



## The effects of lentil hydro alcoholic extraction blood parameters in mice

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### ABSTRACT

Lentil with the generic name of *Securigera securidac* has many therapeutic effects. One of the most important is the strengthening of hematopoiesis. In this study, the effects of lentils hydro alcoholic extract on hematological parameters in 1 mice (Balb/C) were investigated. This study was conducted in 5 groups of 40 mice. The control group received no extract, Placebo group Received 5 cc normal saline every other day and 3 treated group is injected lentil extract with doses 50,100,200 mg/kg/2day intraperitoneally (IP). Then the mice blood samples were taken from the heart, the main evaluated parameters, were the number of red and white blood cells, Hemoglobin, HCT, MCHC, MCH and MCV with respect of controlled groups. The results with SPSS software at  $P < 0.05$  were analyzed. Results showed that at all doses the number of red blood cells, hemoglobin and MCHC increased, while the MCH and MCV showed no significant difference. The number of white blood cells at doses of 100 and 200 mg / kg increased. Results shows that the dose dependent effect of lentil on blood parameters in mice.

**Keywords:** lentil extract, blood factors, mice.

### INTRODUCTION

In traditional medicine is trying to use the chemical compounds found in plants, Or by mixing different parts of the plant can be introduced as an alternative to synthetic drugs. In other words, the aim in the traditional medicine is improving the performance of life system, and therefore there is a wider range of herbal medicines than chemical drugs and synthetic medications are in medicine [1].

The use of plant extracts has the biological effects that can improve the metabolism of the body, create balance in the digestive system, and provide cardiovascular health through blood pressure regulation and having a positive effect on diabetes and immune system. Lentil is one of the herbs used in traditional medicine which for a long time has a special place [2]. The plant with the scientific name *Securigera securidac* is one-year-old plant and one of leguminous family. The distributions of this plant in Europe, Asia and Australia have been reported. In Iranian traditional medicine, the seed of this plant has been used in treatment of diabetes and high cholesterol. Like other members of the legume family, lentils has low glycemic index and blood sugar does not have saltation and at the same time helps to absorb more iron. It should be noted that the iron in lentil is considered as plant iron that is less absorbed by the body than animal iron [3]. Lentils contain all the B vitamins. These vitamins are great anti-stress. These herbs are rich of antioxidants catechin family. The result of researches showed that the antioxidant to fight high cholesterol and in this way a great service to heart [2]. Like other legumes, lentils are a good source of protein. Lentils contain substantially insoluble fibers in the water that a high level of its consumption stimulates the bowel [4, 5]. Based on that far as scientific research is not accomplished on the effects of lentil extract on blood factors, the purpose of this study is to evaluate the effects of lentil hydroalcoholic extract on hematopoiesis and its related factors.

### EXPERIMENTAL SECTION

In this study, 40 mice (Balb/C) with weight range of  $30 \pm 5$  g is used. The mice in an environment with natural light and stored at  $24-26^{\circ}\text{C}$  and had free access to food and water. This study was approved by ethics committees. To prepare hydro alcoholic extract, grind the prepared lentils in the form of powder and 40 grams of this powder is put into a sterile flask and 40 ml of ethyl alcohol was added and placed for 24 hours in a cool environment, after a day by using a shaker flask contents is mixed for 5 minutes. In this stage, after the sample filtration through Whatman paper, and calculating the amount of extract remaining in solution (2.34 g), Lentil mother solution concentration will be determined, and by diluting of obtained extract with injection physiology serum the desired samples were taken[6].

Lentil extract at concentrations of 50, 100 and 200 mg/kg of body weight was prepared and every other day for 20 days intraperitoneally was injected. A week before the injections the samples were randomly divided into 5 groups. Each of the groups was placed in separate cages. In each group, there were the numbers of 8 laboratory mice (total of 40). So that the average weight of each group was  $30 \pm 3$  grams.

The Groups under test were determined as follows:

- **Control group:** In order to achieve basic levels of blood proteins comparison of this group and the treated group with the same conditions but without the injections were stored during the test.
- **Placebo group:** In order to ensure the ineffectiveness of injections as a result of trial and comparison with the control group, the group's daily rate of 5.0 mL of saline was injected.
- **Treatment group 1:** consisted of 8 mice with a dose 50 mg/kg/2day for 20 days and then every other day were injected intraperitoneally.
- **Treatment group 2:** consisted 8 mice with a dose 100 mg/kg/2day for 20 days and then every other day were injected intraperitoneally.
- **Treatment group 3:** consisted 8 mice with a dose 200 mg/kg/2day for 20 days and then every other day were injected intraperitoneally.

