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Research Article

The adjuvant effect of Myrtle, *Myrtus communis*, extract on hematological, Immuno-physiological, antioxidant responses, and tissue histomorphology of gill and liver in juvenile Siberian sturgeon, *Acipenser baerii*

Barzegar R. 1; Farokhrouz M. 1*; Khara H. 1; Shenavar Masouleh A. 2; Ahmadnezhad M. 3

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Abstract

This study aimed to evaluate the effect of Myrtle, Myrtus communis, extract on hematological, immuno-physiological, antioxidant responses, bactericidal activity, and tissue histomorphology of gill and liver in juvenile Siberian sturgeon, Acipenser baerii. Siberian sturgeon were exposed to 4 doses of myrtle extract including 25% (67.4 mg/L; M_{25}), 50% (134.9 mg/L; M_{50}), 75% (202.0 mg/L; M_{75}), and 100% (269.8 mg/L; M_{100}) of the maximum allowable concentration and a control treatment (without exposure myrtle extract). Hemoglobin and red blood cell values were significantly increased in fish exposed to the myrtle extracts (p<0.05). The white blood cell was lower in M_{25} and M_{75} , while the highest value was found in M_{100} treatment (p<0.05). Myrtle extract did not affect the lymphocyte value in the course of exposure (p>0.05). The highest albumin and total protein levels were observed in M₂₅ and M₅₀ groups. The highest values of lysozyme and total immunoglobulin (Ig) activities were observed in M₅₀, M₇₅ and M₂₅, M₅₀, respectively (p<0.05). Superoxide dismutase and catalase activities of those fish exposed to M_{50} and M_{75} were significantly higher than the control and M_{100} groups (p<0.05). The lowest glutathione peroxidase value was observed in the control group compared to the others (p < 0.05). The severe changes such as adhesion and curling of gill lamella discern were observed in fish exposed to different levels of myrtle extracts. Moreover, in the control group, severe hepatocyte destruction was accompanied by nucleus pyknosis, but the severity of atrophy was observed in M_{75} and M_{100} treatments. Overall, the results suggested that myrtle in the range of 67.4-202 mg/L could be applied as a stimulant agent to Siberian sturgeon aquaculture.

Keywords: Exposure, Myrtle, Immuno-physiological, Siberian sturgeon

¹⁻Department of Fisheries, Lahijan Branch, Islamic Azad University, Lahijan, Iran

²⁻International Sturgeon Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Rasht, Iran

³⁻Inland Waters Aquaculture Research Center. Iranian Fisheries Sciences Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Bandar Anzali, Iran.

^{*}Corresponding author's Email: dr.farokhrouz@gmail.com

Introduction

Sturgeon farming is a fast-growing practice worldwide, and nowadays, noteworthy attention has been paid to this operation (Banavreh et al., 2019a). Siberian sturgeon, Acipenser baerii, is one of the most important sturgeon species with a fast growth rate, feeding adaptability, and stress resistance and has been used as a biological model to initiate investigations (Fontagné-Dicharry et al., 2018; Mirzakhani et al., 2020). The high density of fish in the rearing system provides a stressful environment for the aquatic animal and promote the sensitivity of the fish to suppressing the immune system and ultimately leading to infectious diseases in the animal (Long et al., 2017; Banavreh et al., 2019b). Traditionally, antibiotics and chemotherapeutics are regularly used to prevent aquatic diseases. Their indiscriminate produces resistance pathogens during antibacterial therapy, bioaccumulation in the aquatic animals. environmental deterioration (Banavreh et al., 2019b).

Nowadays, application the butanoic extracts as an immunostimulant in practical modern aquaculture has been recommended as a promising approach for ameliorating health conditions and disease prevention (Mansouri Taee et The medicinal 2017). plant's derivative stimulants of the immune system, growth promotion, improved appetite, and antimicrobial activity due to their natural compounds such as phenolics, oils, alkaloids, essential flavonoids, which steroids, are affordable and bio-compatible, biodegradable and eco-friendly agents without any side-effects (Harikrishnan et al., 2011; Afzali and Wong, 2017; Soltani et al., 2018). Immunostimulants can be applied by three routes of bathing, injection, oral administration. or Traditionally, herbal extracts have been utilized as an appetizer, antibacterial, antiviral, antibacterial anticancer, as well as an antioxidant agent while attenuating the free radicals (Amensour et al., 2010; Mousavi et al., 2011; Safari et al., 2017).

