

The Effect of hydroalcoholic extract of *Astragalus membranaceus*'s root on the hematological parameters and bone marrow cells after the administration of Mitomycin C in male rats

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Abstract

Mitomycin C (MMC) is an anti-cancer drug; but it has a side-effect which is bone marrow depression. *Astragalus membranaceus* is used in traditional Chinese medicine. This plant has various pharmacological effects, including hematopoietic, angiogenesis and immunostimulant effects. This study evaluated the effects of *Astragalus* root hydroalcoholic extract on the hematological and bone marrow cells after administering MMC. Twenty-eight male Sprague-Dawley rats (250±30 g) were divided into four groups: control (without treatment), Mitomycin group (MMC, 2 mg/kg, i.p.), Astragalus extract (*Astragalus* root extract, 500 mg/kg for 14 days) and treated group (MMC, 2 mg/kg, i.p. and *Astragalus* root extract, 500 mg/kg for 14 days). The hematological parameters (hematocrit, hemoglobin, red blood cell (RBC) count, red cells indices, total white blood cells (WBC), platelets, and differential leukocyte count) and bone marrow cells (erythroid and myeloid series) were measured. After receiving MMC, the hematological parameters and bone marrow cells were reduced. The results showed that hydroalcoholic extract of *Astragalus membranaceus* could increase significantly the hematological parameters including hematocrit, hemoglobin, RBC, WBC, and platelets as well as bone marrow cells. This study showed that *Astragalus* root hydroalcoholic extract positively affected anemia signs.

Key words: *Astragalus membranaceus*, Anemia, Mitomycin C, Bone marrow

Introduction

Anemia is a common complication that leads to a decrease of hemoglobin and red blood cells (Patel et al., 2016). Various causes and many factors, such as mutations in the gene for hemoglobin, acute and chronic bleeding, inadequate nutritional intake, inflammation, low levels of erythropoietin, iron deficiency, and failure to produce red blood cells can induce

anemia. Anemia is interference with the oxygen delivery to tissues, weakness, increased risk of preterm delivery, low birth weight, headache, pallor, shortness of breath, poor concentration, psychosis, seizures, strokes, and even death (Kassebaum, 2016). Anemia may also be induced by drugs like isoniazid, sulfonamides, cyclophosphamide, and

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Mitomycin (Barragán-Ibanez, 2016). Mitomycin C is an anticancer drug that could be converted into an alkylating agent and used for treating a range of cancers, including esophagus, breast, bladder, stomach and cervix cancer. Mitomycin C also causes decreased hemoglobin levels, hemolytic anemia, inhibition in the activity of bone marrow, leukopenia, thrombocytopenia, kidney disorders, and hypertension, as side effects (Shah et al, 2013).

The dried root of *Astragalus membranaceus* is one of the oldest and most commonly used treatments in traditional Chinese medicines (Wong, 2006). A wide range of medicinal properties also have been reported for *Astragalus* root, including its blood-forming activity, anti-hyperglycemia, anti-inflammatory, anti-bacterial, and anti-viral effects (Bian and Li, 2009). Saponins, isoflavonoids, polysaccharides, and astragalosides are all biologically active constituents of *Astragalus membranaceus* root (Bian and Li, 2009; Liu et al, 2017). This study aimed to evaluate the effects of *Astragalus* root hydroalcoholic extract on the hematological parameters and bone marrow cells after administering Mitomycin C in rats.

Materials and Methods

Root extraction and drug preparations

Astragalus root powder (255 g) was mixed with ethanol (550 ml of 25%) and stirred for several hours. After 24 h, the mixture was filtered and made smooth. Then, the vacuum distillation to a quarter of the initial volume was condensed; after lyophilization, the powder was kept in the freezer (-20 ° C). In this study, Mitomycin C (2 mg vials) (Kowya, Japan) was used (Kumari et al, 2016).

