



Evaluation of the anti-anemic potential of *Glycyrrhiza glabra* aqueous extract in Phenylhydrazine-treated rats

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ABSTRACT

Glycyrrhiza glabra is one of the popularly grown leafy plants with several therapeutical effects in Iran. In this experiment, we evaluated the anti-anemia potential of aqueous extract of *G. glabra* on Phenylhydrazine-induced anemic rats. In vivo design, 50 rats were used. Induction of hemolytic anemia was done by three injections of Phenylhydrazine in 40 rats. After one day, the rats were divided into five subgroups, including negative healthy control, untreated negative control, and three groups receiving the *G. glabra* at 30, 60, and 120 mg/kg concentrations. At the end of day 15 of treatment, the animals of all groups were sacrificed, and blood samples were taken to analyze the biochemical, hematological, and immunological parameters. Several doses of *G. glabra* significantly ($p \leq 0.05$) enhanced the reduced levels of high-density lipoprotein, total protein, albumin, white blood cell, platelet, red blood cell, hemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, interleukin 4, interleukin 5, interleukin 10, interleukin 13, and interferon alpha and decreased the increased levels of alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyltransferase, ferrous, ferritin, erythropoietin, cholesterol, low-density lipoprotein, triglyceride, total and conjugated bilirubin, urea, creatinine, interleukin 1, interleukin 6, interleukin 12, interleukin 18, interferon gamma, and tumor necrosis factor alpha, as compared to the untreated group. In conclusion, the obtained results revealed the anti-anemia potential of aqueous extract of *G. glabra*. Extraction of active molecules will be the future work to peruse.

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Keywords

Glycyrrhiza glabra,
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INTRODUCTION

Hemolytic anemia is a public health problem extremely frequent in the world which assigns people of any age [1]. The groups at high the risks are infants, pregnant women, in particular, those in age to procreate and old people [2]. In developing countries, hemolytic anemia prevalence is 3 to 4 times higher than that in developed countries.

Hemolytic anemia is associated several situations such as heavy bleeding, nutritional deficiencies, genetic defects, infectious diseases, prolonged use of non-steroids drugs and exposure to toxic chemicals as Phenylhydrazine which reduce in quality and quantity red blood cells and hemoglobin [3-8].

Various treatments are carried out according to hemolytic anemia type. They can act of contribution out iron, vitamin B₁₂ or B₉ by the oral route, treatment with immunosuppressors or corticosteroids, erythropoietin injection, blood transfusion, or osseous marrow transplantation [9]. The low cost, availability, accessibility, and effectiveness are some reasons due to the widespread use of medicinal plants [10].

One of the most important herbal medicines which are widely used for the treatment of several diseases is *Glycyrrhiza glabra* from *Fabales* order, *Fabaceae* family, *Faboideae* subfamily and *Glycyrrhiza* genus. *G. glabra* has been utilized in folk medicine as an antioxidant, anti-inflammatory, antispasmodic, antipyretic, antiparasitic, antibacterial, antifungal, and antiviral agent and for curing diarrhea, gastrointestinal ulcers, and infection [11-14].

It has a long history of use in traditional medicine, but there is a little evidence to indicate *G. glabra* is useful to treat hemolytic anemia. We attempted to survey the therapeutical effect of aqueous extract of *G. glabra* on the hemolytic anemia in rats.

MATERIALS AND METHODS

Animal

This experimental study was conducted on 50 Wistar male rats with the weight of 200±5 gr that were kept in individual cages (Each group in two separate cages) for ten days to adapt to the environment. During the experiments, the temperature of the animal house was adjusted at 22±3 °C under a 12 h dark/light cycle.

Extract preparation method

In this empirical study, 1500 g of *G. glabra* leaves were collected in Kermanshah, Iran (geographical coordinates: 34.3277° N and 47.0778°E). Then, the leaves of the plant were dried in shadow, and after grinding, each time 100 gr of the obtained powder was dissolved in 1000 cc distilled water and put in Soxhlet extractor for eight h. The collected extract was filtered by Whatman filter paper no 1 and steamed into a glass container at the solvent temperature. The remaining dried extract was poured into a glass container and weighed. The powder of the obtained extract weighed as required depending on the dose and dissolved in distilled water.

Experimental design

To induce hemolytic anemia, to all groups, except negative healthy control, were injected intravenously (in the caudal vein) with Phenylhydrazine 20 mg/kg at three different times (Days 1, 3, and 5). Then, the animals were divided into five subgroups, including negative healthy control receiving distilled water, the untreated negative control receiving distilled water, and three groups receiving the *G. glabra* aqueous extract at 30, 60, and 120 mg/kg concentrations (Distilled water was used to prepare different doses of the extract). One day after the last injection of the Phenylhydrazine, the rats underwent oral treatment of

several doses of *G. glabra* aqueous extract for 15 days (Days 6-21). On the 21st, Four hours after oral administration of different doses of *G. glabra*, the rats were sacrificed. Blood samples were taken from the rats' heart to analyze biochemical, hematological, and immunological parameters [15].

