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Original Research Article

Effect of Alcohol Extract of *Zataria multiflora* (Boiss), *Satureja bachtiarica* (Bunge) and *Zaravschanica membranacea* (Boiss) on Immuno-Hematologic Factors in Rats

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Abstract

Purpose: *To determine the effect alcohol extract of Zataria multiflora, Satureja bachtiarica and Zaravschanica membranacea on immunohematologic factors in Wistar rats.*
Methods: *Wistar rats were randomly allocated to seven treatment groups which consisted of control group with water and feed only (1); 200 mg. kg^{-1} Z. membranacea (2); 400 mg. kg^{-1} Z. membranacea (3); 200 mg. kg^{-1} S. bachtiarica (4); 400 mg. kg^{-1} S. bachtiarica (5); 200 mg. kg^{-1} Z. multiflora (6) and 400 mg. kg^{-1} Z. multiflora (7). Erythrocyte counts (RBC), packed cell volumes (PCV), haemoglobin (Hb) concentration, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), Translocation and Assembly Module (Tam) protein, IgM and albumin were measured after 29 days.*
Results: *Z. membranacea at 200 mg kg^{-1} showed the highest level of Tam-protein content ($p < 0.05$). Z. multiflora boiss at 400 mg kg^{-1} produced higher levels of immunoglobulin M (IgM) compared to S. bachtiarica and Z. multiflora ($p < 0.05$). Both Z. membranacea and S. bachtiarica at 200 mg. kg^{-1} caused a significant increase in albumin levels in the rats ($p < 0.05$). Z. multiflora at 400 mg. kg^{-1} had the highest effect on white blood cells (WBC) while S. bachtiarica produced the highest effect on neutrophils (Nut) ($p < 0.05$). Z. membranacea and Z. multiflora at 200 mg. kg^{-1} showed significantly higher levels of monocytes (Mon) % ($p < 0.05$). Z. multiflora and S. bachtiarica at 400 mg. kg^{-1} showed a significant effect on phagocytosis % ($p < 0.05$) whilst S. bachtiarica at 400 mg.kg^{-1} had a significant effect on phagocytosis number ($p < 0.05$).*
Conclusion: *The alcohol extracts of Z. multiflora Z. membranacea and S. bachtiarica extracts are capable of stimulating the immune defense mechanism without causing undesirable effects on hematological parameters.*

Keywords: *Zataria multiflora, Satureja bachtiarica, Zaravschanica membranacea, Immunoglobulin, Serum albumen, Immunohematologic factors*

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INTRODUCTION

The immune system is defined as biological structures and processes responsible for protecting an organism against diseases by identifying and eliminating the pathogen, stemming the emergence of tumours and maintaining constant internal conditions [1]. Enhancement of the immune system is possibly the most vital step in achieving resistance to disease and reducing susceptibility to infections. Immuno-stimulants can be used to activate the immune system through increased secretion and recognition on immuno-hematologic factors. Immuno-stimulants can be plant, viral, bacterial or parasitic derivatives as well as some synthetic compounds [2, 3].

One class of immune modulators is recognized as herbal medicines [4]. Extensive efforts are being invested to identify chemical compounds that are present in herbal products which act as immunostimulant agents to combat infections as therapeutic or prophylactic or treatments, or to enhance host immune mechanisms [5]. Barbour *et al* [6], Wagner [7] and Wichtl [8] reported that different herbal products are efficient in treating irregularities of the immune system and enhancing immune function in cases of toxicosis and chronic infections.

Herbal plants of medicinal importance such as *Zataria multiflora* Boiss, *Satureja bachtiarica* and *Zaravschanica membranacea* (Boiss) modify the physiological functions of living organisms, and thus, they are supposed to be compatibility with the animal body [9]. However, the specific effects of these plant extracts on the immunohematologic factors in animals have not been studied in detail. Therefore, the objective of the study was to determine the effect of alcoholic of extract *Zataria multiflora* Boiss, *Satureja bachtiarica* and *Zaravschanica membranacea* (Boiss) on immunohematologic factors of in rats.

EXPERIMENTAL

All animal experiments were carried out after approval from the ethical committee of the Islamic Azad University of Shahrekord Branch (no. IAUSHK/1393).

