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Acute toxicity and hematological indices and biochemical parameters of giant sturgeon, *Huso huso* after acute exposure to crude oil

Khoshbavar Rostam H.A.[1*]; Yelghi S.[1]

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Abstract

This study was conducted to assess the effect of crude oil on some immunophysiological variables of *Huso huso* weighing 8.5±1g at 22°C. The 96h-LC_{50} value was first measured under static conditions and hematological and biochemical parameters were then assessed in treated fish exposed to 96h-LC_{50}. The LC_{50}- 96h of 17.4 mg L^{-1} was obtained. The leucocyte and erythrocyte counts were declined in the treatments compared to the control, while the mean corpuscular volume and hematocrit were significantly higher than those in the fish exposed to crude oil LC_{50} concentration. An increase in serum glucose was seen in sera samples of the treatments while levels of alanine aminotransferase, alkaline phosphatase and lactate dehydrogenase were lower than those the control. The alterations of the hemato-biochemical parameters can be used as suitable biomarkers in monitoring of crude oil pollution in the aquatic environment and to protect aquatic organisms such as great sturgeon.

Keywords: Beluga, Biochemistry, Crude oil, Hematology, LC_{50}, Pollution.

1-Inland Water Aquatic Stocks Research Center, Iranian Fisheries Science Research Institute (IFSRI), Agricultural Research, Education and Extension Organization (AREEO), Gorgan. Iran.
*Corresponding author's Email: hkr_rostami@yahoo.com

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Introduction

In recent years, oil pollution has become a global environmental issue in oceanic and marine ecosystems and also inland aquatic breeding ecosystems which are threatened greatly. Polycyclic aromatic hydrocarbons (PAHs) are a group of ubiquitous environmental persistent organic pollutants (POPs) with two or more aromatic rings that are usually present in the air, water and aquatic systems, soils and sediments (Neff, 1979). Due to PAHs mutagenic and carcinogenic activity (Tam *et al*., 2001; Harris *et al*., 2011), they constitute a major concern for public and environmental health and proper identification of their source is crucial for aquaculture water quality management (Yunker and Macdonald, 2003; Hu *et al*., 2010).

PAHs can enter the body via many exposure routes including inhalation and ingestion. Exposure to PAHs can also occur by skin contact. Once PAHs enter the body of a living organism, they are metabolized in a number of organs including liver, kidney and gills, excreted in bile, urine or breast milk and stored to a limited degree in adipose tissue. They are metabolized to form highly reactive molecules such as diol epoxides that are PAH intermediate metabolites (Trabelsi and Driss, 2005; Arias*et al*., 2009; Zhang*et al*., 2011). The lipophilic properties of PAHs enable them to actively penetrate cell membranes. Subsequent metabolism makes them more water-soluble that is easier for body to remove. However, PAHs can also be converted to more toxic or carcinogenic metabolites (Munoz and Albores, 2011).

The Caspian Sea basin holds large quantities of both oil and natural gas that could help meet the increasing global demand for energy resources and gain the world's attention of the international oil and gas industry (Effimoff, 2000). The sea is bordered by five states of Azerbaijan, Kazakhstan, Turkmenistan, Iran and Russia. Offshore oil production and land-based sources, notably the Volga River, are responsible as the main sources of pollution (Karpinsky, 1992). High variability in the total PAHs (6-2988 ng g^{-1} dry weight) has been reported in surface sediment samples collected from the coastal zone of the countries bordering the Caspian Sea (Tolosa *et al*., 2004). Also, previous studies reported a detectable contamination of PAHs in different parts of Iranian Coast of Caspian Sea (Pak and Farajzadeh, 2007; Habibi and Hadjmohammadi, 2008). Under such contamination conditions, some commercially valuable fish species such as sturgeon species are under danger (Moghim *et al*., 2006). Some sturgeon characters such as high lipid content in body, long living period, long juvenile stage and benthivorous diet behavior make them to be quite a potent target for exposing to PAHs (Jaric *et al*., 2011). Also, as these fish are opportunistic bottom feeders, they are more frequently in contact with sediments, that most probably contain PAHs pollutant (Billard and Lecointre, 2001; Kajiwara *et al*., 2003; Hosseini *et*

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al., 2008). Therefore, these valuable fish species may be at a high potential risk.

