Astragalus–Angelica* combinations at 5:1, 2.5:1, 1:1, 1:2.5, 1:5, and 1:10 was greater than in *Astragalus* or *Angelica* alone or *Astragalus–Angelica* mixed at 10:1. *Astragalus* combined with *Angelica* at 1:1, 1:2.5, and 1:5 promoted hematopoiesis to a greater extent; *Astragalus* alone and *Astragalus–Angelica* mixed at a 10:1 ratio had no significant effect on promoting hematopoiesis.

**Interaction Analysis of *Astragalus* Combined with *Angelica* on Promoting Hematopoiesis** The interaction of *Angelica* and *Astragalus* on promoting hematopoiesis was analyzed by multiple linear regression. To evaluate the effects of the Chinese herb instead of considering the interaction between *Astragalus* and *Angelica*, the combination dosages of *Astragalus* and *Angelica* were regarded as independent variables. Meanwhile, the total effect was a dependent variable to obtain the standardized partial regression coefficient of *Astragalus* and *Angelica*, which was 0.458 and 1.060, respectively. This result hinted that *Angelica* was more important for promoting hematopoiesis than *Astragalus*. Concerning the interaction between *Astragalus* and *Angelica*, the combination dosages and the interaction value were regarded as independent variables. The total effect was seen as a dependent variable, and the results demonstrated that the standardized partial regression coefficient of *Astragalus* combined with *Angelica* was 0.795, a positive value. This finding clarified that the interaction of the *Astragalus–Angelica* combination fulfilled an indispensable positive impact on promoting hematopoiesis, and the combination of *Astragalus–Angelica* exerted a synergistic effect.

**Comparison of Colony Count of HPC among Each Group (Fig. 7)** Compared with the normal group, CTX reduced CFU-GM, CFU-MK, CFU-E, and BFU-E ($p<0.01$). Compared with the model group, CFU-GM was significantly increased ($p<0.01$) while CFU-MK, CFU-E and BFU-E were not notably
affected in the rhG-CSF group \((p>0.05)\). CFU-GM, CFU-MK, CFU-E, and BFU-E were not changed in the Astragalus alone group \((p>0.05)\), whereas these parameters were increased in the Angelica alone group \((p<0.01)\). Among the different ratio combinations of Astragalus and Angelica, CFU-MK, and BFU-E remarkably increased in the 10:1 combination group \((p<0.05\) or \(p<0.01)\) while CFU-GM did not significantly change \((p>0.05)\). CFU-GM, CFU-MK, CFU-E, and BFU-E were significantly increased in the 5:1, 1:1, and 1:5 combination groups \((p<0.01)\). Compared with the 1:1 combination group, CFU-GM, CFU-E, and BFU-E were lower in Astragalus alone, Angelica alone, and the 10:1, 5:1, and 1:5 combination groups \((p<0.01)\), CFU-MK was also lower in the Astragalus alone and 10:1 and 1:5 combination groups.

Content Changes of Five Components in Different Combinations of Astragalus and Angelica (Fig. 8, Table 4) The content of ferulic acid was lower than its theoretical value, and the contents of calycosin glycoside, calycosin, formononetin and astragaloside IV were close to or below their theoretical values in the combinations of Astragalus–Angelica mixed at 5:1, 2.5:1, 1:1, 1:2.5, 1:5, and 1:10 ratios, fat cells were reduced to various degrees, nucleated cells were increased, and the area of hematopoietic tissues was enlarged compared to the model group. Astragalus alone and Astragalus combined with Angelica at a 10:1 ratio had no role in enhancing the bone marrow hematopoietic tissue area.

