Therapeutic effect of notoginseng saponins before and after fermentation on blood deficiency rats

WENJIE SU[1,2*], ZUGUO LIANG[3*], DAIAN PAN[2], LANCAO ZHANG[1], YUYAO ZHANG[1], TONGYI YUAN[3], XIANG GAO[3], HANG SU[1] and HE ZHANG[1,2]

[1]Northeast Asia Research Institute of Traditional Chinese Medicine, Changchun University of Chinese Medicine, Changchun, Jilin 130117; [2]Research Center of Traditional Chinese Medicine, The Affiliated Hospital to Changchun University of Chinese Medicine, Changchun, Jilin 130021; [3]College of Pharmacy, Changchun University of Chinese Medicine, Changchun, Jilin 130117, P.R. China

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Abstract. Notoginseng saponins (NS) are the active ingredients in *Panax notoginseng* (Burk.) F.H. Chen (PN). NS can be transformed depending on how the extract is processed. Fermentation has been shown to produce secondary ginsenosides with increased bioavailability. However, the therapeutic effect of fermented NS (FNS) requires further study. The present study compared the compositions and activities of FNS and NS in blood deficiency rats, which resembles the symptoms of anemia in modern medicine, induced by acetylphenylhydrazine and cyclophosphamide. A total of 32 rats were randomly divided into control, model, FNS and NS groups. A blood deficiency model was established and then treatment was orally administered for 21 days. The results of component analysis indicated that some saponins transformed during the fermentation process resulting in a decrease of notoginsenoside R1, and ginsenosides Rg1, Rb1 and Re, and an increase in ginsenosides Rd, Rh2, compound K, protopanaxadiol and protopanaxatriol. The animal results showed that both FNS and NS increased the number of white blood cells (WBCs), red blood cells, hemoglobin, platelets and reticulocytes, and the levels of granulocyte-macrophage colony-stimulating factor (GM-CSF), erythropoietin (EPO) and thrombopoietin (TPO), decreased the G_0/G_1 phase and increased G_2/M phase, and decreased the apoptosis rate of bone marrow (BM) cells, which suggested a contribution to the recovery of hematopoietic function of the BM cells. FNS and NS increased the protein expression levels of the cytokines IL-4, IL-10, IL-12, IL-13, TGF-β, IL-6, IFN-γ and TNF-α, and the mRNA expression levels of transcription factors GATA binding protein 3 and T-box expressed in T cell (T-bet). FNS and NS treatment also increased the number of $CD4^+$ T cells, and decreased the enlargement of the rat spleen and thymus atrophy, which indicated a protective effect on the organs of the immune system. The results of the present study demonstrated that compared with NS, FNS showed an improved ability to increase the levels of WBCs, lymphocytes, GM-CSF, EPO, TPO, aspartate aminotransferase, IL-10, IL-12, IL-13 and TNF-α, and the mRNA expression levels of T-bet, and decrease alanine aminotransferase levels. The differences seen for FNS treatment could arise from their improved bioavailability compared with NS, due to the larger proportion of hydrophobic ginsenosides produced during fermentation.

Introduction

In Traditional Chinese Medicine (TCM) it is believed that blood deficiency can be defined as suffering from Qi and blood loss, deficiency in the stomach and spleen, and insufficient hematogenesis. Common symptoms are pale or pale-yellow complexion, pale lips, dizziness, blurred vision, hand and foot numbness, and low menstrual volume in women (1). These symptoms are in line with the symptoms of anemia in modern medicine (2). Patients with malignant tumors are treated with chemotherapy drugs, which usually cause myelosuppression and immunosuppression. The reduction of erythrocytopenia and thrombocytopenia in chemotherapy patients may impede the chemotherapy process, which can affect both the therapeutic outcome and quality of life of patients (3). Therefore, compounds that can treat and/or prevent the side effects of anemia in patients with malignant tumors are of great interest.

Panax notoginseng (Burk.) F.H. Chen (PN) is a well-known traditional herb in China and has been used in TCM for >2,000 years. PN can promote blood circulation, hemostasis, detumescence and relieve pain. Saponins are a general term for a type of glycoside composed of triterpenes or spirostanes. The saponins first found in ginseng that were

Correspondence to: Dr Hang Su or Professor He Zhang, Northeast Asia Research Institute of Traditional Chinese Medicine, Changchun University of Chinese Medicine, 1035 Boshuo Road, Changchun, Jilin 130117, P.R. China
E-mail: suhang0720@live.cn
E-mail: 13843148161@163.com

*Contributed equally

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named as ginsenosides Rb1, Rg1, Rd, etc. and in PN were named as notoginsenosides R1, Ft1, and etc. The main active components of PN are saponins, including notoginsenoside R1 (nR1), and ginsenosides Rb1, Rg1 and Rd (4). These saponins are used as anti-inflammatory and antitumor treatments and are also considered to support the immune system, provide cardiovascular protection and promote blood circulation (5,6). Traditionally, PN has two different forms, raw and processed. The processed PN, known as steamed PN, is the key component in generating blood (7), which increases the production of blood cells and is involved in the activation of immune cells through the JAK-STAT signaling pathway, finally promoting hematopoiesis in anemia (8). The process of steaming PN increases the amount and activities of certain ginsenosides. For example, the ginsenoside Rb1 is converted into the more bioactive ginsenoside compound K (CK) and protopanaxadiol (PPD) (9). Although PN is typically processed by steaming, it can also be processed by fermentation. The enzymes produced by microorganisms in the fermentation process hydrolyze the carbohydrate side chains at C-3, C-6 and C-20, which changes the composition and contents of saponins (10). Previous studies (9,10) have reported that the processed PN generates a large number of effective ginsenosides, which differ from the ones found in raw PN. Ginsenosides Rk3, Rh4, Rk1, Rg5, F4, 20(S/R)-Rg3, CK and 20(S/R)-Rh1 are unique saponins that only exist in processed PN but not in raw PN (11). The microbial transformation method is stable in the production of PN or NS (12,13) and can also improve drug efficacy and reduce toxicity. Several studies have reported that the β-glucosidase enzyme produced by *Lactobacillus plantarum*, *Lactobacillus delbrueckii*, *Lactobacillus fermentum*, *Bifidobacterium longum* or *Leuconostoc mesenteroides* could transform the ginsenosides into rare ginsenosides, which are the deglycosylated secondary metabolic derivatives of major ginsenosides and function as active substances (14,15). In the present study, the chemical composition and content of the total saponins from PN before and after fermentation with *Lactobacillus plantarum* were compared.

In anemia, functional recovery of hematopoietic organs is a key process. Hematopoiesis is the differentiation of a small pool of self-renewing pluripotent hematopoietic stem cells (HSCs) to produce blood cells, including white blood cells (WBCs), red blood cells (RBCs), platelets (PLTs) and reticulocytes (Rets) (16). HSCs can produce a new blood cell count to resist the reduction caused by blood deficiency. Hematopoietic cytokines, such as thrombopoietin (TPO), erythropoietin (EPO) and granulocyte-macrophage colony-stimulating factor (GM-CSF), perform vital roles in the progress of hemopoiesis (17). Cytokines are crucial in the inflammatory response to anemia and are necessary for re-establishing flow to the afflicted organs (18). Decreased levels of cytokines suggest organ pathology, which inhibits the development of T cells and other immune cells (19). The effect of saponins on hematopoiesis and immunity should be evaluated for use in treating anemia. However, to the best of our knowledge, no studies have previously reported the therapeutic effect of notoginseng saponins (NS) and fermented NS (FNS) on blood deficiency.

In the present study, the total saponins from PN were fermented with *Lactobacillus plantarum*, and the changes in saponin content were evaluated. Blood deficiency was induced in rats using acetylphenylhydrazine (APH) and cyclophosphamide (CP), and the effects of FNS and NS on blood deficiency parameters were assessed. The present study aimed to provide a useful theoretical basis for the future clinical treatment of blood deficiency.

Materials and methods

Materials. The saponins [notoginsenoside R1 (nR1), ginsenosides Rg1, Rb1, Re, Rd, Rh2, CK, PPD and protopanaxatriol (PPT)] and NS were purchased from Shanghai Yuanye Biotechnology Co., Ltd. The saponins were supplied with a purity of >99.0%. APH and CP were purchased from MilliporeSigma. The ELISA kits for interleukin(IL)-4, IL-6, IL-10, IL-12, IL-13, transforming growth factor-β (TGF-β), interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), thrombopoietin (TPO), erythropoietin (EPO), granulocyte-macrophage colony-stimulating factor (GM-CSF), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), direct bilirubin (DBIL), lactate dehydrogenase (LDH), and transferrin (TRF) were purchased from Nanjing Jiancheng Bioengineering Institute. Cell cycle and apoptosis kits were supplied by BD Biosciences. A blood routine reagent kit was purchased from IDEXX Laboratories Inc.

Preparation of FNS. NS was inoculated with *Lactobacillus plantarum* culture (10% MRS broth (Beijing Solarbio Science & Technology Co., Ltd).; 3.1% of inoculation amount; pH 7.0) and fermented for 3.2 days at 37.6˚C. After fermentation, the culture was freeze-dried and smashed. The FNS and the raw NS powders were stored at -20˚C for later use.

HPLC analysis. A mixed standard solution containing nR1, ginsenosides Rg1, Rb1, PPD, Re, Rd, PPT, CK and Rh2 at 1.5 mg/ml was dissolved in methanol and filtered through a 0.22-μm filter membrane. Sample solutions of FNS and NS (2 mg/ml) were dissolved in methanol and filtered. The saponin contents of FNS and NS were analyzed using the LC-2030 HPLC system (Shimadzu Corporation) and a C18 column (Agilent, 150x4.6 mm; 5 μm). The mobile phase comprised acetonitrile (A) and water (B). The elution gradient was as follows: 0-10 min, 18-23% A; 10-30 min, 23-44% A; 30-38 min, 44-68% A; 38-45 min, 68% A; 45-55 min, 68-100% A; 55-60 min, 100% A; and 60-65 min, 100-18% A. The flow rate was 1.0 ml/min, the detection wavelength was 203 nm, the column oven was maintained at 25˚C and injection volume was 10 μl.

Animal model. A total of 32 male Wistar rats (weight, 200.0±20.0 g; age, 8 weeks) were supplied by Changchun Yisi Experimental Animal Technology Co., Ltd. [animal license no. SCXK (Ji)-2021-0003]. The rats had *ad libitum* access to food and water and were kept in an environment with controlled light (12 h light/dark cycle), temperature (25±1˚C) and relative humidity (60±5%). This experiment was authorized by the Bioethics Committee of Changchun University of Chinese Medicine and the Institutional Animal Care (approval no. 2022156; Changchun, China), and was performed based on

the NIH guide for the care and use of laboratory animals (20). After a 3-day period of acclimation, 32 rats were divided into the control, model, FNS and NS groups (n=8). To induce the blood deficiency model, the rats (model, FNS and NS groups) were subcutaneously injected in the neck with 2% APH normal saline (20 and 10 mg/kg) on days 1 and 4, respectively. At 2 h after injection on day 4, the rats were intraperitoneally injected with CP normal saline (20 mg/kg), this was repeated on days 5, 6 and 7 (21). After APH and CP treatment for 24 h, which started on day 8, the rats were intragastrically administered FNS (250 mg/kg) and NS (250 mg/kg), and the rats in the control and model groups were intragastrically administered 0.9% normal saline (1 ml/100 g body weight), once a day for 21 consecutive days. The safe clinical NS dosage was 2.5-10 mg/kg per day (22), which could be transformed into 15.75-63 mg/kg daily for rats (obversion coefficient=6.3). A dose of 250 mg/kg is four times the amount of the maximum clinical dose (22). The body weight of each rat was measured daily. After 21 days of drug treatment, all rats were euthanized by cervical dislocation under anesthesia with 30 mg/kg pentobarbital sodium via intraperitoneal injection after fasting for 24 h.

Routine blood tests. After rats were anesthetized as aforementioned, blood from the abdominal aorta was collected in a tube with K_2-EDTA. The WBC, RBC, hemoglobin (HGB), PLT and Ret parameters of rats were detected using the XT-2000i automated hematology analyzer (Sysmex Corporation) (n=6). In the remaining rats, the blood was used in other tests. HCT parameter was calculated as follows: HGB (%)=RBC (10^{12}/l) x MCV (fl).

Cytokine and biochemical parameter assays in serum. Blood prepared with K_2-EDTA as aforementioned was centrifuged at 4˚C at 5,760 x g for 5 min to collect serum. Hematopoiesis-related cytokines EPO (cat. no. H051), TPO (cat. no. H482-1) and GM-CSF (cat. no. H060), and inflammatory cytokines IL-4 (cat. no. H005-1-2), IL-6 (cat. no. H007-1-2), IL-10 (cat. no. H009-1-2), IL-12 (cat. no. H010-1-2), IL-13 (cat. no. H011), TGF (cat. no. H034-1-2), IFN-γ (cat. no. H025-1-2) and TNF-α (cat. no. H052-1-2) were analyzed using ELISA kits.

The whole blood of rats was collected from the abdominal aorta in a tube with an additive clot activator and then centrifuged at 4˚C at 5,760 x g for 5 min to collect the serum. The biochemical parameters, such as alkaline phosphatase (ALP; cat. no. A059-2-2), alanine aminotransferase (ALT; cat. no. C009-2-1), aspartate aminotransferase (AST; cat. no. C010-2-1), direct bilirubin (DBIL; cat. no. C019-2-1), lactate dehydrogenase (LDH; cat. no. A020-2-2) and transferrin (TRF; cat. no. H130-1-2), were detected using ELISA kits.