Myrtle, *Myrtus communis* L, is a medicinal plant with a perennial shrub and dispersed in the Mediterranean regions, especially in Iran. The leaves of this plant contain essential oil, myrtenyl acetate, limonene, polyphenolic compounds like saponins, flavonoids, and tannins (Mansouri Taee *et al.*, 2017; Safari *et al.*, 2017).

However, in our knowledge, there is no information on the effects of myrtle on the Siberian sturgeon. Hence, the objective of the current study was to determine the effects of biological activities and phytochemical composition of myrtle on immunophysiological and tissue histomorphological responses and antimicrobial properties of Siberian sturgeon.

Materials and methods

Preparation of myrtle

The myrtle leaves were obtained from the central region of Iran (Yazd province). The leaves were dried at 37°C in an incubator for 4 days and mailed by a grinder. After that, leaves were powdered (10 g) and blended with ethanol (100 mL, 70%) and then shaked by rotary shaker for 24 hour (h). The collected extract was preserved in a dark container at 4°C, pending to use (Amensour *et al.*, 2010).

Total phenolic and flavonoid content The content of total phenol quantified according to the Folin-Ciocalteu method (Stocker et al., 2004). In brief, an aliquot (0.25 mL of extract) was added with Folin-Ciocalteu reagent (1.25 mL) and distilled water (0.5 mL). The compound was agitated and allowed to stand for 5 min before adding 1.25 mL of sodium carbonate solution (7%). The absorbance of the resulting dilution was measured at 760 nm. The total flavonoid content was determined based on the method summarized by Wannes and Marzouk (2016). Briefly, 250 mL of diluted extract was blended with 75 mL NaNO₂ (5%). After 6 minutes, 500 mL of NaOH, and 150 mL of AlCl3 were added to this mixture. Then, the mixture remains stable at room temperature for 15 min; the absorbance was quantified at 510 nm. The condense tannin was (2% detected with Ferric ferric ammonium sulphate in 2 N HCl) and HCL-Butanol reagents (Porter et al., 1986). The total flavonoid values of extract were displayed as milligram catechin equivalents per gram (mg CE/g) through a calibration curve with catechin (3 replicates).

Fish and experimental conditions

A total number of 500 Siberian sturgeon (average weight: 15.1±1.03 g) were distributed into 15 circular fiberglass tanks (350 liters) at the International Sturgeon Research Institute (Rasht, Iran). Water quality indices including dissolved oxygen, water temperature, pH, nitrite, and NH₃ were measured as 7.19±0.5 mg/L, 19.1±1.52°C, 7.35±0.65, <0.1 mg/L and <0.05 mg/L, respectively.

Determination of trial dose and preparation of the experiment

The acute toxicity was tested for myrtle extract followed the guidelines for chemical tests approved by the OECD (1998). The experiment was carried out in a static system without water renewal. Sturgeon mortality at each myrtle extract concentration was recorded at 24, 48, 72, and 96 h. LC₅₀-96 h and maximum allowable concentration (MAC) were quantified in 269.8 mg/L by a computer program (CEAM, 1999) using Finney (1952) Probit Analysis. In the next step, 4 doses of myrtle extract were used including 25% (67.4 mg/L), 50% (134.9 mg/L), 75% (202.0 mg/L), and 100% (269.8 mg/L) of the MAC value and a control treatment (without exposure myrtle extract), hereafter named as M₂₅, M_{50} , M_{75} . M_{100} , and control. respectively. Each treatment contained 3 replications. During the test experiment, the water inlet was stopped. The tanks were continuously aerated with air pumps. Siberian sturgeon was exposed to the suspected doses for 96 h.

Sampling and blood collection

At the end of the 4th day, blood sampling was performed. It should be noticed that tranquilizer was not utilized in the samplings because they can be an influence on the blood indices. In order to solve this bottleneck, fish were sacrificed by a sharp blow to the head. After that, the blood samples were randomly collected via the caudal vein of three fish per replicate. An aliquot of the blood sample in the heparinized vial was used for hematological assays, and second aliquot in the nonheparinized vial was centrifuged at room temperature. The sera preserved at -20°C until further analysis.