All data were analyzed by One-way variance analysis (ANOVA), using the SPSS 18 software package. Data were considered statistically significant at $p \leq 0.05$.

RESULTS

Effects of aqueous extract of *G. glabra* on the concentrations of biochemical parameters

The estimated values of the biochemical parameters are indicated in Figures 1-8. Phenylhydrazine-induced toxicity increased the concentrations of ferrous (Fe), ferritin, erythropoietin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), cholesterol, low-density lipoprotein (LDL), triglyceride, total and conjugated bilirubin, urea, and creatinine and decreased high-density lipoprotein (HDL), total protein, and albumin, significantly ($p \leq 0.05$) as compared to the control group. Several doses of aqueous extract of *G. glabra* could significantly ($p \leq 0.05$) improved above parameters as compared to the untreated group. There were no significant differences ($p \leq 0.05$) in the concentrations of GGT, triglyceride, albumin, conjugated bilirubin, and creatinine among different groups of *G. glabra* and control group. Administration of G120 could significantly ($p \leq 0.05$) ameliorate the concentrations of Fe, HDL, LDL, total bilirubin, and urea similar to the control group.

Effect of aqueous extract of *G. glabra* on the levels of hematological parameters

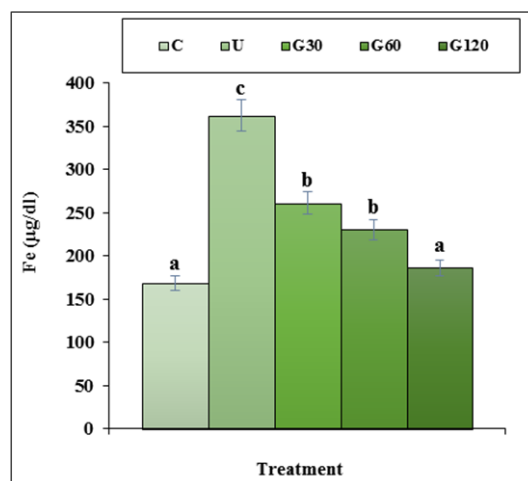


Figure 1. The level of Fe in several groups.

C: Control, U: Untreated, G: *Glycyrrhiza glabra*, Fe: Ferrous. Different letters represent the statistical difference ($p \leq 0.05$) between tested groups of animals.

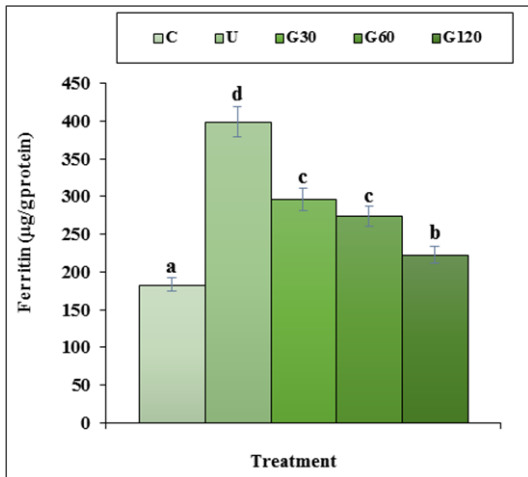


Figure 2. The level of ferritin in several groups. C: Control, U: Untreated, G: *Glycyrrhiza glabra*. Different letters represent the statistical difference ($p \leq 0.05$) between tested groups of animals.

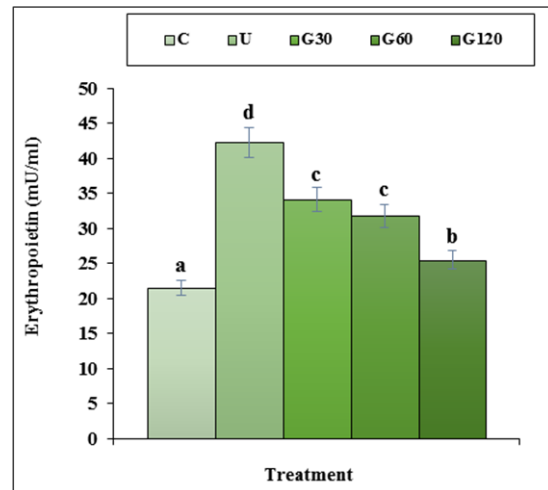


Figure 3. The level of erythropoietin in several groups. C: Control, U: Untreated, G: *Glycyrrhiza glabra*. Different letters represent the statistical difference ($p \leq 0.05$) between tested groups of animals.

The numbers of WBC, platelet, and RBC, the percentage of lymphocyte and neutrophil, and the levels of Hb, PCV, MCV, MCH, and MCHC were significantly ($p \leq 0.05$) reduced in the untreated group. The treatment with aqueous extract of *G. glabra* significantly ($p \leq 0.05$) enhanced the above parameters. There weren't significant differences

($p \leq 0.05$) in percentages of lymphocyte, neutrophil, monocyte, eosinophil, and basophil among several doses of *G. glabra* and control group. No significant differences ($p \leq 0.05$) were found among all groups in the percentages of monocyte, eosinophil, and basophil (Figs. 9-16).

