Some hematological and biochemical changes in blood serum of Beluga (*Huso huso*) after chronic exposure to diazinon

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Abstract: Hematological and biochemical changes were studied in beluga (*Huso huso*) weighing 450±50g in the presence of β,1-3, D-glucan as a single intraperitoneal injection (0.3mg/kg body weight) once, and thereafter a chronic exposure to organophosphate, diazinon at concentration of 1.5 mg/L as a continuous bath for 63 days at 22±1°C. Results of erythrocyte profile in fish treated with glucan, but without diazinon, generally showed significant higher values of RBC ($P<0.05$) compare to fish treated with glucan plus diazinon bath, while there was no significant difference ($P>0.05$) in the values of HCT, Hb, MCH and MCHC between the two groups. Also, fish treated with glucan, but without diazinon bath, showed significant higher values of RBC than that untreated fish up to 3 weeks post-treatment ($P<0.05$), while no significant differences was found in other erythrocytic indices between these two groups ($P>0.05$). Furthermore, fish treated with diazinon showed higher levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) than other three groups for 14-28 days post-treatment and then they decreased to the lower level for the rest of experiment. Also, fish treated with glucan-diazinon showed insignificant higher levels of these enzymes up to 21 days post-exposure compare to fish treated with glucan but without diazinon bath ($P>0.05$). However, such levels reduced to a lower level in this group for the rest of experiment ($P>0.05$). Also, fish treated with glucan-diazinon showed generally lower and higher levels of total protein and glucose concentrations in blood plasma, respectively compared to fish treated with glucan but without diazinon bath ($P<0.05$).

Keywords: *Huso huso*, diazinon, Glucan, Hematology, Biochemistry

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Introduction

Fish hematopoietic organ has been known to be seriously affected by the harmful environmental toxicants. For instance, alterations in hematocrit and leukocrit, MCH, and MCV due to the presence of chemicals have been noted by a number of workers (e.g. Gill *et al.*, 1990, Benarji & Rajendranath, 1990; Khattak & Hafeez, 1996; Sancho *et al.*, 1997; Tavares *et al.*, 1999; Luskova *et al.*, 2002,). Because many of the contaminants affecting protective immune systems in the environment are nonlethal, the problem is to show if a chronic exposure to toxicants makes fish more susceptible to diseases. (Anderson & Zeeman, 1996).

Fish blood parameters are suitable biomarkers for evaluating the potential risk of chemicals (Roche & Boge, 1996). Since chemical intoxication may be considered as a potential source of stress thus, plasma hormones, metabolic substrates, blood cell volumes and enzyme activities are among the first elements to be measured (Adams *et al.*, 1989; Panduranga-Rao *et al.*, 1990). Particularly, demonstration of some stress indicators such as plasma cortisol, glucose, blood hemoglobin (Hb), packed cell volume (PVC), mean corpuscular hemoglobin concentration (MCHC), total plasma proteins and alkaline phosphatase (ALP) activity are important (Roche & Boge ,2000). Also, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) are enzymes frequently used in the diagnosis of damages caused by pollutants in various tissues such as liver, muscle, and gills (De La Torre *et al.*, 1999, 2000).

During the past forty years, especially in the last decade, the level of contamination in the Caspian Sea has increased, subsequently the anthropogenic pressures on the coastal and marine ecosystems have grown progressively. The main sources of pollution of the sea are land-based and offshore sources (Murty, 1988). Diazinon in amount of >10000 tonnes is one of the major herbicidal/ insecticidal chemicals currently used mostly in the northern part of Iran each year (Khoshbavar-Rostami *et al.*, 2004). This chemical substance is also currently being used in aquaculture industry for prevention and treatment of some parasitic crustaceans such as *Lerna* (Hoole *et al*, 2001). This organophosphate compound enters to the sea through the both land-based and offshore sources. In previous study by Khoshbavar-Rostami *et al.* (2004) the acute toxicity of diazinon in this species was carried out indicating a remarkable adverse effect on some fish hematogical parameters. The objective of this study was to determine the effect of long-term (63 days) exposure of sublethal concentration of diazinon on some hematological and biochemical variables of giant sturgeon. This was done because the greatest risks due to long-term exposures to small doses of such pollutant chemical can be resulted in a decline in the population, retardation of reproduction in second generation, damage to life cycle and enhancing of fish susceptibility to infectious diseases.