Fig. 6. Effects on Bone Marrow Hematopoietic Tissue Area with Different Combinations of Astragalus and Angelica

A. Morphology change of bone marrow among the groups \((\times 400, \text{Bar}=20 \mu m)\). B. Comparison of bone marrow hematopoietic tissue area among the groups \((\bar{x}\pm s, \ n=18)\). *\(p<0.01\), vs. normal group; *\(p<0.01\), vs. model group. In the normal group, the morphology and structure of the bone marrow were normal, and a large number of nucleated cells were observed. In the model group, the bone marrow was filled with fat cells, and the hematopoietic tissues were markedly diminished. Compared with the model group, fat cells were barely detected in the rhG-CSF group. In Angelica alone and the combinations of Astragalus–Angelica mixed at 5:1, 2.5:1, 1:1, 1:2.5, 1:5, and 1:10 ratios, fat cells were reduced to various degrees, nucleated cells were increased, and the area of hematopoietic tissues was enlarged compared to the model group. Astragalus alone and Astragalus combined with Angelica at a 10:1 ratio had no role in enhancing the bone marrow hematopoietic tissue area.
IV was close to the theoretical value. In the combination of Astragalus and Angelica at the 1:5 ratio, the contents of ferulic acid, calycosin glycoside, calycosin and astragaloside IV were all more than their theoretical values, and the content of formononetin was less than its theoretical value. These results revealed that Astragalus mixed with Angelica at 1:1 and 1:5 ratios could prompt the dissolution of the effective components of Astragalus and Angelica.

DISCUSSION

Previous studies report that the promotion of hematopoiesis by Danggui Buxue Decoction (DBT) combined with Astragalus and Angelica at a 5:1 ratio is best. In the mouse model of bone marrow suppression, DBT can enhance the expression of EPO, TPO, and GM-CSF and the proliferation of bone marrow HSC or HPC, thereby promoting hematopoiesis.10) DBT can stimulate the proliferation of BFU-E and CFU-GM and increase the BMNC count in immune-mediated aplastic anemia mice.18) However, in clinical application, other combination ratios of Astragalus and Angelica besides the 5:1 ratio can also promote hematopoiesis in the treatment of anemia, suggesting that Astragalus and Angelica may play a better role promoting hematopoiesis at a certain range of combination ratios. Our results showed that Astragalus alone had no effect on peripheral hemogram, hematopoietic tissue or HGF. Angelica alone could prompt the recovery of peripheral hemogram and increase the bone marrow hematopoietic...
tissue area while having no significant effect on the contents of HGF and BMNC. This result suggested that *Angelica* provided some effect in promoting hematopoiesis. Among different ratio combinations of *Astragalus* and *Angelica*, the combination at 10:1 only slightly elevated the number of RBC and the content of EPO while the other combinations, including 5:1, 2.5:1, 1:1, 1:2.5, 1:5, and 1:10, not only increased the numbers of RBC, WBC, and PLT in blood but also raised the HGF content, BMNC count and bone marrow hematopoietic tissue area and decreased SI. The combination ratios of 1:1, 1:2.5, and 1:5 exerted the strongest effect in promoting hematopoiesis. The comprehensive effect analysis

Fig. 8. Chromatography and Mass Spectrometry of Five Chemical Components in the Combination of *Astragalus* and *Angelica*

A. UPLC fingerprints of five components in *Astragalus*–*Angelica* combination. Five components in the *Astragalus*–*Angelica* combination could be detected. B. Mass spectrometry of five components in the mixture reference substance. C. Mass spectrometry of five components in the *Astragalus*–*Angelica* combination.
on the promotion of hematopoiesis by *Astragalus* combined with *Angelica* also highlighted that promoting hematopoiesis using the combinations was more effective than *Astragalus* alone or *Angelica* alone, and the comprehensive effect of the combinations with 1:1, 1:2.5, and 1:5 was more obvious. In addition, the interaction analysis showed that the combinations of *Astragalus* and *Angelica* had a synergistic effect on promoting hematopoiesis. These results implied that *Angelica* had the most important role in promoting hematopoiesis when mixed with *Astragalus*, which was strengthened by the combinations; in addition, the best combination ratio was not the traditional 5:1, but the range of 1:1, 1:2.5, and 1:5.

