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Effectiveness of hydroalcoholic extract *Hyssopus Angustifolius* in amelioration of hematology and immune response indices in rat

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ABSTRACT. Medicinal plants due to their diverse active ingredients have a high potential to become safe and effective drugs for humans and animals. One of the most important sources of medicinal plants in Iran is *Hyssopus officinalis*, which *Hyssopus angustifolius* (*Hyssop*) species is specific to Iran and grows in different parts of Iran. Numerous therapeutic properties of *Hyssop* such as antibacterial, antifungal, anti-inflammatory, and analgesic have been reported. However, there is limited information on its effects on the immune system. Therefore, the aim of this study was to investigate the effect of hydroalcoholic extract of *Hyssop* on blood and immunological parameters in Wistar rats, such as: complete blood cell count (packed cell volume, hemoglobin, red and white blood cells and percentage of each them), immunology parameters in blood (phagocytosis percentage and average phagocytic bacteria) and in serum (total protein, Albumin and Immunoglobulin M). For this purpose, 32 male Wistar rats were used. The rats were divided into a control and 3 experimental groups. The experimental groups received 50, 100 and 150 mg kg^{-1} of hydroalcoholic extract of *Hyssop* for 21 days. At the end of experiment period blood samples were taken to measure the blood and immunological parameters. The results showed that concentration of 50 mg kg^{-1} of the extract increased phagocytosis percentage, average phagocytic bacteria and increased immunity. A concentration of 100 mg kg^{-1} of *Hyssop* was led to an increase in blood Platelets. Also, this concentration was increased white blood cells and was decreased neutrophils and immunoglobulin M. Concentration of 150 mg kg^{-1} increased the level of immunity and number of white blood cells. In general, the results of this study showed that the effect of *Hyssop* on blood and immunological parameters is different depending on the type of indicator and *Hyssop* extract concentration.

Keywords: Medicinal plants; *Hyssop*; Extract; immunology indices.

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Introduction

Herbal plants have been long used as a valuable natural source for obtaining medicines with no side effect to man (Gholami-Ahangaran et al., 2021). According to global statistics, the effective ingredients of more than 50% of the drugs on the market, are of plant origin (Sharma et al., 2020). Among different countries, Iran due to high diversity of climatic conditions is one of the richest sources of herbal medicines in the world (Gholami & Karimi Dehkordi, 2019). One of the most important and valuable herbs in Iran is *Hyssopus angustifolius* (*Hyssop*). *Hyssop* is a perennial plant from the lamiaceae family that is a self-growing plant and is one of the most important medicinal plants and spices (Pirbalouti et al., 2019).

The origin of this plant is reported to be Anatolia and it grows in the Caspian Sea, to the Black Sea, as well as in the sandy areas of the Mediterranean (Özer et al., 2006). The useful parts of the plant are the branches with flower; while in some regions, all aerial parts of the plant are used (Pour et al., 2019). The chemical compounds found in the plant vary depending on the place and condition of growth (Karimi-Dehkordi et al., 2024). Different compounds like flavonoids, tannin, thymol, charoechrol, mirtnic acid, and pinic acid are found in the plant extract. Numerous therapeutic properties of *Hyssop* such as antibacterial, antifungal, anti-inflammatory, and analgesic as well as change of biochemical parameters (Etminan et al., 2021) have been reported (Hatipoğlu et al., 2013). Salehi and Setorki (2018) examined analgesic and anti-inflamatory effects of *Hyssop* ethanol essence in mice. The results showed that ethanol extract of *Hyssop* can control pain and inflammation in small lab mice (Salehi & Setorki, 2018). Hou et al. (2009) found that long-term use of Zofa