Blood processing and analyses

The number of white blood cells (WBCs) and red blood cells (RBCs) were counted by hemocytometer after diluting blood samples by adding Turk solution and Hayem solution (isotonic solution), respectively (Blaxhall and Daisley, 1973). The estimation of the differential leukocyte counts, including lymphocyte, neutrophil, eosinophil, and monocyte were manually counted and determined using a light microscope. The standard microhematocrit method was utilized to measure hematocrit (Hct), and the values expressed as a percentage of erythrocytes. The hemoglobin (Hb) concentration was determined using spectrophotometry with (540)nm) the cyanomethahemoglobin method, blood mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and

corpuscular hemoglobin mean concentration (MCHC) were quantitated based on the method described by Blaxhall and Daisley (1973). Hematobiochemical factors including albumin, glucose, globulin glucose, cortisol, and hepatic attributes of serum such as alanine aminotransferase (ALT). aspartate aminotransferase (AST), and alkaline phosphatase (ALP), as well as immunological indices such as lysozyme total immunoglobulin, and quantified by an autoanalyzer instrument (Perstige 24i, Boeki, Japan) using commercial kits (Pars-Azmoon, Karaj, Iran) according to the manufacturer's protocol.

Antioxidant activities

The assay of catalase (CAT) in serum samples was accomplished, according to Aebi (1984), by assaying the reduction of H₂O₂ value at 240 nm. One unit of CAT activity was defined as the quantity of CAT needed to transform 1 µmol of H₂O₂/min. The superoxide dismutase (SOD) activity was defined using the method of Beauchamp and Fridovich (1971). SOD value was calculated using the sample that catalyzes the breakdown of umol of O⁻² to hydrogen peroxide and oxygen/min. Glutathione peroxidase (GPx) activity was detected using the technique illustrated by Adel et al. (2017).

Gill and liver histopathology

At the end of the experiment, three fish per treatment euthanized with 300 mg/L clove powder (Barij Essence, Iran;

Banavreh et al., 2019a) to remove the gills (second-gill arches of the left side) and liver under aseptic conditions for histopathological analysis. The respective specimens were fixed in Bouin's solution for 18-24 h, dehydrated in soaring concentrations of ethanol, cleaning with xylene, and then paraffin embedding, and prepared histological studies. The combined tissues were sectioned with a thickness of 4-6 µm using a microtome (Leitz-1512, Germany) (Rezakhani et al., 2020). Thereafter, these segments were stained with H&E. Histopathological modifications were appraised by a light microscope (Nikon, Ni-U, Japan).

Statistical analysis

After normality verification using a Shapiro-Wilk test, data were analyzed by a one-way ANOVA followed by Tukey's test. All comparisons were accomplished by SPSS software version no. 22.0 (Chicago, USA), and variations were considered significant at p<0.05. The quantitative analyses of the data were displayed as mean±SD.

Results

Phenolic contents

The phenolic compounds of the extract of myrtle are described in Table 1.

Table 1: Phenolic compounds of myrtle extract.

	leaves				
Sample	Total phenols	Proanthocyanidins ¹	Flavonoids		
Myrtle extract	22.63±0.03	0.52±0.02	0.56±0.01		

Total phenolic was expressed by mg gallic acid/g dry matter; flavonoids and proanthocyanidin values were expressed by mg catechin/g dry matter.

Hematological indices

As shown in Table 2, Hb and RBC values were significantly increased in fish exposed to the myrtle extracts (M_{25} , M_{50} , and M_{75}) compared with those of the control treatment (p<0.05; Tab 2). A similar tendency was obtained for MCV values, while most of the values were observed in M_{50} treatment. Hct values showed no variation in different groups (p>0.05). The WBCs was lower in M_{25} and M_{75} , while the highest value was recognized in M_{100} treatment (p<0.05).