Cell cycle and apoptosis analysis of bone marrow (BM) cells. BM from the left femur was flushed with sterile PBS. A single cell suspension (1x10^6 cells/ml) in PBS was centrifuged at 300 x g for 5 min at room temperature. The BM cells were fixed with 70% ethanol at 4˚C overnight. After washing twice with PBS, the cells were resuspended with 1 ml PI/Triton X-100 staining solution with RNase A (Beyotime Institute of Biotechnology) and incubated for 30 min at room temperature (n=6). The cells were quantified using a DxFLEX flow cytometer (Beckman Coulter, Inc.). The cell cycle distribution of the BM cells was analyzed using ModFit LT 5.0 software (Verity Software House, Inc.).

The apoptosis rate of the BM cells (1x10^6 cells/ml) prepared as aforementioned was measured using an Annexin V-FITC/PI apoptosis kit (Beyotime Institute of Biotechnology) according to the manufacturer's instructions and a DxFLEX flow cytometer with CytExpert software 5.0 (Beckman Coulter, Inc.) (n=6). In the remaining rats, the tissue was used in other tests.

Western blotting. Single suspension cells from rats for each group were collected from the left femur, total protein was obtained by using RIPA lysis buffer containing protease/phosphatase inhibitor cocktail (Beyotime Institute of Biotechnology). The total protein concentration was determined using a BCA Protein Assay Kit (Beyotime Institute of Biotechnology) and 30 μg protein per lane was separated by 10% SDS-PAGE and transferred to PVDF membranes. The membranes were blocked with 5% (w/v) nonfat dried milk for 2 h at room temperature and incubated with specific primary antibodies at 4˚C overnight. The primary antibodies used were as follows: Cyclin A polyclonal antibody (1:500; cat. no. BS1083; Bioworld Technology, Inc.), cyclin D1 polyclonal antibody (1:500; cat. no. BS1741; Bioworld Technology, Inc.), Bcl-2 (1:500; cat. no. BS80057; Bioworld Technology, Inc.), Bax (1:500; cat. no. BS79682; Bioworld Technology, Inc.) and β-actin monoclonal antibody (1:5,000; cat. no. BS6007M; Bioworld Technology, Inc.). The membranes were subsequently washed in TBS with 0.1% Tween-20 (TBST) and incubated with secondary antibodies, HRP-labelled goat anti-mouse IgG (H+L) (1:5,000; cat. no. ZJ2020-M; Bioworld Technology, Inc.) or HRP-labelled goat anti-rabbit IgG (H+L) (1:5,000; cat. no. ZJ2020-R; Bioworld Technology, Inc.), at room temperature for 1.5 h. Following washing with TBST (0.1% Tween-20), protein bands were visualized using a BeyoECL Plus Kit (Beyotime Institute of Biotechnology). Immunoreactive protein bands were quantified by using a ChemiDocTM MP imaging system (Bio-Rad Laboratories, Inc.).

Splenic T-lymphocyte (LYMPH) subpopulation assay. Splenocytes (1x10^6 cells/ml) from rat spleen were prepared using sterile PBS according to the aforementioned method used for BM cells. Splenocytes were labeled with FITC-conjugated anti-rat CD4 (2.5 μg/ml final concentration; cat. no. 201505; BioLegend, Inc.) and phycoerythrin-conjugated anti-rat CD25 antibodies (2.5 μg/ml final concentration; cat. no. 202105; BioLegend, Inc.). The labeled splenocytes were washed twice with PBS and resuspended in the PBS buffer, and the expression of CD4 and CD25 by splenocytes was detected using a DxFLEX flow cytometer with CytExpert software 5.0 (Beckman Coulter, Inc.).

Thymus and spleen indexes. After the rats were sacrificed, the spleen and thymus were collected and weighed. Thymus and spleen indexes were calculated as follows: Organ index=organ weight (g)/body weight (g).

H&E staining. Spleen and thymus specimens were fixed in 10% formalin solution for 48 h at room temperature, paraffin embedded and cut into 4 μm sections. The paraffin slices underwent H&E staining, with hematoxylin for 3 min and eosin for 30 sec at room temperature, for routine morphological analysis. Images were captured using a fluorescence microscope (Nikon Corporation) at a magnification of x400.

RNA extraction and reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from the rat spleens with TRIzol® reagent (Thermo Fisher Scientific, Inc.). Total RNA was reverse transcribed into cDNA using the FastKing RT Kit (Tiangen Biotech Co., Ltd.) according to the manufacturer's protocol. qPCR was performed using the CFX Connect Real-Time PCR Detection System (Bio-Rad Laboratories, Inc.) with SuperReal PreMix Plus SYBR Green reagent (Tiangen Biotech Co., Ltd.). The primer sequences used for qPCR were as follows: β-actin forward (F), 5'-CTGTCCCTG TATGCCTCTG-3' and reverse (R), 5'-ATGTCACGCACG ATTTCC-3'; EPO F, 5'-GGGGGTGCCCGAACG-3' and R, 5'-GGCCCCCAGAATATCACTGC-3'; TPO F, 5'-GAACCC AGCTTCCTCCACAG-3' and R, 5'-CCTTTCCCCGAAGCA GTTGT-3'; GM-CSF F, 5'-TCCTAAATGACATGCGTGCT-3' and R, GCCATTGAGTTTGGTGAGGT; IL-4 F, 5'-CTT GCTGTCACCCTGTTC-3' and R, 5'-CATGGAAGTGCA GGACTGC-3'; IL-6 F, 5'-GAGTTCCGTTTCTACCTG-3' and R, 5'-CTCTGGCTTTGTCTTTCT-3'; IFN-γ F, 5'-CGT CTTGGTTTTGCAGCTC-3' and R, 5'-ACTCCTTTTCCG CTTCCTT-3'; GATA binding protein 3 (GATA-3) F, 5'-CTG GCTGGATGGCGGCAAAG-3' and R, 5'-TGGGCGGGA AGGTGAAGAG-3'; and T-bet F, 5'-AACCAGTATCCTGTT CCCAGC-3' and R, 5'-TGTCGCCACTGGAAGGATA-3'. The thermocycling conditions were as follows: 95˚C for 15 min, followed by 40 cycles of 95˚C for 10 sec, 55˚C for 20 sec and 72˚C for 30 sec. The transcript levels were quantified and normalized to the internal reference gene β-actin using the $2^{-\Delta\Delta Cq}$ method (23).

Statistical analysis. All experiment data are presented as the mean ± SD. The significance of differences was analyzed using one-way ANOVA with Tukey's post hoc test using GraphPad Prism 8.0 software (Dotmatics). $P<0.05$ was considered to indicate a statistically significant difference.

Results

HPLC analysis of FNS and NS. Saponins were the main active ingredients in FNS and NS with nine saponins detected using HPLC. As shown in Fig. 1 and Table I, the levels of the ginsenosides PPD, Rd, PPT, CK and Rh2 were increased in FNS, and nR1, Rg1, Rb1 and Re levels were decreased in FNS compared with NS. In summary, there was a marked difference in saponin content between FNS and NS.

Effect of FNS and NS on blood cell parameters of blood deficiency rats. APH and CP treatment in the model rats significantly reduced the WBC, RBC, HGB, PLT and Ret levels compared with those in the control group. After FNS and NS treatment for 21 days, most blood cell parameters were significantly increased compared with the model group (Fig. 2).

WBC parameters. For the WBC parameters, the model group exhibited significantly decreased WBC, LYMPH and monocyte (MONO) levels compared with the control group. A decrease in neutrophil (NEUT) levels was observed compared with the model group; however, this was not statistically significant. FNS treatment significantly increased WBC, LYMPH, NEUT and MONO levels, and NS significantly increased WBC, LYMPH and NEUT levels compared with the model group. MONO levels were also increased in the NS group; however, this was not statistically significant compared with the model group. Furthermore, the WBC and LYMPH levels of rats treated with FNS were significantly higher than those of rats in the NS group (Fig. 2A).

RBC parameters. In terms of the RBC parameters, the RBC levels of rats in the model group were significantly reduced, and the mean corpuscular volume (MCV), red cell distribution width-standard deviation (RDW-SD) and red cell distribution width-coefficient of variation (RDW-CV) levels were significantly increased compared with the control group. No significant decrease in MCV and RDW-SD was seen in the FNS and NS treatment groups compared with the model group. However, a significant increase in RDW-CV levels was seen in FNS-treated rats compared with the model group, and these were also significantly higher than the levels in the NS-treated group. The hematocrit (HCT) was calculated according to the RBCs and MCV. According to the results, the HCT of the model rats demonstrated no significant difference compared with the control group. Both FNS and NS significantly increased the RBC and HCT levels compared with the model group. (Fig. 2B).

HGB parameters. The HGB and mean corpuscular hemoglobin concentration (MCHC) levels of rats were significantly decreased and mean corpuscular hemoglobin (MCH) levels were significantly increased in the model group compared with the control group. Both FNS and NS significantly elevated the HGB level compared with the model group; however, there was no significant difference in MCH and MCHC levels compared with the model rats (Fig. 2C).

PLT parameters. PLT and plateletcrit (PCT) levels of model rats were significantly decreased, and the platelet distribution width (PDW), mean platelet volume (MPV) and platelet larger cell ratio (P-LCR) were significantly increased in the model group compared with the control group. FNS and NS treatment significantly increased PLT levels, and significantly reduced MPV and P-LCR levels compared with the model group. However, there was no statistically significant difference in PCT and PDW levels compared with the model group (Fig. 2D).

Ret parameters. The Ret level of model rats was significantly reduced compared with the control group, and the model group exhibited no significant change in immature Ret fraction (IRF), low fluorescence ratio (LRF), middle fluorescence ratio (MRF) and high fluorescence ratio (HRF) compared with the control group. FNS significantly increased the Ret, IRF and HRF levels of rats, and NS significantly elevated the Ret and IRF levels compared with the model group. NS and FNS also significantly decreased the LRF level of rats compared with the model group (Fig. 2E).

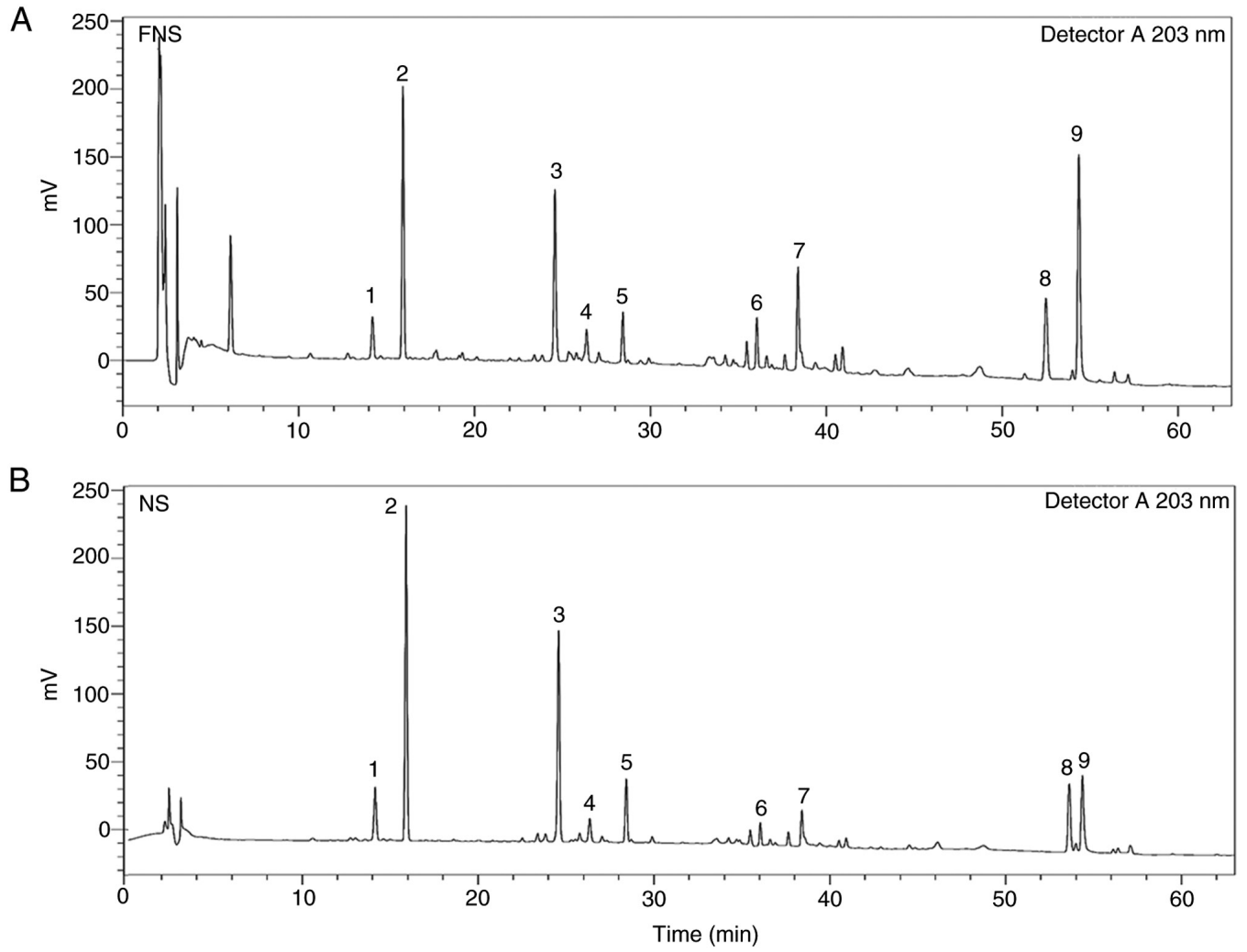

Figure 1. Chromatograms of (A) FNS and (B) NS from high-performance liquid chromatography. 1, notoginsenoside R1; 2, Rg1; 3, Rb1; 4, protopanaxadiol; 5, Re; 6, Rd; 7, protopanaxatriol; 8, compound K; and 9, Rh2. FNS, fermented notoginseng saponins; NS, notoginseng saponins.

