The hematopoietic function of medicinal wine Maoji Jiu revealed in blood deficiency model rats

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Abstract

Background: Maoji Jiu (MJ), a medicinal wine, has been used commonly by Chinese to enrich and nourish the blood. In this study, the aim is to examine the hematopoietic function of MJ and investigate its anti-blood deficiency mechanism.

Methods: 36 rats were randomly divided into six groups with six rats in each group. The blood deficiency model was induced by injecting with N-acetylphenylhydrazine (APH) and injected intraperitoneally with cyclophosphamide (CTX), and treatment drugs were given by oral gavage twice each day for continuous 10 days from the start of experiments.

Results: The administration of MJ improved the levels of white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB) and hematocrit (HCT) in the blood deficiency model rats. Hematopoietic effect involves in regulating the antioxidant activity in the liver and the levels of Bcl-2, Bax, erythropoietin (EPO), transforming growth factor-beta-1 (TGF-β1) and macrophage colony-stimulating factor (M-CSF) mRNA in spleen tissues to enhance extramedullary hematopoiesis.

Conclusion: This study suggests that MJ has a beneficial effect on blood deficiency model rats.

Background

Anemia, the most common disease of blood, is a condition associated with an insufficient number of healthy red blood cells resulting in lower hemoglobin levels. It is often induced by increased erythrocyte damage, decreased or faulty RBC production, and massive blood loss.[1] Physiological conditions, such as menstruation, pregnancy and postpartum, occur in the female and therefore the anemic syndrome is more common in women than men and widely affects women's health. In addition, malignant tumor chemotherapy also has a common adverse reaction on hematopoietic function, which can exhibit leucopoenia and thrombocytopenia in patients.[2] This is a major adverse effect when treating malignant tumor patients by using chemotherapy. Therefore, improving hematopoietic function in anemic patients and preventing the side-effects of malignant tumor chemotherapy is a major goal of research in this field of medicine. In the recent years, it has been reported that many traditional Chinese medicines (TCMs) could treat blood deficiency because of their unique effect and reduced toxicity.[3,4]

The blood deficiency model is usually induced by N-acetyl phenylhydrazine (APH) and Cyclophosphamide (CTX) in previous studies.[5,6] APH is a strong oxidizing agent that decreases the production of glutathione and destroys the stability of red blood cell membranes.[7] CTX is an anti-tumor drug commonly used in clinic, which has a primary adverse effect on the immune organs and the peripheral routine blood.[8] Therefore, the curative effect evaluation of hemopoietics can be from peripheral routine blood, antioxidant capacity and immune organs. Previous study showed that haemopoiesis is the process by which haematopoietic stem cells develop into mature blood cells through

the stimulation of various cytokines in the haematopoietic microenvironment, including EPO, TGF-β_1, and M-CSF.[9]

Maoji Jiu (MJ) is a Chinese health-care wine used commonly for treating women's ailments. It has notable effects on nourishing the blood and regulating menstruation and these were recorded in "Guangxi Zhong Yao Zhi" (published in 1959) and "Quan Guo Zhong Cao Yao Hui Bian" (published in 2014). It consists of *Centropus sinensis* (Hong-mao-ji or He-chi-ya-juan), *Angelica sinensis* Radix (Danggui), Chuangxiong Rhizoma (Chuanxiong), *Angelicae dahuricae* Radix (Baizhi), *Carthami* Flos (Honghua), *Homalomenae* Rhizoma (Qian-nian-jian), *Poria cocos* (Fuling), *Paeoniae lactiflora Pall* Radix (Chishao), *Semen persicae* (Taoren) and some wine in the ratios of 32: 32: 32: 32: 32: 32:4: 3: 3, respectively, with a final ratio of medical materials to wine of 1:17. The beneficial hematopoietic effect of the components of these TCMs has been proved in several previous studies.[5,6,10–13] Dating back to the late Qing Dynasty and the early Republic of China, MJ was first used in Guangdong and Guangxi provinces, and in the next few decades has been used extensively all over the world. Recently, MJ has a well-deserved reputation in China and Southeast Asian Nations because of its notable quality and efficacy.

Although MJ has been used commonly and widely for the treatment of blood deficiency for many years, there is no any scientific study, to our best knowledge, on its potential pharmacological property. Therefore, it is necessary to confirm the hematopoietic function of MJ and further explore its mechanism using a scientific approach, which will provide the basis for its clinical application. In this study, blood deficiency model rat is used to investigate the mechanism of hematopoietic function of MJ. To evaluate the hematopoietic activity of MJ, Fufang Ejiao Jiang (FEJ), a drug has been shown to possess powerful hematopoietic effects,[14] was used as the positive control drug in this study.

Materials And Methods

Chemicals and reagents

N-acetyl phenylhydrazine (APH) was purchased from Shanghai Ziyi Reagent Factory (Shanghai, China, No. ZY150623). Cyclophosphamide (CTX) was purchased from Baxter Oncology GmbH (Germany, No. 5J078A). The superoxide dismutase (SOD), total antioxidant activity (T-AOC) and malondialdehyde (MDA) kits were purchased from Nanjing Jiancheng Bioengineering Research Institute (Nanjing, China). Erythropoietin (EPO) ELISA kit was obtained from Wuhan Boster Bio-engineering Co. Ltd. (Wuhan, China).

Medical materials and extraction protocol

Centropus sinensis was obtained from Pingnan, Guigang (Guangxi, China), and *Angelica sinensis* Radix, Chuangxiong Rhizoma, *Angelicae dahuricae* Radix, *Carthami* Flos, *Homalomenae* Rhizoma, *Poria cocos*, *Paeoniae lactiflora Pall* Radix, and *Semen persicae* were purchased from Yancheng Buyi Pharmacy Co. Ltd. (Jiangsu, China). All of them were identified by Dr. Li (Guangxi Botanical Garden of Medicinal Plants, Guangxi, China). The voucher specimens were deposited in Guangxi Botanical Garden of Medicinal Plants. In this study, we prepared MJ in a traditional and classical way according to a folklore practice.