Materials and methods

Fish

Two hundreds fish weighting 450±50g supplied by Rajai fish farm in Mazandran province were used for the experiments. Fish were held in 2000 L tanks containing 25 fish each tank at 22±1°C, dissolved oxygen of 7.5-9mg/L and pH 7.5. Other water quality parameters including ammonia, nitrite and total hardness consisted of <0.02mg/L, <0.1mg/L and 145 mg/L, respectively. Fish were acclimated to new conditions a few weeks prior to the experiments. Fish were fed with a commercial trout pellet (Chineh Company, Karaj, Iran) with ingredients: protein 36%, lipid 14%, ash 11%, fiber 3.5%, phosphorus 1%, wet 11%, carbohydrate 22.5% and fish meal 50%.

Application of glucan and diazinon

Two groups of fish (each group 50 fish) were intraperitonealy injected with β 1-3 D-glucan (Sigma) at 0.3 mg/kg body weight once. Two weeks later, diazinon was applied to the water of one glucan injected group (n=50 fish) at sublethal concentration of 1.5mg/L at 22±1°C for nine continuous weeks. Fifty untreated glucan fish were also exposed to the toxicant at the same concentration for the same duration. The second glucan received group and also the second glucan untreated group were kept in clean water separately.

Sample collection and assays

Samples were collected from all groups once a week for 9 weeks post-exposure to the toxicant. Five fish per treatment were used at each sampling time.

Hematology

Blood (about 3ml) was collected by caudal puncture. Samples were taken from the tail vein with a heparinized and nonheparinized syringe after anesthetizing fish with clove oil at 100mg/L. The haematological parameters consisting of erythrocyte count (RBC), hematocrit (PCV), haemoglobin (Hb), mean corpuscular haemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were measured using the procedure described by Klontz (1994).

Plasma biochemistry

The non-heparinized blood samples were centrifuged for 15 minutes at 400×g and separated sera were used to determine the levels of: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), glucose and total protein spectophotometrically using an automatic biochemistry analyzer (Eppendorf, Epos 5060) and optimized tests of Boehring Manaheim GmBH.

Statistical analysis

Data were statistically analyzed by ANOVA analysis. Data were considered as significant at $P<0.05$.

Results

Haematology

Fish exposed to a constant sublethal exposure of diazinon (group D) had generally significantly lower values of RBC, Hct, Hb, MCV and MCH ($P<0.05$) than fish without any treatment (group A) (Table 1). Also, comparing the erythrocyte profile of glucan injected fish without diazinon bath (group B) and glucan injected fish exposed to diazinon bath (group C) resulted in no significant difference (P>0.05) in the values of HCT, Hb, MCH and MCHC between these two groups. However, value of RBC in group B was significantly higher than group C (P<0.05). In addition, comparing such values between group A and B showed that there was no significant difference (P>0.05) between these two groups except for RBC values that was significantly higher (P<0.05) in group B than group A up to 3 weeks post-treatment (Table 1).

Table 1: Erythrocyte profile of *Huso huso* following exposure to a constant sublethal concentration of diazinon at 1.5mg/L at 22°C. (Mean ±SD). A= Fish without diazinon bath, B=Glucan injected fish without diazinon bath, C= Glucan injected fish with diazinon bath, D= Fish with diazinon bath.