Monocyte value was higher in more than 50% of the MAC value compare to the control and M₂₅ groups. However, an

increasing percentage of lymphocyte was observed in M_{25} , M_{50} , and M_{75} groups, but no significant differences were detected between any groups (p>0.05; Table 3). Unlike monocyte, the lowest eosinophils and neutrophils were found in M_{25} , M_{50} groups (p<0.05).

Fish in M_{25} and M_{50} groups had the highest albumin, which differs significantly from the other treatments (Table 4).

¹As condense tannins equivalents

Table 2: Hematological values of juvenile Siberian sturgeon exposed to different concentrations of myrtle.

-	Treatments				
Indices	Control	M_{25}	M_{50}	M ₇₅	M_{100}
Hb (g/dL)	5.17±0.15 ^a	6.13±0.21 ^b	6.37±0.15 ^b	6.17±0.25 ^b	5.13±0.25 ^a
RBC ($\times 10^3$ /mm ³)	510.01 ± 0.04^{a}	595.00 ± 0.01^{d}	598.1±0.01e	584.99±0.01°	531.01±0.02 ^b
WBCs	4.90 ± 0.08^d	4.09 ± 0.05^{a}	4.80 ± 0.07^{c}	4.29 ± 0.04^{b}	5.89 ± 0.06^{e}
$(\times 10^3 / \text{mm}^3)$					
Hct (%)	24.67±2.52	30.33 ± 0.57	30.01±3.61	30.06 ± 1.02	25.67±1.53
MCV (fl)	$489.67{\pm}10.07^a$	506.01 ± 4.58^{ab}	519.67±7.02°	511.33 ± 10.60^{bc}	491.67 ± 3.79^{ab}
MCHC (g/dL)	20.83 ± 0.06^{b}	20.57 ± 0.25^{b}	20.30 ± 0.10^{b}	20.20 ± 0.50^{ab}	19.57 ± 0.15^{a}

Notes. M_{25} : 25% of MAC; M_{50} : 0% of MAC; M_{75} : 75% of MAC; M_{100} : 100% of MAC. Data are presented as mean±SD. The presence of different superscript letters denotes significant variation between treatments (p<0.05). The absence of letters indicates no significant difference between treatments (p>0.05)

Table 3: Differential leukocyte counts of juvenile Siberian sturgeon exposed to different concentrations of myrtle.

			Treatments		
Indices	Control	M_{25}	M_{50}	M_{75}	M_{100}
Monocyte (%)	4.03±0.15 ^a	4.07±0.12a	5.03±0.15 ^b	5.10±0.10°	5.33±0.35°
Lymphocyte (%)	79.67±1.53	84.33 ± 2.8	84.67 ± 2.52	85.01 ± 3.04	81.67±1.53
Eosinophil (%)	0.97 ± 0.25^{b}	0.97 ± 0.15^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.97 ± 0.15^{C}
Neutrophil (%)	15.13±0.35°	10.17±0.29a	11.97±0.55 ^b	11.13 ± 38^{ab}	10.97 ± 0.45^{ab}

Notes. M_{25} : 25% of MAC; M_{50} : 0% of MAC; M_{75} : 75% of MAC; M_{100} : 100% of MAC. Data are presented as mean \pm SD. The presence of different superscript letters denotes significant variation between treatments (p<0.05). The absence of letters indicates no significant difference between treatments (p>0.05).

Table 4: Effect of various concentrations of myrtle extracts on hemato-biochemical parameters in juvenile Siberian sturgeon

	Treatments					
Indices	Control	M ₂₅	M_{50}	M_{75}	M_{100}	
Albumin (g/dL)	0.75±0.03a	0.93±0.00 ^d	0.89±0.01 ^{cd}	0.82 ± 0.02^{b}	0.84±0.01bc	
Total protein (g/dL)	1.76 ± 0.01^{c}	1.95 ± 0.01^{e}	1.93 ± 0.00^{de}	1.81 ± 0.01^{c}	1.79 ± 0.00^{c}	
Glucose (mg/dL)	49.01 ± 2.65	47.33 ± 2.58	52.33±1.53	52.46±2.65	56.67±3.53	
Cortisol (µg/L)	104.33 ± 5.13^{a}	110.21±5.01a	108.11 ± 7.02^a	117.52 ± 4.08^{b}	122.32 ± 7.37^{b}	
ALP (U/L)	711.67±0.41°	865.01 ± 4.36^{d}	659.33±4.04b	602.00±5.19a	945.01 ± 4.58^{e}	
ALT (U/L)	18.00 ± 1.73^{a}	34.67±2.08°	28.03±3.61b	22.33 ± 2.52^{ab}	37.67 ± 1.53^{c}	
AST (U/L)	394.33±4.23b	377.00±5.29°	326.02±5.19a	482.33±1.16°	531.20±3.61 ^d	
LDH (U/L)	984.70±1.53e	661.03±9.54b	826.72±5.69°	887.7 ± 14.01^{d}	984.71±1.53e	