All injections were performed every other day between the hours of 10-12 am. After 20 days, blood samples were taken from each groups and transported to the laboratory for required tests, and obtained samples were used for CBC.

Results mean (Mean  $\pm$  SEM) using the Tukey, Duncan statistical tests and variance Analyze were analyzed in SPSS software version11.5.

### RESULTS AND DISCUSSION

The comparison of the average amount of red blood cells and hemoglobin was done between the control group and the experimental group using Duncan test at the level of  $p < 0.05$  and represents a significant increase in the amount of red blood cells in the experimental group compared to the control group. Figure 1 and 2 represents the comparison of the average number of red blood cells and haemoglobin between the control group and the experimental group and the placebo groups.

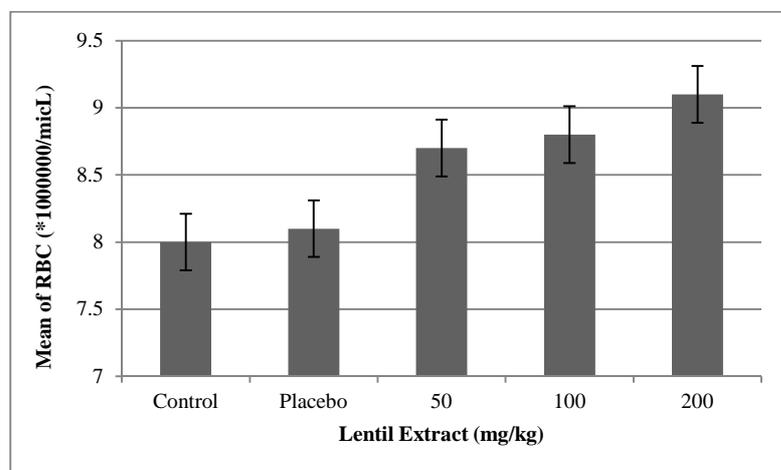
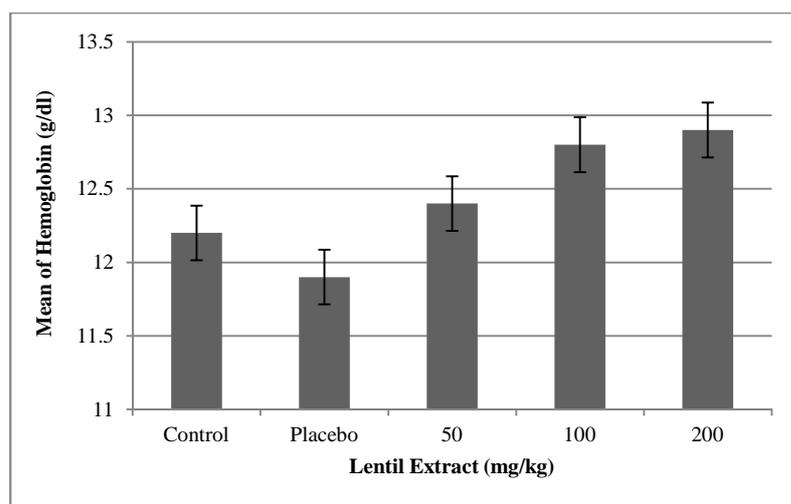


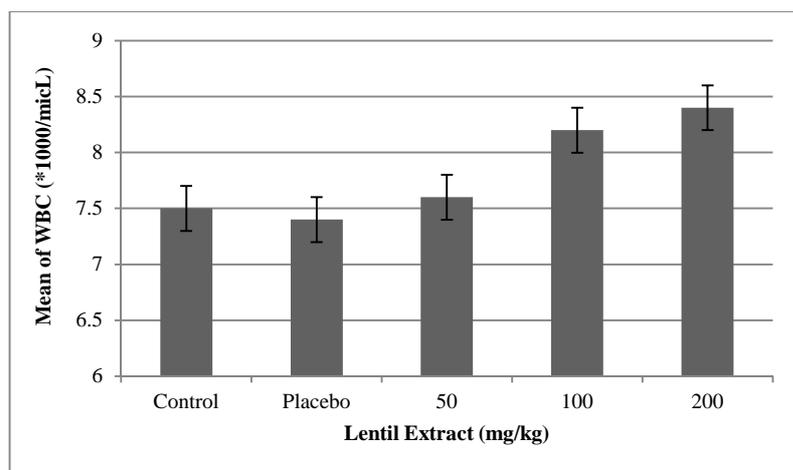
Figure1. Effect of Lentil extract on mean comparison of RBC in all groups