Animals

Twenty-eight male Sprague Dawley rats (250±30) were divided randomly into four groups (n=7) and kept in separate cages as in the following groups:

1. The control group received no treatment.

2. Mitomycin group: 2 mg/kg Mitomycin C (1 mL) was injected intraperitoneal (i.p.) and after four weeks, for 14 days, distilled water gavage was done.

3. *Astragalus* extract group: distilled water (1 mL) was injected (i.p.), and after four weeks, 500 mg/kg of *Astragalus* root extract was given for 14 days.

4. Treated group: 2 mg/kg Mitomycin C was injected (i.p.), and after four weeks, 500 mg/kg *Astragalus* root extract was given for 14 days (Shah et al, 2013).

Animal ethics

The experiment was performed under the approval of the state Committee on Animal Ethics, Shiraz University, Shiraz, Iran (IACUC no: 4687/63). In addition, the recommendations of the European Council Directive (86/609/EC) of Nov 24, 1986, regarding protecting animals used for experimental purposes were considered.

Blood and bone marrow analysis

At the end of the test, the rats were anesthetized by CO₂ and blood samples were taken from the heart and collected in EDTA tubes. Blood parameters including hematocrit, hemoglobin concentration, white blood cells, red blood cells, differential leukocyte count, and platelet count were measured. The femur was selected for bone marrow, and the smears were prepared. Bone marrow slides were used to evaluate the cellularity, particle distribution and average megakaryocyte numbers per 20 fields and to classify the erythroid and myeloid precursors (Voigt and Swist, 2011; Latimer, 2011). Bone marrow cell counts, erythroid and myeloid cell counts, and the proportion of the myeloid cells to erythroid cells (M/E ratio) were also measured.

Statistical analysis

The one-way analysis of variance (ANOVA) and Duncan test were

conducted. P values ≤ 0.05 were considered significant.

Results

Blood factors

The amount of hemoglobin and red blood cells in the Mitomycin group showed a significant decrease compared to the

amounts observed in the control group. Hemoglobin and the number of red blood cells in the Astragalus extract group increased compared to the control group, but this difference was insignificant. Hemoglobin in the treated group increased compared to the Mitomycin group (Table 1).

Table 1: Comparison of erythrocytes in different groups (Mean \pm SEM)

Factors Groups	Hct (%)	Hb (g/dl)	WBC (μ l)	RBC $\times 10^6$ (μ l)	PLT $\times 10^5$ (μ l)	MCV (fl)	MCH (pg)	MCHC (g/dl)
Group 1(control)	47.57 \pm 0.48 ^b	15.83 \pm 0.15 ^b	8917.29 \pm 354.90 ^b	8.64 $\times 10^6$ \pm 2 ^b	5.33 $\times 10^5$ \pm 5758.75 ^b	55.16 \pm 0.96 ^b	18.36 \pm 0.33 ^b	33.27 \pm 0.04 ^b
Group 2(C1)	40.83 \pm 3.59 ^a	13.51 \pm 1.19 ^a	7414.50 \pm 179.76 ^a	5.76 $\times 10^6$ \pm 5.59 ^a	4.30 $\times 10^5$ \pm 7846.09 ^a	71.39 \pm 1.27 ^a	23.62 \pm 0.44 ^c	32.92 \pm 0.18 ^b
Group 3(C2)	49 \pm 0.44 ^b	17.47 \pm 0.57 ^b	1.12 $\times 10^4$ \pm 552.6 ^c	8.80 $\times 10^6$ \pm 1.07 ^b	5.43 $\times 10^5$ \pm 25539.89 ^b	55.71 \pm 0.39 ^b	19.86 \pm 0.63 ^b	35.64 \pm 1.04 ^b
Group 4(T)	45.43 \pm 2.10 ^{ab}	13.59 \pm .28 ^a	8988.14 \pm 504.37 ^b	8.32 $\times 10^6$ \pm 3.40 ^b	5.47 $\times 10^5$ \pm 9413.77 ^b	58.71 \pm 1.41 ^b	16.45 \pm .57 ^a	29.50 \pm 1.64 ^a

Control: control group; C1: Mitomycin group; C2: Astragalus extract group; T: treated group treated with Mitomycin C and Astragalus root extract. Lowercase letters in each column show a significant difference in various groups' p < 0.05.