Extraction

The whole plant of *Zataria multiflora* Boiss, *Satureja bachtiarica* and *Zaravschanica membranacea* obtained from a herbarium in Tehran Iran were ground and dried under shade for 10 days and ground through a 1 mm sieve before the addition of 96 % ethanol solvent. The mixture was left for 72 h at 45 °C before the filtrate was removed through a filter and dried by rotary evaporator. The weighed extract was dissolved in water to make two doses of 200 mg kg^{-1} and 400 mg kg^{-1} for the investigation.

Animals

A total of 35 mature female white Wistar rats weighing 180 ± 20 g were randomly allocated to seven groups. The rats were kept under well-ventilated cages in an air-controlled room, fed with normal mice chow, and water was provided *ad libitum* over 28 days.

Group1: control treatment with water and food only.
Group 2: rats on 200 mg/kg *Z. membranacea*.
Group 3: rats on 400 mg/kg *Z. membranacea*.
Group 4: rats on 200 mg/kg *S. bachtiarica*.
Group 5: rats on 400 mg/kg *S. bachtiarica*.
Group 6: rats on 200 mg/kg *Z. multiflora* boiss.
Group 7: rats on 400 mg/kg *Z. multiflora* boiss.

Anesthesiology and sampling

At the end of study (day 29), ketamine drug (100 mg kg^{-1}) Netherland Alpha sun and vezayla zin (10 mg kg^{-1}) Netherland Alpha sun were injected intramuscularly to anaesthetize the rats. Blood was collected directly from heart, 2 mL of blood into a tube with EDTA for hematology tests and 2 mL into a tube without an anticoagulant (separating serum and albumin biochemical factors, IgM and Tam-protein). Blood cell count was done by Gimsa coloring and PCV hematocrit by microhematocrit method (centrifuging microhematocrit tube in 12000 round for two minutes). The biofactors albumin, IgM and Tam protein, were analysed from blood without any anticoagulant material. The blood factors were measured spectrophotometrically using kits of Pars Azmoon Company with Italian BT3000 auto analyzer in Almehdi Clinical Laboratory.

Statistical analysis

Data were statistically analyzed using SPSS® software (version 19.0) and presented as mean ± standard deviation (SD). Differences were considered statistically significant at $p < 0.05$.

RESULTS

The results in Table 1 show the effect of alcohol extract of *Zataria multiflora* boiss, *Satureja bachtiarica* and *Zaravschanica membranacea* on Tam-protein, IgM and albumin. Significantly ($p < 0.05$) higher level of Tam-protein was observed in *Z. membranacea* treatment at 200 mg kg^{-1} compared to the rest of the treatments. Higher levels of IgM were observed in *Z. multiflora* boiss treatment at 400 mg kg^{-1} and it was statistically significant from the other treatments ($p < 0.05$). Both *Z. membranacea* and *S. bachtiarica* treatments at 200 mg kg^{-1} showed a significant increase in albumin levels in rats ($p < 0.05$).

Table 2 shows effect of alcoholic extract of *Zataria multiflora* boiss, *Satureja bachtiarica* and *Zaravschanica membranacea* on biofactors and hematologic indices in the rats. *Zataria multiflora* boiss, *Satureja bachtiarica* and *Zaravschanica membranacea* showed no significant effect on erythrocyte counts (RBC), packed cell volumes (PCV), haemoglobin (Hb) concentration, mean corpuscular volumes (MCV) and mean corpuscular haemoglobin (MCH) ($p > 0.05$).

Table 3 shows effect of alcoholic extract of *Zataria multiflora* boiss, *Satureja bachtiarica* and *Zaravschanica membranacea* on factors and hematologic indices in the rats. *Z. multiflora* boiss at 400 mg kg^{-1} showed higher WBC and *S. bachtiarica* had the highest effect on Nut ($p < 0.05$). All the treatments had no significant effect on lymphocytes (Lymp) %, eosinophils (Eos) and basophils (Baso) ($p > 0.05$). *Z. membranacea* and *Z. multiflora* at 200 mg kg^{-1} showed significant effect on Mon % ($p < 0.05$). Table 4 shows effect of alcoholic extract of *Zataria multiflora* boiss, *Satureja bachtiarica* and *Zaravschanica membranacea* on phagocytosis number and percent in rat. *Z. multiflora boiss* and *S. bachtiarica* at 400 mg kg^{-1} showed a significant effect on phagocytosis % ($p < 0.05$) whilst, *S. bachtiarica* at 400 mg kg^{-1} had a significant effect on phagocytosis number ($p < 0.05$).