Effect of FNS and NS on hematopoiesis-related cytokines and biochemical parameters of blood deficiency rats. In model rats, the GM-CSF, EPO and TPO levels were significantly reduced compared with the control group. Treatment with FNS and NS significantly elevated the GM-CSF, EPO and TPO levels of rats compared with the model group. The GM-CSF, EPO and TPO levels of rats treated with FNS were significantly higher than those of rats treated with NS (Fig. 3A).

CP is metabolized in the liver and can cause liver damage (24) as seen in the model rats where the ALP, ALT and DBIL levels were significantly increased and AST, LDH and TRF levels were significantly decreased compared with the control group. FNS and NS significantly reduced ALP, ALT and DBIL and significantly increased AST, LDH and TRF levels compared with the model group. ALT levels in FNS-treated rats were significantly reduced and AST levels were significantly increased compared with those in the NS-treated rats (Fig. 3B).

Effect of FNS and NS on inflammatory cytokines of blood deficiency rats. APH and CP can reduce the efficiency of the immune system and cause an imbalance between anti-inflammatory and pro-inflammatory cytokines (25,26).

The levels of anti-inflammatory cytokines (IL-4, IL-10, IL-12, IL-13 and TGF-β) and pro-inflammatory cytokines (IL-6, IFN-γ and TNF-α) in the model rats were significantly reduced compared with those in the control group (27,28). Both FNS and NS significantly increased the levels of these indicators compared with those in the model rats. There was a significant increase in IL-10, IL-12, IL-13 and TNF-α levels in FNS-treated rats compared with the NS-treated group (Fig. 4).

Effect of FNS and NS on the cell cycle and apoptosis of BM cells from blood deficiency rats. Chemotherapy causes myelosuppression, damages the DNA, affects normal hematopoiesis and changes the proportions of cells in different cell cycle phases (29).

The percentage of BM cells in the G_0/G_1 phase was significantly increased and the percentage of BM cells in the G_2/M phase was significantly decreased in model rats compared with the control group (Fig. 5A and B). No significant difference in the percentage of cells in the S phase was seen compared with the control. After treatment with FNS and NS, the percentage of BM cells in the G_0/G_1 phase was significantly decreased and the percentage of BM cells in G_2/M was significantly increased compared with the model group. An increase in the

Table I. Saponin contents of FNS and NS.

Peak no.	Ginsenosides	Type of saponin	FNS, g/100 g	NS, g/100 g
1	nR1	PPT	5.36±0.68	7.58±0.75
2	Rg1	PPT	12.42±0.93	19.84±1.31
3	Rb1	PPD	16.25±2.35	23.34±2.22
4	PPD	PPD	1.37±0.21	0.87±0.19
5	Re	PPT	2.64±0.37	3.15±0.32
6	Rd	PPD	1.93±0.29	0.57±0.08
7	PPT	PPT	5.50±0.67	2.27±0.38
8	CK	PPD	14.08±1.97	10.80±1.21
9	Rh2	PPD	11.23±1.33	5.65±0.83

nR1, notoginsenoside R1; CK, compound K; PPT, protopanaxatriol; PPD, protopanaxadiol; NS, notoginseng saponins; FNS, fermented notoginseng saponins.

proportion of cells in S phase was also seen after treatment with FNS and NS; however, the difference was not statistically significant compared with the model group (Fig. 5B). These results demonstrated that FNS and NS effectively improved the recovery of hemopoiesis in blood deficiency rats by increasing the progression of BM cells from G_0/G_1 phase arrest into G_2/M and S phases.

The protein expression levels of cyclin A and cyclin D1 in BM cells were significantly decreased in blood deficiency model rats compared with the control group. FNS and NS treatment significantly increased the protein expression levels of cyclin A and cyclin D1 compared with the model group. Furthermore, a significant increase in cyclin A and cyclin D1 protein expression levels was seen in FNS-treated rats compared with NS-treated rats (Fig. 5C).

Apoptosis and proliferation of BM cells are coupled and are responsible for the maintenance of hematopoiesis in the hematopoietic system (30). The apoptosis rates of BM cells in the model group, including the early, late and total apoptosis rates, were significantly increased compared with the control group. The early, late and total apoptosis rates of BM cells were significantly decreased after FNS and NS administration compared with the model group. Furthermore, the late and total apoptosis rates of BM cells isolated from rats treated with FNS were significantly decreased compared with the NS group (Fig. 6A and B).

Furthermore, the expression levels of anti-apoptotic protein Bcl-2 and pro-apoptotic protein Bax were detected. These proteins can prevent or promote cell apoptosis and prolong or shorten cell lifespan, respectively (31,32). In model rats, the relative expression levels of Bcl-2 were significantly decreased and the relative expression levels of Bax were significantly increased compared with the control group. FNS and NS significantly increased Bcl-2 protein expression and FNS significantly reduced Bax protein expression compared with the model group. The reduction of Bax protein expression in the NS group was not statistically significant compared with the model group. Furthermore, the relative protein expression levels of Bcl-2 were significantly increased in FNS-treated rats and the relative protein expression levels of Bax were significantly reduced compared with the NS-treated group (Fig. 6C). These results suggested that APH and CP could accelerate the apoptosis of BM cells and that the anti-apoptotic effect of FNS was superior to NS in the relative expression of Bcl-2 and Bax.

Effect of FNS and NS on T cells of blood deficiency rats. APH and CP can damage immunological self-tolerance and homeostasis, and significantly influence the function of T cells (33). The levels of $CD4^+$, $CD25^+$ and $CD4^+CD25^+$ T cells from the spleen were measured using flow cytometry. In the model group, $CD4^+$ T cell levels were significantly decreased and the levels of $CD25^+$ and $CD4^+CD25^+$ T cells were significantly increased compared with those in the control group. FNS treatment significantly increased the percentage of $CD4^+$ T cells compared with the model group. There was a significant decrease in the percentages of $CD25^+$ and $CD4^+CD25^+$ T cells in the NS-treated group compared with the model group. There was no significant difference in the percentage of $CD4^+$, $CD25^+$ and $CD4^+CD25^+$ T cells between the FNS and NS groups (Fig. 7).

Effect of FNS and NS on body weight, organ indexes, and the morphology of the spleen and thymus of blood deficiency rats. APH and CP seriously affect immune organs, such as the spleen and thymus (33,34). In the model group, there was a significant decrease in the body weight and thymus index, and a significant increase in the spleen index compared with the control group. FNS and NS treatment significantly increased the body weight and thymus index and significantly decreased the spleen index compared with the model group. However, there was no statistically significant difference in the body weight, spleen index or thymus index between the FNS and NS groups (Fig. 8A-C).

APH and CP damage the histological structure changes of the rat spleen and thymus, including induction of disorganization in splenic structures, thymic apoptosis, hypocellularity and atrophy (35,36). H&E staining showed that the splenic cord, splenic sinus and trabecula in the red pulp (RP) were displayed clearly in the control group. However, in the model group, RP expansion, white pulp (WP) and central artery shrinking were visible, which indicated neutrophil accumulation and a decreasing level of LYMPHs, respectively. The marginal zone

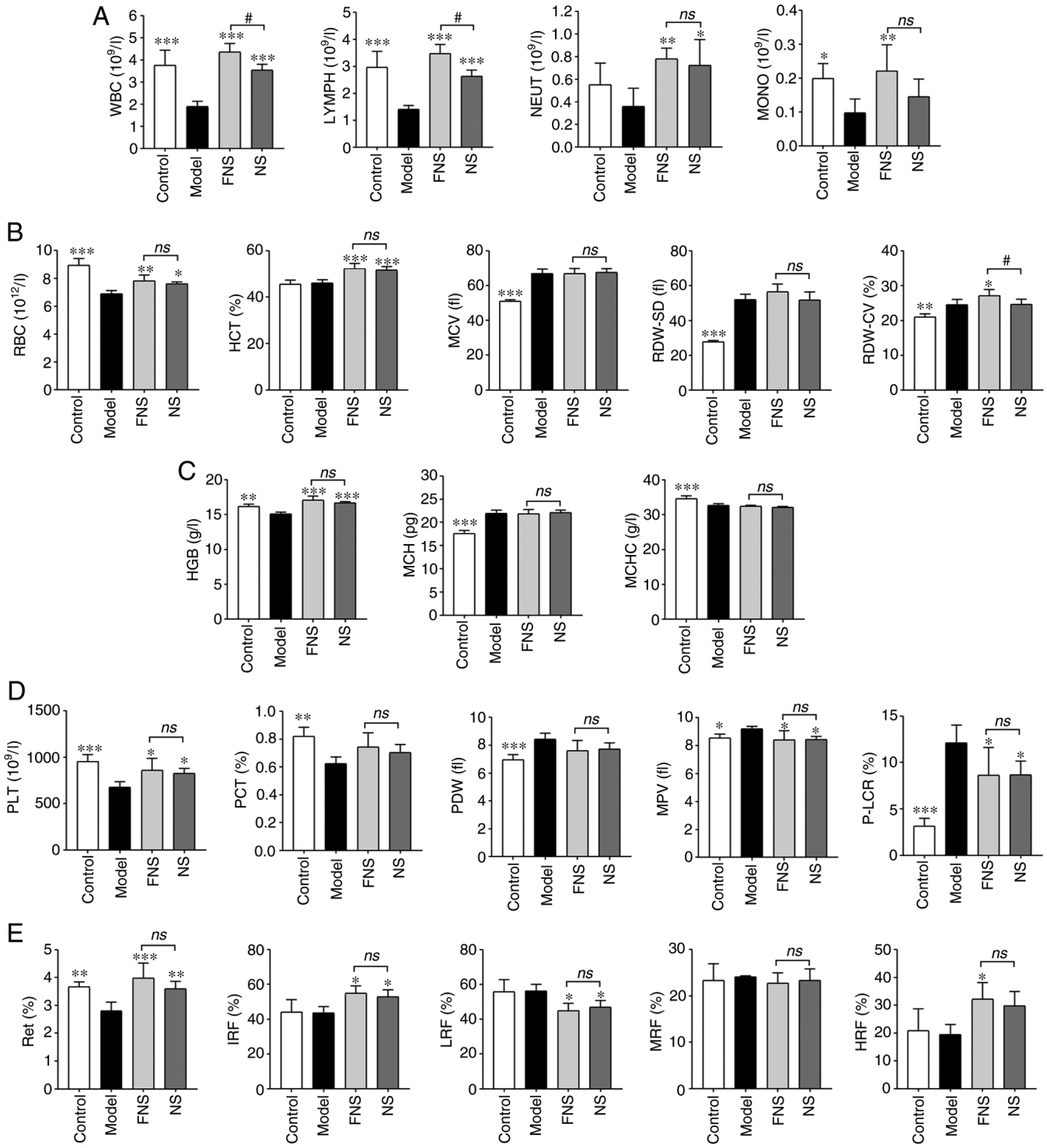

Figure 2. Effect of FNS and NS on (A) WBC, (B) RBC, (C) HGB, (D) PLT and (E) Ret parameters in blood deficiency rats. The data are presented as the mean ± SD (n=6). *P<0.05, **P<0.01 and ***P<0.001 compared with the model group; and $^{\#}$P<0.05 compared with the NS group. NS, notoginseng saponins; FNS, fermented notoginseng saponins; WBC, white blood cell; LYMPH, lymphocyte in the plasma; NEUT, neutrophil; MONO, monocyte; RBC, red blood cell; HCT, hematocrit; MCV, mean corpuscular volume; RDW-SD, red cell distribution width-standard deviation; RDW-CV, red cell distribution width-coefficient of variation; HGB, hemoglobin; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet; PCT, plateletcrit; PDW, platelet distribution width; MPV, mean platelet volume; P-LCR, platelet larger cell ratio; Ret, reticulocyte; IRF, immature reticulocyte fraction; LRF, low fluorescence ratio; MRF, middle fluorescence ratio; HRF, high fluorescence ratio; ns, not statistically significant.

was ambiguous between RP and WP. These results showed a decreasing level of LYMPHs and an increasing level of macrophages. FNS and NS improved the histological structure of the rat spleens compared with the model group. The WP was darker and had extensive distribution which related to an increasing level of macrophages, and the marginal zone was clear in the FNS and NS groups (Fig. 8D).