Hedayati *et al*. (2012) did an acute toxicological test to determine LC_{50} of crude oil in some main cultured cyprinidae species. Common carp (*Cyprinus carpio*) has higher LC_{50} of crude oil than silver carp (*Hypophthalmicthys molitrix*) and Caspian roach. Jahanbakhshi *et al*. (2012) in a similar research determined LC_{50} of crude oil in beluga, *Huso huso* (24.80+0.23 ppm) using probit analysis. Direct infusion of water fraction of crude oil causes a progressive variations in hematological (Jahanbakhshi and Hedayati, 2013) and biochemical (Jahanbakhshi and Hedayati, 2013) parameters in great sturgeon. However, little research has been done on the effects of crude oil and its products on fish (Di Giulio and Hinton, 2008), considering the growing cases of environmental and health risk of crude oil for biota in recent decades (Kajiwara *et al*., 2003), the aim of this study was to assess some hematological and biochemical parameters of giant sturgeon after exposing to crude oil.

Material and methods

LC_{50} detection

The experiment was conducted on juvenile giant sturgeon (N=210) weighing 8.5±1 g obtained from Rajaei fish farm in Mazandaran Province, Iran. Fish were acclimated to laboratory conditions for two weeks in 160-L fiberglass with dechlorinated tap water. During acclimatization, fish were fed with commercial feed twice a day. Water temperature, dissolved oxygen, pH and total hardness were 22±1°C, 8.2±0.8 mg L^{-1}, 7.5±0.1 and 145±5 mg L^{-1}, respectively. Also, ammonia <0.02 mg L^{-1}, nitrite <0.1mg L^{-1} and phosphate <0.285 mg L^{-1} were measured frequently. Fish were maintained under natural photoperiod (L/D=14/10). Groups of 10 fish were exposed to 12.5, 15, 20, 25, 30, 50 mg L^{-1} of crude oil.

To detect LC_{50} fish (9 fish per concentration) were then subjected to different crude oil-tested concentrations of 16, 17, 18, 19 and 20 mg L^{-1} crude oil under static conditions (test medium was not renewed during the assay and no food was provided for animals). Three replicates were carried out for each concentration (Hotos and Vlahos, 1998). Dead fish were immediately removed with special plastic forceps to avoid possible deterioration of the water quality (Gooley *et al*., 2000). Values of mortalities were measured at time 24, 48, 72 and 96 h (Hedayati *et al*., 2010). The LC_{50} values were calculated from the data obtained in acute toxicity bioassays by SPSS computer statistical software.

Hematological and biochemical analysis

Blood samples were taken from the caudal vein of the surviving fish in LC_{50} toxicity tests and transferred to heparinized and non-heparinized tubes (Hedayati and Safahieh, 2011). The blood indices were performed using heparinized blood samples. Leukocyte

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population size (WBC) and erythrocytes (RBC) were counted by diluting heparinized blood with Giemsa stain at 1:30 dilution and cells were counted using a hemocytometer Neubauer cell counting under light microscope (Stevens, 1997). The leukocyte differential count was made in blood smears stained by Merck Giemsa (Beutler *et al.*, 2001). Hematocrit values (PCV), hemoglobin (Hb) were determined according to methods described Goldenfarb *et al*. (1971) and Lee *et al*. (1998). Erythrocytes indices including mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) were calculated from RBC, PCV, and Hb values (Lee *et al*., 1998).

The levels of glucose, total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) in sera samples were carried out by Pars Azmoon, Iran Diagnostics kits using a Technicon Ra1000 auto-analyzer (Technicon Ltd, USA).

Statistical analysis

For each index, the data were evaluated for normality and homogeneity of variances. T-test was used to determine significant differences to evaluate the effect of crude oil on the parameters. The differences between means were analyzed at 5 % probability level. Data are reported as means ± standard deviation. The software SPSS, version 17 (SPSS, Chicago, Illinois state) was used as described by Dytham (1999).

Results

In this study all control groups resulted in mortalities less than 5%, which indicated the acceptability of the experiments procedure and conditions provided for the performance of the test. The mortality of fish exposed to different concentrations of crude oil is shown in Table 1. By increasing in oil concentration, a significant increase was observed in mortality. Considering the crude oil bioassay, LC_5 and LC_{50} of 24, 48, 72 and 96 h were 20.1, 17.8, 15.8 and 15.7 ppm and 22, 19.7, 18.2 and 17.4 mg L^{-1}, respectively.