DISCUSSION

Z. multiflora boiss showed significant effect on WBS, IgM, monocytes (Mon) and phagocytosis percent. This effect caused by *Z. multiflora* boiss might be due to the presence of carvacrol, thymol as main phenolic compounds and p-cymeneas main non-phenolic compounds in the plant extract [10].

Table 1: Effect of alcoholic extract of *Zataria multiflora boiss*, *Satureja bachtiarica* and *Zaravschanica membranacea* on Tam protein, IgM and albumin in rat

Groups	Dosage	Tam protein (SD ± Mean)	IgM (SD ± Mean)	Albumin (SD ± Mean)
1 Control	-	7.1 ± 2^{b}	0.24 ± 0.16^{b}	2.9 ± 0.60^{b}
2 (*Z. membranacea*)	200mg. kg^{-1}	10.3 ± 0.96^{a}	0.27 ± 0.07^{b}	3.7 ± 0.20^{a}
3 (*Z. membranacea*)	400 mg. kg^{-1}	6.7 ± 0.83^{b}	0.39 ± 0.07^{b}	3 ± 0.43^{b}
4 (*S. bachtiarica*)	200 mg. kg^{-1}	8.4 ± 0.39^{b}	0.34 ± 0.08^{b}	3.7 ± 0.15^{a}
5.(*S. bachtiarica*)	400 mg. kg^{-1}	6.06 ± 0.50^{b}	0.039 ± 0.10^{b}	3.4 ± 0.30^{b}
6 (*Z. multiflora boiss*)	200mg. kg^{-1}	6.8 ± 1.6^{b}	0.31 ± 0.19^{b}	3.4 ± 0.30^{b}
7 (*Z. multiflora boiss*)	400mg. kg^{-1}	8.1 ± 1^{b}	0.50 ± 0.12^{a}	3 ± 0.15^{b}

In each column numbers that have similar letters the difference is not significant (p > 0.05)

Table 4: Effect of alcoholic extract of *Zataria multiflora boiss*, *Satureja bachtiarica* and *Zaravschanica membranacea* on number and phagocytosis percent in rat

Groups	Dosage	Phagocytosis (%) (SD ± Mean)	Phagocytosis number (SD ± Mean)
1 Control	-	16.8 ± 5.5^{b}	12.8 ± 2.8^{b}
2 (*Z. membranacea*)	200 mg. kg^{-1}	20.5 ± 7.1^{b}	15.7 ± 5.8^{b}
3 (*Z. membranacea*)	400 mg. kg^{-1}	2.5 ± 1.9^{b}	17.2 ± 2.8^{b}
4 (*S. bachtiarica*)	200 mg. kg^{-1}	19.2 ± 1.9^{b}	15.8 ± 4.4^{b}
5.(*S. bachtiarica*)	400 mg. kg^{-1}	27.2 ± 4.5^{a}	23.5 ± 6.8^{a}
6 (*Z. multiflora boiss*)	200 mg. kg^{-1}	228 ± 4.2^{b}	19.5 ± 3.3^{b}
7 (*Z. multitlora boiss*)	400 mg. kg^{-1}	28.2 ± 2.9^{a}	15.8 ± 12.7^{b}

In each column numbers that have similar letters the difference is not significant (p > 0.05)

Table 2: Effect of alcoholic extract of *Zataria multiflora boiss*, *Satureja bachtiarica* and *Zaravschanica membranacea* on hematologic indices in rat

