Effects of oral administration of *Zataria multiflora* essential oil on some blood and serum parameters in *Acipenser persicus*

Sharif Rohani M. [*1] ; Masoumzadeh M.[2] ; Haghighi M.[1] ; Jalilpoor J.[2] ; Pourdehghani M.[2] ; Shenavar Masouleh A.[2] ; Alizadeh M.[2] ; Bazari Moghaddam S.[2]

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Abstract

In order to does this study, 150 Persian sturgeons having 75g mean weight transferred to 500 liter tanks. After dividing the fishes into two groups (experimental and control), the fingerlings fed by concentrate food that contained different levels of *Zataria multiflora* essential oil (15, 25, 50, 100 g/kg) for 8 weeks. Finally, sampling carried out on blood and serum of fishes. After preparing the samples based on standard methods, hematology and serology studies, performed. The results showed significant difference in mean of monocytes between experimental and control groups ($P<0.05$). In treatment 25g/kg, monocyte count was more than other treatments. But there were no significant differences in mean and standard deviation of other white blood cells. The results of serologic studies showed no significant differences in serum indices (C3, C4 and total protein) ($P>0.05$), but there is significant difference in mean and standard deviation of albumin. So that, the average of serum parameters in treatments 15 and 25g/kg and control group was more than treatments 50 and 100g/kg of *Zataria multiflora* essential oil.

Keywords: *Zataria multiflora*, essential oil, hematology, serology, Persian Sturgeon

1- Iranian Fisheries Research Organization, Tehran, 14155-6116, Iran.

2-International Sturgeon Research Institute, P.O. Box 41635-3464, Rasht, Iran

*Corresponding Author[s] email: rohani@ifro.ir

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Introduction

Mal effects of drugs and chemical disinfectants which leads to resistance in body of farmed aquatics, makes it necessary to be replaced with herbal therapeutics having natural resources and it is one of the most important priorities of researchers in fish medicine arena. *Zataria multiflora* belongs to Labiatae family, is one of the most well-known herbal drugs in Iran and traditional medicine. Its antimicrobial effects approved in different studies. Therapeutic parts of this plant include its top branches and dried leaves. Its extract is Thymol. Results of photochemistry experiments showed components such as alkaloid, saponin, flavonoid and tannin in *Z. ataria multiflora.*

Up to know, several studies performed on using *Zataria multiflora* on aquatics in Iran. These studies are on usage of herbal essence in controlling fungal pollutions on trout eggs (Sharif Rohani et al., 2006), research on antibacterial effects of some herbal extracts on *Aeromonas hydrophyla* which is a septicemia factor in fish (Alishahi et al., 2008) and the effects of *Z. multiflora* essential oil on hatching rate of eggs in rainbow trout and survival rate of larvae in oxygenated water and malachite green (Soltani et al., 2009). Regarding the importance of sturgeons, this study carried out to determine the effects of different doses of oral administration of *Z. multiflora* essential oil on some hematological and serum factors in Persian sturgeon.

Materials and methods

Fish and rearing condition: A Total of 150 specimens of Persian sturgeon fingerlings with 75g mean weight randomly collected from Shahid Beheshti Rearing and Propagation Complex and transferred to the International Sturgeon Research Institute (ISIR), for further adaptation with new condition in 500 liter fiberglass tanks including 10 fishes per tank for a 10 days period. The fishes fed by the diet formulated by ISRI. The water used for rearing was a mixture of well and river water in equal ratio, in this study physicochemical factors of water, including temperature, oxygen and pH were measured daily. During experiment the mean water temperature was 18±2°C and other factors were in acceptable range.

Essential oil: The pure essential oil of *Zataria multiflora* with special weight prepared from medicinal Company of Barij Essence (Kashan, Iran) and added to fish food in different doses.

Experimental treatments: The treatment determined according to dose of *Z. multiflora* essential oil in the diet in department of Rearing and propagation in ISRI. In this regard, the fishes randomly selected and divided into 4 treatments including 15, 25, 50 and 100 g/kg *Zataria multiflora* essential oil and one control group. Each treatment and control group administered triplicates. The fishes fed as one percent of their weight for 8 weeks daily in three times. At the end, blood and tissue samples collected to determine haematological and serum parameters.