Centropus sinensis was sacrificed; the viscera were wiped off, and then the whole body was dried in an oven. After steaming with a boiler for 15 minutes, *Centropus sinensis* (160g) was placed in a glass jar and appropriate wine (the ethanol content was 40 percent) was poured into the jar (inundated with *Centropus sinensis*), and then the jar was sealed for 25 days. The rest of the medical materials, *Radix Angelica sinensis* (160g), *Radix Ligusticum chuangxiong Hort* (160g), *Radix Angelicae dahuricae* (160g), *Flos Carthami* (160g), *Rhizoma Homalomenae* (160), *Poria cocos* (20g), *Radix Paeoniae lactiflora Pall* (15g), *Semen persicae* (15g) and the remaining wine, with the total volume up to16800 ml, was added into the same jar. The jar was sealed again for additional 55 days and then filtered through gauze. The accumulated filtrate was evaporated with a rotary evaporator under vacuum at 50℃. The ethanol content of the concentrate was adjusted to 40 percent (v/v), and the total volume was 8400 ml. The wine was stored at room temperature till further use.

Animals

Female Sprague-Dawley rats, with weight between 180 and 220g, were provided by Guangxi Medical University Laboratory Animal Centre (Nanning, China, License No. SCXK [Gui] 2014-0002). All rats were kept in a case at a temperature of 20-25℃ and a relative humidity of 40-60% under a 12-h light/dark cycle with free access to tap water and standard diet. Rats were allowed to acclimatize for five days before the experiment. All procedures were carried in accordance with the ARRIVE (Animal Research: Reporting In Vivo Experiments) guidelines and approved by Ethics Committee of Guangxi Botanical Garden of Medicinal Plants (No. 20170301).

Blood deficiency model construction and administration

After acclimatization, the rats were randomly divided into six groups, each with six rats. The groups are control group (CG), model group (MG), FEJ group, high dose group of MJ (MJ-H), middle dose group of MJ (MJ-M) and low dose group of MJ (MJ-L). The rats in model group, FEJ, MJ-H, MJ-M and MJ-L groups were hypodermically injected with 2% APH saline solution on the 1st and 4th day at the dose of 20 mg/kg and 10 mg/kg, respectively; 2 hours after injections on the 4th day, the rats were intraperitoneally injected with CTX saline solution at a dose of 15 mg/kg for continuous four days. The rats in the control group were hypodermically or intraperitoneally injected with an equal volume of normal saline (NS). Thus, the blood deficiency model was created. The rats in MJ-H, MJ-M and MJ-L groups were intragastrically given 10ml/kg, 5 ml/kg and 2.5 ml/kg body mass of MJ, respectively, twice a day for 10 continuous days. The rats in FEJ group were intragastrically given 6ml/kg body mass of FEJ twice a day for 10 continuous days. Simultaneously, rats in the control group and model group were given an equal volume of NS by oral gavage. All rats were sacrificed on the 11th day. Blood, thymus, spleen, liver and lung samples were obtained for further examination.

Blood routine examination and visceral index calculation

The blood samples of inner canthus 0.5 ml were collected to assay the routine blood tests using a SysmexXT-2000iv full-automatic blood cell analyzer (Sysmex Company, Japan), which included white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT) and platelets (PLT) levels.

The visceral index, including the thymus, spleen, liver and lung, was calculated according to the formula: visceral index = visceral weight (mg)/rat weight (g).

Assessment of antioxidant activity and lipid peroxidation

The antioxidant activities commonly include measurements of SOD and T-AOC detected by using commercially available kits. In brief, liver tissues were homogenized on ice with NS (10% homogenate). Then the homogenates were centrifuged at 2500×g for 10 min at 4 ℃ to obtain the supernatants, which were analyzed for antioxidant activities and lipid peroxidation. The activities of SOD and T-AOC were measured by using commercially available kits according to the instructions of the manufacturer. The content of lipid peroxidation was detected by measurement of MDA formation employing a commercially available kit.

Histopathological examination

The spleen tissue was obtained and a piece was immediately fixed in phosphate buffered saline containing 4% formaldehyde. After fixation, the sample was embedded in paraffin, sectioned in 5 μm thick and mounted on slides. The paraffin-embedded sections were rehydrated using xylene and alcohol series, then stained with hematoxylin-eosin (H&E) to observe histological changes and these evaluated by using optical microscopy (Olympus, China).

Immunohistochemistry

The paraffin-embedded sections of spleen samples were also rehydrated using xylene and alcohol series, then labeled separately with rat monoclonal antibody Bcl-2 (1:100, Bioss, Beijing, China) and Bax (1:1000, Santa Cruz, China) as the primary antibodies and streptavidin-peroxidase as a secondary antibody respectively to observe the presence of these antigens under optical microscopy (Olympus, China), and Image-pro-plus 6.0 was used to analyze the Bcl-2 and Bax expressions semi-quantitative.

Cytokine secretion

The spleen tissues together with 10 times NS were homogenized using a homogenizer (Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China), and then the total proteins of the homogenates were detected using a nucleic acid detector (Thermo, America). Erythropoietin (EPO) concentration in the homogenates was assayed by using the avidin-biotin peroxidase complex Enzyme-Linked Immunosorbent Assay (ABC-ELISA) kits according to the manufacturer's instructions.