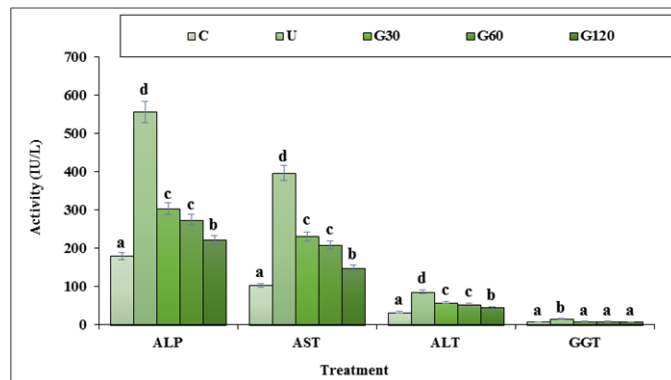


Figure 4. The levels of ALP, AST, ALT, and GGT in several groups. C: Control, U: Untreated, G: *Glycyrrhiza glabra*, ALP: Alkaline phosphatase, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: Gamma-glutamyl transferase. Different letters represent the statistical difference ($p \leq 0.05$) between tested groups of animals.

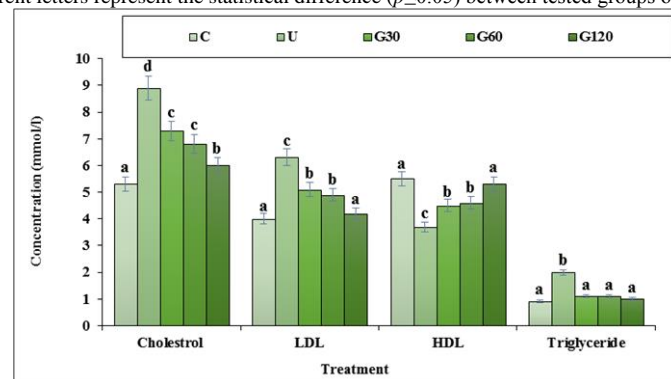


Figure 5. The levels of cholesterol, LDL, HDL, and triglyceride levels in several groups. C: Control, U: Untreated, G: *Glycyrrhiza glabra*, LDL: Low-density lipoprotein, HDL: High-density lipoprotein. Different letters represent the statistical difference ($p \leq 0.05$) between tested groups of animals.

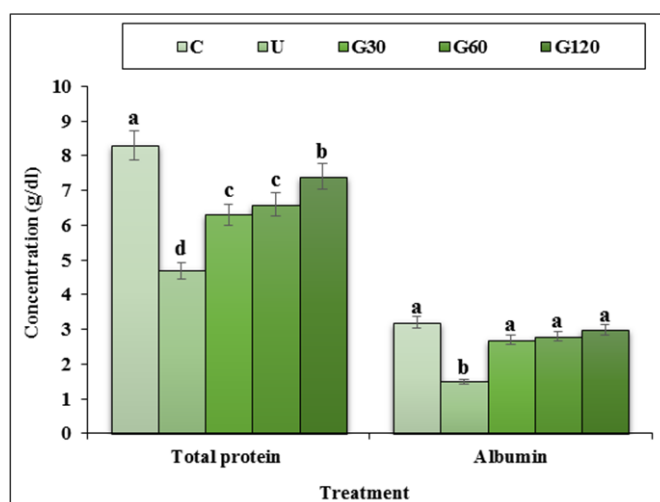


Figure 6. The levels of total protein and albumin in several groups.

C: Control, U: Untreated, G: *Glycyrrhiza glabra*. Different letters represent the statistical difference ($p \leq 0.05$) between tested groups of animals.

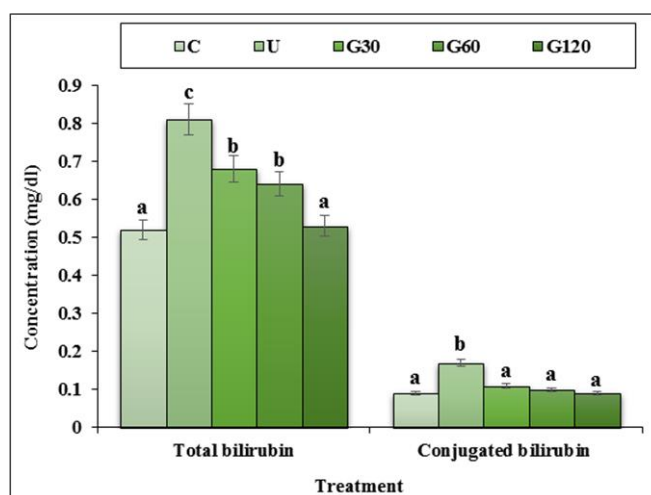


Figure 7. The levels of total and conjugated bilirubin in several groups.