The cortex (COR) and medulla (MED) of the model rat thymus, which related to the change of thymus morphology and atrophy, became fused in some areas with no apparent

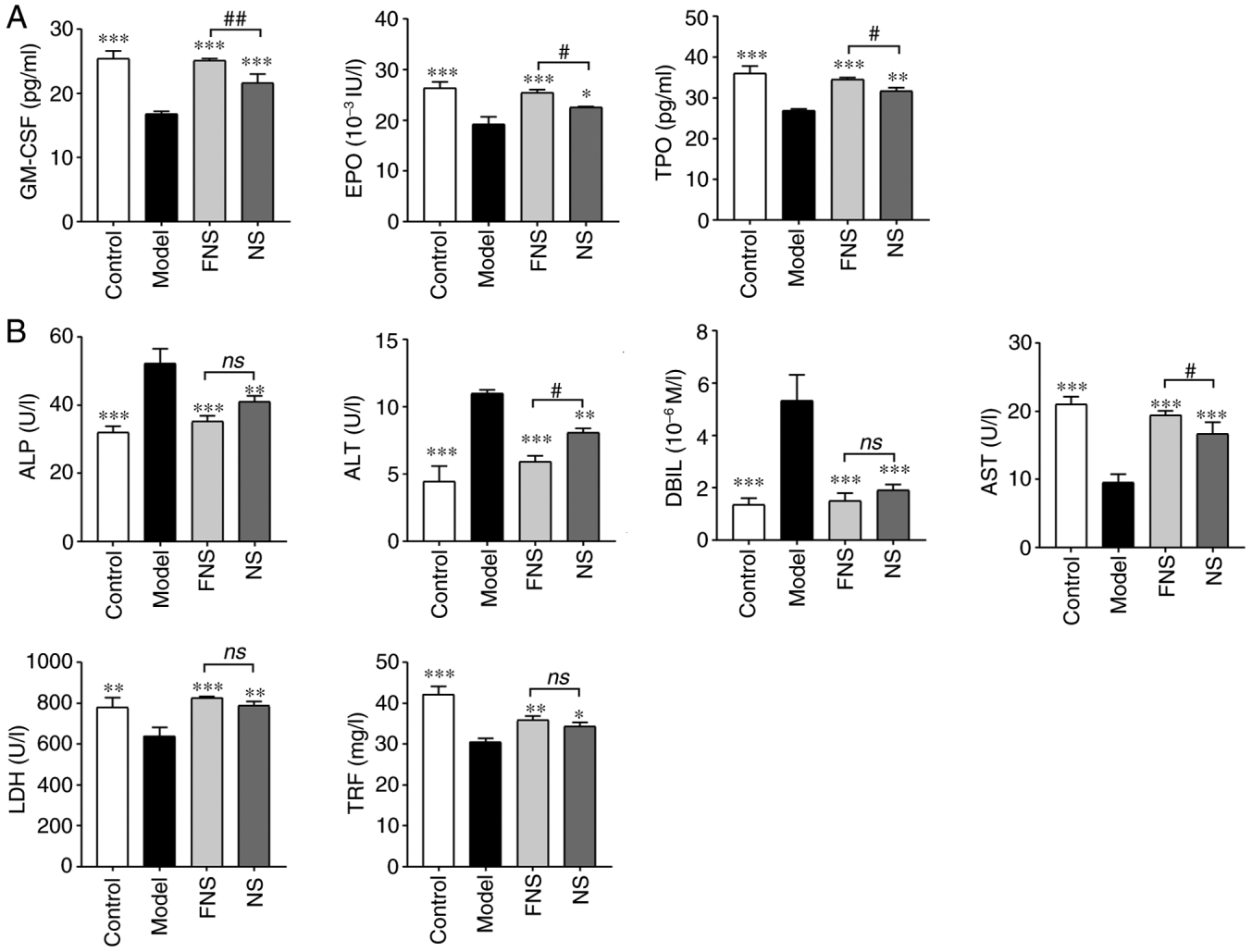

Figure 3. Effect of FNS and NS on (A) hematopoiesis-related cytokines and (B) biochemical parameters of blood deficiency rats. The data are presented as the mean ± SD (n=8). *P<0.05, **P<0.01 and ***P<0.001 compared with the model group; and $^{\#}$P<0.05 and $^{\#\#}$P<0.01 compared with the NS group. GM-CSF, granulocyte-macrophage colony-stimulating factor; EPO, erythropoietin; TPO, thrombopoietin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; DBIL, direct bilirubin; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; TRF, transferrin; NS, notoginseng saponins; FNS, fermented notoginseng saponins; ns, not statistically significant.

marginal zones between them. In the model group, the number of Lcs and epithelial reticular cells (ERCs) was reduced compared with the control, and ERCs provided a scaffold in the COR. The medullary epithelial cells (MECs) and thymic corpuscle (TC) are important to T cell development, and became expanded and irregular in the MED. In the FNS and NS groups, the number of Lcs, ERCs and MECs increased, the COR had deep staining, and round or oval TCs were seen in the MED. Both FNS and NS appeared to reduce tissue damage induced by APH and CP in the model group (Fig. 8E).

Effect of FNS and NS on mRNA expression levels in blood deficiency rats. APH and CP break the balance of body immunity, affect immune organs and the release of immunity cytokines and transcription factors and prevent stem cells from differentiating into hematopoietic cell lineages by affecting the release of cytokines and transcription factors (37,38). In the model group, the mRNA expression levels of hematopoietic cytokines (GM-CSF, EPO and TPO), inflammatory cytokines (IL-4, IL-6 and IFN-γ) and transcription factors (GATA-3 and T-bet) were significantly reduced compared with those in the control group. With NS and FNS treatment, the mRNA expression levels of GM-CSF, EPO, TPO, IL-4, IL-6, IFN-γ, GATA-3 and T-bet were significantly increased compared with the model group. FNS treatment significantly increased GM-CSF, TPO, IL-4, IL-6 and T-bet mRNA expression compared with NS treatment (Fig. 9).

Discussion

According to the theory of TCM, deficiency of viscera and insufficiency of Qi and blood are attributed to blood deficiency. The effect of PN in promoting hemostasis is known as 'the raw materials eliminate and the steamed ones tonify' and steamed PN is considered to possess the function of warming and toning viscera, benefiting Qi and nourishing blood in TCM (39). In TCM it is believed that there are marked differences in composition, activities and efficacy between raw PN and the steamed PN (8). For example, in TCM raw PN is considered to primarily stop bleeding, promote apokatastasis,

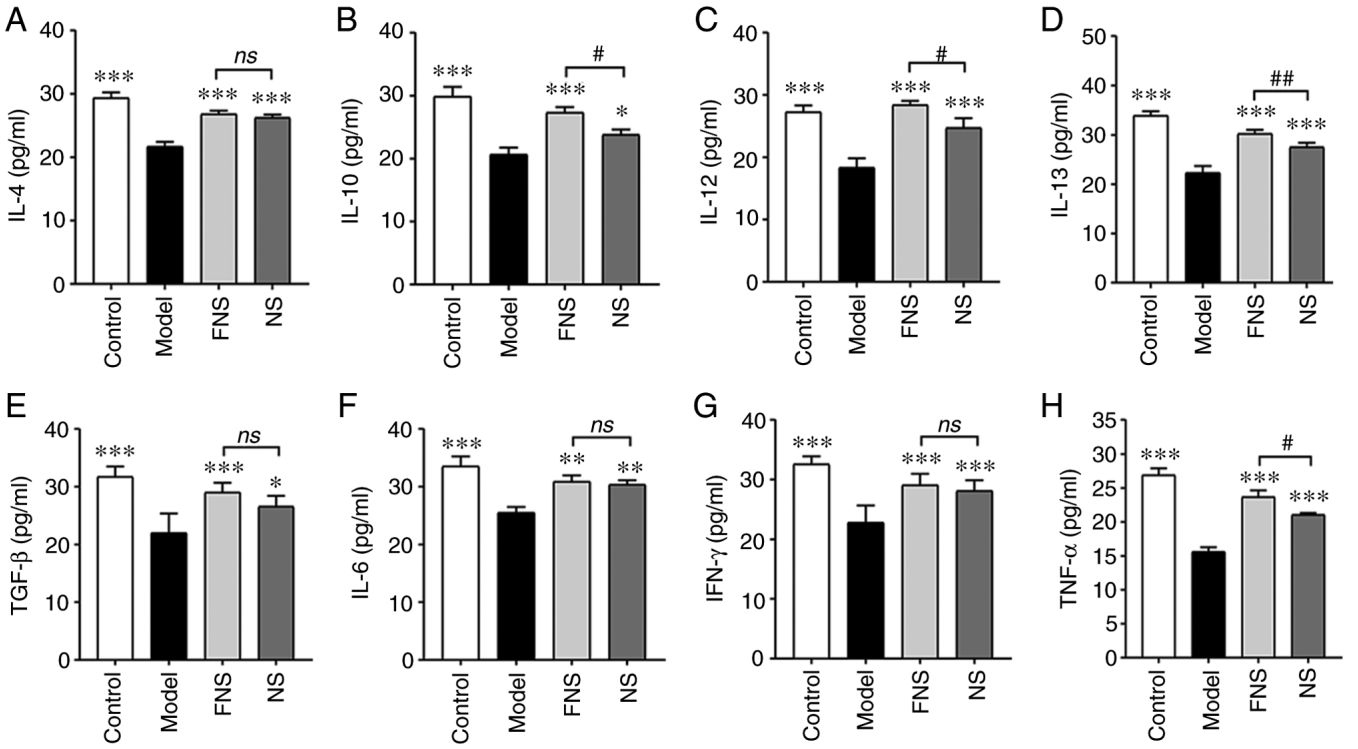

Figure 4. Effect of FNS and NS on inflammatory cytokines of blood deficiency rats. Levels of (A) IL-4, (B) IL-10, (C) IL-12, (D) IL-13, (E) TGF-β, (F) IL-6, (G) IFN-γ and (H) TNF-α were detected using an ELISA. The data are presented as the mean ± SD (n=8). *P<0.05, **P<0.01 and ***P<0.001 compared with the model group; and $^{\#}$P<0.05 and $^{\#\#}$P<0.01 compared with the NS group. NS, notoginseng saponins; FNS, fermented notoginseng saponins; ns, not statistically significant.

strengthen the heart and provide pain relief. Whereas steamed PN is used in TCM to nourish blood, regulate circulation and improve immune function (40).

Previous research has shown that fermentation of PN extracts produces a similar ginsenoside profile to steamed PN (41). *L. plantarum* metabolizes ginsenosides mainly through deglycosylation and dehydration (42). NS contains Rb1, Rg1, Re and nR1 as the major active compounds, which are metabolized by β-glycosidases produced by the gut microbiota (43). The C-20 glucosides of Rb1/Rb2/Rc are deglycosylated to form Rd, which further be converted to Rg3. The C-20 of Rg3 is dehydrated to form Rk1 or Rg5 (44). The C-3 glucoside of Rg3 is deglycosylated to form Rh2 which is then transformed into PPD (45). In addition, the C-3 glucoside of Rb1 is deglycosylated to convert X VII to LXXV/F2, CK and PPD (46,47).

Previous studies have reported on the metabolic pathway for the elimination of the C-20 sugar moieties in Re and Rg1 produce 20(*S*)-Rg2 and 20(*S*)-Rh1, respectively (48,49). It has been suggested that the C-6 rhamnose of Rg2 is eliminated to generate Rh1 (50,51). The elimination of the C-6 glucoside of Re produces Rg1. Rg1 is transformed into Rh1, which further changed into PPT by *L. plantarum* fermentation. Furthermore, the C-20 glucoside of Re is deglycosylated to produce Rg2, which is further dehydrated to Rh4, F4 or Rg6. The C-6 glucoside of Rg6 and nR1 can also be deglycosylated to produce Rk3 and Rg1, respectively (51). In the present study, according to HPLC analysis, *L. plantarum* fermentation transformed nR1, Rg1, Rb1 and Re into their corresponding metabolites, and increased the PPD, Rd, PPT, CK and Rh2 content in FNS compared with unfermented NS.

Hypodermic injection of APH and intraperitoneal injection of CP were used to establish a blood deficiency rat model (52). Hua *et al* (53) and Li *et al* (54) reported that Sprague Dawley rats were hypodermically injected with 2% APH saline solution on days 1 and 4 at a dose of 20 and 40 mg/kg, respectively; 2 h after the hypodermic injection with 2% APH saline solution on day 4, the rats were intraperitoneally injected with CP saline solution on days 4, 5, 6 and 7 at a dose of 20 mg/kg. A similar modeling method has been previously reported in mice; however, the dose of CP was 40 mg/kg on days 4, 5, 6 and 7 (55). Then, the blood deficiency model was created. APH and CP decreased WBCs, RBCs, HGB, PLTs and Rets, and model rats exhibited mental sluggishness, movement retardation, peripheral blood cell count reduction and weight loss (25,53). Liu *et al* (56) reported that PN extract treated with a microwave processing method increased the WBCs and HGB of blood deficiency model mice induced by APH and CP compared with raw PN. In an additional study, steamed PN was demonstrated to elevate the levels of WBCs, RBCs, HGB and PLTs in mice with blood deficiency induced by APH and CP. The PN contents were examined and the main saponins included the notoginsenoside R1, Rg1, Re, Rh1, Rb1, Rd, Rk3, Rh4 and Rg3 (7). The results of the present study demonstrated that FNS and NS significantly improved the blood cell parameters. Notably, the levels of WBCs and LYMPHs of rats treated with FNS were increased compared with those of rats treated with NS.