Real-time PCR

Total spleen RNA was extracted from frozen tissue samples using Trizol reagent according to the instructions of the manufacturer. Complementary DNA (cDNA) was synthesized using 1 μg of total RNA and a SuperScript® □ Reverse Transcriptase Kit (Takara, China). RT-PCR amplification was performed on a 7300 real time PCR detection system (Applied Biosystems, Foster City, CA, USA) and SYBR® Green PCR Master Mix (Applied Biosystems). Sample cDNAs (equivalent to 2 μg of total RNA) were regarded as

templates with gene-specific primers. The PCR primer sequences were set as follows: TGF-β_1: 5'-CATTGCTGTCCCGTGCAGA-3' (forward) and 5'-AGGTAACGCCAGGAATTGTTGCTA-3'(reverse); M-CSF: 5'-GAATACTGAAC-CTGCCT GCTGAA-3'(forward) and 5'-AGGCCAGCTCAGTGCAAGAA-3' (reverse); β-actin: 5'-GGAGATTACTGCCCTGGCTCCTA-3' (forward) and 5'-GACTCATCGTACTCCT-

GCTTGCTG-3' (reverse). β-actin was used as the housekeeping gene. The expression levels of target mRNAs were normalized to β-actin in arbitrary units.

Statistical analysis

Statistical analysis was performed by using SPSS 16.0 and the results are expressed as mean ± SD, where SD represents standard deviation. Statistical differences between groups were determined using a one-way analysis of variance. A value of $p < 0.05$ was considered to be statistically significant.

Results

Appearance and histopathological examination of the blood deficiency model rats

The appearances of rats were changed after given APH and CTX. As showed in Fig. 1, the rats in the control group appeared to be in a good condition; their ears, noses, faces and tails were pink, and their hairs were tight and glossy. However, the rats in the model group appeared to be in a bad condition; their hairs were fluffy and shed easily, and their ears, noses, faces and feet were pale. Especially the ears of the model group were easily observed. After administration, the above features of the rats in the FEJ, MJ-H, MJ-M and MJ-L groups were similar to those in the control group. Among those, the MJ-H was the closest.

Histopathological changes of spleen sections were observed and evaluated by HE staining (Fig. 2). The spleen tissues in the rats of the control group showed a normal spleen architecture clearly with regular distribution of red pulps (RP) and white pulps (WP), tight arrangement of lymphocytes and no obvious existence of segmentation and differentiation. In contrast, the structure of white pulps in splenocytes of the model group were destroyed. Crypta (CR) was increased and lymphocytes were decreased. In the FEJ (6ml/kg) treatment group, the rats appeared to be better, with less connective tissue and more lymphocytes. These characteristics of rats in the model group were also observed after treatment with MJ (10ml/kg, 5ml/kg or 2.5ml/kg), but they were significantly improved compared with those in the model group.

Effects of MJ on peripheral routine blood and visceral index of the blood deficiency model rats

The indexes of peripheral routine blood directly reflect the curative effect of replenishing blood. As showed in Table 1, the WBC, RBC, HGB, HCT and PLT levels in the model group were significantly decreased compared with those in the animals in the control group ($p < 0.01$), suggesting the model was copied successfully. The WBC, RBC, HGB and HCT levels in MJ and FEJ treatment groups showed an obvious increasing trend compared with those in the animals in the model group.

The changes of visceral indexes can be reflected injure and recover of organs in a certain extent. As showed in Table 2, the liver index, thymus index and spleen index in rats in the model group were reduced significantly compared with those in the control group ($p < 0.01$). Compared with the model group, the liver index and spleen index in MJ-treated groups and FEJ showed a decreasing trend and the thymus index in the MJ and FEJ treatment groups showed an obvious increasing trend. The lung index in the control and treatment groups had no statistical difference compared with the animals in the model group.

Effects of MJ on antioxidant activity and lipid peroxidation of the blood deficiency model rats

The indicators of antioxidant capacity and lipid peroxidation mainly include SOD, T-AOC and MDA. As showed in Table 3, the activity of T-AOC presented a significant increase in rats in the control group and the blood deficiency model rats treated with MJ or FEJ compared with the animals in the model group ($p < 0.05$). In addition, the level of MDA in the animals in the model group was significantly decreased compared with those in the control group ($p < 0.01$). Compared with model group, the levels of MDA in the animals in the MJ-H, MJ-M and FEJ groups were reduced significantly ($p < 0.05$), but in the MJ-L group there was little impact on this parameter. The activity of SOD in the rats in the model group showed a little decrease compared with the other groups, but this was not statistically significant.

Effects of MJ on apoptosis regulatory proteins and hematopoietic growth factors in spleen tissues of blood deficiency model rats

Bcl-2 and Bax are two proteins that play important roles in regulating cell apoptosis. In this study, we found that the expression levels of Bcl-2 and Bax, and the Bcl-2/Bax ratio decreased significantly ($p < 0.05$) in blood deficiency model rats that was induced by APH and CTX. After the administration of MJ and FEJ, Bcl-2 expression level and the Bcl-2/Bax ratio of the animals in the FEJ, MJ-H and MJ-M groups increased significantly ($p < 0.05$); however Bax expression level of FEJ and MJ-H groups increased significantly ($p < 0.05$) compared with that in the model group (Fig. 3).

In present study, we detected the EPO level in spleen tissues of blood deficiency model rats, and the results are showed in Fig. 4. The EPO level in the model group was decreased significantly ($p <0.01$) compared with in the control group. However, the EPO level of the FEJ and MJ-H groups was increased

significantly (p <0.05), and the EPO level of MJ-M and MJ-L groups was also shown an increasing trend compared with in the model group.