- Hct: Hematocrit
- Hb: Hemoglobin
- WBC: White Blood Cell
- RBC: Red Blood Cell
- PLT: Platelets
- MCV: Mean Corpuscular Volume
- MCH: Mean Corpuscular Hemoglobin
- MCHC: Mean Corpuscular Hemoglobin Concentration

The MCV decreased significantly in the treated group compared to the Mitomycin group, with no significant differences with the control group. MCH and MCHC in the treated group showed a significant decrease compared with the Mitomycin group (Table 1).

White blood cell counts in the treated group showed a significant increase compared to the Mitomycin group. However, it showed no significant difference compared to the control group (Table 1). Lymphocytes in the treated group showed a significant decrease compared to

that of the Mitomycin group, but it showed no significant difference compared to the control group. The number of neutrophils in the treated group showed a significant increase compared to that observed for the Mitomycin group. However, it showed no significant difference compared to the control group (Table 2).

The platelet counts in the treated group significantly increased compared to those of the Mitomycin group, but it showed no significant difference compared to the control group (Table 1).

Table 2: Comparison of blood leukocytes in different groups (Mean ± SEM)

Factors Groups	BAS (%)	BAS (/μl)	EOS (%)	EOS (/μl)	MON (%)	MON (/μl)	LYM (%)	LYM (/μl)	NUT (%)	NUT (/μl)
Group 1(control)	0	0	0.14 ± 0.14 ^a	12.48 ± 12.48 ^a	5.35 ± 0.73 ^a	477.07 ± 65.09 ^a	63.18 ± 1.99 ^a	5633.94 ± 177.45 ^a	28.42 ± 2.12 ^b	2534.29 ± 189.04 ^b
Group 2(C1)	0	0	0.17 ± 0.17 ^a	12.60 ± 12.60 ^a	4.59 ± 0.80 ^a	340.32 ± 59.31 ^a	78.07 ± 2.37 ^b	5788.50 ± 175.72 ^b	17.17 ± 2.63 ^a	1273.06 ± 195 ^a
Group 3(C2)	0	0	0 ^a	0 ^a	5.85 ± 0.78 ^a	655.2 ± 87.36 ^a	61.36 ± 3.04 ^a	6872.32 ± 340.48 ^a	31.36 ± 2.85 ^b	3512.32 ± 319.2 ^b
Group 4(T)	0	0	0.14 ± 0.14 ^a	12.58 ± 12.58 ^a	3.74 ± 0.48 ^a	336.15 ± 43.14 ^a	61.61 ± 1.75 ^a	5597.59 ± 157.29 ^a	36.31 ± 1.74 ^b	3263.59 ± 156.39 ^b

Control: control group; C1: Mitomycin group; C2: Astragalus extract group, T: treated group treated with Mitomycin C and Astragalus root extract. Lowercase letters in each column show a significant difference in various groups' p < 0.05.

A: absolute
 BAS: Basophils
 EOS: Eosinophils
 MON: Monocytes
 LYM: Lymphocytes
 NUT: Neutrophils

Bone marrow factors

After administering Mitomycin C in all groups compared to the negative control group, all cells of the myeloid line were significantly reduced, except the band cell. Myeloid cell lines including myeloblast (MBS), promyelocyte (PMS), myelocyte (MS), meta myelocyte (MTMS) and erythroid cell lines including rubriblast (RRS), prorubricyte (PRS), basophilic rubricyte (RS), and polychromatic rubricyte

(MRS) demonstrated in Figure 1. In the test group, all the cells of the myeloid line (except the band cell) and all the erythroid cell lines increased compared to the positive control group. The proportion of the myeloid cell line to the erythroid cell line in the positive control, comparative control, and test groups increased compared to the proportion observed for the negative control group (Table 3).