Fish exposed to crude oil for 96 h showed a significant change in the PCV, Hb, RBC, WBC, MCV, and MCHC levels ($p \leq 0.01$; $p \leq 0.05$) (Table 2). The level of PCV and MCV increased while Hb, RBC, WBC and MCHC values decreased significantly after exposing fish to crude oil.

Differential leucocyte counting showed that lymphocyte and eosinophil cells percent decreased in fish exposed to LC_{50} (96 h) of crude oil compared to the control group ($p<0.01$). In contrast, the neutrophil ($p \leq 0.01$) and monocyte ($p \leq 0.05$) numbers in the treatment groups were significantly higher than those of the control (Table 2).

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Table 1: Mortality of great sturgeon after acute exposure to crude oil (n=10, each concentration)

Concentration (ppm)	Mortality (%)			
	24 h	48 h	72 h	96 h
Control	0	0	0	0
16	0	0	7	20
17	0	0	7	27
18	0	7	40	53
19	3	7	53	67
20	7	71	90	100

Table 2: Hematological parameters of great sturgeon after exposing to crude oil (N=10, concentration=17.4 ppm)

Parameter	Control	Treatment	T value	P value
PCV (%)	13.3±1.6	18.8±2.4	-3.338	0.029^{*}
Hb (g dL^{-1})	4±0.4	2.8±0.5	3.246	0.031^{*}
RBC $\times 10^{4}$ (mm^{-3})	68.7±7.8	46.3±3.6	4.506	0.010^{**}
WBC $\times 10^{3}$ (mm^{-3})	50.3±6.464	27.9±5.404	4.508	0.010^{**}
MCV (fl)	197.2±41.2	405.7±57.7	-5.094	0.007^{**}
MCH (Pg)	59.4±8.7	61.8±12.4	-0.274	0.797^{ns}
MCHC (%)	30.7±4.5	15.4±3.3	4.749	0.009^{**}
Lymphocyte (%)	33.2±2.4	11.3±3.6	8.767	0.001^{**}
Neutrophil (%)	32.5±3.9	71.1±4.6	-11.086	0.000^{**}
Eosinophil (%)	33.1±3.1	15±5.3	5.106	0.007^{**}
Monocyte (%)	1.2±0.4	2.6±0.8	-2.611	0.05^{*}

Data presented as mean ± standard deviation. $^{**}p \leq 0.01$, $^{*}p \leq 0.05$.

Total protein, ALP, ALT and LDH levels showed a significantly lower level in fish exposed to LC_{50} 96 h than those of the control ($p \leq 0.01$; $p \leq 0.05$), whereas serum glucose was significantly higher in the control group ($p \leq 0.05$) (Table 3). AST enzyme activity did not show any difference between control and test groups.

Table 3: Biochemical parameters of great sturgeon after exposing to crude oil (N=10, concentration=17.4 ppm)

Parameter	Control	Treatment	T value	*p* value
Total protein (g dL^{-1})	3.5±0.7	1.1±0.4	5.156	0.007^{**}
Serum glucose (g dL^{-1})	33.3±3.2	60±11	-4.037	0.016^{*}
ALP (IU L^{-1})	285.3±11.6	237.7±17.7	3.896	0.018^{*}
ALT(IU L^{-1})	21.1±5.7	10.7±2.6	2.875	0.045^{*}
AST (IU L^{-1})	22.6±5	21.6±7.8	0.187	0.861^{ns}
LDH (IU L^{-1})	341.9±19.9	237±19.5	6.521	0.003^{**}

Data presented as mean ± standard deviation. $^{**}p \leq 0.01$, $^{*}p \leq 0.05$.

Discussion

Biochemical and physiological parameters appraisal is a commonly used diagnostic tool in aquatic toxicology and biomonitoring (McDonald and Milligan, 1992; Folmar, 1993; Soimasue *et al*., 1995; Kang *et al*., 1999; Kang *et al*., 2003). In addition, various hematological indices have been shown to be proper and sensitive indicators of changes in ecobiological condition (Payne *et al*.,

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1978; Sancho *et al*., 2000). Although it is known that some PAHs are immunotoxic and have adverse effects on immunophysiological function, there is little research related to hematological responses in fish exposed oil pollutants (Sancho *et al*., 2000).