Sampling and experiments: Eight weeks after feeding of Persian sturgeon by special diet in control and test groups, blood samples prepared from fished in each group (from 3 fishes in each replicate). Blood samples

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collected from caudal vein and instantly 2 ml of blood transferred to tubes containing Heparin anticoagulation. Also, 1 ml of blood was centrifuged at 3000 rpm in laboratory temperature for 10 minutes and the serum separated in order to serologic studies.

Determination of blood parameters: In order to determine leukocyte counts, the methods of which suggested by Svobodova et al. (1991) and Feldman et al. (2000), were used and differential counting of white blood cells (WBC) of samples carried out using Neobar Lamella.

Determination of some serum parameters: The method of Biuret and estimation kit of biochemistry Company and auto analyzer unit are used to determine total protein. Wave length 560 nm is used according to measurement method. Additionally, method of Bromocresol green and estimation kit of biochemistry Company and auto analyzer unit are used to measure the concentration of serum albumin. In this regard, albumin reacted with Bromocresol green and a blue complex resulted. Its color intensity is related to albumin concentration. This color is measured in 630nm.

In order to measure C3 and C4 of serum, method of Immuno Turbidimetric was used. Regarding this, C3 and C4 of the samples with anti C3 and C4 polyclonal antibody in solution create complex which leads the solution became turbid. Intensity of turbidity is directly related to amount of C3 and C4. This turbidity is measured in 340nm.

Statistical analysis: The data related to Hematological and serum indices, analyzed by one way Anova and Duncan's test, comparing means in 95% confidence level among different treatments. SPSS applied in order to analyze the data.

Results

Counts of Hematological indices: The results showed that the variation range of white blood cells such as lymphocyte, monocyte, neutrophil and eosinophil is 68.8-81.33%, 1.0-2.5%, 14.66-25.6% and 2.33-4.6%, respectively (Table 1).

Statistical analysis carried out by one way ANOVAs on fish WBC in treatment and control groups, showed no significant differences in number of blood lymphocyte, neutrophil and eosinophil in experimental (15, 25, 50 and 100 g/kg) and control group.

Also, the results showed that the number of monocytes in group of 25g/kg were more than other experimental and control group. The results of one way variance analysis exhibited significant differences ($P<0.05$) in mean (±SD) of monocyte number in experimental and control groups (Fig. 2).

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Table 1: Comparing the mean of WBC in Persian sturgeon fed by formulated food containing different doses of *Zataria* multiflora essential oil

Parameter	Treat. 15g/kg		Treat. 25g/kg		Treat. 50g/kg		Treat. 100g/kg		Control group	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Lymphocyte(%)	74.50	13.78	78.16	15.96	72.33	10.46	68.8	17.97	81.33	1.52
Monocyte(%)	1.16	0.16	2.50	0.61	1.16	0.16	1.00	-	1.00	-
Neutrophil(%)	20.50	5.48	17.0	6.77	24.16	3.91	25.60	7.62	14.66	1.58
Eosinophil(%)	3.83	1.01	2.33	0.61	2.33	0.55	4.60	1.12	3.00	0.68

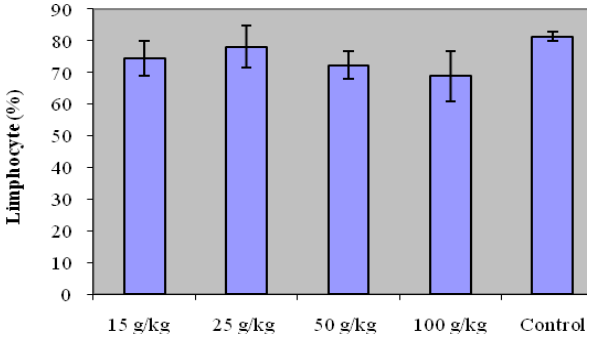


Fig. 1: Comparing the lymphocyte percent in experimental and control group of using *Zataria* multiflora essential oil