BM is a key site of hematopoiesis and is responsible for producing new blood cells (57). A series of hematopoiesis-related cytokines, including GM-CSF, EPO and TPO are

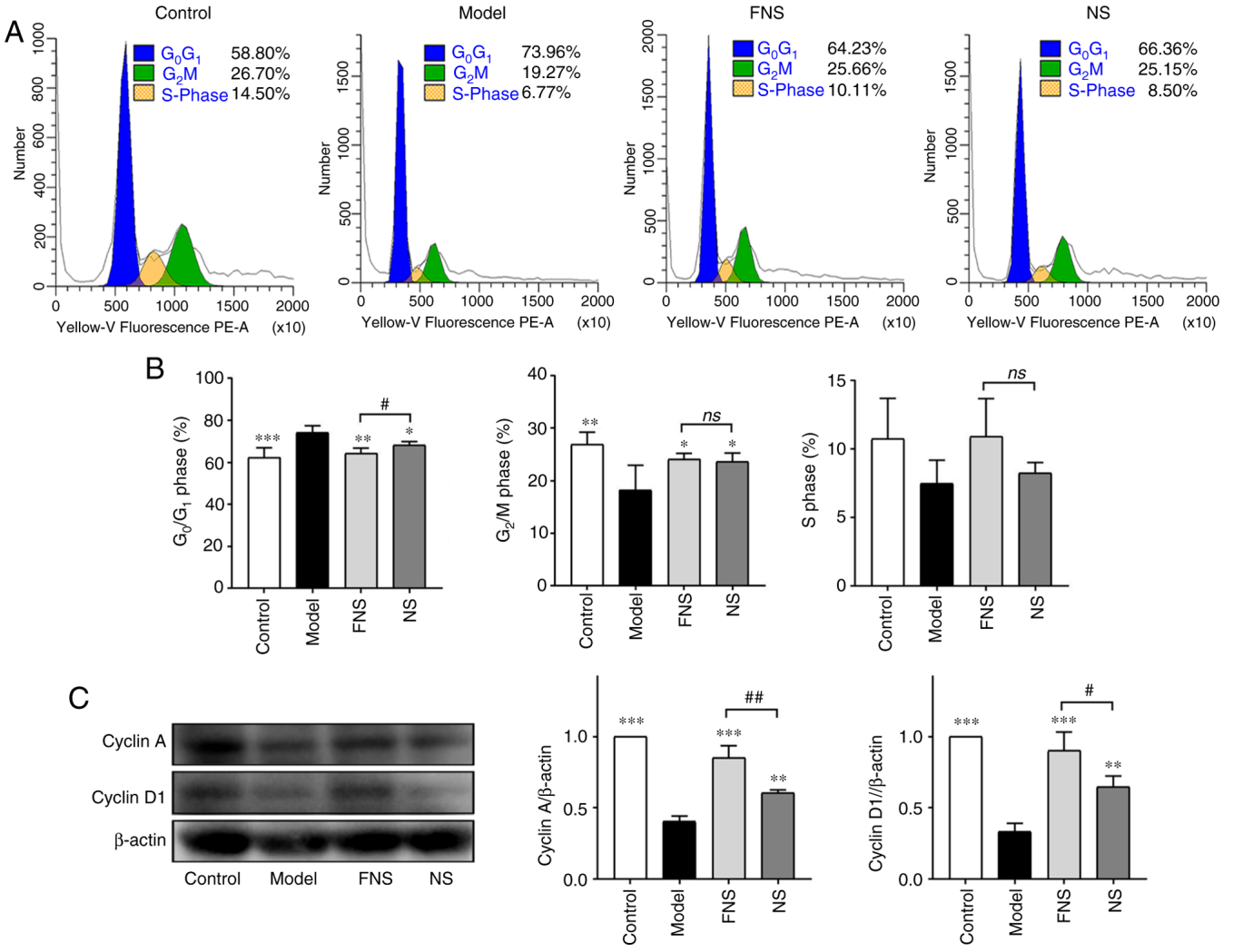

Figure 5. Effect of FNS and NS on the cell cycle of BM cells of blood deficiency model rats. (A) Flow cytometry analysis of the cell cycle. (B) Quantification of each cell cycle phase. (C) Western blotting and semi-quantification of cyclin A and cyclin D1 protein expression in BM cells normalized to β-actin. The data are presented as the mean ± SD (n=6). *P<0.05, **P<0.01 and ***P<0.001 compared with the model group; and $^{\#}$P<0.05 and $^{\#\#}$P<0.01 compared with the NS group. BM, bone marrow; NS, notoginseng saponins; FNS, fermented notoginseng saponins; ns, not statistically significant.

required for blood cell formation (58). TPO has been reported to improve thrombocytopenia and markedly augment megakaryopoiesis (59). EPO and GM-CSF have been suggested to promote erythropoiesis and the generation of myeloid cell subsets, respectively (60). CP damage the BM and cause cell apoptosis by increasing the expression of the pro-apoptotic protein Bax and decreasing the expression of anti-apoptotic proteins in the model mice, such as Bcl-2 (61). CP induce G_0/G_1 phase arrest of BM (62) and inhibit the protein expressions of Cyclin D1 (63). NS has previously been reported to decrease the apoptosis rate, Bax expression and caspase-3 activity of BM stromal cells induced by hydrogen peroxide (64,65). Ginsenoside CK could control apoptosis and promote cells to enter the normal cell cycle via the Bcl-2/Bax and MEK/ERK signaling pathways in myelosuppression mice induced by CP (66). Ginsenoside Rg1 increased the number of hematopoietic stem and progenitor cells and restored the function of BM in CP-treated myelosuppressed mice.

The results of the present study demonstrated that both FNS and NS reduced the cell apoptosis rate, recovered the normal pattern of the cell cycle of BM cells and increased the levels of GM-CSF, EPO and TPO. Furthermore, treatment with FNS further increased the levels of WBCs, LYMPHs, GM-CSF, EPO and TPO, and the protein expression levels of cyclin A and D1 compared with NS treatment. FNS treatment further decreased the total apoptosis rate of BM cells compared with NS treatment.

The liver stores blood and regulates the quantity of blood in circulation. CP is converted to phosphoramide mustard and acrolein by the liver cytochrome P450, which can result in liver damage (24). ALT and AST indicate the degree of liver damage. A high level of ALT suggests liver damage (67). DBIL represents the liver metabolic capacity, acting as an indicator of liver damage, and the level of DBIL is increased in patients with hepatitis and cirrhosis (68). ALP is released from the liver and bones, and its levels are increased in certain liver diseases and bone disorders (69). TRF is responsible for transporting iron from the digestive tract and degrading RBCs that enter the BM as a complex of TRF-Fe^{3+} (70). Due to the barrier of iron utilization by RBCs, the TRF is reduced during anemia (71). NS can improve hepatic function in non-alcoholic fatty liver disease and acute ethanol-induced liver injury (72,73).

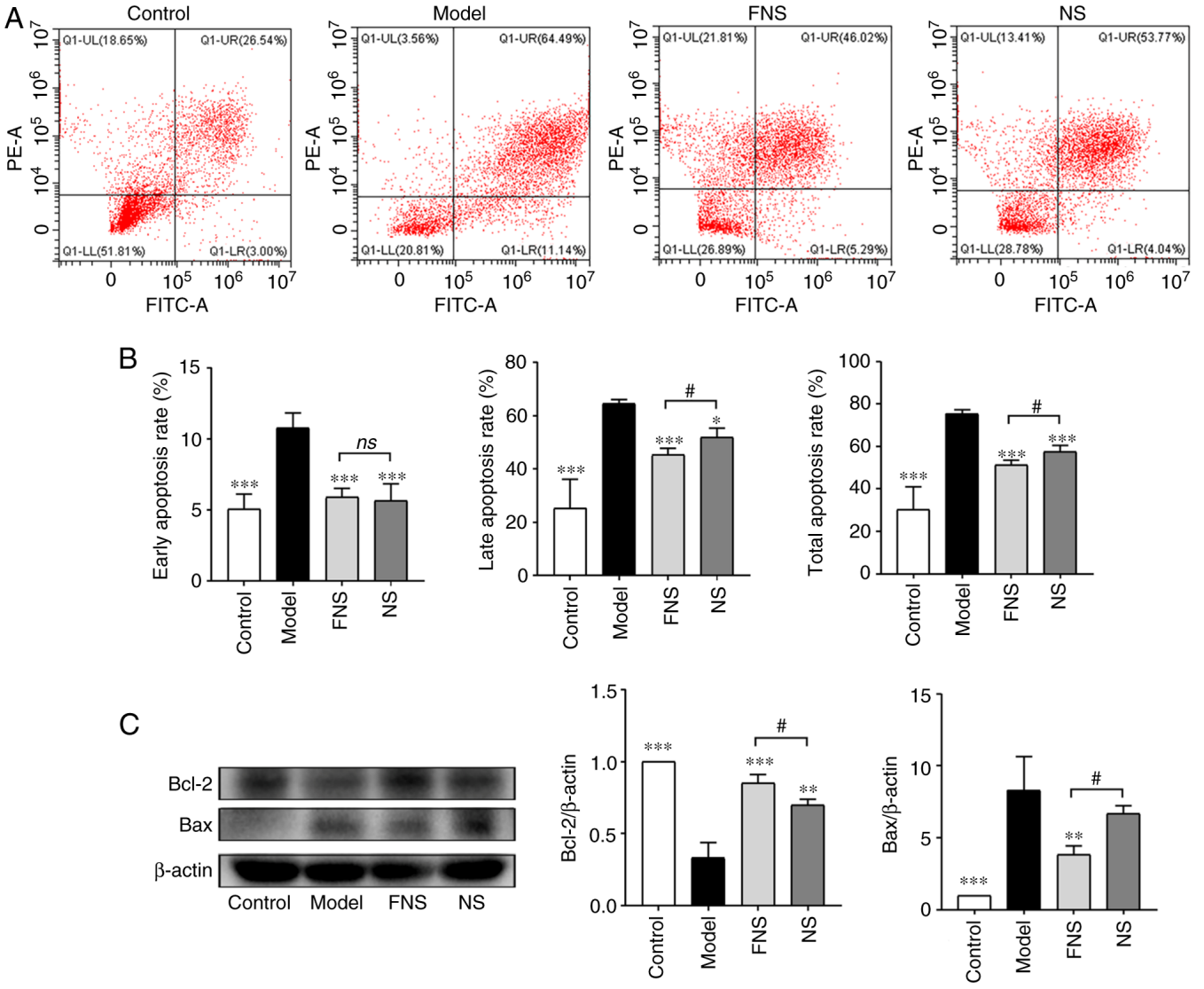

Figure 6. Effect of FNS and NS on the apoptosis of BM cells of blood deficiency rats. (A) Flow cytometry analysis of the apoptosis rate in BM cells. (B) Early, late and total apoptosis rate. (C) Western blotting and semi-quantification of Bcl-2 and Bax protein expression in BM cells normalized to β-actin. The data are presented as the mean ± SD (n=6). *P<0.05, **P<0.01 and ***P<0.001 compared with the model group; $^{\#}$P<0.05 compared with the NS group. BM, bone marrow; NS, notoginseng saponins; FNS, fermented notoginseng saponins; ns, not statistically significant; PE, phycoerythrin.

Zhong *et al* (74) reported that NS promoted liver regeneration through activation of the PI3K/AKT/mTOR cell proliferation pathway and upregulation of the AKT/Bad cell survival pathway in mice. The results of the present study indicated that FNS and NS protected the liver and maintained normal biochemical parameters in blood deficiency rats by reducing the ALP, ALT and DBIL levels and increasing the AST, LDH and TRF levels. The effect on ALT and AST levels in FNS rats was greater than that in NS treated rats.

APH and CP can damage the spleen and thymus, immune cells, such as T cells, B-LYMPHs and granulocytes, and cause a reduction in levels of inflammatory cytokines, such as IL-2, IL-4 and IL-6 (27,75). Lcs are produced in the BM and mature in the thymus gland or BM (76), and are the key cells involved in the regulation of immune function throughout the body (77). $CD4^{+}$ T cells are activated by antigen-presenting cells and regulate immune responses via the production of cytokines and helper T (Th) cells, such as Th1, Th2, Th17 and regulatory T cells (78). T-bet can induce Th1 cells to produce IL-2, IFN-γ and TNF-α, which are pro-inflammatory cytokines. These cytokines enhance antigen presentation and facilitate phagocytic function by macrophages (79). GATA-3 can induce Th2 cells to secrete IL-4, IL-10 and IL-13, which are anti-inflammatory cytokines involved in humoral immunity (80). Th1 and Th2 serve essential roles in the coordination and intercellular communication of lymphoid, inflammatory and hematopoietic cells in the immune system (81). CP inhibits the expression of TNF-α, IFN-γ, IL-4 and IL-10, and decreases the Th1/Th2 cytokine secretion ratio (17,82). Both APH and CP decrease the levels of TNF-α and IL-6 (38). In radiation-induced aplastic anemia mice, NS regulates Th1 and Th2 immune responses by downregulating the production of Th1 cytokines and T-bet protein expression, and upregulating the production of Th2 cytokines and expression of GATA-3 (83). Furthermore, Rd can promote the Th1 and Th2 immune responses by increasing IL-2, IFN-γ, IL-4 and IL-10 mRNA expression in mice splenocytes (84).