C: Control, U: Untreated, G: *Glycyrrhiza glabra*. Different letters represent the statistical difference ($p \leq 0.05$) between tested groups of animals.

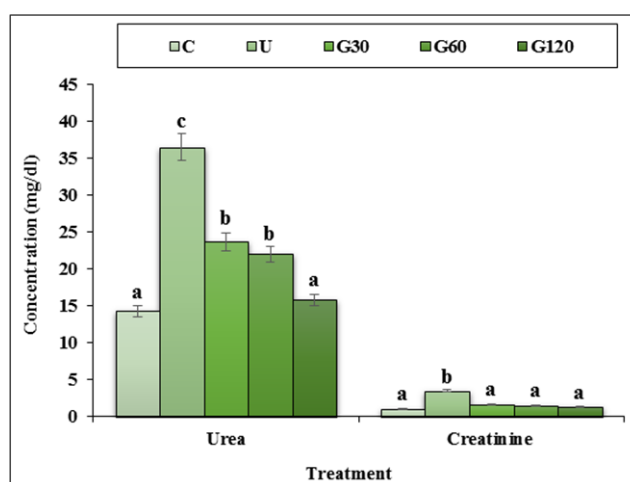


Figure 8. The levels of urea and creatinine in several groups.

C: Control, U: Untreated, G: *Glycyrrhiza glabra*. Different letters represent the statistical difference ($p \leq 0.05$) between tested groups of animals.

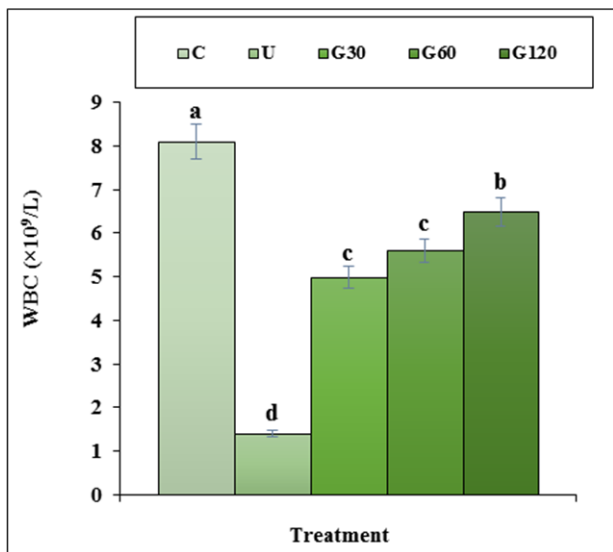


Figure 9. The number of WBC in several groups. C: Control, U: Untreated, G: *Glycyrrhiza glabra*, WBC: White blood cell. Different letters represent the statistical difference ($p \leq 0.05$) between tested groups of animals.

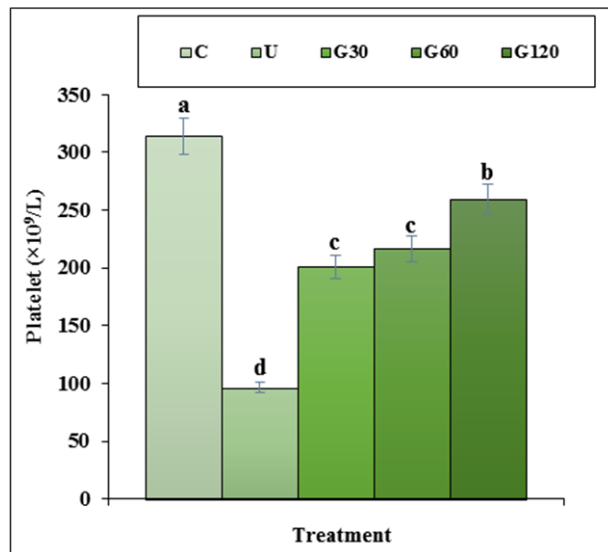


Figure 11. The number of platelet in several groups. C: Control, U: Untreated, G: *Glycyrrhiza glabra*. Different letters represent the statistical difference ($p \leq 0.05$) between tested groups of animals.

Effect of aqueous extract of *G. glabra* on the concentrations of immunological parameters

As shown in Figures 17 and 18, the concentrations of anti-inflammatory cytokines (IL4, IL5, IL10, IL13, and IFN- α) reduced significantly ($p \leq 0.05$) and pro-inflammatory cytokines (IL1, IL6, IL12, IL18, IFN-Y, and TNF- α) enhanced significantly ($p \leq 0.05$) in untreated rats compared to the control ones. Several doses of aqueous extract of *G. glabra* significantly ($p \leq 0.05$) ameliorated the above parameters. Administration of G120 could significantly ($p \leq 0.05$) ameliorate the concentrations of IL1, IL4, IL13,

IL18, IFN- α , and TNF- α similar to the control group.