Notes. M₂₅: 25% of MAC; M₅₀: 0% of MAC; M₇₅: 75% of MAC; M₁₀₀: 100% of MAC. Data are presented as mean±SD. The presence of different superscript letters denotes significant variation between treatments (*p*<0.05).

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase.

Albumin and total protein levels wereparalleled each other. The glucose level was not significantly different in fish exposed to the tested concentrations, while cortisol value increased more than 202 mg/L in M₇₅ group. The lowest ALP and ALT activity was recognized in the

 M_{75} group, while the inferior AST value was observed in the M_{50} group (p<0.05). LDH was decreased significantly in the M_{25} and then increased subsequently. The lysozyme activity of serum was elevated significantly in M_{50} and M_{75} groups compared to the control and M_{100}

groups (p<0.05; Fig. 1). Serum total Ig content was higher in fish exposed to

 M_{25} and M_{50} than control and M_{100} groups (p<0.05; Fig. 2).

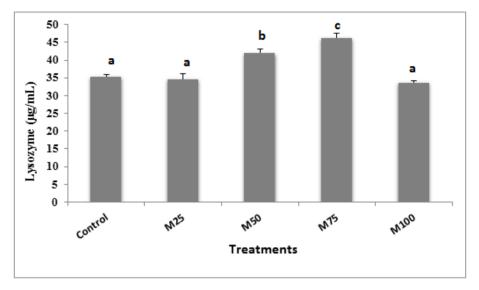


Figure 1: Lysozyme activity of juvenile Siberian sturgeon exposed to different concentrations of myrtle.

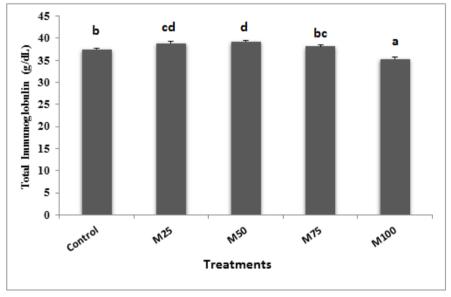


Figure 2: Total Ig of juvenile Siberian sturgeon exposed to different concentrations of myrtle.

Antioxidant enzymes

The antioxidant enzyme activities of serum are presented in Table 5. SOD and CAT activities of those fish exposed to M_{50} and M_{75} were significantly higher in

comparison to the control and M_{100} groups (p<0.05). The lowest GPX activity was observed in the control group (p<0.05).

Table 5: Antioxidant enzyme activities in serum of juvenile Siberian sturgeon exposed to different concentrations of myrtle.

	-		Treatments			
Indices	Control	M ₂₅	M_{50}	M ₇₅	M_{100}	
SOD (U/mL)	55.67±0.58b	52.13±1.73 ^b	63.02±3.12°	66.33±2.89°	40.67±1.53a	
GPX (U/mL)	324.67 ± 1.53^a	551.67 ± 5.69^{d}	404.33 ± 5.13^{b}	478.33±5.69°	621.33±3.51e	
CAT (U/mL)	63.33±1.53a	71.00 ± 2.00^{b}	80.33±0.58°	85.00 ± 1.00^{d}	74.33 ± 1.16^{b}	

Notes. M_{25} : 25% of MAC; M_{50} : 0% of MAC; M_{75} : 75% of MAC; M_{100} : 100% of MAC. Data are presented as mean±SD. The presence of different superscript letters denotes significant variation between treatments (p<0.05).

Abbreviations: SOD, Superoxide dismutase; GPX, glutathione peroxidase; CAT; Catalase.