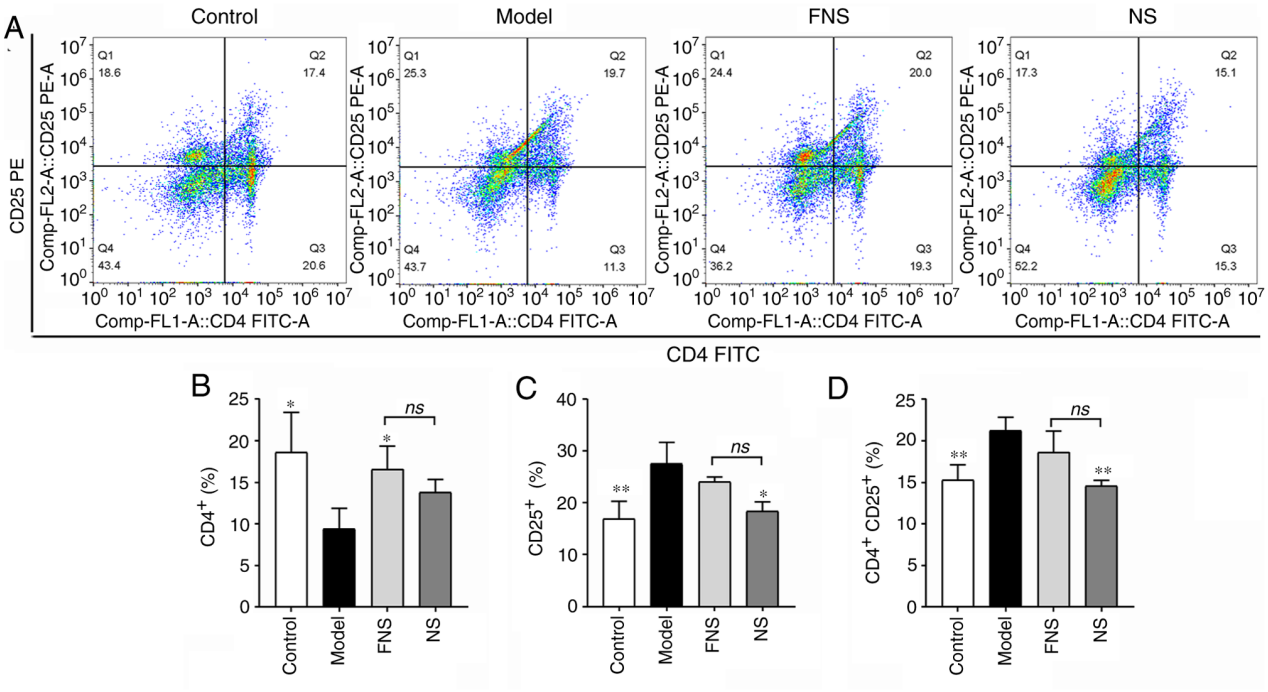

Figure 7. Effect of FNS and NS on T cells of blood deficiency rats. (A) Flow cytometry analysis of the distribution of $CD4^+$, $CD25^+$ and $CD4^+CD25^+$ T cells in the rat spleen and the percentage of (B) $CD4^+$, (C) $CD25^+$ and (D) $CD4^+CD25^+$ T cells. The data are presented as the mean ± SD (n=8). $^*P<0.05$ and $^{**}P<0.01$ compared with the model group. NS, notoginseng saponins; FNS, fermented notoginseng saponins; ns, not statistically significant; PE, phycoerythrin.

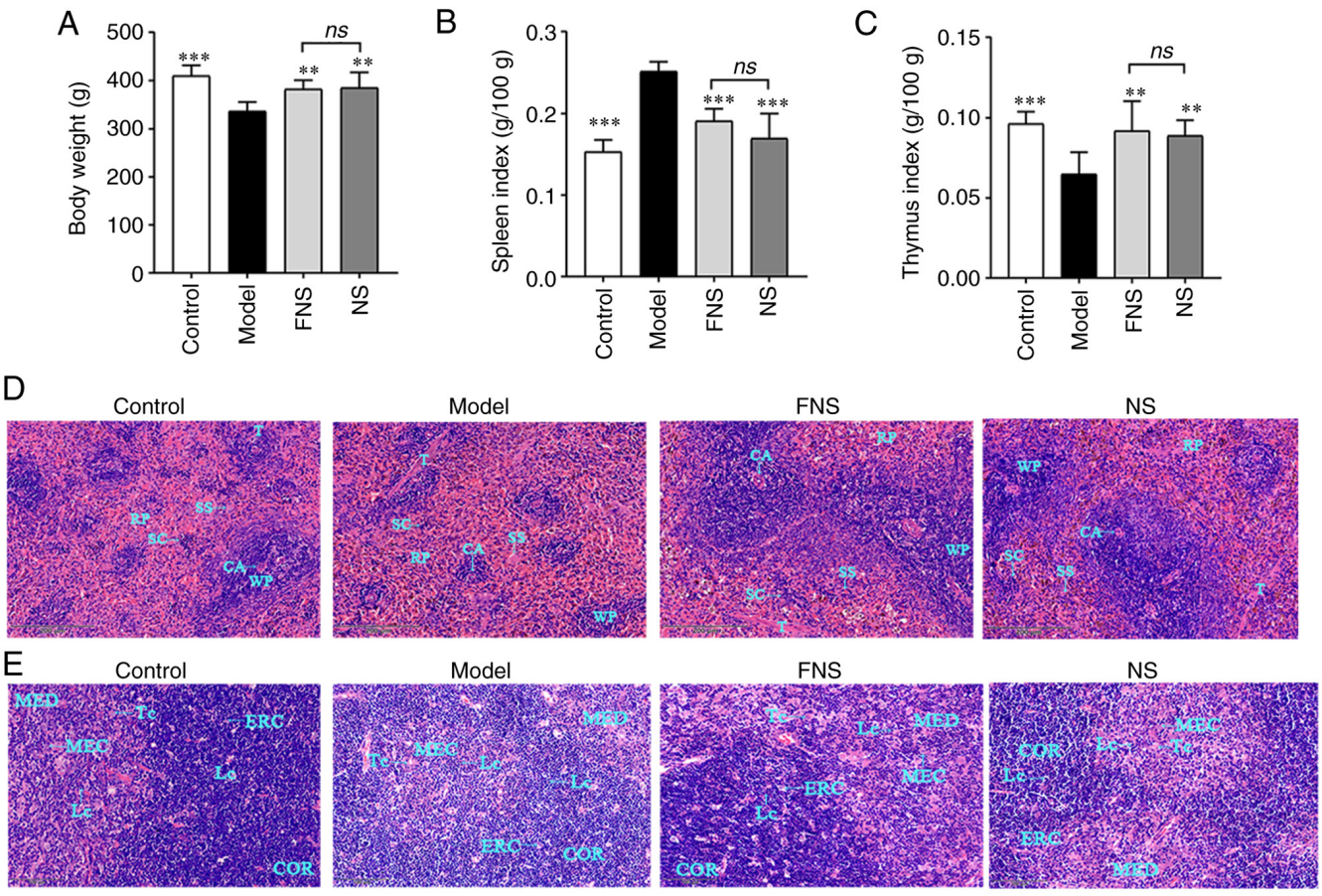

Figure 8. Effect of FNS and NS on (A) body weight, (B) spleen index and (C) thymus index, and hematoxylin-eosin staining of histological structure of blood deficiency rat (D) spleen and (E) thymus at x400 magnification. The data are presented as the mean ± SD (n=8). $^{**}P<0.01$ and $^{***}P<0.001$ compared with the model group. WP, white pulp; RP, red pulp; CA, central artery; SS, splenic sinus; SC, splenic cord; T, trabecula; MED, medulla; MEC, medullary epithelial cell; Lc, lymphocyte in tissues; TC, thymic corpuscle; ERC, epithelial reticular cell; COR, cortex; NS, notoginseng saponins; FNS, fermented notoginseng saponins; ns, not statistically significant.

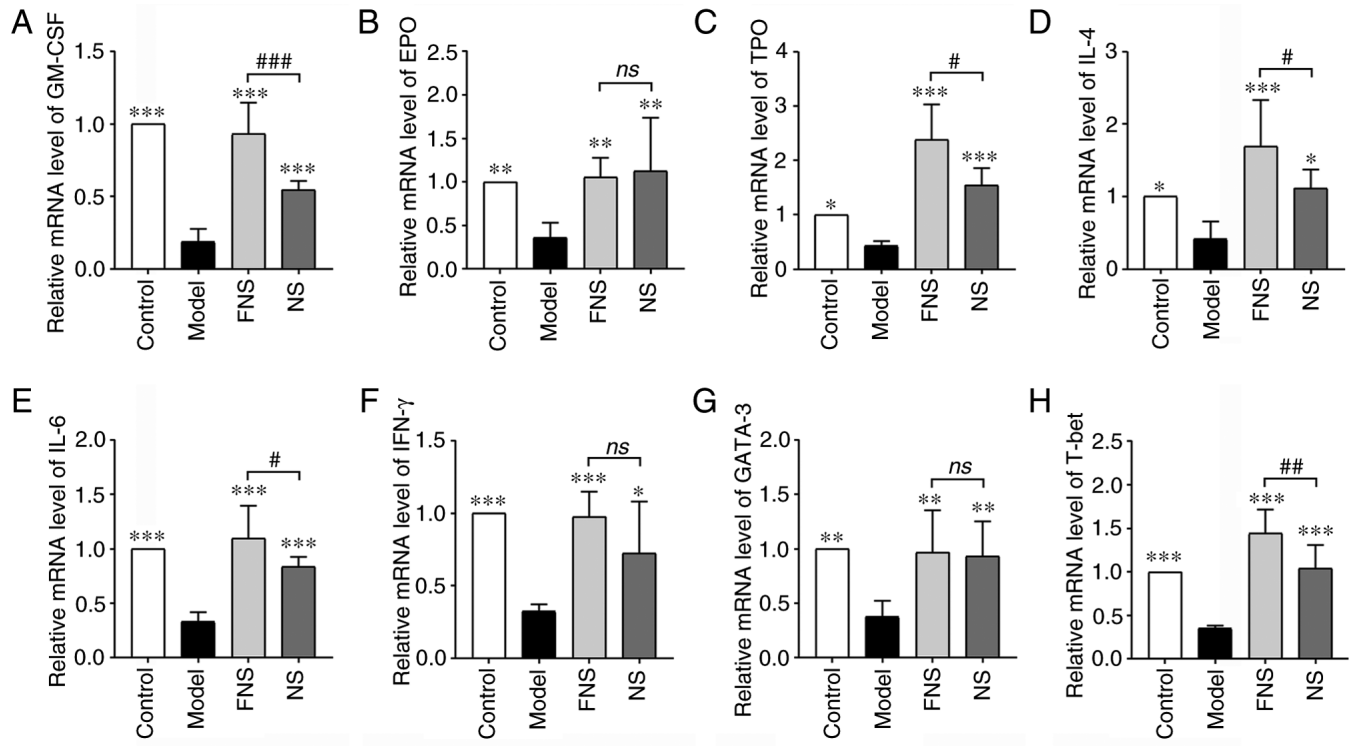

Figure 9. Effect of FNS and NS on mRNA expression levels in the spleens of blood deficiency rats. mRNA expression levels of (A) GM-CSF, (B) EPO, (C) TPO, (D) IL-4, (E) IL-6, (F) IFN-γ, (G) GATA-3 and (H) T-bet. The data are presented as the mean ± SD (n=8). *P<0.05, **P<0.01 and ***P<0.001 compared with the model group; and $^{\#}$P<0.05, $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001 compared with the NS group. GM-CSF, granulocyte-macrophage colony-stimulating factor; EPO, erythropoietin; TPO, thrombopoietin; GATA-3, GATA binding protein 3; T-bet, T-box expressed in T cell; NS, notoginseng saponins; FNS, fermented notoginseng saponins; ns, not statistically significant.

The results of the present study demonstrated that FNS and NS increased the protein expression levels of IL-4, IL-10, IL-12, IL-13, TGF-β, IL-6, IFN-γ and TNF-α, and regulated Th1 and Th2 immune responses by increasing the protein expression levels of GATA-3 and T-bet. Furthermore, FNS treatment significantly increased the levels of immune cytokines (IL-10, IL-12, IL-13 and TNF-α) and transcription factor T-bet compared with NS treatment. This difference may be due to the increase of certain ginsenosides during fermentation.

Most ginsenosides in NS have low oral bioavailability (85). In *L. plantarum* fermentation, the hydrophilic ginsenosides (nR1, Rb1, Rg1, Rc, Re and R1) are deglycosylated and converted into hydrophobic ginsenosides (Rd, Rh2, CK, PPT and PPD). This increased hydrophobicity allows passage through cell membranes and increases bioavailability (86-88). In the present study, the total contents of nR1, Rg1, Rb1 and Re, as the main bioactive components in NS (89,90), were reduced from 53.91 g/100 g to 36.67 g/100 g, and the total content of Rd, Rh2, CK, PPT and PPD was increased from 20.16 g/100 g to 34.11 g/100 g, during the NS *Lactobacillus* fermentation process. Zhu *et al* (91) reported that the levels of Rb1, Rd, Rk1, Rg5, Rk3, Rh4 and 20(S)-PPD increased in steamed PN and could be used as pharmacokinetic markers of the steamed PN based on their elevated levels in the plasma. The ginsenosides in PN are classified as oleanane type, protopanaxadiol (PPD) and protopanaxatriol (PPT) types, according to the chemical structure. The PPD-type ginsenosides showed improved absorption compared with PPT-types in rat gastrointestinal systems. The peak concentration (C_{max}) and area under the concentration-time curve (AUC) of PPD-type ginsenosides Fa, Rb1, Rd, Rk1 Rg5 and PPD were higher than PPT-type ginsenosides R1, Re, Rg1, Rg2, F4, Rh1 and PPT; the peak time (T_{max}) of the PPT-types ginsenosides Rg1 (0.83 h), R1 (1.17 h), Re (1.33 h), Rg2 (1.00 h), Rh1 (0.63 h) and 20(R)-Rh1 (0.79 h) was shorter than that of the PPD-types ginsenosides Fa (8.00 h), Rb1 (8.00 h), Rb2 (8.00 h), Rd (9.33 h), CK (12.00 h), Rk1 (3.67 h), Rg5 (3.67 h) and PPD (12.00 h) (91).