**Figure2. Effect of Lentil extract on mean comparison of hemoglobin in all groups**

Review and comparison of the average of MCHC between the control group and the experimental group represented that the MCHC in three experimental groups compared with the control group is increased. But the comparison of the mean percentage of MCH, MCV at  $P < 0.05$  has no significant differences.

The comparison between the average number of white blood cells in the control group and the experimental group shows that the amount of white blood cells in the experimental group 2 (treatment with a dose 100 mg/kg) and 3 (treatment with a dose 200 mg/kg) increased significantly compared to the control group (Figure3).



**Figure3. Effect of Lentil extract on mean comparison of WBC in all groups**

In this study, the increase of the average number of red blood cells in the three experimental groups of 50, 100 and 200 as compared to the control group is significant. Blood storage is result of the proliferation and differentiation of blood cells that are ongoing at the same time and come from stem cells. According to research conducted and the mechanisms of increase the number of white blood cells by the blood of oregano extract, it may be because:

- The effect of stem cells of (powerful, myeloid and lymphoid more power) is increased mitotic divisions.
- The effect on committed progenitor cells and increase in the mitotic divisions in them. So after effect of lentil extract in three experimental doses 50, 100 and 200 on the hematopoietic multifunctional stem cells in the bonemarrow, proliferation of cells is increased. The division of these cells produces erythrocyte colony forming units (CFU-E), which ultimately produce red blood cells [7].

The mean cell hemoglobin parameter is affected by changes in hemoglobin and red blood cell count. Therefore, anything on any of the above mentioned factors can, change in the level of hemoglobin cells, In the study by increasing the number of red blood cells, hemoglobin, also shows a significant increase. At the same time lentil extract concentration 100 200 mg/kg increase in the average number of white blood cells and subsequently increase

phagocytosis neutrophils and thereby strengthens and stimulates the immune system. After cell divisions in the myeloid bone marrow, CFU - GEM arise, these colony forming cells which is divided among the progenitor cells and other committed progenitor cells including (CFU - GEM , CFU - Baso<sub>2</sub> , CFU - EO ) makes. That affected by stimulating factor influencing their growth Granulocyte-monocyte colony (GM - CSF 4, IL-3), the stem cell factor (CSF ,FLt - 3L), is stimulated and reproduced. And according to this lentil extract probably by stimulating mitosis division in ancestral cells increases white blood cells in this category [8].

### CONCLUSION

Considering the results, Results shows that the dose dependent effect of *Securigera securidac* has on blood parameters in mice.

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### REFERENCES

- [1]Garjani A, Fathiazad F, Zakheri A, et al. *Journal of ethnopharmacology*. **2009**, 126(3): 525-32.
- [2]Hosseinzadeh H, Ramezani M, Danaei A. *Phytotherapy Research*.**2002**, 16(8): 745-7.
- [3]Mard S, Bahari Z, Eshaghi N, Farbood Y. *Pak J Biol Sci*. **2008**,11(23): 2619-23.
- [4]Hajzadeh M, Rajaei Z, Ghamami G, Tamiz A. *Pharmacologyonline*.**2011**,1: 213-220
- [5]Behbahani M, Shanehsazzadeh M, Shokoohinia Y. *Pharmaceuticalbiology*.**2011**, 13:123-126
- [6]Modaresi M. *J Ani. Vet. Adv*.**2012**, 11(4):458-461
- [7]Sarker , SD . Nahar , L . Current Medicine Chemistry, Phytopharmaceutical Research Laboratory, The Robert Gordon – University, schoolhill , Aberdeen AB , Scotland , UK . **2004**, 11 (11) 1479 – 500
- [8]Fariba Sharififar, Shirin Pournourmohammadi, Moslem Arabnejad. *Indian Journal of Experimental Biology*.**2009**,47,668-671.