Table 3: Comparison of erythroid and myeloid cells in the bone marrow in different groups (Mean ± SEM)

Factors Groups	MBS (%)	PMS (%)	MS (%)	MTMS (%)	BAND (%)	RRS (%)	PRS (%)	RS (%)	MRS (%)	M/E
Group 1(control)	0.372 ± 0.26 ^a	2.2 ± 0.38 ^a	16.82 ± 0.99 ^b	18.37 ± 0.86 ^b	17.31 ± 3.88 ^a	0.82 ± 0.34 ^{ab}	5.62 ± 0.77 ^b	27 ± 1.49 ^b	11.05 ± 5.33 ^b	1.23 ± 0.05 ^a
Group 2(C1)	0.26 ± 0.21 ^a	2.3 ± 0.56 ^a	13.8 ± 1.69 ^a	16.66 ± 0.99 ^a	0.34 ± 3.16 ^c	0.63 ± 0.31 ^a	4.83 ± 0.48 ^a	0.20 ± 1.06 ^a	6.56 ± 2.90 ^a	2.08 ± 0.04 ^c
Group 3(C2)	0.34 ± 0.42 ^a	2.25 ± 0.48 ^a	17.2 ± 0.53 ^b	18.62 ± 0.80 ^b	17.68 ± 5.12 ^a	0.97 ± 0.34 ^b	5.74 ± 0.86 ^b	27.4 ± 1.17 ^b	9.86 ± 5.15 ^b	1.28 ± 0.05 ^a
Group 4(T)	0.28 ± 0.29 ^a	2.22 ± 0.40 ^a	16.94 ± 0.97 ^b	17.14 ± 0.92 ^a	26.8 ± 7.60 ^b	0.86 ± 0.53 ^a	5.31 ± 0.72 ^b	21.4 ± 6.61 ^a	8.91 ± 1.46 ^{ab}	1.74 ± 0.10 ^b

Control: control group; C1: Mitomycin group; C2: Astragalus extract group; T: treated group treated with Mitomycin C and Astragalus root extract. Lowercase letters in each column show a significant difference in various groups' p < 0.05

RRS: Rubriblast; PRS: Prorubricyte; RS: Basophilic Rubricyte; MRS: Polychromatic rubricyte
 MBS: Myeloblast; PMS: Promyelocytes; MS: Myelocytes; MTMS: Meta myelocytes

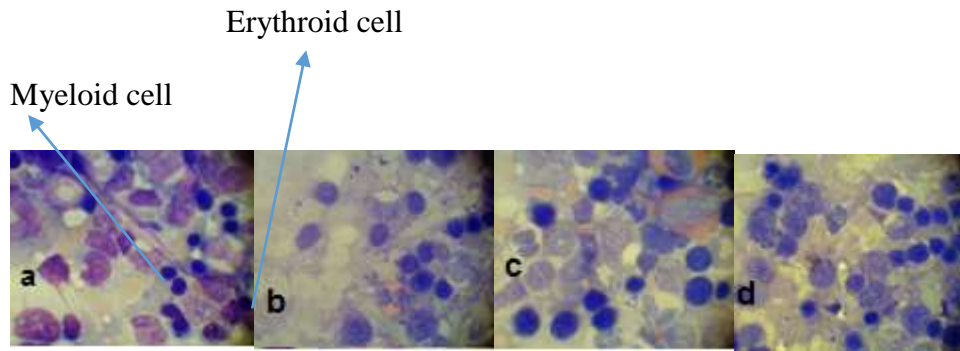


Figure 1: Pathology image of bone marrow cells
a): bone marrow cells in the control group (100 ×).
b): bone marrow cells in the Mitomycin group (100 ×).
c): bone marrow cells in the Astragalus extract group (100 ×).
d): bone marrow cells in the treated group (100 ×).