The 96-h LC_{50} tests are performed to assess the vulnerability and survival potential of organisms to particular toxic component such as oil pollution. The relationship between the degrees of response of test organisms and the quantity of exposure to chemical almost always assumes a concentration of a chemical (Di Giulio and Hinton, 2008). LC_{50} value obtained in present study was 17.4 ppm. The corresponding values of crude oil published in the literature for other species of fish were 22.4±0.03 ppm for *Cyprinus carpio*, 16.7±0.06 ppm for *Hypophthalmicthys molitrix*, 20.4±0.7 ppm for *Rutilus rutilus caspicus* (Hedayati *et al*., 2012), 24.80 ppm for *Huso huso* (200 g) (Jahanbakhshi *et al*., 2012); and for water soluble fraction of crude oil it was 34.87 ppm for *Huso huso* (Khodabash *et al*., 2014), 37.5 ppm for *Mugil Liza* (Moreira *et al*., 2014) and 33.95 ppm for *Rutilus kutum* (Sharifpour *et al*., 2011). The different levels of acute toxicity of a chemical compound depend on the size, age and experimental conditions (Hedayati and Safahieh, 2011). The LC_{50}-96h obtained in this study shows that great sturgeon has mainly a lower susceptibility to crude oil than other tested species. However our results are similar to Jahanbakhshi *et al*. (2012) who reported the lower values of LC_{50} for beluga (*Huso huso*) when compared with most species showing sensitivity of beluga to low diesel oil doses.

Our results declared increase in the MCV, and PCV, and decrease of the RBC, WBC, Hb and MCHC after acute exposure (p<0.01). Jee *et al*. (2004) reported similar hematological responses in RBC, PCV, MCHC and Hb of olive flounder (*Paralichthys olivaceus*) exposed to single PAH, phenanthrene. A progressive decrease in RBC, WBC counts and Hb level resulted in subsequent physiological stress. Our data provide evidence that crude oil can affect RBC hemolysis because the level of RBC was decreased compared to control fish. Also erythrocyte indices including MCH and MCV showed that the crude oil stimulates erythropoiesis in fish after acute exposure. These parameters reflect increased production and release of reticulocytes that are larger in size and have decreased hemoglobin content compared to mature RBCs (Haschek *et al*., 2010).

Based on the results of leukocyte counts, the occurrence of neutrophilia and lymphopenia in fish exposed to acute dosage of crude oil warranted further studies. Hlavek and Bulkley (1980) found a transient neutrophilia in rainbow trout 24h after treatment with malachite green, but this decreased after 4 days. Darwish *et al*. (2001) also found an increase of neutrophil counts in channel catfish exposed to high doses of potassium permanganate.

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Normally, after exposure to a stressor agent, a significant increase in glycaemia occurs (Jahanbakhshi *et al*., 2013). In the present study, the animals showed a hyperglycemic response after 96-h exposure to crude oil, indicating the provision of energy reserves for immediate utilization (Oliveira *et al*., 2011). Similarly, Alkindi *et al*. (1996) observed a significantly elevated plasma glucose concentration in flounder after 3-h exposure to the water-soluble fraction of crude oil with an increase above 50 % after 48 h. Our results are in accordance with Hoseini and Tarkhani (2013) who stated an increase in glucose and cortisol levels in gold fish, *Carassius auratus* exposed to short term treatment with potassium permanganate.

AST, ALT, ALP and LDH are the enzymes that have been applied for evaluating hepatocellular damage (Gad, 2007). Results showed crude oil inhibited these enzymes activities. Gabriel *et al*. (2012) confirms metabolic enzymes activities (AST, ALT, ALP and LDH) in gill, muscle, kidney, liver and plasma of *Clarias gariepinus* were inhibited by different concentrations of cypermethrin. The lower values of AST, ALT and ALP enzyme activities when compared to the controls showed that inactive transamination and oxidative deamination occurred. Our results showed crude oil-induced changes in the activities of AST, ALT, ALP and LDH in liver tissue of fish. These alterations in hepatic enzymes are biomarkers of hepatic injury and indicated severe hepatic damage caused by crude oil.

In conclusion, the present results showed that exposing great sturgeon to crude oil at acute toxic level can adversely affect some fish hemato-serological features including immunocompetent cells and some serum enzymes. Such negative effects make fish become suppressed followed by an increase in fish susceptibility to secondary infections. Therefore, such hemato-serological indices caused by crude oil in sturgeon can provide useful data if fish are exposed to crude oil pollution in water bodies.

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