Gill and liver histopathology

After 4th day of exposure, the histopathological variation in gill and liver are shown in Tables 6 and 7. The severity of changes in the

histopathological attributes were specified as lack of tissue lesion (-), mild (+), moderate (++), and severe (+++).

Table 6: Semi-quantitative scouring of histopathology in the gill of Siberian sturgeon after 96 h exposure to different levels of myrtle extract.

Apoptosis	Control	M ₂₅	M ₅₀	M ₇₅	M ₁₀₀
Edema in the lamella epithelium	+++	++	+	+	++
Detachment of lamella epithelium	-	+	-	-	+
Adhesion of the lamellae	+	+++	+++	+++	++
Curling of lamella	+	+++	+++	++	+++
Dilation of filamentous capillaries	+++	-	-	-	++
Telangiectasia	-	-	-	-	+
Hemorrhages	-	-	-	++	+
Necrosis of the epithelial cell	-	-	-	++	-
Epithelial hypertrophy	-	+++	+++	+	+++
Hyperplasia	-	-	-	-	++

Score: Lack of alteration (-), Mild alteration (+), moderate alteration (++), severe alteration (+++).

Table 7: Semi-quantitative scouring of histopathology in the liver of Siberian sturgeon after 96 h exposure to different levels of myrtle extract

Apoptosis	Control	M_{25}	M_{50}	M ₇₅	M_{100}
Atrophy	-	++	++	+++	+++
Nuclear pyknosis	+++	+	++	++	++
Necrosis	+++	-	++	++	++
Hyperemia in sinusoids	-	-	-	-	+
Accumulation of	-	-	-	-	-
melanomacrophages					

Score: Lack of alteration (-), Mild alteration (+), moderate alteration (++), severe alteration (+++).

The severity of lamella edema and filamentous dilation were observed in the control group compared to the other groups (Fig. 3). Juvenile fish exposed to the M_{25} and M_{50} treatments had severe

adhesion and curling of lamella as well as epithelial hypertrophy. Overall, most of the number of gill changes was observed in fish exposed to the M_{100} group.

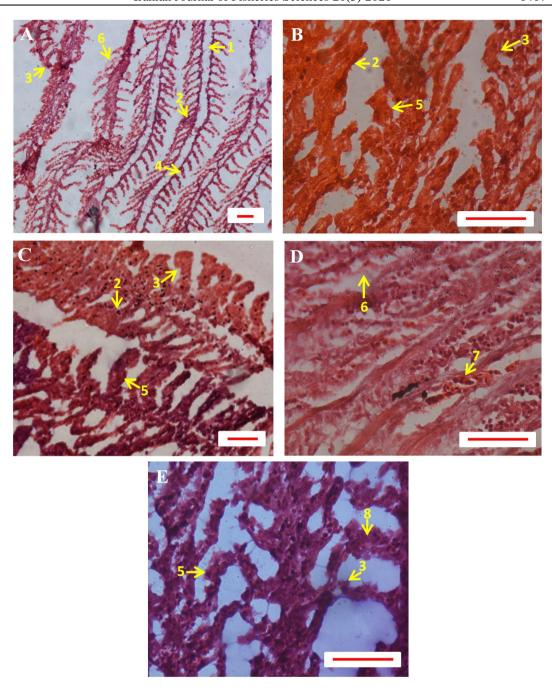


Figure 3: Longitudinal pathological section of the gill in juvenile Siberian sturgeon using H&E staining. A (control), B (M_{25}), C (M_{50}), D (M_{75}), E (M_{100}).1, dilation of filamentous capillaries; 2, adhesion of the lamellae; 3; curling of lamella; 4, edema in the lamella epithelium; 5, epithelial hypertrophy; 6, necrosis of the epithelial cell; 7, hemorrhages; 8, hyperplasia. Scale bar= 50 μ m.

In the control group, severe hepatocyte destruction was accompanied by nucleus pyknosis (Table 7). The severity of atrophy was observed in M_{75} and M_{100} treatments (Fig. 4). Nevertheless,

hyperemia in sinusoids was not an apparent discrepancy between treatments. Accumulation of melanomacrophages was not found in any treatments.