DISCUSSION

In this study, phenylhydrazine was used to induce hemolytic anemia. Phenylhydrazine is toxic for the body and impairs several tissues while entering the body [16-17]. Studies have revealed that Phenylhydrazine causes oxidative stress, production of free radicals, lipid peroxidation, oxidative degradation of spectrum cell membrane, and lysis of red blood cells [16-18]. It causes the deficiency in the Glucose-6-Phosphate Dehydrogenase to cause favism [19]. It demonstrated that Phenylhydrazine caused the conversion

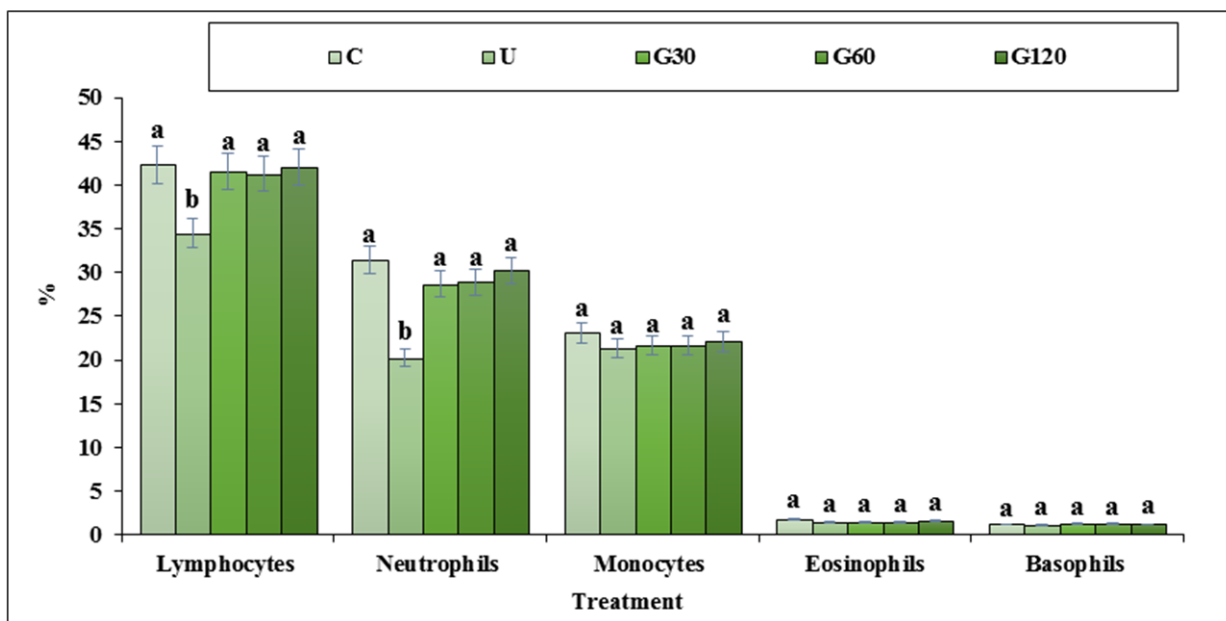


Figure 10. The percentages of lymphocyte, neutrophil, monocyte, eosinophil, and basophil in several groups. C: Control, U: Untreated, G: *Glycyrrhiza glabra*. Different letters represent the statistical difference ($p \leq 0.05$) between tested groups of animals.

of hemoglobin to methemoglobin. Therefore it plays a major role in forming the Heinz bodies [19-21]. Studies have indicated Phenylhydrazine, by enhancing hemolysis (enhanced the levels of ferrous, ferritin, and erythropoietin), causes liver enlargement and chronic failure (through hypertrophy of liver cells), spleen enlargement and chronic failure (through hypertrophy of spleen cells), reduction of mean number of testicular sperms (through atrophy of testicular structure), and chronic and acute renal failure (by destruction of structures such as proximal, distal renal cells, and glomeruli) [18, 22-23]. Also, it reported that Phenylhydrazine decreased body weight and enhanced the weight and volume of the liver, spleen, kidneys, and adrenal glands. In the previous study showed that Phenylhydrazine with degenerating of hepatocytes, glomeruli, proximal convoluted tubules, and distal convoluted tubules, enhanced

the biochemical parameters of the liver and kidneys in the blood [24-25]. It indicated that Phenylhydrazine as a toxin increased the levels of pro-inflammatory cytokine and reduced the concentration of anti-inflammatory cytokine [24-25]. According to the above studies, in our study showed that Phenylhydrazine significantly ($p \leq 0.05$) reduced the levels of HDL, total protein, albumin, WBC, platelet, RBC, Hb, PCV, MCV, MCH, MCHC, IL4, IL5, IL10, IL13, and IFN- α and increased the levels of ALP, AST, ALT, GGT, Fe, ferritin, erythropoietin, cholesterol, LDL, triglyceride, total and conjugated bilirubin, urea, creatinine, IL1, IL6, IL12, IL18, IFN- γ , and TNF- α . In spite of the above toxicity properties of Phenylhydrazine, the treatment with several doses of aqueous extract of *G. glabra* could significantly ($p \leq 0.05$) improve the levels of biochemical, hematological, and immunological parameters.