M-CSF and TGF-β_1 are two important cytokines in promoting hematopoiesis. Here, we determined and compared the M-CSF and TGF-β_1 mRNA levels in spleen tissues of blood deficiency model rats (Fig. 5). Compared with the control group, the M-CSF mRNA level in the other groups was decreased significantly (p<0.05), but the TGF-β_1 mRNA level in the model group was increased significantly (p<0.05). Compared with animals in the model group, the M-CSF mRNA level in FEJ and MJ-H groups was increased significantly (p<0.05), but the TGF-β_1 mRNA level in FEJ and MJ-H groups was decreased significantly (p<0.05). These results demonstrated that MJ could regulate hematopoiesis via M-CSF and TFG-β_1, with similar effect as FEJ if MJ dose is high.

Discussion

The aim of this study was to evaluate the hematopoietic function of MJ and investigate its mechanism. APH, as a strong oxidizing agent, decreases the production of glutathione and destroys the stability of red blood cell membranes.[7] CTX, as an anti-tumor drug commonly used in clinic, has a primary adverse effect on immune organs and the total number of reduced RBC, WBC and PLT in peripheral blood.[8] Therefore, we induced rats into a blood deficiency model by hypodermically injecting with APH and intraperitoneally injecting with CTX. We detected peripheral routine blood parameters of blood deficiency model rats using a fully automated blood cell analyzer. The results shown that the rats given MJ-H and MJ-M of MJ had significantly higher WBC, RBC, HGB and HCT levels than the animals in the model group (p < 0.05), which suggests that MJ could improve blood deficiency status of rats.

Having confirmed that MJ could improve hematopoietic function, we investigated its mechanism further. Blood deficiency syndrome may be connected with the damages of non-oxidation function.[15,16] The activities of SOD and T-AOC, and the level of MDA are commonly detected to assess the anti-oxidative property in previous studies.[8,17,18] In this study, the results show that the activity of T-AOC in animals in the MJ-H, MJ-M and MJ-L groups was significantly higher than those in the model group (p < 0.01), and the level of MDA in MJ-H and MJ-M groups was lower than the rats in the model group (p < 0.05) (Table 3). The results suggested that MJ could improve the anti-oxidative property via SOD, T-AOC and MDA in blood deficiency model rats.

Bcl-2 and Bax gene are two apoptosis regulatory proteins that belong to the Bcl-2 gene family, which plays an important role in regulating cell apoptosis. Previous studies shown that cell viability can be monitored by measuring the ratio of Bcl-2 and Bax after activation of apoptosis.[19,20] In this study, injury and apoptosis of spleen cells in the model group were observed, which were induced by APH and CTX. We also observed that when FEJ or MJ was administered, this phenomenon in the treatment groups was alleviated when compared to the model group. To better elucidate the cause behind the injury and apoptosis, we used immunohistochemical methods to determine the Bcl-2 and Bax expression levels in

spleen. Compared with the model group, the Bcl-2 and Bax expression levels and the value of Bcl-2/Bax ratio in the FEJ, MJ-H and MJ-M groups were significantly increased ($p < 0.05$). These results indicate that MJ may reduce spleen cells injury and apoptosis by regulating Bcl-2 and Bax expression.

Hematopoietic growth factors from spleen cells, bone-marrow stromal cells and other cells [21] play an important role in the growth and differentiation of various blood cells.[22] EPO and M-SCF are two essential HGFs that participate in hematopoietic regulation.[5,23] EPO regulates the erythropoiesis production and stimulates the proliferation of early erythroid precursors and the differentiation of late erythroid precursors.[24] TGF-β_1, a member of TGF-β, plays an important role in regulating hematopoiesis. Hematopoiesis could be inhibited when it is at a high level. In the present study, the M-CSF mRNA level in the model group, FEJ, MJ-H MJ-M and MJ-L groups were higher than in animals in the control group ($p < 0.01$) and this may be connected with a self-healing mechanism when rats are in a blood deficiency status. However, the M-CSF mRNA level in the FEJ and MJ-H groups were higher than in animals in the model group ($p < 0.05$). Similarly, the EPO level in the control group, FEJ and MJ-H groups were higher than in the model group of animals ($p < 0.05$). However, the TGF-β_1 mRNA level in the control group, FEJ and MJ-H groups were lower than in the model group ($p < 0.05$). These results indicate that MJ may increase hematopoietic activity by increasing the EPO and M-CSF mRNA levels and decreasing the TGF-β_1 mRNA.

Conclusions

In this study, we have shown that MJ, a traditional Chinese health-care wine, has hematopoietic function *in vivo* in a rat model. The findings indicated that MJ administered at high and middle dose groups significantly increased the level of WBC, RBC, HGB and HCT ($p <0.05$), which confirms its hematopoietic effect. In this study, we observed that MJ can significantly increase antioxidant capacity of liver, Bcl-2/Bax ratio, the levels of EPO and M-CSF mRNA of spleen, and significantly decrease the level of TGF-β_1 mRNA in spleen. Therefore, the hematopoietic effect of MJ may be connected with antioxidant ability, apoptosis regulatory proteins and hematopoietic growth factors. It is of interesting to confirm the mechanism of hematopoietic function of MJ in further investigation.

Abbreviations

MJ: Maoji Jiu; APH: N-acetylphenylhydrazine; CTX: Cyclophosphamide; WBC: White blood cell; RBC: Red blood cell; HGB: Hemoglobin; HCT: Hematocrit; PLT: Platelet; EPO: Erythropoietin; TGF-β1: Transforming growth factor-beta-1; M-CSF: Macrophage colony-stimulating factor; TCMs: Traditional Chinese medicines; FEJ: Fufang Ejiao Jiang; SOD: Superoxide dismutase; T-AOC: Total antioxidant activity; MDA: Malondialdehyde; ELISA: Enzyme-linked Immunosorbent assay; CG: Control group; MG: Model group; MJ-H: High dose group of Maoji Jiu; MJ-M: Middle dose group of Maoji Jiu; MJ-L: Low dose group of Maoji Jiu; NS: Normal saline; H&E: Hematoxylin-eosin; ABC-ELISA: Avidin-biotin peroxidase complex Enzyme-

Linked Immunosorbent Assay; PCR: Reverse transcription polymerase chain reaction; cDNA: complementary DNA; RP: Red pulps; WP: White pulps; CR: Crypta.