extract can inhibit secretion of eotaxin 2, which is one of significant factors of inflammatory response in asthma patients. Therefore, the plant attenuates inflammation response in these patients (Hou et al., 2009). *Hyssop* contains mucilage compounds and volatile compounds like pinkokamphan, alpha, betapinin, Kamphan, and sezkoitrephen alcohol that are good for the respiration system. The plant can be used to prevent respiratory infection, asthma, influenza and bronchitis (Stanković et al., 2016). In addition, the presence of flavonoid compounds in the plant has caused its antioxidant properties (Džamić et al., 2013). In this regard, Dzamic et al. (2013) with in vitro evaluation showed that *Hyssop* plant has desirable antioxidant properties that can be used in food and pharmaceutical industries (Džamić et al., 2013). Tehranipour and Lagzian (2017) showed that alcoholic extract of *Hyssop* leaves has healing effect on the anterior horn neurons of the spinal cord in the rat after creating a lesion. This effect can be explained by antioxidant and anti-inflammation effects of *Hyssop* (Tehranipour & Lagzian 2017). Tannin, several phenol compound, and various organic acid in *Hyssop* create considerable antibacterial effect (Kizil, 2010). Another study examined antibacterial effect of *Hyssop* extract on E-coli, Staphylococcus aureus, and listeria monocytogenes and showed the positive effect of *Hyssop* on the growth inhibition of these bacteria (Nasirpour et al., 2014). Kizil et al. (2010) examined antibacterial effect of *Hyssop* collected from the southeast of Anatolia and reported that the essence of Zofa has a notable antibacterial activity against microorganisms (Kizil, 2010). Variety of compounds like myrcene, humulene (Başer et al., 2016), and antioxidants in *Hyssop* make it a good option to affect cellular factors and immunity system homeostasis either directly or indirectly (Ma et al., 2014). Despite several reports on antioxidant and antibacterial effects of *Hyssop*, there is a paucity of studies on the effects of *Hyssop* on the immunity system. The present study is an attempt to examine the effect of hydroalcoholic extract of *Hyssop* on immunohematology indices in rats. Hematologic parameters such as packed cell volume (PCV), hemoglobin (Hb), red (RBC) and white blood cells (WBC) and percentage of each them, neutrophil (Neu), lymphocyte (Sartz et al., 2008), Monocyte (Mono), eosinophil (Eos) and basophil (Baso). Immunology parameters in blood as phagocytosis percentage and average phagocytic bacteria and in serum such as total protein (TP), Albumin (Alb) and Immunoglobulin M (IgM). The results of this study, in addition to along with showing the effect of *Hyssop* on the indicators of the immune system, provide researchers with basic information about the practical use of this plant in order to boosting immune system.

Material and method

To prepare the hydroalcoholic extract, *Hyssop* was prepared from medicinal herb market and authenticity of the plant was confirmed by Herbal Plants Research Department, Shahrekord Azad University through comparing with herbarium samples. In order to extract, the method presented by Alinezhad et al. (2013) was used (Alinezhad et al., 2013). To this end, the samples were first wetted in a solution of water and alcohol and then the extract was extracted using a rotary device and a clevenger. Then, the extracted extract was dried out and 50, 100, 150 mg concentrations were prepared (Etminan et al., 2021).

Laboratory animals

Totally, 32 male Wistar rats, approximately 3-month-old, weighing 250 ± 20 g were purchased from lab animal house, the Islamic Azad University, Shahrekord Branch. The rats were kept with 12/12 day/night cycle and temperature of 22.2°C, with standard feeding programs during the experiment.

Grouping and implementing lab treatments

To conduct the experiment, 32 male Wistar rats were randomly allocated into four groups of 8 (Aydemir et al., 2016; Castrogiovanni et al., 2019; Shokri et al., 2019; Wang et al., 2012). Including control (They did not receive any extract, they only received distilled water), treatment 1: Receive the extract at a rate of 50 mg kg^{-1} by gavage, treatment 2: Receive the extract at a rate of 100 mg kg^{-1} by gavage, and treatment 3: Receive the extract at a rate of 150 mg kg^{-1} by gavage. The experiment took 21 days and the treatment were implemented every day in a specific hour

Sampling, blood and immunological parameters

At the end of experiment, the animals were anaesthetized using ketamine (10 mg kg^{-1}) and xylazine (10 mg kg^{-1}) administered via intraperitoneal (i.p.) injections with observation of all ethical codes (Nelissen et al.,

