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Effects of traditional Chinese herbal medicines on blood cell count and immunity in chickens

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To investigate the effects of traditional Chinese herbal medicines (TCHM) on hematological parameters and immunity in chickens, *Astragalus membranaceus* (AM), *Angelicae sinensis* extract (ASE) and Danggui Buxue San (DBS) were used for the study of immunity and hematinic mechanism in order to provide basis for clinical reference. Three hundred 56-week-old hens were randomized into 10 groups. AM were added into the diets of the animals at the dosage of 5, 10 and 15 mg/kg, respectively from group I to group III; ASE were added from group IV to group VI and DBS were added from VII to group IX in the same way. The hens of group X, as of control, were fed with the diet without TCHM. At 1 day of test, all hens of each group were injected against Newcastle disease virus (NDV)-IV vaccine. The added TCHM period was 15 days. At 8 and 15 days of the test, blood of 1 hen of each group per replication was collected by wing-vein picks to measure the blood index. At 8, 15, 22, and 29 days of the test, blood of 1 hen of each group per replication was collected by wing-vein picks to measure the hens Newcastle disease (ND) and hemagglutination inhibition (HI) antibody titer. The results showed that DBS could significantly increase the count of white blood cell (WBC) ($p<0.05$) and red blood cell (RBC) ($p<0.01$), the content of hemoglobin (Hb) ($p<0.01$) and packed cell volume (PCV) ($p<0.01$) in 10 mg/kg group; DBS (15 mg/kg) had significant increase on the count of WBC and RBC and the content of Hb ($p<0.05$), but had no more effect on PCV ($p>0.05$); ASE (15 mg/kg) could significantly increase the count of RBC and the content of Hb ($p<0.05$), but had no more effect on WBC and PCV ($p>0.05$). At the same time, DBS (10 mg/kg) could significantly decrease the dropping rate of blood cell at 30 ($p<0.01$), 45 and 60 min ($p<0.05$). The other groups had no more effect on blood physiological index than the control group ($P>0.05$). In addition, DBS (10 mg/kg) could improve the antibody level than control group at 8, 15, 22 and 29 days ($p<0.05$); DBS (5 mg/kg) could improve the antibody level at 15 and 22 days ($p<0.05$); but the other groups had no more effect on the antibody level ($p>0.05$). This indicated that DBS had the ability on improving hemopoietic function and immunity in chickens. At the same time, DBS was added into the diets of the animals at the recommended dosage of 10 mg/kg.

Key words: *Astragalus membranaceus*, *Angelicae sinensis* extract, chickens, blood cell count, immunity.

INTRODUCTION

Based on the traditional Chinese veterinary medicine, syndrome of “blood” and “Qi” deficiency are morbid condition of insufficient “blood” and “Qi” supply to the visceral organs and channels for their nutrition. Studies showed

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that syndrome of “blood” and “Qi” deficiency were inevitable for aged animals, because of physiological characteristics. The main symptoms were pale tongue, sparse hair, dullness, thready-weak pulse, decreased egg production and laying rate, etc (Liang et al., 1999). It is necessary to adjust “Blood” and “Qi” for aged animals by traditional Chinese veterinary medicine.

Danggui Buxue Tang (DBT) is an ancient traditional Chinese herbal formula composed of Huangqi (Radix Astragali) and Danggui (*Radix Angelica sinensis*) with a weight ratio of 5:1 (Gao et al., 2007). DBT was first described in Neiwaishang Bianhuo Lun by Li Dongyuan in China in AD 1247. According to Chinese medicinal theory, the daily intake of DBT could raise the “Qi” and nourish the “blood” of menopausal women. Pharmacological actions indicated that DBT had the effects of promoting hematopoietic functions, preventing osteoporosis, stimulating cardiovascular circulation, increasing anti-oxidation activity, stimulating immune response and mimic estrogen (Jinous and Fereshteh, 2012; Dong, 2006; Gao, 2007). DBT has a good function of nourishing Qi (energy flow) and Xue (blood), as well as resolving extravasated blood, and has been used traditionally to treat menopausal disorders (Gao et al., 2007). It is worthwhile to note that DBT can stimulate the production of erythropoietin which is a specific hematopoietic growth factor (Zheng et al., 2010).