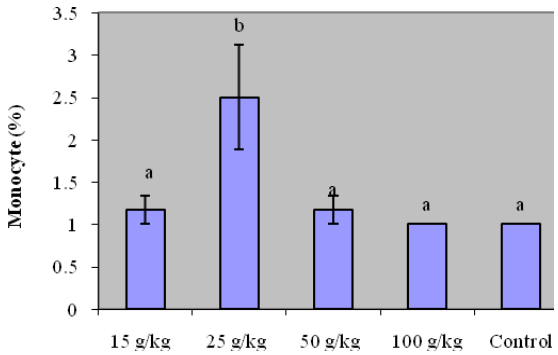


Fig. 2: Comparing the monocyte percent in experimental and control group of using *Zataria multiflora* essential

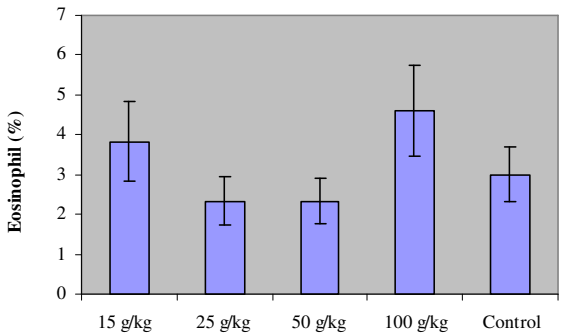


Fig. 3: Comparing the neutrophil percent in experimental and control group of using *Zataria multiflora* essential oil

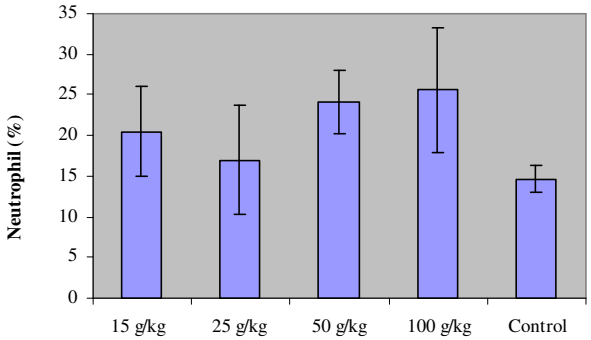


Fig. 4: Comparing the eosinophil percent in experimental and control group of using *Zataria multiflora* essential

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Ratio of serum indices: The results for determination of some most important factors of serum indices showed varieties in C3 and C4 and serum total protein and albumin as 29.0-54.66, 28.60-53.83, 1.48-2.42 and 0.84-1.22%, respectively (Table 2).

Statistical analysis on mean of some serum factors in juvenile Persian sturgeon fed by diet containing *Zataria multiflora* essential oil for 8 weeks, showed significant differences in all serum parameters (such as C3, C4 and serum total protein) except albumin in experimental and control group ($P<0.05$). Thus, the mean and standard deviation in doses of 15 and 25g/kg were more than doses of 50 and 100g/kg (Table 2).

Table 2: Comparing the mean of WBC in Persian sturgeon fed by formulated diet containing different doses of *Zataria multiflora* essential oil

Parameter	Treat. 15g/kg		Treat. 25g/kg		Treat. 50g/kg		Treat. 100g/kg		Control group	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C3 (%)	54.66	3.65	47.50	4.21	30.00	6.21	29.00	8.26	50.20	2.31
C4 (%)	55.83	3.90	52.00	5.00	34.00	7.30	28.60	5.97	55.80	2.03
Total protein (%)	2.42	0.14	2.11	0.11	1.66	0.21	1.48	0.12	2.28	0.07
Albumin (%)	1.22	0.11	1.03	0.08	0.86	0.11	0.84	0.21	1.08	0.11