2019). Then, blood samples (4 ml) were collected from the rats' hearts (Al-Afifi et al., 2018; Goorani et al., 2019; Padmini & Kumar 2012; Parasuraman et al., 2010). Then, 2 ml of each sample was poured into a tube containing EDTA anticoagulation to check hematology indices; white blood cell (WBC), neutrophil (NEU), lymphocyte (Sartz et al., 2008), monocyte (Mono), eosinophil (Eos), basophil (Baso), red blood cell (RBC), hemoglobin (Hb), and packed cell volume (PCV). In addition, 2 mL of each sample was poured into another tube without anticoagulation to obtain the serum and measure immunological indices (albumin, total protein, immunoglobulin M (IgM), phagocytose percentage, heterophils, and average phagocytic bacteria). Blood samples were manually tinted to estimate the number of white blood cells using Giesma method. To calculate the percentage of phagocytosis, fresh blood was mixed with an equal volume of medium containing staphylococcus bacteria (10^7 CFU mL^{-1}). After half an hour at 37°C, blood spread was prepared and Giemsa staining was done. In order to calculate average phagocytic bacteria, 100 neutrophils were counted and the number of neutrophils containing bacteria was reported as the percentage of phagocytosis (Gershwin et al., 1995). In order to calculate the average phagocytic bacteria, randomly 10 neutrophils that had phagocytosed were examined with a light microscope and the average number of phagocytosed bacteria was expressed (Maqsood et al., 2010). To measure albumin and total protein, Pars Azmon kits were used.

Data analysis

The collected data were analyzed in SPSS 25 using a fully random design. To compare the mean of data, tukey's method was used (P=0.05).

Ethical approval

The study design was previously reviewed and approved by the Ethics Committee of the Shahrekord Branch, Islamic Azad University, Shahrekord, Iran (Ethic approval number: IR.IAU.SHK.REC.1400.024) for animal care and use in research.

Results

Tables 1, 2 and 3 list the results of hematological indices. As listed in Table 1, despite numerical difference between the groups in terms of PCV, RBC and Hb, there was no significant difference between them ($p > 0.05$).

The white blood cell counts of study groups are listed in Table 2. As can be seen in the WBC index, there is a significant difference between the T2 and T3 groups with the control and T1 groups. ($p < 0.05$).

Changes in immunological indices between different groups are listed in Table 3. The highest phagocytic index was in T1 group, which was significantly higher than the other groups ($p < 0.05$). The difference in other groups was not significant ($p > 0.05$).

Table 1. Changes in red blood cells and platelets in different groups.

Treatments Group (mg kg^{-1})	PCV[1] (%)	RBC[2] (×10^6 µLit^{-1})	Hb[3] (gr dL^{-1})	Plt[4] (number µLit^{-1})
Control	42.38 ± 3.07	8.14 ± 0.75	12.66 ± 0.98	193.38 ± 35.09[a]
T1	39.63 ± 5.40	7.66 ± 1.12	13.05 ± 0.90	252.75 ± 35.33[b]
T2	41.63 ± 5.12	8.24 ± 1.28	12.80 ± 1.12	327.88 ± 54.04[c]
T3	41.13 ± 2.70	7.89 ± 0.85	12.77 ± 0.71	304.00 ± 35.40[ab]
Significance level	0.62	0.68	0.87	0.001

Control: received no extract (zero/Control), T1: received 50 mg kg^{-1} bw *Hyssop* extract, T2: received 100 mg kg^{-1} bw *Hyssop* extract, T3: received 150 mg kg^{-1} bw *Hyssop* extract. [1]Packed cell volume, [2]Red blood cell, [3]Hemoglobin, [4]Platelete

Table 2. Changes in the parameters of white blood cells in the treatment groups.