In addition, ASE and AS are commonly used in treating the age-related diseases, which have been demonstrated in stimulating bone cell proliferation, increasing bone formation and reducing bone re-sorption in patients (Yang et al., 2002). Treatment of BALB/c mice spleen cells with Angelica polysaccharide (100 µg/ml) increased the production of IL-2 and interferon (IFN) and decreased IL-4 (Yang et al., 2006). Angelica polysaccharide treatment rescued BALB/c mice from retro-orbital bleeding induced anemia and increased IL-6, the concentration of granulocyte macrophages colony stimulating factor (GM-CSF) in spleen cells (Liu et al., 2010). ASE induces the proliferation of ICR murine bone marrow mononuclear cells by activating ERK1/2 and P38 MAPK protein (Chen et al., 2006). Astragalus polysaccharides (APS) have been most widely studied, mainly on their immunopotentiating properties, such as stimulation of murine cytokine production and B-cell proliferation cytokine production (Shao et al., 2004). Clinical studies showed that APS could counteract the side effects of chemotherapeutic drugs, and improved medulla-suppressed of cancer patient to some extent (Tin et al., 2007). APS is a bioactive chemical in *Astragalus membranaceus* which has been used in medicine for centuries in China and has the effects of exhibiting immune-stimulatory, anti-oxidation, anti-viral and anti-tumor (Dang et al., 2004; Yuan et al., 2008; Li et al., 2008; Sima et al., 2012). Danggui can be used for anemia due to chronic renal failure (Bradley et al., 1999) and can enhance hematopoiesis by stimulating macrophages, lymphocytes in hematopoietic inductive microenvironment (Mak et al., 2006). Some new poly heterocyclic fused ring systems containing pyridazine nuclei, and tested them for analgesic, anti-inflammatory and anti-microbial activities that have been synthesized, in comparison to some reference drugs (Naif et al., 2013). Angelica polysaccharide had been reported to possess anti-tumor effects (Shang et al., 2003; Tsai et al., 2005) and also exhibit immunostimulating activities both *in vitro* and *in vivo* (Cho et al., 2000) in cancer cells.

Previous research showed DBT had effects on raising the “Qi” and nourishing the “blood” in mice/human with “blood-Qi” deficiency. However, there is less reports on its effects on raising the “Qi” and nourishing the “blood” of DBT or Danggui Buxue San (DBS) in aged poultry. In this study, according to the measurement of blood cell count and immunity of AM, ASE and DBS in chickens, the best way and dosage were selected and the mechanisms on enriching “blood” and nourishing “Qi” discussed.

MATERIALS AND METHODS

Treatment of animals

56-week-old Hyline Brown laying hens were purchased from Hebei Laboratory Animal Center, housed in cages. The hens were given free access to feedstuff and water. All the experimental animals were treated in accordance with the guidelines of the Chinese Council for Animal Care.

Preparation of reagents

AM and ASE were purchased from Beijing Biochem Co., Ltd. (China). AM and ASE were smashed, respectively to 200 mesh sieve. DBS consisted of AM and ASE in a 5:1 ratio.

Experiment design

Three hundred 57-week-old normal hens were randomly divided into ten groups with the same number and similar body weight. The hens in groups I, II and III were given three gradient dosages (5, 10, and 15 mg/kg) of AM, respectively. Hens in groups IV, V and VI were given three gradient dosages (5, 10, and 15 mg/kg) of ASE, respectively. In groups VII, VIII and IX hens were given DBS by the same gradient dosages, respectively. Hens in group were fed basal diet as control. All medicines X were given for 15 days in groups. On the first day of this experiment, all hens of each group were injected against Newcastle disease virus (NDV)-IV vaccine.

Blood specimen collection and examination

At 8 and 15 days of test, each blood sample was collected from wing-vein of every replication per group for blood parameter tests. The steps were followed with the reference to those of previous study (Bain et al., 1995).

At 8, 15, 22, and 29 days of test, each blood sample was collected from wing-vein of every replication per group for the measurement of Newcastle disease (ND) antibody titer. Hemagglutination test and hemagglutination inhibition test in 96-well U plates were used for measuring ND titer. RBCs from both horse and turkey were used for HA and HI tests (WHO, 2002).

Table 1. Effects of traditional Chinese herbal medicines on count of WBC and RBC, Hb content, and PCV in chickens.