Chinese herbs can affect the dissolution of effective components after being combined and decocted together. The mutual influence among chemical components can impact absorption, metabolism and therapeutic drug targets, thereby playing a synergistic role. This study demonstrated that the content of ferulic acid in the decoction was increased when *Astragalus* was combined with *Angelica* at a 5:1 ratio. Ferulic acid derived from *Angelica* can improve the dissolution of active components of *Astragalus* and enhance the function of DBT. *Angelica* oil can reduce the dissolution of active components in *Astragalus*, such as astragaloside IV, calycosin and formononetin, and inhibit the biological activity of DBT. In *vitro*, ferulic acid can prompt the permeability of calycosin and formononetin in Caco-2 cells, thereby boosting the absorption. Following DBT in a rat model of anemia induced by blood loss, the contents of five major components (ferulic acid, caffeic acid, calycosin-7-O-β-glucoside, ononin and astragaloside IV) of DBT in the liver were increased more than in other tissues, which were higher in anemic rats than in normal rats. This result implies that these components of DBT can improve the tissue perfusion in the blood deficient animal. Given the recent finding that the erythropoietic abilities were markedly decreased in a calycosin-depleted DBT decoction, calycosin alone did not show significant responses, revealing that calycosin had a synergistic effect on DBT. In our study, pharmaceutical chemical component analysis demonstrated that the dissolution of five chemical components was not significantly changed, and some chemical components were lower than their theoretical values when *Astragalus* was combined with *Angelica* at 10:1 and 5:1 ratios. However, the contents of ferulic acid, calycosin glycoside, calycosin, formononetin and astragaloside IV were increased to various levels when *Astragalus* was combined with *Angelica* at the ratios of 1:1 and 1:5, especially 1:1. This result suggested that the combinations of *Astragalus* and *Angelica* at 1:1 and 1:5 prompted the dissolution of the active components. Therefore, pharmaceutical chemistry demonstrated that *Astragalus* combined with *Angelica* at 1:1 and 1:5 had a good effect on promoting hematopoietic function.

The generation of blood cells is a dynamic equilibrium process where HSCs proliferate and differentiate into a variety of mature blood cells under the control of a hematopoietic regulatory network. HGF, the major factor in the regulation of hematopoiesis in vivo, induces macrophage and granulocyte stimulating factor (GM-CSF), erythropoietin (EPO), thrombopoietin (TPO), and so on. HGF can stimulate the proliferation and differentiation of HSCs in different differentiation periods and different types, the hematopoiesis process is concluded by the hematopoietic cells under the regulation of multiple HGF, and DBT can up-regulate the expression of EPO in Hep3B cells in *vitro*. Following the transfection of fibroblast kidney cells (HEK293T) with a plasmid containing a hypoxia response element (HRE), DBT triggers the expression of hypoxia-inducible factor-1α (HIF-1α) and EPO, which is relevant to the signaling pathway of mitogen-activated protein kinases. Our research also noted that *Astragalus* alone slightly up-regulated the level of EPO but did not have a distinct effect on GM-CSF or TPO; GM-CSF, TPO and EPO were not affected by *Angelica* alone. These results demonstrated that the effect of *Astragalus* or *Angelica* alone on HGF was slight. To some extent, *Astragalus* combined with *Angelica* could increase the content of HGF in blood, especially in the combinations of *Astragalus* and *Angelica* at 1:1, 2.5:1, 1:2.5, 1:5, and 1:10. HPC determination results also showed that *Astragalus* alone had no significant effect on CFU-GM, CFU-MK, CFU-E, or BFU-E while *Angelica* alone could markedly increase their levels. The combinations of *Astragalus* and *Angelica* at 5:1, 1:1, and 1:5 could enhance the colonies of CFU-GM, CFU-MK, CFU-E, and BFU-E, and the effects of 1:1 and 1:5 were greatest. In the combination of *Astragalus* and *Angelica* at a 1:1 ratio, the numbers of CFU-GM, CFU-E, and BFU-E were significantly greater than those in *Astragalus* alone, *Angelica* alone and the other combinations. This result also indicated that the effects of *Astragalus* combined with *Angelica* at 1:1 and 1:5, instead of the traditional 5:1 ratio, prompting the synthesis and secretion of HGF as well as the proliferation of HPC were greater than *Astragalus* alone, *Angelica* alone and the other combinations. These results revealed that spurring the expression of HGF and the proliferation of HSC or HPC might be one of the mechanisms for the reasonable combinations of *Astragalus* and *Angelica* in promoting hematopoiesis.

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Conflict of Interest  The authors declare no conflict of interest.

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