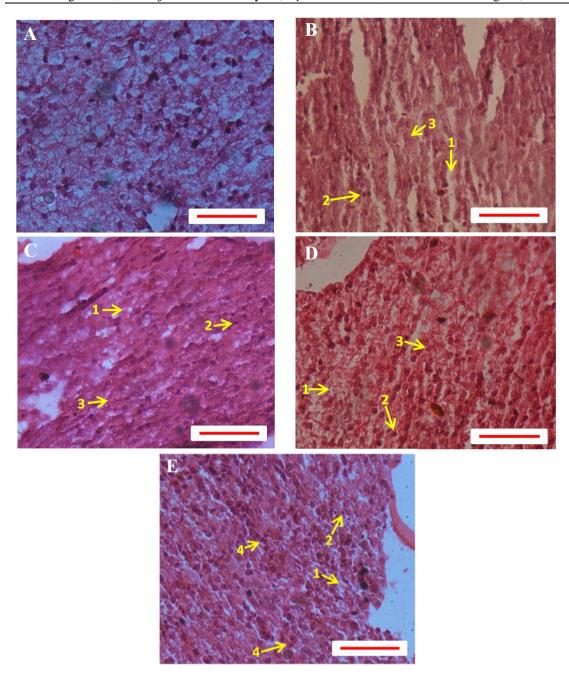


Figure 4: Longitudinal pathological section of the liver of juvenile Siberian sturgeon using H&E staining. A (control), B (M_{25}), C (M_{50}), D (M_{75}), E (M_{100}).1, necrosis; 2, nuclear pyknosis; 3, atrophy; 4, hyperemia in sinusoids. Scale bar= 50 μ m.

Discussion

In the current study, increase in Hb and RBC were observed in fish exposed to the myrtle extract M₇₅ (202 mg/L). In line with this, Goda (2008) stated that dietary immunostimulant (ginseng herb)

levels in a dose-dependent manner lead to increase hematological indices such as RBC, Hb, and Hct in Nile tilapia (*Oreochromis niloticus*) fingerlings. The author believed that ginseng derivative enhances the hematological function in

Nile tilapia. Moreover, Moghaddam et al. (2017) demonstrated that Siberian sturgeon fed supplemental Aloe vera extract had a significantly higher amount of hemoglobin than the control group. In contrast, the WBCs value in the mentioned above studies was higher compare with the control group, while in the present study, this trend in the eosinophils and neutrophils were lower up to 50% of the MAC value. Inconsistency results may be related to the routes of administration of extracts, species-specific, and fish age (Van Hai, 2015). The higher monocyte in the bloodstream is a crucial indicator of fish health (de Moraes et al., 2018). In the present study, monocyte was elevated in higher myrtle extract groups. This can be ascribed to an increase fish immunocompetence in the exposed groups. In our finding, although the results revealed that lymphocytes did not variations, manifest any however, decreased with rising exposure to 202 mg/L (M₇₅) of myrtle extract in juvenile Siberian sturgeon. Dadras et al. (2016) found that dietary administration of rosehip and safflower ended strengthening the nonspecific defense system to pathogens and bacteria in beluga (Huso huso).

It is demonstrated that, an increase in the biomolecules, such as total protein and albumin value, triggers the fortified immune response of fishes, stress level, and physiology welfare of fish (Damusaru *et al.*, 2019; Mukherjee *et al.*, 2019). Our finding revealed that the levels of albumin and total protein were

fortified in M₂₅ and M₅₀ groups. In accordance with our result. Roychowdhury et al (2020) postulated that failures in liver function might lead to lower serum protein and albumin. Further, Harikrishnan et al. (2009) advocated that dip treatment with triherbal solvent extract from Azadirachta indica, Ocimum sanctum, and Curcuma longa aqueous leaf extract displayed a notable boost in serum total protein in goldfish, Carassius auratus.

Glucose and cortisol in blood serum are indices that are widely used as stress responses (Naderi, et al., 2019). Cortisol level was increased in fish exposed to more than 134.8 mg/L (M₅₀) of myrtle extract, but these differences had disappeared to this level of myrtle extract. This finding demonstrated a higher inclusion of extract or more prolonged exposure lead to higher levels of cortisol in the bloodstream, thereby inflammatory low immunity and responses and indicating that the fish were under stress.