Group	N	Dosage (mg/kg)	Count of WBC ($\times10^9$/L)	Count of RBC ($\times10^{12}$/L)	Hb content (g/L)	PCV (%)
(I) Low dosage of AM	5	5	17.55±0.71	2.42±0.05	70.03±2.01	21.99±0.57
(II) Middle dosage of AM	5	10	17.62±0.69	2.45±0.03	71.19±1.72	22.12±0.71
(III) High dosage of AM	5	15	17.89±0.43	2.45±0.07	72.06±1.33	22.37±0.43
(IV) Low dosage of AMS	5	5	17.59±0.67	2.43±0.09	70.66±0.97	22.07±0.61
(V) Middle dosage of ASE	5	10	17.89±0.51	2.49±0.05	71.91±1.44	22.31±0.45
(VI) High dosage ASE	5	15	18.03±0.47	2.57±0.07*	72.86±2.00*	22.72±0.55
(VII) Low dosage of DBS	5	5	17.91±0. 46	2.48±0.08	72.34±1.58	22.84±0.41
(VIII) Middle dosage of DBS	5	10	19.13±0. 83*	2.95±0.09**	82.59±1.37**	25.63±0.91**
(IX) High dosage of DBS	5	15	18.85±0. 53*	2.54±0.05*	72.85±2.05*	22.78±0.59
(X) Control	5	0	17.56±0. 69	2.41±0.06	70.36±1.03	22.06±0.36

**Superscript differs significantly ($p<0.01$) compared with group X. *Superscript differs notably ($p<0.05$) compared with group X.

Table 2. Effects of traditional Chinese herbal medicines on the dropping rates of blood cells in chickens.

Group	N	Dosage (mg/kg)	Time of determination			
			15 min	30 min	45 min	60 min
(I) Low dosage of AM	5	5	0.55±0.30	1.47±0.11	2.40±0.20	3.72±0.34
(II) Middle dosage of AM	5	10	0.59±0.27	1.67±0.30	2.37±0.11	3.68±0.23
(III) High dosage of AM	5	15	0.62±0.19	1.45±0.28	2.41±0.42	3.70±0.41
(IV) Low dosage of AMS	5	5	0.69±0.25	1.44±0.16	2.35±0.19	3.66±0.22
(V) Middle dosage of ASE	5	10	0.51±0.30	1.40±0.42	2.30±0.22	3.60±0.35
(VI) High dosage ASE	5	15	0.47±0.17	1.38±0.32	2.34±0.30	3.71±0.28
(VII) Low dosage of DBS	5	5	0.50±0.17	1.42±0.28	2.41±0.20	3.71±0.16
(VIII) Middle dosage of DBS	5	10	0.46±0.20	1.24±0.37**	2.21±0.20*	3.56±0.22*
(IX) High dosage of DBS	5	15	0.72±0.32	1.39±0.16	2.31±0.20	3.65±0.37
(X) Control	5	0	0.59±0.20	1.45±0.32	2.38±0.18	3.70±0.25

**Superscript differs significantly ($p<0.01$) compared with group X. *Superscript differs notably ($p<0.05$) compared with group X.

Data statistics

All data had a normal distribution presented as mean ± standard deviation (SD) and analysed by Statistical Package for Social Sciences (SPSS) 13.0 statistical software. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by student's *t*-test. A probability of less than 0.01 was considered to be statistically significant.

RESULTS

Effects of traditional Chinese herbal medicines on blood cell count

As shown in Table 1, the count of WBC and RBC in control group X is much lower than that in normal adult hens. The counts of WBC and RBC in normal adult hens are 30×10^9 and 3.5×10^{12} L^{-1}, respectively. The results indicated that the hematological parameter and immunity were abnormal in chickens. DBS in group VIII (10 mg/kg) could significantly increase the count of RBC ($p<0.01$), the content of Hb ($p<0.01$) and PCV ($p<0.01$) and at the same time notably increase the count of WBC ($p<0.05$) than the control group. DBS in group IX (15 mg/kg) had no more effect on PCV, but had notably effects on the count of WBC, RBC and the content of Hb ($p<0.05$) than the control group X. ASE in group VI (15 mg/kg) could notably increase the count of RBC and the content of Hb ($p<0.05$), but had no more effect on WBC and PCV ($p>0.05$) than control group X.

However, AM in groups I, II and II (5, 10, and 15 mg/kg), ASE in groups IV and V (5 and 10 mg/kg) and DBS in group VII (5 mg/kg) had no more change on the blood cell count than those of the control group. In a word, DBS (10 mg/kg) could increase the count of WBC and RBC, the content of Hb, PCV, and the effects were better than other groups.

Effects of traditional Chinese herbal medicines on the dropping rates of blood cells

Table 2 shows that there was a significant decrease in the dropping rate of blood cells at 30 min ($p<0.01$) and

Table 3. Effects of traditional Chinese herbal medicines on antibody level of ND index in chickens.