Treatments Group (mg kg^{-1})	WBC[1] (number µLit^{-1})	Neu[2] (%)	Lym[3] (%)	Mono[4] (%)	Eos[5] (%)	Baso[6] (%)	Band[7] (%)
Control	12119 ± 1088 [a]	20.13 ± 2.47 [ab]	72.25 ± 3.20	4.63 ± 1.41	1.75 ± 0.71 [a]	0.63 ± 0.52	0.75 ± 0.46
T1	13431 ± 1159 [a]	21.88 ± 1.88 [b]	68.75 ± 2.19	4.75 ± 0.71	2.75 ± 0.71 [b]	1.00 ± 0.53	0.88 ± 0.64
T2	16812 ± 1601 [b]	18.75 ± 2.25 [a]	72.37 ± 1.06	4.88 ± 0.83	2.25 ± 0.71 [ab]	1.13 ± 0.64	0.75 ± 0.46
T3	16081 ± 1886 [b]	19.75 ± 2.05 [ab]	71.88 ± 3.60	4.50 ± 0.93	2.38 ± 0.52 [ab]	0.75 ± 0.46	0.75 ± 0.71
Significance level	0.001	0.05	0.055	0.89	0.043	0.26	0.96

Control: received no extract (zero/Control), T1: received 50 mg/kg bw *Hyssop* extract, T2: received 100 mg kg^{-1} bw *Hyssop* extract, T3: received 150 mg/kg bw *Hyssop* extract. [1]Wight blood cell, [2]Neutrophil, [3]Lymphocyte, [4]Monocyte, [5]Eosinophil, [6]Basophil, [7]Immature neutrophil

Table 3. Changes in immunology parameters in different groups.

Treatments Group (mg kg^{-1})	Phagocytosis[1] (%)	average phagocytic bacteria[2]	TP[3] (gr dL^{-1})	Alb[4] (gr dL^{-1})	IgM[5] (mg dL^{-1})
Control	11.75 ± 3.06 [a]	7.62 ± 3.20 [a]	6.09 ± 0.10	3.49 ± 0.32	31.50 ± 3.82 [bc]
T1	16.87 ± 3.00 [b]	12.00 ± 3.12 [b]	5.99 ± 0.18	3.21 ± 0.11	26.38 ± 5.07 [ab]
T2	10.62 ± 2.45 [a]	6.12 ± 2.03 [a]	6.11 ± 0.22	3.29 ± 0.18	24.38 ± 3.20 [a]
T3	8.75 ± 2.71 [a]	4.87 ± 1.64 [a]	6.15 ± 0.15	3.30 ± 0.31	35.63 ± 5.97 [c]
Significance level	0.001	0.001	0.27	0.16	0.001

Control: received no extract (zero/Control), T1: received 50 mg kg^{-1} bw Hyssop extract, T2: received 100 mg kg^{-1} bw Hyssop extract, T3: received 150 mg kg^{-1} bw Hyssop extract. [1]phagocytosis percentage, [2]the average number of phagocytosed bacteria by 10 neutrophils, [3]Total protein, [4]Albumin, [5]Immunoglobulin M.

Discussion

Medicinal plants are very valuable natural resources that due to having various compounds with different medicinal properties, their recognizing and exploiting has a very important role in the development of new medicinal products and improving the properties of existing synthetic drugs. *Hyssop* is one of the plants that has attracted the attention of many researchers due to the variety of compounds in it. Due to the importance of the immune system and the existence of limited studies on the effect of *Hyssop* on this system, the aim of this study was to investigate the effect of hydroalcoholic extract of *Hyssop* on the immunohematological parameters of blood in rats.