Declarations

Acknowledgements

Not applicable.

Ethics approval and consent to participate

Female Sprague-Dawley rats were obtained from the Guangxi Medical University Laboratory Animal Centre (Nanning, China, License No. SCXK [Gui] 2014-0002). All procedures were carried in accordance with the ARRIVE (Animal Research: Reporting In Vivo Experiments) guidelines and approved by Ethics Committee of Guangxi Botanical Garden of Medicinal Plants (No. 20170301).

Consent for publication

Not applicable.

Availability of data and materials

All data generated and analyzed during this study are included in this article. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

ZGY and LL contributed equally to this work. All experiments were performed by FQZ, YLX, and CJZ. FQZ, YLX, JH, and ZGY wrote the manuscript. JPJ and JH were in charge of data analysis and technical graphics and helped revise the manuscript. ZGY and LL helped revise the manuscript. The authors read and approved the final manuscript.

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Tables

Table 1 The changes of peripheral blood routine on blood deficiency model rats ($\bar{x} \pm s$, n = 6).

Group	WBC/×10^9/L	RBC/×10^{12}/L	HGB/g·L^{-1}	HCT/%	PLT/×10^9/L
CG	9.15±1.00	7.18±0.31	135.67±6.12	42.67±1.71	929.0±140.04
MG	2.89±0.45*	3.47±0.32*	89.17±10.82*	31.17±2.72*	646.0±62.66*
FEJ	3.63±0.48#	3.99±0.15##	101.33±5.35#	33.57±2.96	810.5±159.79#
MJ-H	3.74±0.47##	4.20±0.37##	105.83±6.82##	36.57±3.14##	656.0±168.00
MJ-M	3.55±0.54#	3.95±0.34#	100.17±4.22#	35.33±2.18#	679.8±125.57
MJ-L	3.16±0.88	3.80±0.16#	94.83±6.15	33.33±1.53	639.7±99.26

*$P < 0.01$ vs control group.

#$P < 0.05$

##$P < 0.01$ vs model group.

Table 2 The visceral index changes on blood deficiency model rats ($\bar{x} \pm s$, n = 6).

Group	Liver index (mg/g)	Thymus index (mg/g)	Spleen index (mg/g)	Lung index (mg/g)
CG	25.97±1.30	1.79±0.21	2.35±0.12	5.20±0.55
MG	32.52±3.01*	0.74±0.05*	5.41±0.47*	5.48±0.35
FEJ	29.23±1.65#	0.87±0.12#	5.13±1.05	5.16±0.44
MJ-H	29.75±0.52#	0.94±0.12##	4.84±0.40#	5.35±0.23
MJ-M	30.59±0.98	0.87±0.09#	4.91±0.25#	5.33±0.39
MJ-L	30.51±1.62	0.81±0.18	5.24±1.04	5.32±0.69

*$P < 0.01$ vs control group.

#$P < 0.05$

##$P < 0.01$ vs model group.

Table 3 Effects of MJ on the activity of SOD, T-AOC and the level of MDA in livers of blood deficiency model rats ($\bar{x} \pm s$, n = 6).

Group	SOD (U/mg prot)	T-AOC (U/mg prot)	MDA (nmol/mg prot)
CG	197.50±7.92	12.51±1.35	9.45±1.05
MG	195.69±5.78	8.68±1.50**	15.44±2.11**
FEJ	200.53±12.27	10.54±0.85#	12.98±1.02#
MJ-H	196.04±11.24	12.47±1.37##	12.40±1.08##
MJ-M	195.43±6.47	11.55±0.76##	13.25±0.90#
MJ-L	194.50±15.42	11.36±1.29##	13.62±1.22

*$P < 0.01$ vs control group.

#$P < 0.05$

##$P < 0.01$ vs model group.