Group	N	Dosage (mg/kg)	Antibody level of ND at day 8	Antibody level of ND at day 15	Antibody level of ND at day 22	Antibody level of ND at day 29
(I) Low dosage of AM	5	5	3.40±0.45	4.60±0.55	5.70±0.45	4.10±0.55
(II) Middle dosage of AM	5	10	3.40±0.55	4.90±0.45	6.00±0.45	4.50±0.89
(III) High dosage of AM	5	15	3.20±0.45	4.70±0.89	5.90±0.71	4.30±0.55
(IV) Low dosage of AMS	5	5	3.20±0.55	4.40±0.89	5.90±0.45	4.00±0.55
(V) Middle dosage of ASE	5	10	3.20±0.45	4.60±0.45	5.90±0.55	4.10±0.89
(VI) High dosage ASE	5	15	3.20±0.45	4.60±0.71	5.80±0.71	4.00±0.55
(VII) Low dosage of DBS	5	5	3.40±0.55	5.40±0.55*	6.20±0.45*	4.40±0.55
(VIII) Middle dosage of DBS	5	10	3.60±0.55*	5.80±0.45*	7.40±0.55*	5.40±0.89*
(IX) High dosage of DBS	5	15	3.20±0.45	4.90±0.89	6.00±0.71	4.60±0.55
(X) Control	5	0	3.20±0.45	4.40±0.55	5.60±0.55	3.80±0.45

*Superscript differs notably ($p<0.05$) compared with group X.

notable decrease at 45 and 60 min in group VIII (DBS, 10 mg/kg) than the control group. However, there was no change in other groups than the control group ($p>0.05$).

Effects of traditional Chinese herbal medicines on antibody level of ND

Table 3 shows that the antibody level of ND in groups I to VI had no change than the control group ($p>0.05$). DBS in group VIII (10 mg/kg) had notable effects on the antibody level of ND at days 8, 15, 22 and 29 than the control group ($p<0.05$). DBS in group VII (5 mg/kg) had notable effects on the antibody level of ND at days 15 and 22 than the control group ($p<0.05$), but had no more change at days 8 and 22 than the control group ($p<0.05$). Although all groups of ND antibody reached the high level at day 22, DBS (5 and 10 mg/kg) could only notably increase the antibody level of ND than the control group ($p<0.05$). In a word, the different dosages of single AM and ASE had no notable effects on the antibody level of ND, but DBS (10 mg/kg) could improve the antibody level of ND and maintain a high level for a long time.

DISCUSSION

The results of this study clearly demonstrated that DBS (10 mg/kg) could not only increase WBC, RBC, HB, PVC but also improve “blood” deficiency by the dropping rate of blood cells and the antibody level of ND (Tables 1 and 2, and Figure 1). Only AM or ASE had no effect. At the same time, low or high dosages of DBS were not suitable.

Syndrome of “blood” and “Qi” deficiency is inevitable for chickens, because of physiological characteristics. As the laying week is increased, primordial and growing follicles gradually atrophy, but atretic follicles increases in ovarium. So the laying rate will decrease (Palmer and Bahr, 1992). Chickens are liable to diseases, for example ND. Therefore, it is very important to find an approach for improving “blood” and “Qi” deficiency.

Blood cell count is indicators of blood cell production and quality. They are affected by diseases more or less, so blood cell count is used for diagnosis, treatment and prognosis (Morley, 1995; Roberts and EL-Badawi, 1985). Clinically, blood loss and anemia often present with the reduction of erythrocyte and hemoglobin content (Hatano et al., 2004; Teng, 1994). ASE as the traditional Chinese medicine is one of the most popular medicine to treat blood deficient syndrome, and Angelica polysaccharide is the major component of *Angelica sinensis* in enriching blood. Studies demonstrated that Angelica polysaccharide could affect the hemopoietic system of animal and had obvious promoter action on proliferation and differentiation of myelogenous hemopoietic progenitor cell of mouse and human. Angelica polysaccharide could improve the hemopoietic function of radiation injured mice (Hong, 2001). Angelica polysaccharide (APS), as the main component of Herbal medicine Angelica, has the efficacy of enriching blood, purifying blood quality, emmenagoue, acesodyne, lenitive and improving circulation (Varga et al., 2010; Wang et al., 2006).. Induced by Angelica polysaccharide, protein levels of eosinophil peroxidase (EPO), IL-3, and IL-6 expressed by bone marrow macrophage were improved differently (Li and Wang, 2005).