The present study demonstrated that the content of PPD-type ginsenosides Rd, CK and PPD in FNS was increased during the fermentation process, with results suggesting increased blood concentration, prolonged the drug duration and increased activity, and so served an important role in the treatment of blood deficiency rats.

CK is a secondary ginsenoside, which is more bioavailable and soluble than its parent ginsenoside (92). The C_{max} of CK is double that of ginsenoside Rb1, and the T_{max} of CK is higher than that of Rb1 (93). Fukami *et al* (94) reported that the T_{max}, C_{max} and AUC were different between *Lactobacillus paracasei* A221 fermented ginseng (FG) and non-FG (NFG). The T_{max} of CK was 2.2 and 16 h and the C_{max} was 41.5 and 1.16 ng/ml in the FG and NFG group, respectively. The $AUC_{0\text{-}12\,h}$ and $AUC_{0\text{-}24\,h}$ of healthy adults treated with FG were 58.3 and 17.5-fold higher than those in the NFG group (94). Choi *et al* (95) reported that the $AUC_{0\text{-}24\,h}$ and C_{max} of CK from FG were 6.3-fold and 6.0-fold higher than those from NFG in rats. The T_{max} of CK in humans and rats was 2.54 and 3.33 h

for FG and 9.11 and 6.75 h for NFG, respectively. The results of the present study demonstrated that the content of CK in FNS was higher than that in NS. These results suggested that administration of FNS resulted in a higher and faster absorption of CK in blood deficiency rats compared with NS.

In conclusion, both FNS and NS treatment appeared to reduce the changes in the blood deficiency parameters induced by APH and CP. For certain parameters, FNS exhibited a greater impact compared with NS, improving the function of the BM, spleen, thymus and liver.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

WS, ZL, DP, LZ, YZ, TY, XG, HS and HZ contributed to the study conception and design. WS also contributed to project development and data collection; WS, ZL and DP contributed to protocol development and manuscript writing. LZ, YZ and TY contributed to data collection and analysis. XG contributed to data analysis. HS and HZ contributed to data analysis and manuscript editing. WS and HZ confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All procedures in the present study were approved by the Bioethics Committee of Changchun University of Chinese Medicine and the Institutional Animal Care (approval no. 2022156; Changchun, China), and the study was conducted based on the guidelines for the care and use of laboratory animals (20).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Shi XQ, Yue SJ, Tang YP, Chen YY, Zhou GS, Zhang J, Zhu ZH, Liu P and Duan JA: A network pharmacology approach to investigate the blood enriching mechanism of Danggui Buxue decoction. J Ethnopharmacol 235: 227-242, 2019.
2. Li PL, Sun HG, Hua YL, Ji P, Zhang L, Li JX and Wei Y: Metabolomics study of hematopoietic function of Angelica sinensis on blood deficiency mice model. J Ethnopharmacol 166: 261-269, 2015.
3. Huang Q, Feng L, Li H, Zheng L, Qi X, Wang Y, Feng Q, Liu Z, Liu X and Lu L: Jian-Pi-Bu-Xue-formula alleviates cyclophosphamide-induced myelosuppression via up-regulating NRF2/HO1/NQO1 signaling. Front Pharmacol 11: 1302, 2020.
4. Uzayisenga R, Ayeka PA and Wang Y: Anti-diabetic potential of *Panax notoginseng* saponins (PNS): A review. Phytother Res 28: 510-516, 2014.
5. Li YH, Li YY, Fan GW, Yu JH, Duan ZZ, Wang LY and Yu B: Cardioprotection of ginsenoside Rb1 against ischemia/reperfusion injury is associated with mitochondrial permeability transition pore opening inhibitiz. Chin J Integr Med: Jan 6, 2016 (Epub ahead of print).
6. Li L, Wang Y, Qi B, Yuan D, Dong S, Guo D, Zhang C and Yu M: Suppression of PMA-induced tumor cell invasion and migration by ginsenoside Rg1 via the inhibition of NF-κB-dependent MMP-9 expression. Oncol Rep 32: 1779-1786, 2014.
7. Zhang Z, Zhang Y, Gao M, Cui X, Yang Y, van Duijn B, Wang M, Hu Y, Wang C and Xiong Y: Steamed *Panax notoginseng* attenuates anemia in mice with blood deficiency syndrome via regulating hematopoietic factors and JAK-STAT pathway. Front Pharmacol 10: 1578, 2020.
8. Xiong Y, Chen L, Man J, Hu Y and Cui X: Chemical and bioactive comparison of *Panax notoginseng* root and rhizome in raw and steamed forms. J Ginseng Res 43: 385-393, 2019.
9. Quan LH, Piao JY, Min JW, Kim HB, Kim SR, Yang DU and Yang DC: Biotransformation of ginsenoside Rb1 to prosapogenins, gypenoside XVII, ginsenoside Rd, ginsenoside F2, and compound K by *Leuconostoc mesenteroides* DC102. J Ginseng Res 35: 344-351, 2011.
10. Li J, Huang Q, Yao Y, Ji P, Mingyao E, Chen J, Zhang Z, Qi H, Liu J, Chen Z, *et al*: Biotransformation, pharmacokinetics, and pharmacological activities of ginsenoside Rd against multiple diseases. Front Pharmacol 13: 909363, 2022.
11. Wang D, Liao PY, Zhu HT, Chen KK, Xu M, Zhang YJ and Yang CR: The processing of *Panax notoginseng* and the transformation of its saponin components. Food Chem 132: 1808-1813, 2012.
12. Zheng F, Zhang MY, Wu YX, Wang YZ, Li FT, Han MX, Dai YL and Yue H: Biotransformation of Ginsenosides (Rb_1, Rb_2, Rb_3, Rc) in human intestinal bacteria and its effect on intestinal flora. Chem Biodivers 18: e2100296, 2021.
13. Liu Z, Li JX, Wang CZ, Zhang DL, Wen X, Ruan CC, Li Y and Yuan CS: Microbial conversion of protopanaxadiol-type ginsenosides by the edible and medicinal mushroom schizophyllum commune: A green biotransformation strategy. ACS Omega 4: 13114-13123, 2019.
14. Kim BG, Shin KS, Yoon TJ, Yu KW, Ra KS, Kim JM, Kim SY and Suh HJ: Fermentation of Korean red ginseng by *Lactobacillus plantarum* M-2 and its immunological activities. Appl Biochem Biotechnol 165: 1107-1119, 2011.
15. Park SE, Na CS, Yoo SA, Seo SH and Son HS: Biotransformation of major ginsenosides in ginsenoside model culture by lactic acid bacteria. J Ginseng Res 41: 36-42, 2017.
16. Barreda D, Hanington P and Belosevic M: Regulation of myeloid development and function by colony stimulating factors. Dev Comp Immunol 28: 509-554, 2004.
17. Zhang H, Sun Y, Fan M, Zhang Y, Liang Z, Zhang L, Gao X, He X, Li X, Zhao D, *et al*: Prevention effect of total ginsenosides and ginseng extract from Panax ginseng on cyclophosphamide-induced immunosuppression in mice. Phytother Res 37: 3583-3601, 2023.
18. Barone FC and Feuerstein GZ: Inflammatory mediators and stroke: new opportunities for novel therapeutics. J Cereb Blood Flow Metab 19: 819-834, 1999.
19. Sharir R, Semo J, Shaish A, Landa-Rouben N, Entin-Meer M, Keren G and George J: Regulatory T cells influence blood flow recovery in experimental hindlimb ischaemia in an IL-10-dependent manner. Cardiovasc Res 103: 585-596, 2014.

20. National Research Council Committee for the Update of the Guide for the C and Use of Laboratory A: The National Academies Collection: Reports funded by National Institutes of Health. In: Guide for the Care and Use of Laboratory Animals. National Academies Press (US), Copyright © 2011, National Academy of Sciences, Washington (DC), 2011.
21. Li W, Tang Y, Guo J, Shang E, Qian Y, Wang L, Zhang L, Liu P, Su S, Qian D and Duan JA: Comparative metabolomics analysis on hematopoietic functions of herb pair Gui-Xiong by ultra-high-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry and pattern recognition approach. J Chromatogr A 1346: 49-56, 2014.
22. Gao J, Liu J, Yao M, Zhang W, Yang B and Wang G: *Panax notoginseng* saponins stimulates neurogenesis and neurological restoration after microsphere-induced cerebral embolism in rats partially via mTOR signaling. Front Pharmacol 13: 889404, 2022.
23. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
24. Steinbrecht S, Kiebist J, König R, Thiessen M, Schmidtke KU, Kammerer S, Küpper JH and Scheibner K: Synthesis of cyclophosphamide metabolites by a peroxygenase from Marasmius rotula for toxicological studies on human cancer cells. AMB Express 10: 128, 2020.
25. Wang J, Wang F, Yuan L, Ruan H, Zhu Z, Fan X, Zhu L and Peng X: Blood-enriching effects and immune-regulation mechanism of steam-processed polygonatum sibiricum polysaccharide in blood deficiency syndrome mice. Front Immunol 13: 813676, 2022.
26. Liu B, Yang Z, Bo L, Zhao Z, Zhou Q and Sun C: Cytotoxic effects, inflammatory response and apoptosis induction of cyclophosphamide in the peripheral blood leukocyte of blunt snout bream (Megalobrama amblycephala). Fish Shellfish Immunol 93: 174-182, 2019.
27. Cheng Y, Liu L, Mo S, Gao J, Zhang H, Zhang H, Zhang C, Song X, Li L and Geng Z: The immunomodulatory effects of phellodendri cortex polysaccharides on cyclophosphamide-induced immunosuppression in mice. Evid Based Complement Alternat Med 2021: 3027708, 2021.
28. Wang S, Huang S, Ye Q, Zeng X, Yu H, Qi D and Qiao S: Prevention of cyclophosphamide-induced immunosuppression in mice with the antimicrobial peptide sublancin. J Immunol Res 2018: 4353580, 2018.
29. Economopoulou C, Pappa V, Papageorgiou S, Kontsioti F, Economopoulou P, Charitidou E, Girkas K, Kapsimali V, Papasteriadi C, Tsirigotis P, *et al*: Cell cycle and apoptosis regulatory gene expression in the bone marrow of patients with de novo myelodysplastic syndromes (MDS). Ann Hematol 89: 349-358, 2010.
30. Vermeulen K, Berneman ZN and Van Bockstaele DR: Cell cycle and apoptosis. Cell Prolif 36: 165-175, 2003.
31. Czabotar PE and Garcia-Saez AJ: Mechanisms of BCL-2 family proteins in mitochondrial apoptosis. Nat Rev Mol Cell Bio 24: 732-748, 2023.
32. Westphal D, Dewson G, Czabotar PE and Kluck RM: Molecular biology of Bax and Bak activation and action. Biochim Biophys Acta 1813: 521-531, 2011.
33. Raj S and Gothandam K: Immunomodulatory activity of methanolic extract of Amorphophallus commutatus var. Wayanadensis under normal and cyclophosphamide induced immunosuppressive conditions in mice models. Food Chem Toxicol 81: 151-159, 2015.
34. Hou F, Yang H, Yu T and Chen W: The immunosuppressive effects of 10 mg/kg cyclophosphamide in Wistar rats. Environ Toxicol Pharmacol 24: 30-36, 2007.
35. Shrief AI, Hamed WHE, Mazroa SA and Moustafa AM: Histological study of the role of CD34+ stem cells and mast cells in cyclophosphamide-induced thymic injury in rats and the possible attenuating role of melatonin. Histochem Cell Biol 159: 501-512, 2023.
36. Khazaei F, Ghanbari E and Khazaei M: Protective effect of royal jelly against cyclophosphamide-induced thrombocytopenia and spleen and bone marrow damages in rats. Cell J 22: 302-309, 2020.
37. Chen H, Luo Z, Shen H, Ren C, Li Z, Tang J, Wang J and Wu T: Research on the roles of transcription factors T-bet and GATA-3 in aplastic anemia. Clin Lab 60: 291-295, 2014.
38. Zhang H, Wang H, Liu Y, Huang L, Wang Z and Li Y: The haematopoietic effect of Panax japonicus on blood deficiency model mice. J Ethnopharmacol 154: 818-824, 2014.
39. Zhang Y, Fei QQ, Wang J, Zhu FX, Chen Y, Tang DQ and Chen B: Study on blood enrichment mechanism of steamed notoginseng based on metabolomics method. Zhongguo Zhong Yao Za Zhi 44: 2139-2148, 2019 (In Chinese).
40. Zhang Z, Chen L, Cui X, Zhang Y, Hu Y, Wang C and Xiong Y: Identification of anti-inflammatory components of raw and steamed *Panax notoginseng* root by analyses of spectrum-effect relationship. RSC Adv 9: 17950-17958, 2019.
41. Shin NR, Bose S, Choi Y, Kim YM, Chin YW, Song EJ, Nam YD and Kim H: Anti-obesity effect of fermented *Panax notoginseng* is mediated via modulation of appetite and gut microbial population. Front Pharmacol 12: 665881, 2021.
42. Bai Y and Gänzle MG: Conversion of ginsenosides by *Lactobacillus plantarum* studied by liquid chromatography coupled to quadrupole trap mass spectrometry. Food Res Int 76: 709-718, 2015.
43. Chen MY, Shao L, Zhang W, Wang CZ, Zhou HH, Huang WH and Yuan CS: Metabolic analysis of *Panax notoginseng* saponins with gut microbiota-mediated biotransformation by HPLC-DAD-Q-TOF-MS/MS. J Pharmaceut Biomed 150: 199-207, 2018.
44. Wang L, Yang X, Yu X, Yao Y and Ren G: Evaluation of antibacterial and anti-inflammatory activities of less polar ginsenosides produced from polar ginsenosides by heat-transformation. J Agric Food Chem 61: 12274-12282, 2013.
45. Bae Ea, Han MJ, Kim EJ and Kim DH: Transformation of ginseng saponins to ginsenoside Rh2 by acids and human intestinal bacteria and biological activities of their transformants. Arch Pharm Res 27: 61-67, 2004.
46. Akao T, Kanaoka M and Kobashi K: Appearance of compound K, a major metabolite of ginsenoside Rb1 by intestinal bacteria, in rat plasma after oral administration-measurement of compound K by enzyme immunoassay. Biol Pharm Bull 21: 245-249, 1998.
47. Choi HS, Kim SY, Park Y, Jung EY and Suh HJ: Enzymatic transformation of ginsenosides in Korean Red Ginseng (Panax ginseng Meyer) extract prepared by Spezyme and Optidex. J Ginseng Res 38: 264-269, 2014.
48. Lee SY, Jeong JJ, Eun SH and Kim DH: Anti-inflammatory effects of ginsenoside Rg1 and its metabolites ginsenoside Rh1 and 20(S)-protopanaxatriol in mice with TNBS-induced colitis. Eur J Pharmacol 762: 333-343, 2015.
49. Liu Z, Wen X, Wang CZ, Li W, Huang WH, Xia J, Ruan CC and Yuan CS: Remarkable impact of amino acids on ginsenoside transformation from fresh ginseng to red ginseng. J Ginseng Res 44: 424-434, 2020.
50. Wang HY, Hua HY, Liu XY, Liu JH and Yu BY: In vitro biotransformation of red ginseng extract by human intestinal microflora: Metabolites identification and metabolic profile elucidation using LC-Q-TOF/MS. J Pharm Biomed Anal 98: 296-306, 2014.
51. Lee BH, You HJ, Park MS, Kwon B and Ji GE: Transformation of the glycosides from food materials by probiotics and food microorganisms. J Microbiol Biotechn 16: 497-504, 2006.
52. Li W, Tang Y, Guo J, Huang M, Li W, Qian D and Duan J: Enriching blood effect comparison in three kinds of blood deficiency model after oral administration of drug pair of Angelicae Sinensis Radix and Chuanxiong Rhizoma and each single herb. Zhongguo Zhong Yao Za Zhi 36: 1808-1814, 2011 (In Chinese).
53. Hua YL, Ma Q, Yuan ZW, Zhang XS, Yao WL, Ji P, Hu JJ and Wei YM: A novel approach based on metabolomics coupled with network pharmacology to explain the effect mechanisms of Danggui Buxue Tang in anaemia. Chin J Nat Med 17: 275-290, 2019.
54. Li S, Lin H, Qu C, Tang Y, Shen J, Li W, Yue S, Kai J, Shang G, Zhu Z, *et al*: Urine and plasma metabonomics coupled with UHPLC-QTOF/MS and multivariate data analysis on potential biomarkers in anemia and hematinic effects of herb pair Gui-Hong. J Ethnopharmacol 170: 175-183, 2015.
55. Hua Y, Yao W, Ji P and Wei Y: Integrated metabonomic-proteomic studies on blood enrichment effects of Angelica sinensis on a blood deficiency mice model. Pharm Biol 55: 853-863, 2017.
56. Liu H, Pan J, Yang Y, Cui X and Qu Y: Production of minor ginenosides from *Panax notoginseng* by microwave processing method and evaluation of their blood-enriching and hemostatic activity. Molecules 23: 1243, 2018.
57. Zhao E, Xu H, Wang L, Kryczek I, Wu K, Hu Y, Wang G and Zou W: Bone marrow and the control of immunity. Cell Mol Immunol 9: 11-19, 2012.
58. Sieff C: Hematopoietic growth factors. J Clin Invest 79: 1549-1557, 1987.