Groups	Dosage	PVC (SD ± Mean)	RBC (SD ± Mean)	Hb (SD ± Mean)	MCV (SD ± Mean)	MCH (SD ± Mean)	MCHC (SD ± Mean)
1 Control	-	33.5 ± 2.4^{b}	6.5 ± 0.93^{b}	12.8 ± 1.2^{b}	52.5 ± 1.5^{b}	20 ± 0.87^{b}	38.2 ± 1.3^{b}
2 (*Z. membranacea*)	200 mg. kg^{-1}	31.2 ± 3.2^{b}	6.4 ± 0.8^{b}	12.1 ± 1.1^{b}	49.9 ± 6.4^{b}	17.9 ± 3.1^{b}	38.9 ± 2.5^{b}
3 (*Z. membranacea*)	400 mg. kg^{-1}	35.8 ± 1.6^{b}	6.8 ± 0.57^{b}	13.7 ± 0.59^{b}	56.9 ± 6.8^{b}	20.5 ± 3.1^{b}	38.1 ± 2.1^{b}
4 (*S. bachtiarica*)	200 mg. kg^{-1}	36.8 ± 1.2^{b}	6.8 ± 0.75^{b}	14 ± 0.61^{b}	55.1 ± 1.1^{b}	20.9 ± 0.61^{b}	$37.9+1.1^{b}$
5.(*S. bachtiarica*)	400 mg. kg^{-1}	33.5 ± 4.1^{b}	6.6 ± 0.95^{b}	13.4 ± 11^{b}	52.1 ± 4^{b}	20.9 ± 0.75^{b}	40.2 ± 2.5^{b}
6 (*Z. multiflora boiss*)	200 mg. kg^{-1}	35.8 ± 1.5^{b}	6.9 ± 0.61^{b}	13.3 ± 0.61^{b}	52.8 ± 0.56^{b}	19.5 ± 0.96^{b}	36.9 ± 1.6^{b}
7 (*Z. multiflora boiss*)	400 mg. kg^{-1}	37.1 ± 2.3^{b}	6.9 ± 0.85^{b}	13 ± 0.87^{b}	54.8 ± 2.5^{b}	19.1 ± 0.71^{b}	34.8 ± 0.95^{b}

In each column numbers that have similar letters the difference is not significant (p> 0.05). Erythrocyte counts (RBC), packed cell volumes (PCV), hemoglobin (Hb) concentration, mean corpuscular volumes (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC)

Table 3: Effect of alcoholic extract of *Zataria multiflora boiss*, *Satureja bachtiarica* and *Zaravschanica membranacea* on factors and hematologic indices in rat

Groups	Dosage	WBC (SD ± Mean)	Nut (%) (SD ± Mean)	Lym(%) (SD ± Mean)	Mon (SD ± Mean)	EOS (SD ± Mean)	Baso (%) (SD ± Mean)	Band (SD ± Mean)
1 Control	-	10766 ± 275b	26.5 ± b	69.2 ± 8.1b	2.2 ± 0.75b	2.2 ± 0.76b	0.5 ± 0.54b	0.5 ± 0.54
2 (*Z.membranacea*)	200 mg. kg^{-1}	11483 ± 740b	29.5 ± 1.9b	62.5 ± 5b	4.5 ± 1a	3.2 ± 1.2b	0.83 ± 0.75b	0.5 ± 0.54
3 (*Z. embranacea*)	400 mg. kg^{-1}	11433 ± 2110b	29.5 ± 4.1b	63.8 ± 3.6b	2.5 ± 0.54b	3 ± 1.7b	1.5 ± 2.3b	1.2 ± 1.2
4 (*S. bachtiarica*)	200 mg. kg^{-1}	11816.7 ± 384b	27.2 ± 1.2b	66.2 ± 2.8b	3.1 ± 0.75b	3.5 ± 1b	0.83 ± 0.75b	0.33 ± 0.52
5.(*S. bachtiarica*)	400 mg. kg^{-1}	10600 ± 651.9b	38.5 ± 8.5a	61.4 ± 3.3b	2.8 ± 0.75	1.2 ± 0.75b	0.33 ± 0.51b	0.5 ± 0.54
6 (*Z. multiflora boiss*)	200 mg. kg^{-1}	12133 ± 501b	26.8 ± 1.9b	64.8 ± 7.2b	4.5 ± 1a	2.2 ± 1.5b	1.5 ± 1b	1.2 ± 0.75
7 (*Z. multiflora boiss*)	400 mg. kg^{-1}	13733.3 ± 889a	25.8 ± 1.9b	69.5 ± 1b	3.2 ± 1.5b	1.1 ± 1b	1 ± 1.2b	0.67 ± 0.82