Discussion

This study showed that *Astragalus* root hydroalcoholic extract positively affected the treatment of anemia. Plants have always been a suitable source for the production of pharmaceuticals (Ashraf et al, 2016). *Astragalus* contains active biological compounds in its root (Ionkova et al., 2010). In the present study, bone marrow erythroid and myeloid categories decreased after the administration of Mitomycin C. These results show that Mitomycin C is a bone marrow suppressor.

Cytotoxicity and the side - effects of Mitomycin C are inhibiting the metabolism and forming free radicals in the cells (Siegel et al, 1990). The drug acts by two mechanisms, namely bioreductive alkylation and generation of free radicals such as superoxide and hydroxyl radicals through its metabolic activation (Bass et al, 2013; Dusre et al, 1989). One of the side - effects of Mitomycin C is bone marrow depression (Croke and Bradner, 1976). Mitomycin C induces genotoxicity and cytotoxicity in healthy cells (Siegel et al, 1990). The extract of *Dorema aucheri* on hematologic factors in rats showed no significant difference in the total number of red blood cells, white blood cells, lymphocytes percentage, basophils, eosinophils and monocytes (Khoshvaghti et al, 2013). *Dorema aucheri* extract is rich in flavonoids and flavonoid compounds and has strong antioxidant properties

(Khoshvaghti et al, 2013; Tavana et al, 2015). The intraperitoneal injection of aqueous and organic extracts of *Eukalyptoseucalyptose globulus* on blood biochemical factors was studied, and the results showed that ethanol extract could have a significant effect on the level of white blood cells, red blood cells, hemoglobin, and hematocrit but no significant effect on the platelet count (Sheikhzadeh et al, 2011).

Zataria multiflora is a grassy and annual plant that contains different constituents, including carvacrol, and can increase the number of white blood cells, and the percentage of neutrophils, monocytes, eosinophils, and lymphocytes (Boskabady and Jalali, 2016). In the present study with *Astragalus* root extract, the increase in the number and percentage of leukocyte parameters are less.

In a study on oxidative stress, *Trifolium* extract showed an antioxidant effect on platelet count. *Trifolium* has polyphenolic compounds, phenolic acids, flavonoids, isoflavones, clovamide, and had powerful antioxidant properties. The extract increased in platelet levels (Kolodziejczyk-Czepas et al, 2013).

The roots of *Astragalus membranaceus*, known pharmaceutically as radix *Astragali*, are rich in saponins, polysaccharides, isoflavonoids, γ -aminobutyric acid, and various trace elements (Wagner et al.,

1997). *Dorema aucheri* extract also has flavonoid compounds; however, future studies should identify effective compounds (Tavana et al, 2015).

Hydro-alcoholic propolis extract showed a Hindi protective effect on gene toxicity and cytotoxicity of Mitomycin C (Kumari et al, 2016). Mitomycin C can cause a significant increase in cellular apoptosis in bone marrow (the polar and non-polar compounds). The propolis extract is an antioxidant and inhibits the product of radicals induced by the Mitomycin C (Kumari et al., 2016). The reduction of erythroid lineage cells and decreased bone marrow myeloid cells, myelocytes and metamyelocytes class by Mitomycin C also corresponded with the findings of the present study and corroborated our findings (Kumari et al, 2016).

Polysaccharides of Angelica root are the most important compound with anti-tumor properties (Hui et al, 2006). They also correspond with the growing diversity of plant species because the most important part is the root of many of the compounds. After suppressing of the bone marrow cells using cyclophosphamide, Angelica extract can improve deficiently (Hui et al., 2006). Cyclophosphamide reduced the number of white blood cells and the number of blood vessels. The Astragalus extract polysaccharides stimulate and increase the production of stem cells in bone marrow (Li et al, 2014). It also enhances the hematopoietic cells, but this difference is not significant. This is probably due to the

same effect in both plant polysaccharides, a finding consistent with the present study (Li et al, 2014). A study found that Astragalus root could treat bone diseases such as osteoporosis (Bian and Li, 2009). Astragalosides can initiate osteoblast proliferation and differentiation and may also be from early to late-stage osteoblast differentiation (Bian and Li, 2009).