Tables 1, 2 and 3 list the results of hematological indices. As listed in Table 1, despite numerical difference between the groups in terms of PCV, RBC and Hb, there was no significant difference between them ($p > 0.05$). The numerical results indicated that hyssop lowered PCV, RBC and among different concentrations, 50 mg kg^{-1} had the highest effect. In addition, concentrations 100 and 150 mg kg^{-1} did not have a significant difference in decreasing of these indices. Akbarizadeh et al. (2020) studied the effects of *Hyssop* on immunity and performance of broiler chicken under cold stress. They reported that treatments received *Hyssop* powder, had a decrease in hematocrit level and treatments that received 0.5 g of *Hyssop* powder demonstrated a significantly lower hematocrit level compared to the control group (Akbarizadeh et al., 2020). The results indicated that the decrease in hematocrit level by using *Hyssop* depended on the concentration of *Hyssop*. This is consistent with our findings to some extent. Despite the numerical increase of RBC in the group receiving the concentration of 50 *Hyssop* extracts, no significant difference was observed between it and the control group. Akbarizadeh et al. (2020) also reported that the chicken that received *Hyssop* powder in their feed did not have a significant difference with control group in terms of red blood cell and hemoglobin, which is consistent with our results (Akbarizadeh et al., 2020). There was a significant difference in PLT index among the groups; so that the lowest and highest levels of blood platelets were in control (193.38±35.09) and 100 mg kg^{-1} group (T2) (327.88±54.04) respectively. Also, no significant difference was observed between control groups with T3 and also T1 and T2 groups ($p > 0.05$). In general, there are a few chemical and herbal drugs that can increase the number of platelets. Therefore, the observed increase in this regard is a notable finding.

The white blood cell counts of study groups are listed in Table 2. As can be seen in the WBC index, there is a significant difference between the T2 and T3 groups with the control and T1 groups. ($p < 0.05$). So that, with an increase in the concentration of extract, WBC count increases. Although, the difference in WBC count between 100 and 150 mg kg^{-1} groups was not significant, 100 mg kg^{-1} of hyssop had the strongest effect on the number of WBC. Akbarizadeh et al. (2020) reported no significant difference between the number of white blood cells in chickens that received *Hyssop* powder and control group (Akbarizadeh et al., 2020), which is not consistent with the present study. In terms of NEU percentage, only T1 and T2 groups had a significant difference ($p < 0.05$), and the difference in other groups was not significant ($p > 0.05$). By using *Hyssop*, NEU percentage increased, while with increasing the concentration of *Hyssop*, the percentage had a declining trend. These results indicate that the effect of *Hyssop* on NEU depends on NEU percentage. Ekim et al. (2011) examined the effect of *Hyssop* on asthma inflammatory response and found that the groups that received *Hyssop* had a significantly lower NEU percentage compared to control group, which is consistent with our findings (Ekim et al., 2011). A comparison among the groups in terms of Eos percentage showed a significant difference between the control and T1 groups ($p < 0.05$), and there was no significant difference between other groups. These results show the effect of *Hyssop* on increasing Eos percentage, which depends on its concentration so that the lower concentration of *Hyssop* (50 mg kg^{-1}), the higher its effect. Despite the fact that the effect of higher concentration of *Hyssop* (100 and 150 mg kg^{-1}) on Eos percentage was not significant

compared to the control group, any change in Eos percentage in blood is notable given its low percentage in blood. Ekim et al. (2011) studied the effects of *Hyssop* on asthma inflammatory responses in mice and showed that the number and percentage of Eos in the group treated with *Hyssop* was significantly lower than the control asthma group. This finding is consistent with the present study (Ekim et al., 2011). Ma et al. (2014) examined the effects of *Hyssop* on inhibition of airway inflammation and adjusting immunity system in mice with chronic asthma and showed that Eos in the group treated with *Hyssop* was is similar to the result observed in the normal group (Ma et al., 2014). This result is inconsistent with the present study. In terms of Lym index, there was no significant difference between the groups ($p > 0.05$); however, comparing the numerical values between the groups indicated that 50 mg kg^{-1} of *Hyssop* decreased Lym compared to the control group. Ekim et al. (2011) studied the effect of *Hyssop* on mice with asthma and found that treated mice had a significantly lower percentage and number of Lym in comparison with the control group (Ekim et al., 2011). There was no significant difference between the groups in terms of Mono percentage. Hangano et al. (2016) studied anti-inflammatory effects of *Hyssop* in comparison with diclofenac in rats and found that the count and percentage of monocytes had a notable decrease, which is not consistent with the present study (Hanganu et al., 2016). Dehkordi et al. (2015) studied the effect of alcoholic extract of *Zataria multiflora*, Satureja hortensis, and *Peucedanum officinale* on immunohematological factors in rats. Monocyte level did not have a significant change after consumption of *Zataria multifl*ora hydroalcoholic extract compared to the control group (Dehkordi et al., 2015). In terms of Baso index, there was no significant difference between the groups ($p > 0.05$). However, groups T1 and T2 had a limited increase in basophil percentage compared to the control group. Dehkordi et al. (2015) reported that basophil level after using hydroalcoholic extract of Zataria multiflora did not have any significant change, which is consistent with the present study (Dehkordi et al., 2015).