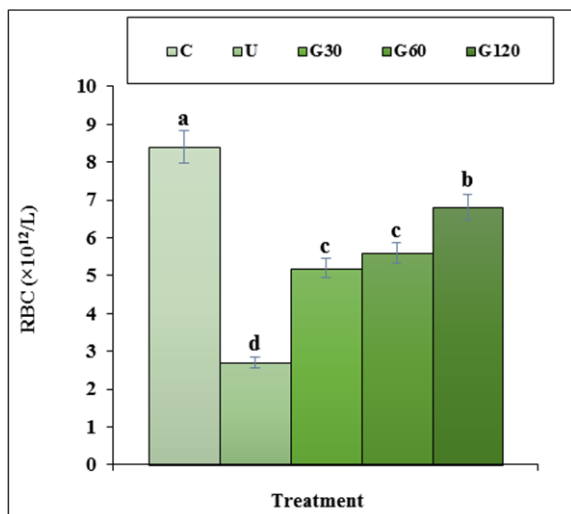


Figure 12. The number of RBC in several groups. C: Control, U: Untreated, G: *Glycyrrhiza glabra*, RBC: Red blood cell. Different letters represent the statistical difference ($p \leq 0.05$) between tested groups of animals.

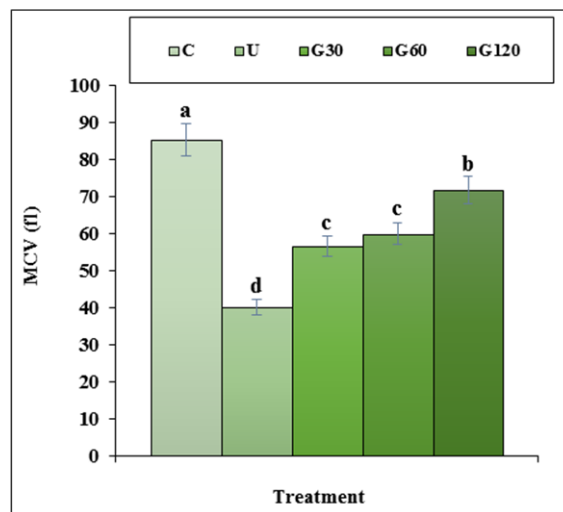


Figure 14. The level of MCV in several groups. C: Control, U: Untreated, G: *Glycyrrhiza glabra*, MCV: Mean corpuscular volume. Different letters represent the statistical difference ($p \leq 0.05$) between tested groups of animals.

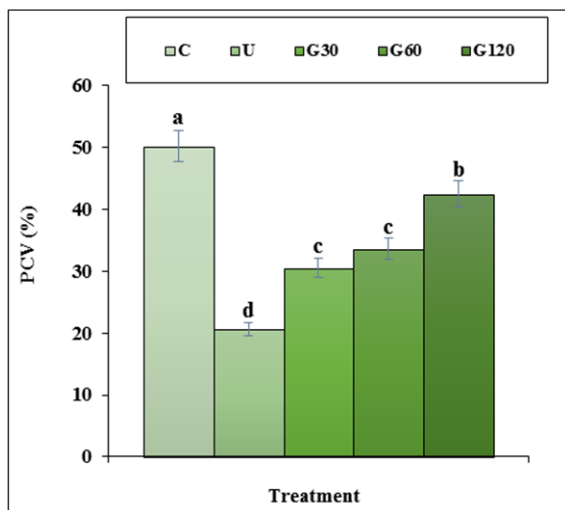


Figure 13. The level of PCV in several groups. C: Control, U: Untreated, G: *Glycyrrhiza glabra*, PCV: Packed cell volume. Different letters represent the statistical difference ($p \leq 0.05$) between tested groups of animals.

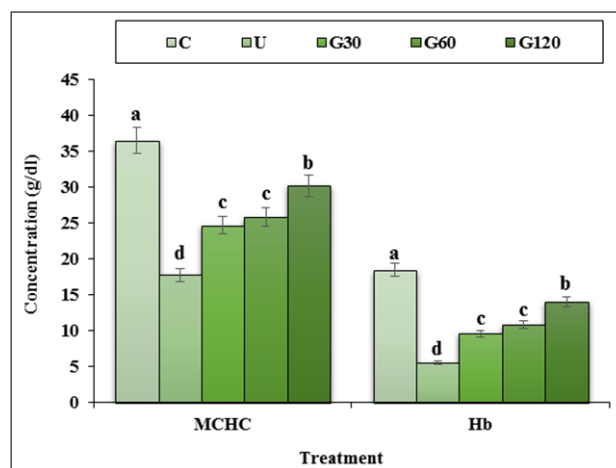


Figure 15. The levels of MCHC and Hb in several groups. C: Control, U: Untreated, G: *Glycyrrhiza glabra*, MCHC: Mean corpuscular hemoglobin concentration, Hb: Hemoglobin. Different letters represent the statistical difference ($p \leq 0.05$) between tested groups of animals.