Figures

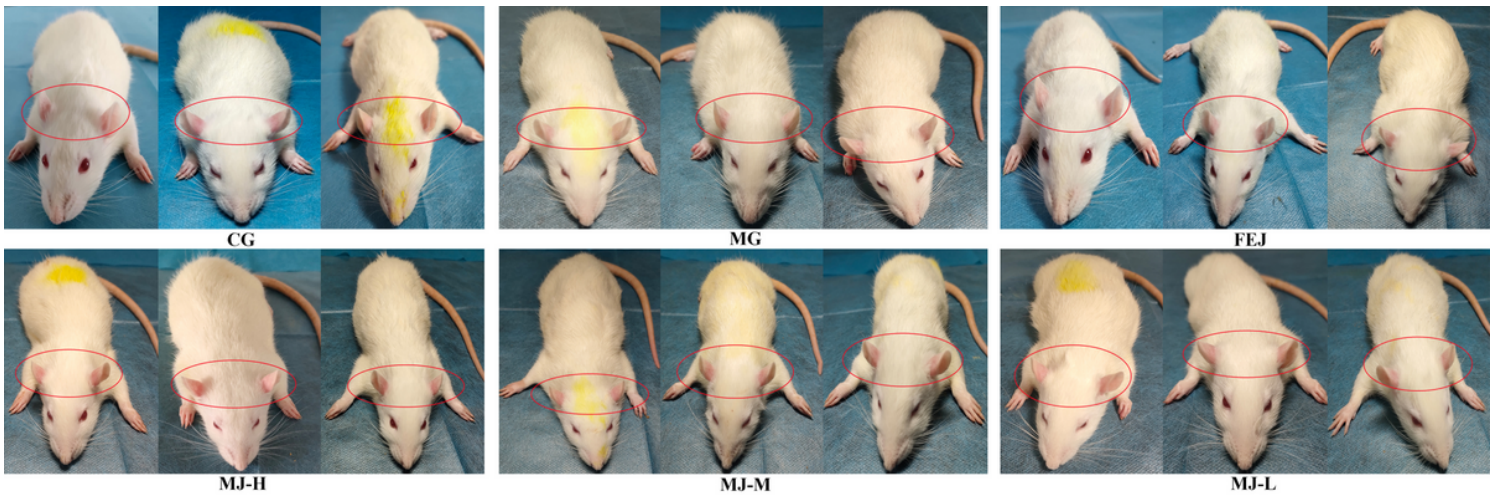

Figure 1

Change of MJ on appearance of the blood deficiency model rats.

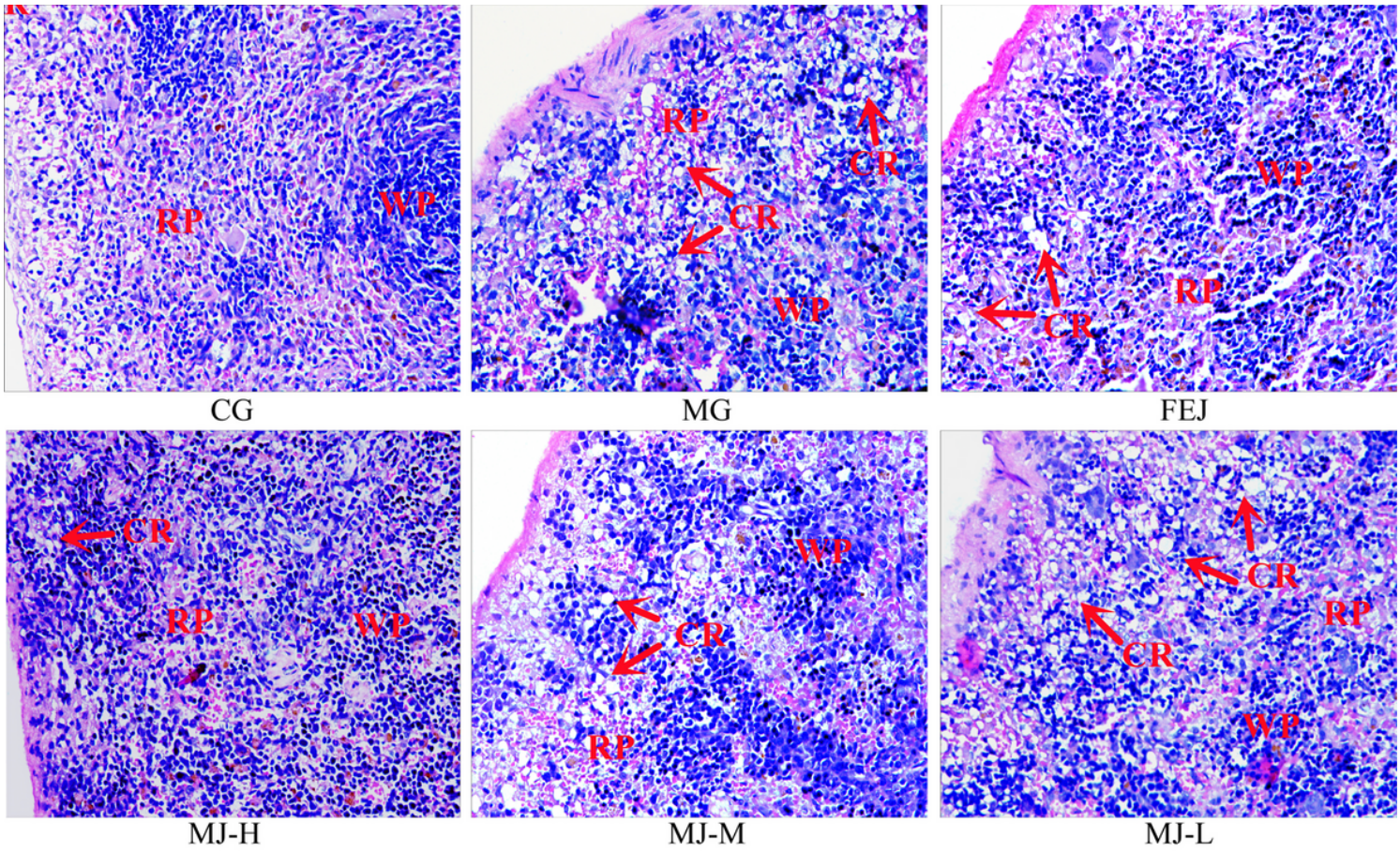

Figure 2

Effects of MJ on the histological structure in spleen tissues of blood deficiency model rats (HE ×200).