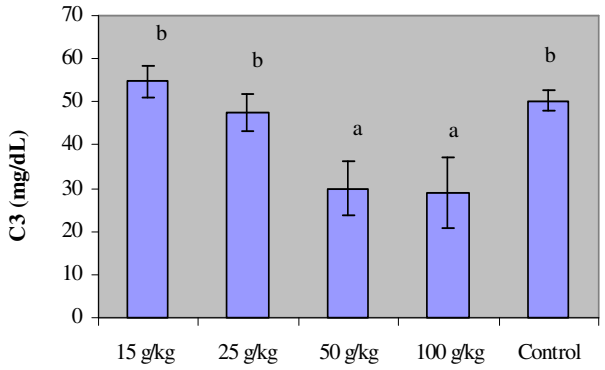


Fig. 5: Comparing the C3 percent in experimental and control treated groups of *Z. multiflora* essential oil

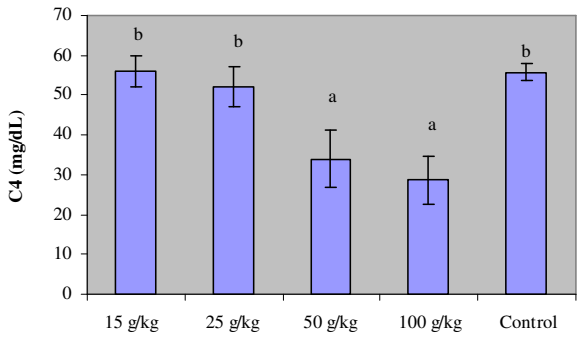


Fig. 6: Comparing the C4 percent in experimental and control treated groups of *Z. multiflora* essential oil

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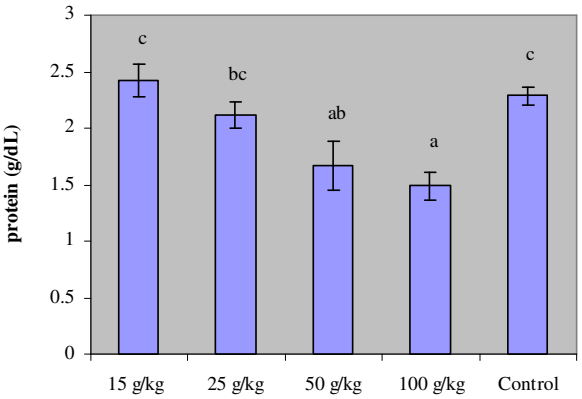


Fig. 7: Comparing total protein percent in experimental and control treated groups *Z. multiflora* essential oil

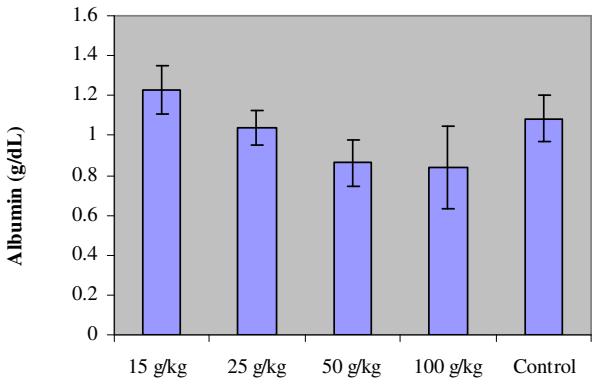


Fig. 8: Comparing albumin percent in experimental and control treated groups *Z. multiflora* essential oil

Discussion

Increase in productivity, also health of consumers and preventing environmental pollution makes it necessary to use natural and medicinal herbal plants to be replaced with chemotherapeutics to control pathogenic factors in aquaculture industry specially sturgeon rearing in recent decades.

The number of WBC is one of the most important health and immune system indices (Shalaby et al., 2006). In this study regarding similar rearing condition in all experimental and control groups and canceling the factors effective in blood indices such as type of species, temperature variation and change in other physicochemical factors of water, also existence of environmental stress and seasonal changes (Roberts et al., 1998), sexual cycle and other physiologic factors (Krajnovic, 1991), there is no significant differences in containing 15, 25, 50 and 100 g/kg *Z. multiflora* essential oil for 8 weeks can not create significant change in number of different white blood cells.