In a study indicated that the ethanolic extract of *G. glabra* at 250 and 500 mg/kg doses reduced the concentrations of the AST and ALT against CCl_4 -induced acute hepatotoxicity in rats [26]. In the study of Yin et al. (2011) revealed the hepatoprotective activity of the 2.5, 5, and 10 $\mu g/ml$ doses of *G. glabra* aqueous extract against CCl_4 -induced hepatotoxicity [27]. In the previous study, the levels of AST and ALT decreased as compared to the untreated group. In other study demonstrated that *G. glabra* aqueous extract (at doses of 100, 150, and 300 mg/kg) reduced the raised levels of hepatic biochemical parameters includes ALP, ALT, and AST, and increased total protein, albumin, and globin as compared to the CCl_4 -treated group in rats [28]. In the similar study, Jung et al. (2016) reported that aqueous extract of the root of *G. uralensis* Fischer

(Another species of the *Glycyrrhiza* genus) decreased the concentrations of the AST and ALT in the alcohol-induced fatty liver in mice [29].

About immunoprotective property of *G. glabra*, in the study of Samadnejad et al. (2012) indicated that it has strong anti-inflammatory potential by decreasing of pro-inflammatory cytokines. In the previous study, *G. glabra* reduced the concentrations of $TNF-\alpha$, NO, and IL-6 [30]. In other studies demonstrated that anti-inflammatory activity of *G. glabra* related to 3 triterpenes (included 18β -glycyrrhetic acid, 18α -glycyrrhizin, and 18β -glycyrrhizin) and 13 flavonoids (included dehydroglyasperin D, dehydroglyasperin C, licorisoflavan A, licoricidin, isoangustone A, glabridin, echinatin, isoliquiritigenin, licochalcone E, licochalcone D, licochalcone C, licochalcone

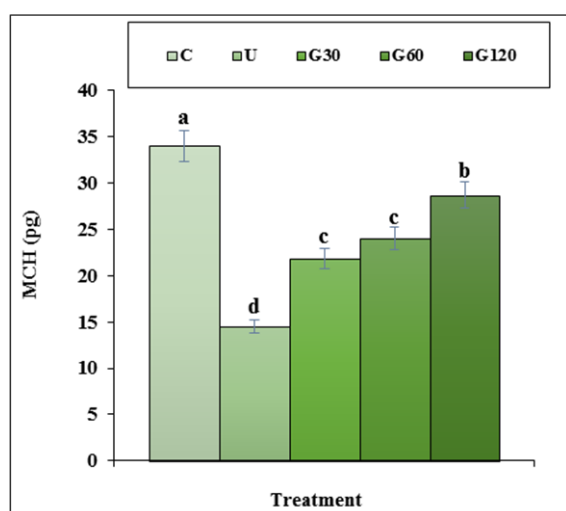


Figure 16. The level of MCH in several groups. C: Control, U: Untreated, G: *Glycyrrhiza glabra*, MCH: Mean corpuscular hemoglobin. Different letters represent the statistical difference ($p \leq 0.05$) between tested groups of animals.

B as well as licochalcone A) [31-32]. A large number of metabolites revealed that *G. glabra* was a remedial option for obtaining anti-inflammation compounds [31-32].

It is noted that antioxidant compounds played a very important role in hematoprotective effect [33-34]. Antioxidants can play the main role in the destruction of free radicals and toxic materials and maintenance of hemostasis because free radicals intervene with biological cell membrane such as red blood cells through peroxidation of unsaturated fatty acids and bring about pathological changes [33-34]. Li et al. (2011) and Liu et al. (2013) indicated that *G. glabra* is rich of triterpenes and flavonoids (Antioxidant compounds), so it was normal that the plant had the hematoprotective effect [31-32].

CONCLUSION

Based on the above findings, it can be deduced that the aqueous extract of *G. glabra* exhibits remarkable hematoprotective effect against hemolytic anemia. This extract also revealed amelioration in biochemical and immunological parameters. It is offered that clinical trials be done to gain this remedial property in human.