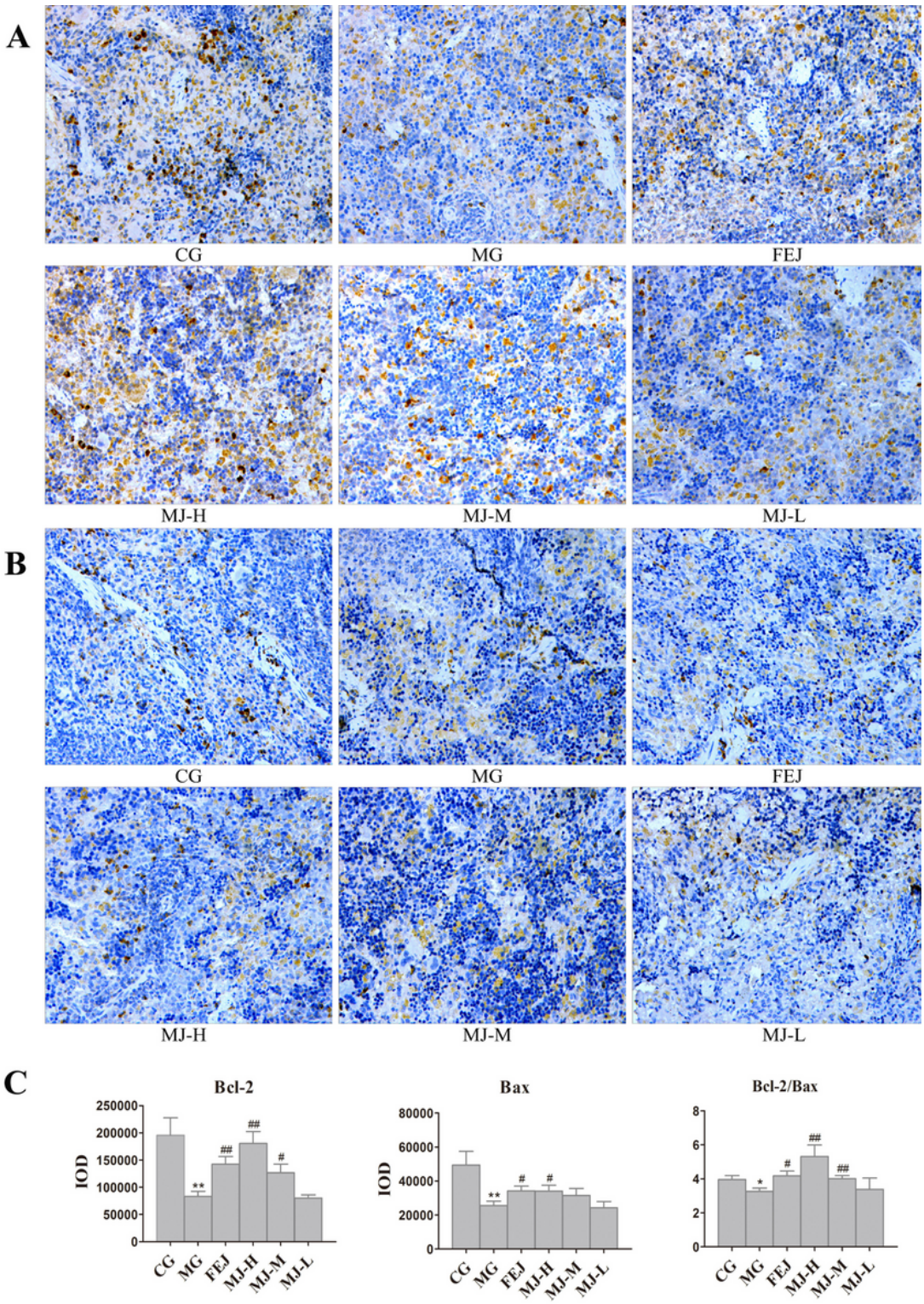

Figure 3

Effects of MJ on expression of Bcl-2 and Bax in spleen tissues of blood deficiency model rats. (A)Representative photomicrographs of the SABC-stained histological slides of Bcl-2 in rat spleens (SABC ×200). (B)Representative photomicrographs of the SABC-stained histological slides of Bax in rat spleens (SABC ×200). Using this staining method, the positive cell cytoplasm and cell nucleus are stained with

claybank colour by SABC. (C)The means±SD of the average absorbance values (IOD) of the splenic Bcl-2 and Bax proteins from the experimental animals are showed under the figure.

*P<0.05, **P<0.01 vs control group; #P<0.05, ##P<0.01 vs model group.