The amount of serum indices is one of the factors that are representative of animal health including fish health. Our results revealed that there is significant difference in all serum indices such as C3, C4 and total protein except the albumin ratio in experimental and control group. So that they were more in doses 15, 25g/kg and control group than doses 50 and 100 g/kg (Figs. 5, 6, 7 and 8). Experiments of many researchers on the effects of natural material on blood parameters show different results. Sahu et al. (2006) studied on seed of *Magnifera indica* as a diet supplement for improvement of immunity system in fingerlings of *Labeo rohita* and no significant change observed in number of white and red

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blood cells. Kumar Jha et al. (2007) studied on some natural immune stimulants on fingerlings of *Catla catla* and their effects on hemoglobin and RBC number and albumin rate in serum. The results of study carried out by Soltani et al. (2004) on the effects of *Daphne indica* extract on common carp by bath method, showed no significant differences in number of WBC in experimental and control group. While the studies carried out by Sheikhzadeh et al. (2009) showed low concentration of *Eucalyptus* extract in both oral and bath method can be lead to increase in number of WBCs compared to control group.

Comparing the results of this investigation with some other studies such as study on the effect of *Zataria multiflora* essential oil on some Hematological indices of *Astronatus ocellatus* (Alishahi et al., 2009) and effects of oral administration of *Allium sativum* on farmed *Huso huso* juveniles (Tangestani et al., 2011) are in agreement.

According to results of this study and other experiments, *Zataria multiflora* essential oil especially with low concentration which will not change blood indices, can be used as antimicrobial compounds to control pathogenic factors in farmed aquatics such as sturgeons.

Acknowledgement:

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اثر مصرف خوراکی اسانس آویشن شیرازی بر روی برخی پارامترهای خون و سرم در تاسماهی ایرانی (*Acipenser persicus*)

شریف روحانی م.[1*]؛ معصوم‌زاده م.[2]؛ حقیقی م.[1]؛ جلیل‌پور ج.[2]؛ پوردهقانی م.[2]؛ شناورماسوله.[2]؛ علی‌زاده م.[2]؛ بازاری مقدم س.[2]

تاریخ دریافت: مرداد ۱۳۹۱ تاریخ پذیرش:بهمن ۱۳۹۱

چکیده:

به منظور بررسی مطالعه اثر مصرف خوراکی اسانس آویشن شیرازی بر روی برخی پارامترهای خون و سرم در تاسماهی ایرانی، ۱۵۰ تاسماهی ایرانی با میانگین وزن ۷۵ گرم به تانک های ۵۰۰لیتری منتقل شدند. پس از تقسیم ماهی به دو گروه (آزمون و شاهد) ، بچه ماهیان انگشت قدی توسط غلظت های غذایی از سطوح مختلف اسانس آویشن شیرازی (۱۵ ، ۲۵، ۵۰ ، ۱۰۰ g/kg)به مدت ۸ هفته تغذیه شدند.

در نهایت، نمونه گیری از خون و سرم ماهی ها انجام گرفت، پس از تهیه نمونه ها بر اساس روش های استاندارد ، مطالعات خون شناسی و سرولوژی، انجام پذیرفت، نتایج نشان داد که بین گروه آزمون و شاهد تفاوت معنی داری ($P<0.05$) در میانگین مونوسیت ها وجود داشت. در تیمار ۲۵ g/kg ، تعداد مونوسیت بیشتر از سایر تیمارها بود. اما تفاوت معنی داری در میانگین و انحراف معیار آن با سایر سلول های سفید خون وجود داشت. نتایج مطالعات سرولوژیک نشان داد تفاوت معنی داری در شاخص های سرمی از قبیلC3، C4 و پروتئین کل وجود نداشت ($P>0.05$) ، اما درمقدار میانگین و انحراف معیار آلبومین تفاوت معنی داری مشاهده شد .نتایج تحقیق حاضر نشان داد میزان متوسط پارامترهای خونی و سرم در تیمارهای۱۵ و ۲۵ g/kg و گروه کنترل کنترلی بیشتر از تیمارهای ۵۰ و ۱۰۰ g/kg از آویشن شیرازی آویشن اسانس بود.

کلمات کلیدی:آویشن شیرازی، اسانس، تاسماهی ایرانی، خون شناسی، سرولوژی.

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آدرس الکترونیکی نویسنده مسئول: rohani@ifro.ir

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