Changes in immunological indices between different groups are listed in Table 3. The highest phagocytic index was in T1 group, which was significantly higher than the other groups ($p < 0.05$). The difference in other groups was not significant ($p > 0.05$). Changes in phagocytosis percentage depended on the concentration of *Hyssop*, so that *Hyssop* in lower concentrations had a positive effect and in higher concentration had a negative effect on phagocytosis percentage. Hanganu et al. (2016) studied anti-inflammation effects of *Hyssop* on rats and found that the extract notably decreased phagocytosis percentages, which is consistent with the present study (Hanganu et al., 2016). Dehkordi et al. (2015) studies the effects of alcoholic extract of *Zataria multiflora*, *Satureja hortensis*, and *Peucedanum officinale* on immunohematologic factors in rats and showed that phagocyte count in *Zataria multiflora* group was significantly lower than the other groups (Dehkordi et al., 2015). In terms of average phagocytic bacteria, the highest value was observed in T1 group, which was significant ($p < 0.05$) and there was no significant difference between other groups ($p > 0.05$). Similar to phagocytosis percentage changes, the study of average phagocytic bacteria between treatments shows that its changes are dependent on *Hyssop* concentration, so that *Hyssop* at low concentrations has a positive effect and at high concentrations has a negative effect on average phagocytic bacteria.

According to other studies, increasing the average phagocytic bacteria is interpreted as improving the immune system, so according to the results of this study, consumption of *Hyssop* at a rate of 50 mg kg^{-1} can have a positive effect on improving immune function. There was no significant difference between the groups in terms of TP and ALB indices ($p > 0.05$). These results indicate that the concentrations used in this study did not have a notable effect on these indices. Akbarizadeh et al. (2020) studied the effect of *Hyssop* on immunity system of broiler chicken under cold stress and found that treatments that used *Hyssop* had a higher TP value compared to the control group. This finding is not consistent with the present study (Akbarizadeh et al., 2020). Dehkordi et al. (2015) also reported a significant increase in albumin level (Dehkordi et al., 2015). Comparison of IGM concentration between different groups showed that despite observing a significant difference between groups T2 and T3, no specific trend was observed in comparing treatments with control. Ma et al. (2014) studied the effects of *Hyssop* on controlling inflammation of airways and regulating immunity system in mice with chronic asthma. They showed that the level of immunoglobulins decreased in the treatment group, which is consistent with the present study (Ma et al., 2014). In addition, Dehkordi et al. (2015) examined IgM level in rats and found that Zataria multiflora increased it significantly compared to the control group.

Conclusion

In general, the results showed that the effects of hydroalcoholic extract of *Hyssop* depended on the concentration and type of immunohematology index studied. For example, with 100 mg kg^{-1} of *Hyssop*, there

was a notable increase in platelets. The use of this concentration also increased white blood cells, decreased neutrophils and decreased immunoglobulins. Concentration of 50 mg kg^{-1} of the extract increased phagocytes, average phagocytic bacteria and increased the level of immunity, and concentration of 150 mg kg^{-1} increased the level of immunity and increased the number of white blood cells.

Data availability

All data generated or analyzed during this study are included in this published article.

Acknowledgment

This study was approved by the Islamic Azad University, Shahrekord Branch, Iran.

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