59. Hokom MM, Lacey D, Kinstler OB, Choi E, Kaufman S, Faust J, Rowan C, Dwyer E, Nichol JL, Grasel T, *et al*: Pegylated megakaryocyte growth and development factor abrogates the lethal thrombocytopenia associated with carboplatin and irradiation in mice. Blood 86: 4486-4492, 1995.
60. Kumar A, Taghi Khani A, Sanchez Ortiz A and Swaminathan S: GM-CSF: A double-edged sword in cancer immunotherapy. Front Immunol 13: 901277, 2022.
61. Liu H, Yan Y, Zhang F and Wu Q: The immuno-enhancement effects of tubiechong (eupolyphaga sinensis) lyophilized powder in cyclophosphamide-induced immunosuppressed mice. Immunol Invest 48: 844-859, 2019.
62. Chen QQ, Han X, Wang WM, Zhao L and Chen A: Danggui sini decoction ameliorates myelosuppression in animal model by upregulating thrombopoietin expression. Cell Biochem Biophys 71: 945-950, 2015.
63. Wang LF, Xu ZY, Jin CJ, Sha HF, Wang ZQ, Zhou WD, Zhang M, Wu J and Bai B: Dual regulation of cell cycles by Shuanghuang Shengbai Granule in Lewis-bearing mice with chemotherapy-induced myelosuppression and its mechanism. Zhong Xi Yi Jie He Xue Bao 7: 453-457, 2009 (In Chinese).
64. Qiang H, Wang KZ, Shi ZB and Fan LH: *Panax notoginseng* saponins protect rabbit bone marrow stromal cells from hydrogen peroxide-induced apoptosis. Zhong Xi Yi Jie He Xue Bao 8: 131-137, 2010.
65. Qiang H, Zhang C, Shi ZB, Yang HQ and Wang KZ: Protective effects and mechanism of *Panax notoginseng* saponins on oxidative stress-induced damage and apoptosis of rabbit bone marrow stromal cells. Chin J Integr Med 16: 525-530, 2010.
66. Han J, Wang Y, Cai E, Zhang L, Zhao Y, Sun N, Zheng X and Wang S: Study of the effects and mechanisms of ginsenoside compound K on myelosuppression. J Agric Food Chem 67: 1402-1408, 2019.
67. Senior J: Alanine aminotransferase: A clinical and regulatory tool for detecting liver injury-past, present, and future. Clin Pharmacol Ther 92: 332-339, 2012.
68. Lee CH and Kim IH: Direct hyperbilirubinemia as a predictor of mortality in patients with liver cirrhosis. Gut Liver 15: 490-491, 2021.
69. Vimalraj S: Alkaline phosphatase: Structure, expression and its function in bone mineralization. Gene 754: 144855, 2020.
70. Gkouvatsos K, Papanikolaou G and Pantopoulos K: Regulation of iron transport and the role of transferrin. Biochim Biophys Acta 1820: 188-202, 2012.
71. Boshuizen M, van der Ploeg K, von Bonsdorff L, Biemond BJ, Zeerleder SS, van Bruggen R and Juffermans NP: Therapeutic use of transferrin to modulate anemia and conditions of iron toxicity. Blood Rev 31: 400-405, 2017.
72. Ding RB, Tian K, Cao YW, Bao JL, Wang M, He C, Hu Y, Su H and Wan JB: Protective effect of *Panax notoginseng* saponins on acute ethanol-induced liver injury is associated with ameliorating hepatic lipid accumulation and reducing ethanol-mediated oxidative stress. J Agric Food Chem 63: 2413-2422, 2015.
73. Xu Y, Wang N, Tan HY, Li S, Zhang C and Feng Y: Gut-liver axis modulation of *Panax notoginseng* saponins in nonalcoholic fatty liver disease. Hepatol Int 15: 350-365, 2021.
74. Zhong H, Wu H, Bai H, Wang M, Wen J, Gong J, Miao M and Yuan F: *Panax notoginseng* saponins promote liver regeneration through activation of the PI3K/AKT/mTOR cell proliferation pathway and upregulation of the AKT/Bad cell survival pathway in mice. BMC Complem Altern Med 19: 122, 2019.
75. Ahlmann M and Hempel G: The effect of cyclophosphamide on the immune system: Implications for clinical cancer therapy. Cancer Chemother Pharmacol 78: 661-671, 2016.
76. Travlos GS: Normal structure, function, and histology of the bone marrow. Toxicol Pathol 34: 548-565, 2006.
77. Kar UK and Joosten LAB: Training the trainable cells of the immune system and beyond. Nat Immunol 21: 115-119, 2020.
78. Dong C: Cytokine regulation and function in T cells. Annu Rev Immunol 39: 51-76, 2021.
79. Oestreich KJ and Weinmann AS: T-bet employs diverse regulatory mechanisms to repress transcription. Trends Immunol 33: 78-83, 2012.
80. Elenkov IJ and Chrousos GP: Stress hormones, proinflammatory and antiinflammatory cytokines, and autoimmunity. Ann N Y Acad Sci 966: 290-303, 2002.
81. Mei YX, Chen HX, Zhang J, Zhang XD and Liang YX: Protective effect of chitooligosaccharides against cyclophosphamide-induced immunosuppression in mice. Int J Biol Macromol 62: 330-335, 2013.
82. Zhu G, Luo J, Du H, Jiang Y, Tu Y, Yao Y and Xu M: Ovotransferrin enhances intestinal immune response in cyclophosphamide-induced immunosuppressed mice. Int J Biol Macromol 120: 1-9, 2018.
83. Zhao Y, Sun X, Yu X, Gao R and Yin L: Saponins from *Panax notoginseng* leaves improve the symptoms of aplastic anemia and aberrant immunity in mice. Biomed Pharmacother 102: 959-965, 2018.
84. Yang Z, Chen A, Sun H, Ye Y and Fang W: Ginsenoside Rd elicits Th1 and Th2 immune responses to ovalbumin in mice. Vaccine 25: 161-169, 2007.
85. Won HJ, Kim HI, Park T, Kim H, Jo K, Jeon H, Ha SJ, Hyun JM, Jeong A, Kim JS, *et al*: Non-clinical pharmacokinetic behavior of ginsenosides. J Ginseng Res 43: 354-360, 2019.
86. Han M and Fang XL: Difference in oral absorption of ginsenoside Rg1 between in vitro and in vivo models. Acta Pharmacol Sin 27: 499-505, 2006.
87. Paek IB, Moon Y, Kim J, Ji HY, Kim SA, Sohn DH, Kim JB and Lee HS: Pharmacokinetics of a ginseng saponin metabolite compound K in rats. Biopharm Drug Dispos 27: 39-45, 2006.
88. Xu QF, Fang XL and Chen DF: Pharmacokinetics and bioavailability of ginsenoside Rb1 and Rg1 from *Panax notoginseng* in rats. J Ethnopharmacol 84: 187-192, 2003.
89. Peng Y, Wu Z, Huo Y, Chen Y, Lu F, Peng Q and Liang Y: Simultaneous determination of ginsenosides Rg1, Re, and Rb1 and notoginsenoside R1 by solid phase extraction followed by UHPLC-MS/MS and investigation of their concentrations in various kinds of cosmetics. Anal Methods 9: 5441-5448, 2017.
90. Li W, Wu Y, Wan M, Chu Y, Wang X, Li S, Liu Z, Chen X, Polachi N, Zhou S and Sun H: Simultaneous determination of three saponins in human plasma after oral administration of compound danshen dripping pills by LC-MS/MS and its application in a pharmacokinetic study. J Pharm Biomed Anal 169: 254-259, 2019.
91. Zhu D, Zhou Q, Li H, Li S, Dong Z, Li D and Zhang W: Pharmacokinetic characteristics of steamed notoginseng by an efficient LC-MS/MS method for simultaneously quantifying twenty-three triterpenoids. J Agric Food Chem 66: 8187-8198, 2018.
92. Sharma A and Lee HJ: Ginsenoside compound K: Insights into recent studies on pharmacokinetics and health-promoting activities. Biomolecules 10: 1028, 2020.
93. Kim HK: Pharmacokinetics of ginsenoside Rb1 and its metabolite compound K after oral administration of Korean Red Ginseng extract. J Ginseng Res 37: 451-456, 2013.
94. Fukami H, Ueda T and Matsuoka N: Pharmacokinetic study of compound K in Japanese subjects after ingestion of Panax ginseng fermented by *Lactobacillus paracasei* A221 reveals significant increase of absorption into blood. J Med Food 22: 257-263, 2019.
95. Choi ID, Ryu JH, Lee DE, Lee MH, Shim JJ, Ahn YT, Sim JH, Huh CS, Shim WS, Yim SV, *et al*: Enhanced absorption study of ginsenoside compound K (20-O-β-(D-Glucopyranosyl)-20(S)-protopanaxadiol) after oral administration of fermented red ginseng extract (HYFRG™) in healthy Korean volunteers and rats. Evid Based Complement Alternat Med 2016: 3908142, 2016.