Astragalus root as a medicinal plant has all of the above-described compounds, in turn, today, as a new drug used to treat a variety of diseases (Kim et al., 2009). Because of the superior efficacy and completeness of *Astragalus membranaceus* to other plants, the composition is effective as a whole compound (Liu et al, 2017). In the present study, Astragalus root extract could increase the erythroid and myeloid lineage cells in the bone marrow and blood. Different compounds in the Astragalus root extract (Liu et al, 2017) can improve the toxic effects of Mitomycin C on blood factors and bone marrow cells, and therefore suggested that this extract has hematopoietic activity.

Astragalus root extract positively affected the blood and bone marrow factors. In other words, this extract could increase the erythroid and myeloid lineage cells in the bone marrow and blood. Further research is needed in the immune, cardiovascular, and reproductive systems so that the types of compounds in the Astragalus root could be identified.

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Conflict of interest

There is no conflict of interest to disclose.

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تأثیر عصاره هیدروالکلی ریشه گون بر پارامترهای خونی و سلول‌های مغز استخوان پس از تجویز میتومایسین سی در موش‌های صحرایی نر

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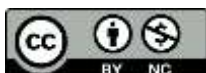
چکیده

میتومایسین C (MMC) یک داروی ضد سرطان است، اما یک عارضه جانبی دارد و آن افسردگی مغز استخوان است. *Astragalus membranaceus* در طب سنتی چینی استفاده می‌شود. این گیاه دارای اثرات فارماکولوژیک مختلفی از جمله خون‌سازی، رگ‌زایی و تحریک ایمنی است. این مطالعه اثرات عصاره هیدروالکلی ریشه گون را بر سلول‌های خونی و مغز استخوان پس از تجویز MMC نشان داد. بیست و هشت موش صحرایی نر نژاد اسپراگ-داولی (به وزن 250 ± 30 گرم) به چهار گروه کنترل (بدون دارو و درمان)، میتومایسین (MMC، ۲ میلی‌گرم در کیلوگرم، داخل صفاقی)، عصاره ریشه گون (عصاره ریشه گون، ۵۰۰ میلی‌گرم در کیلوگرم به مدت ۱۴ روز) و درمان (تجویز میتومایسین ۲ میلی‌گرم در کیلوگرم، داخل صفاقی و درمان با عصاره ریشه گون ۵۰۰ میلی‌گرم در کیلوگرم به مدت ۱۴ روز) تقسیم شدند. پارامترهای هماتولوژیک (هماتوکریت، هموگلوبین، تعداد گلبول‌های قرمز (RBC)، شاخص‌های گلبول‌های قرمز، تعداد پلاکت‌ها، تعداد گلبول‌های سفید (WBC) و تشخیص افتراقی گلبول‌های سفید) و سلول‌های مغز استخوان (سری اریثروئید و میلوئید) اندازه‌گیری شدند. پس از دریافت MMC، پارامترهای خونی و سلول‌های مغز استخوان کاهش یافت. نتایج نشان داد که عصاره هیدروالکلی گون می‌تواند پارامترهای خونی شامل هماتوکریت، هموگلوبین، تعداد گلبول‌های قرمز، تعداد گلبول‌های سفید و پلاکت‌ها و همچنین سلول‌های مغز استخوان را افزایش دهد. این مطالعه نشان داد که عصاره هیدروالکلی ریشه گون تأثیر مثبتی بر نشانه‌های کم خونی دارد.

کلمات کلیدی: ریشه گیاه گون، کم خونی، میتومایسین سی، مغز استخوان

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