The results indicated that the count of WBC were notably increased by DBS (10 and 15 mg/kg) compared with the control group. The changes of WBC counts are important to clinical diagnosis and treatment of disease. DBS could increase immunity, however, AM or ASE had no more effect on the count of WBC. The count of RBC and the content of Hb can display status of hematopoietic functions. In this experiment, the results also indicated that AM (15 mg/kg), DBS (10 mg/kg) and (15 mg/kg) could increase the count of RBC and the content of Hb. It showed that AM could strengthen status of hematopoietic

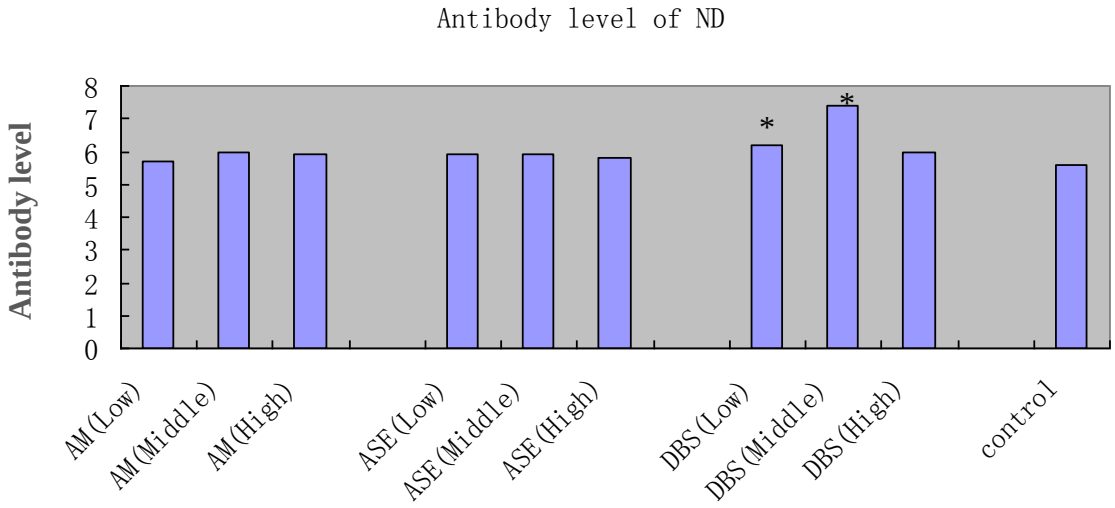

Figure 1. Effects of traditional Chinese herbal medicines on antibody level of ND index in chickens at D22.*Superscript differs notably ($p<0.05$) compared with group control.

functions, and then nourish “blood”. The results also indicated that DBS (10 mg/kg) could improve “blood” deficiency by the dropping the rate of blood cells.

Antibody level of ND can reflect humoral immunity of animals. It was proven by modern pharmacology that polysaccharide is the main components to enhance immunity in AM and ASE. Humoral immunity is a kind of important specific immune response mediated by B lymphocytes, and also one of the main factors in resisting infectious diseases. Angelica polysaccharide can promote cellular immunity and then promote the immune response of lymphocyte (Xia and Peng, 1999). It can be seen that Angelica polysaccharide does not only promote T lymphocytes to produce IL-2, but also increase macrophage to secrete TNF-α.

Angelica polysaccharide could strengthen secondary erythrocyte immunity and affect the coordination of immune function directly or indirectly. The investigators clearly stated that the immunomodulatory effects of APS were extensive, including promoting both cellular and humoral immunity, enriching the immune functions in a murine immunosuppressive model, and modulating the production of cytokines (Tu et al., 1995). DBS (10 mg/Kkg) could improve the antibody level of ND, which displayed the coordination between AM and ASE. However, only AM or ASE had no effect. At the same time, low or high dosages of DBS were not suitable.

The AM fills the gas of lienal lung and promotes blood source. AM used in combination with ASE nourish “Qi” and “blood” and help to keep abundance in “Qi” and “blood”. In a word, the results indicated that DBS (10 mg/kg) could improve the symptoms of “blood” and “Qi” deficiency and were suitable for the clinical manifestations. The results were the implication to chickens in several farms for two years. It increased egg production and laying rate and reduced medical expenses due to diseases, which will bring huge economic and social benefits. One of the limitation of this study is that there was no research on the stability of hemopoietic function and immunity of DBS, but with the development of scientific, technological and traditional Chinese medicine theory, the stability and mechanism of DBS on enriching “blood” and “Qi” will be deepened and systematized gradually.

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