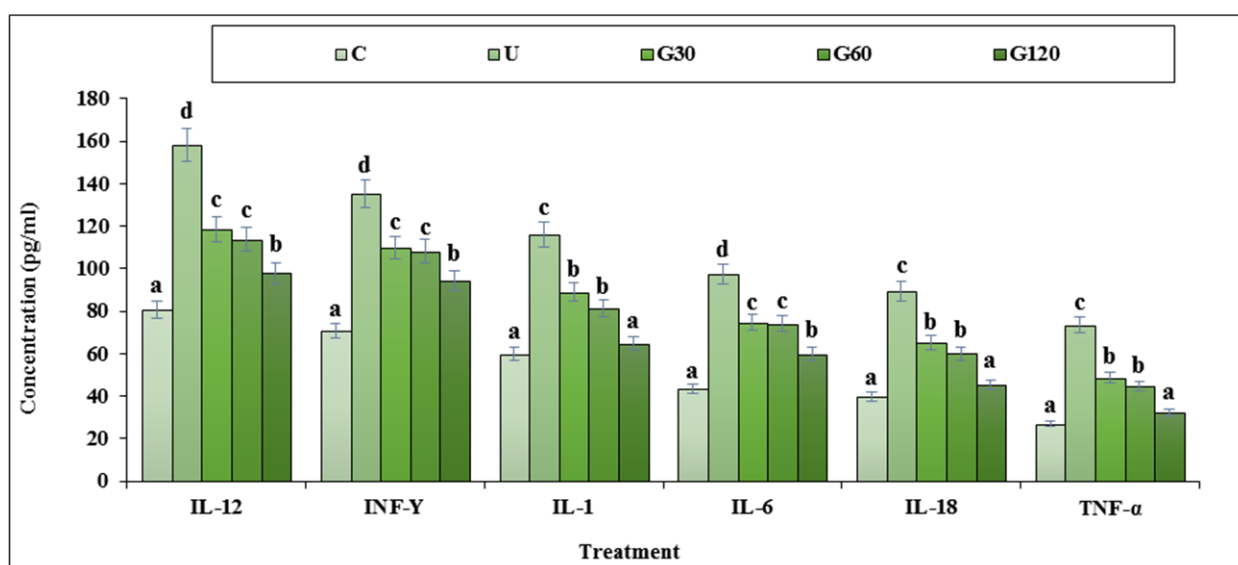


Figure 17. The levels of pro-inflammatory cytokines in several groups. C: Control, U: Untreated, G: *Glycyrrhiza glabra*, IL1: Interleukin 1, IL6: Interleukin 6, IL12: Interleukin 12, IL18: Interleukin 18, IFN-γ: Interferon gamma, TNF-α: Tumor necrosis factor alpha. Different letters represent the statistical difference ($p \leq 0.05$) between tested groups of animals.

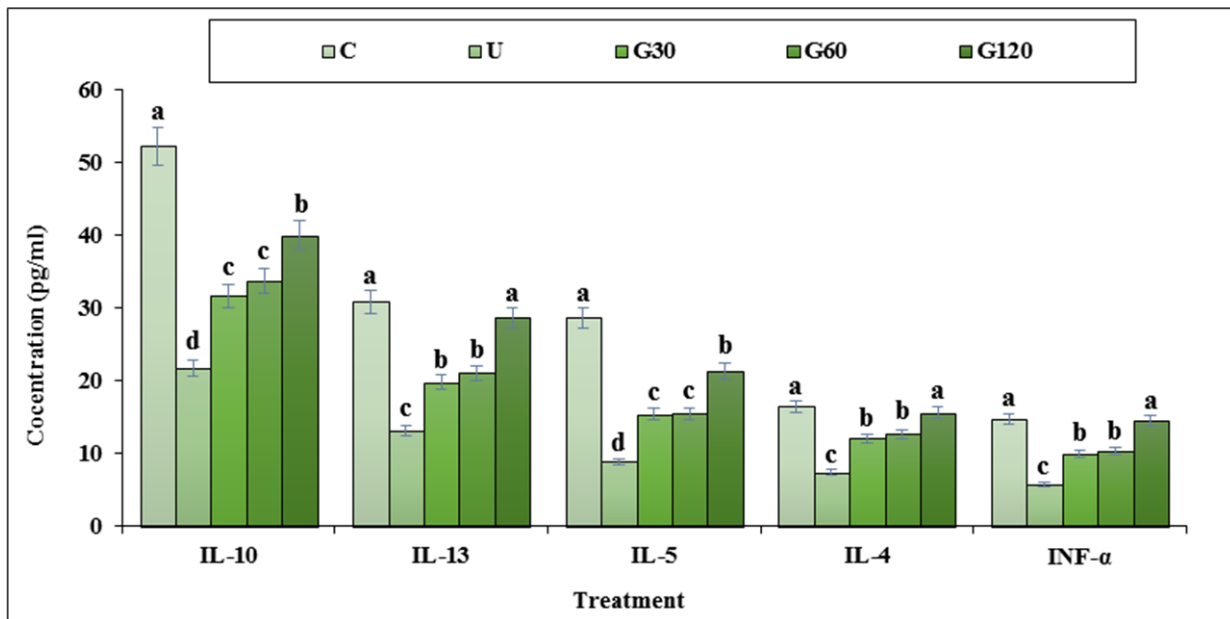


Figure 18. The levels of anti-inflammatory cytokines in several groups.

C: Control, U: Untreated, G: *Glycyrrhiza glabra*, IL4: Interleukin 4, IL5: Interleukin 5, IL10: Interleukin 10, IL13: Interleukin 13, INF- α : Interferon alpha. Different letters represent the statistical difference ($p \leq 0.05$) between tested groups of animals.

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