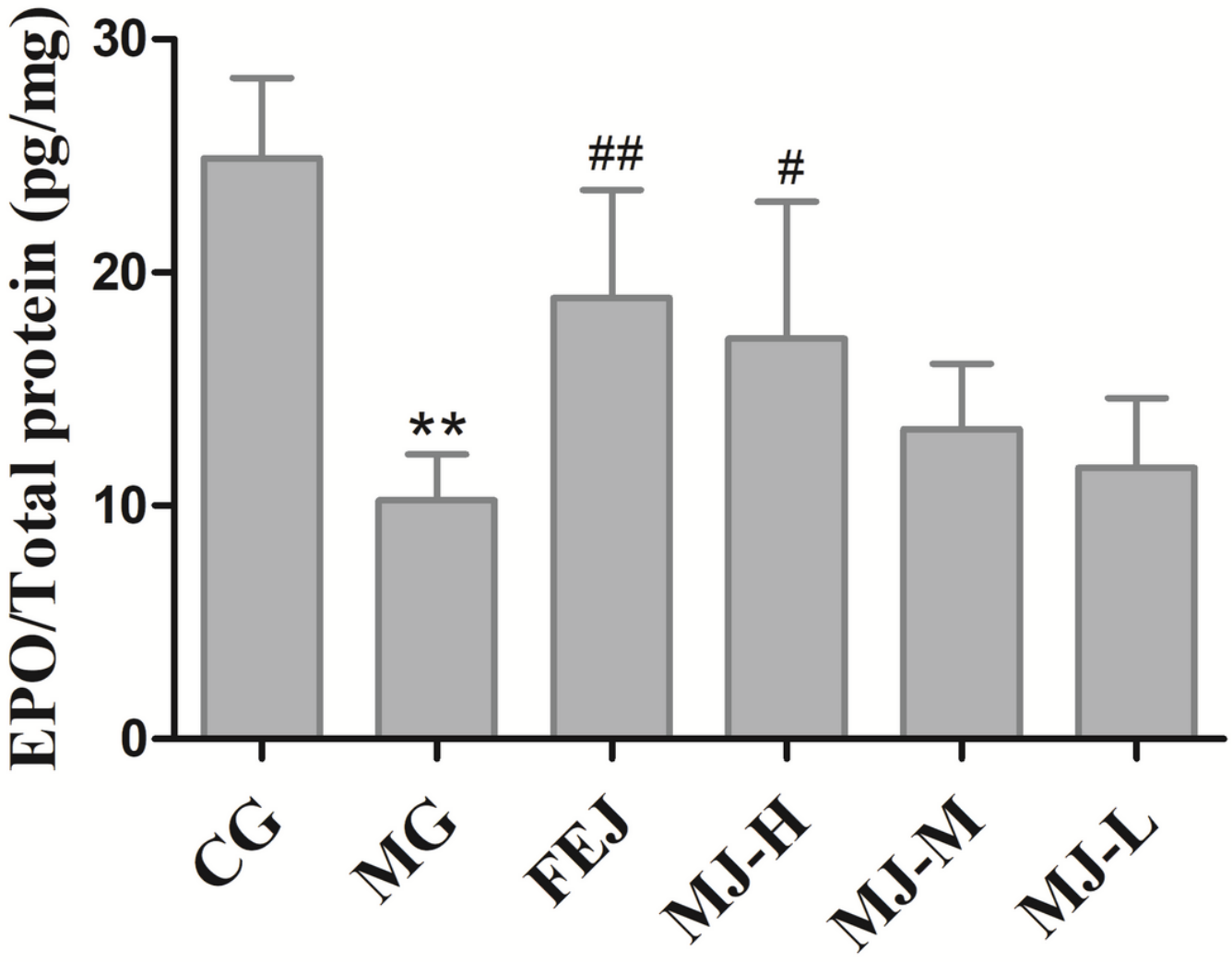

Figure 4

Effects of MJ on EPO level of spleen tissues of blood deficiency model rats.

*P<0.05, **P<0.01 vs control group; #P<0.05, ##P<0.01 vs model group.

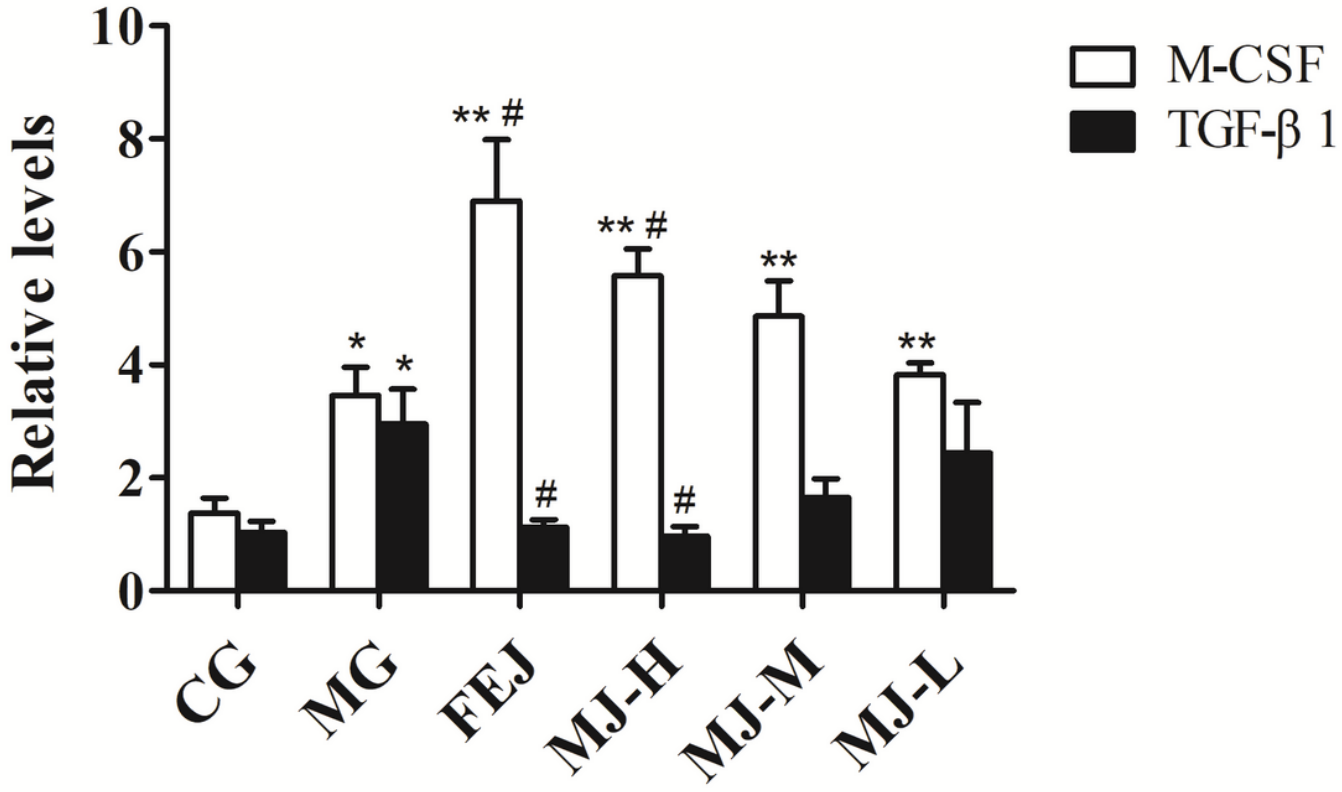

Figure 5

Effects of MJ on M-CSF and TGF-β1 mRNA level of spleen tissues of blood deficiency model rats.

**P<0.01 vs control group; #P<0.05 vs model group.