In each column numbers that have similar letters the difference is not significant (p > 0.05). Leukocytes counts (WBC), neutrophils (Nut) and lymphocyte (Lym), monocytes (Mon), eosinophils (Eos), basophils (Baso)

The compounds present in *Z. multiflora* boiss have antioxidant properties and are able to inhibit linoleic oxidation [10,11]. *Z. multiflora* essential oil has also been used for antimicrobial purposes in food [12,13].

Shokri *et al* [5] reported that *Z. multiflora* boiss has immuno-regulatory effects. The same result on the effect of *Z. multiflora* boiss on WBC observed in this study was also reported by Dehkordi *et al* [14]. *Z. multiflora* boiss is capable of increasing human mononuclear cells as well as Tam-protein and differential WBC count and endothelin level in blood which may confirm the immune-regulatory and anti-inflammatory effects of the plant [14].

In the present study, IgM in *Satureja bachtiarica* was significantly less than in *Z. multiflora*. Sturkie [15], observed an increase in lymphocytes percent and decrease in heterophils percent that indicates positive effect the on enhancement of body immune system by *Satureja bachtiarica*. In present study *Satureja bachtiarica* extract did not show a significant increase in IgM compared to control group. *Satureja bachtiarica* showed a significant increase in neutrophils and phagocytosis number and this relative increase can create increase in lymphatic immune system performance and phagocytes and overall cascade of immune system performance as observed by Khodadadi *et al* [16]. The increase in albumin observed in *Satureja bachtiarica* was also observed by Iranpour Mobarakeh *et al* [17], who concluded that the plant can lead to increase in blood serum proteins. Thomke and Elwinger [18] suggested that *Satureja bachtiarica* stimulates increase in liver performance marked by decrease in aminotransferase concentration. *Satureja bachtiarica* is capable of triggering increase in amount of complement system protein and as result a relative increase in complement system components were observed in the present study.

An experiment by Khodadadi *et al* [16] showed that *Z. membranacea* caused a relative increase in IgM when 200 and 400 mg kg^{-1} was fed to animals in two treatment groups for 30 days. However, no significant increase in IgM was observed at similar concentrations in this study whilst, significant increases were in Tam-protein, albumin, phagocytosis and Mon. *Z. membranacea* is known of increasing serum proteins as a result improving anticancer immune system and control pathogens growth [19, 20]. Bobadilla *et al* [21] conducted a study in fish and the results obtained showed that 50 % *Z. membranacea* boiss plant has significant effect on activity level of complement factor.

Shokri *et al* [5] observed that *Z. multiflora* boiss extract can significantly stimulate natural immunity function and it can be used as a treatment by itself or in concoction with other immune-stimulatory agents. *Satureja bachtiarica* has antidiabetic, antioxidant, anticoagulant and antibacterial property and is also responsible for triglyceride and weight reduction in animals [22]. Moreover, *Satureja bachtiarica* is used in treatment of spasms, colic, runny nose, otitis and sclerosis [23].

There was no significant effect of different concentrations of *Zataria multiflora* boiss, *Satureja bachtiarica* and *Zaravschanica membranacea* on erythrocyte counts (RBC), packed cell volumes (PCV), haemoglobin (Hb) concentration, mean corpuscular volumes (MCV) mean corpuscular haemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). All the plant extracts are capable of maintaining a stable condition and had no undesirable effects on hematological parameters. It is therefore possible that use of *Zataria multiflora boiss*, *Satureja bachtiarica* and *Zaravschanica membranacea* may not contribute to the risk for cardiovascular syndrome.

CONCLUSION

Zataria multiflora boiss, *Satureja bachtiarica* and *Zaravschanica membranacea* plant extracts improved the levels of lymphocytes, WBC, IgM, Mon, albumin, phagocytosis, neutrophils and Tam-protein indicating the stimulation of immune defense mechanism. They are also capable of maintaining haematological parameters in rat.

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