Serum hepatic enzymes are very sensitive indices applied in diagnosing hepatic impairment because they are cytoplasmic enzymes and are delivered into the bloodstream after cellular damage (Sadeghi *et al.*, 2013). Sadeghi *et al.* (2013) stated that myrtle oil extract added to diets containing aflatoxin of broiler chicks could decrease ALT, AST, and ALP activities. Likewise, Sen *et al.* (2016) declared that hepatic damage in the rats was markedly ameliorated by feeding with dietary myrtle extracts. In corroborated with our

result, except for AST, the lowest amount of liver enzymes was observed in M₅₀ treatment. Similar results were reported that the lower levels of hepatic enzymes might be due to the medicinal herbs' hepatoprotective influence (Dadras et al., 2016). Our finding revealed that LDH has a decreasing trend in M25, M50, and M75 groups enzymes following the mentioned above.

It has been illustrated that sturgeons have superior levels of lysozyme activities (Banavreh et al., 2019b). Lysozyme is a main first-line host fish defense agent that is responsible for destroying pathogens and is commonly measured as an important sign of innate immune function in fish (Verlhac et al., 1998; Dadras et al., 2016). Lysozyme hydrolysis of β (1-4) glycosidic bonds in the peptidoglycan of bacterial cell walls (Mirghaed et al., 2019). It is well documented that herbs bioactive compounds, like polyphenols flavonoids, could be elevated lysozyme and immunity reinforcement in fish (Hwang et al., 2013; Van Hai, 2015; Soltani et al., 2018; Banavreh et al., 2019b). By far, little information is available regarding exposure of fish to herbal extracts. The present result revealed that juvenile sturgeons exposed to myrtle extract significantly affected lysozyme activity in M₅₀ and M₇₅ groups, while Mansouri Taee et al. (2017) suggested that rainbow trout fed with myrtle supplemented diet failed influenced on skin mouse lysozyme activity. The conflicting result may be related to the mode of administration, species, and fish exposure duration. In addition, many authors announced that phytoimmunostimulant could improved Ig (Dadras *et al.*, 2016; Hoseinifar *et al.*, 2016). In this vein, serum Ig of juvenile Siberian sturgeon increased in M₂₅ and M₅₀ groups; this can be attributed to the slight increase, but not significant changes of lymphocyte percentage.

CAT, SOD, and GPX activities usually imply an improved antioxidative defense system in fish and the initial step of the enzymatic antioxidative defense system that removes the reactive oxygen species preserve tissues oxidative damage (Liu et al., 2019). In rabbits, Sepici-Dincel et al. (2007) found an ameliorating effect of myrtle on the SOD and CAT activities in the liver. Similarly, Safari et al. (2017) reported that Zebrafish (Danio rerio) fed with myrtle powder led to positive effects of antioxidant enzymes gene expression. In our study, antioxidants activities were higher in fish exposed to the M₅₀ and M₇₅ groups, implying the occurrence of a compensatory response to protect against stress-induced impairment.

In piscine, the gill is a multifunctional organ that performs a crucial role in physiological functions likes ammonium excretion, acid-base regulation, and ion transportation. Accordingly, the modifications recognized in gill histomorphology may trigger disorder of the gill's physiological function; thereby, fish is threatened (Chupani *et al.*, 2016). Unfortunately, no data have been

reported on the effects of myrtle on histomorphological parameters of animals. Our findings showed that Siberian sturgeon exposed to myrtle in <202 mg/L does did not appear disruption of the physiochemical function of the gill, but more than this range could lead to disorder function. However, the paucity of data makes it hard to have definitive conclusions, and more investigation is required.

It is demonstrated that liver impairment can influence health status. Our research findings pointed out that there was no significant alteration in the liver in M_{25} and M_{50} groups in the course of exposure.

The results of current study indicated that the concentration of <202 mg/L could be proposed as an adjuvant immunostimulant in juvenile Siberian sturgeon. Thus, it is possible to increase the resistance of fish to diseases and reduce the use of drugs in aquaculture. More researches are needed to assess the potentials effects of physiological and osmoregulation indices in this spices.

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