	Time post-sampling (day)									
Treatment	1	7	14	21	28	35	42	49	56	63
RBC ($x10^4$ cells/ml)										
A	100.8±3.7	101±6.3	101± 3.1	100.6±3.1	100.4±3.1	100.6±1.7	99.6±3.8	99.4±2.3	99±4.3	99.4±3.9
B	110.4±7^{ade}	108.4±7.1	106± 3.2	105.6±8.3	102.4±3.4	102.4±3.8	101.4±4.8	102.2±3.7	100.4±4.4	99.4±3.5
C	99.4±2.1	100.4±2.1	95.4±2.4	92.6±4.5	84.6±8.8	80.8±9.9	80± 9	80.2±12.1	79.2±8.8	79.4±8.4
D	98.2±6.5	91± 4.6^{abcde}	82±2.2^{abcdef}	74.8±3.1^{bcdef}	72.4±1.8^{bcdef}	69.4±3.2^{cdef}	67.2±3.2^{bcdef}	69.4±2.1^{bdef}	64.8±2.6^{cdef}	66.8±1.8^{bcdef}
PCV(%)										
A	22.6±1.1	22± 1.6	21.8±1.2	21.8±0.8	21±1.9	21.2±1.6	21.4±1.6	21.5±1.6	21.2±1.6	20.9±1.1
B	23±1.2	22.4±2.4	22.4±2	22.4±0.6	21.8±1.3	21.6±0.4	22±1.5	20.8±0.6	20.9±0.9	21.6±0.6
C	22.3±0.8	22±1.2	21.2±1.1	20.6±0.6	19.6±0.6	19.1±1.2	19±1	18.7±1.1	19.3±1	19.6±1.1
D	21.5±1.1	20.2±0.6^{e}	19.3±1.1^{bce}	18.1±0.8^{bcde}	16.9±0.7^{bcde}	17.3±0.8^{bdef}	16.7±0.8^{bcdef}	17.9±0.7^{bdef}	17.4±1.5^{be}	17.8±1.7^{be}
Hb (g/dl)										
A	5.3±0.35	5.3±0.28	5.4±0.13	5.2±0.37	5.2±0.28	5.2±0.34	5.1±0.3	5.2±0.3	5.4±0.31	5.2±0.26
B	5.5±0.26	5.4±0.5	5.5±0.2	5.5±0.34	5.5±0.33	5.3±0.26	5.2±0.28	5.1±0.12	5.4±0.31	5.5±0.23
C	5.4±0.59	5.1±0.15	5±0.01	5±0.21	4.9±0.15	5±0.5	4.8±0.16	4.6±0.11	4.7±0.24	4.6±0.22
D	5.3±0.61	5±0.22^{e}	4.6±0.3^{bcde}	4.7±0.35^{e}	4.4±0.21^{bcde}	4.6±0.34^{e}	4.6±0.23^{be}	4.4±0.16^{bde}	4.3±0.13^{bde}	4.4±0.16^{bde}

Table 1 continued:

Treatment	Time post-sampling (day)									
	1	7	14	21	28	35	42	49	56	63
MCV (Fl)										
A	216.6±19.3	214.6±5.1 ^{bf}	212.6±11.0	213.0±16.8	213.6±9.8	213.2±8.8	214.0±6.3	211.8±6.5	214.6±6.7	214.0±30.6
B	222.2±6.1	223.8±13.0	220.4±4.0	220.0±12.4	220.0±12.4	220.6±16.2	220.4±12.6	220.2±8.9	221.6±12.7	219.2±21.0
C	222.0±8.4	222.2±8.6	224.0±13.1	222.8±10.7	221.8±10.1	223.8±9.1	222.0±9.5	223.4±9.8	218.8±9.3	218.2±21.0
D	227.0±35.3	228.2±5.0	229.8±8.7	228.4±7.2	228.2±12.6 ^{b}	227.6±9.6	227.6±6.5	222.8±2.6	222.4±5.3	221.8±7.0
MCH (pg/ml)										
A	58.8±2.4	58.8±2.5	58.4±5.5	59.0±9.8	59.8±9.4	58.4±5.9	58.8±9.9	58.8±3.5	56.8±1.8	57.6±2.7
B	61.6±5.2	60.8±3.0	60.6±2.1	60.0±4.2	61.2±4.3	59.6±4.8	57.8±3.4	59.2±1.6	58.2±2.6	59.8±1.6
C	61.4±4.3	60.6±1.8	60.4±5.1	57.6±1.9	54.4±3.9	55.0±6.4	53.6±6.9	54.0±2.2	53.6±2.4	54.2±2.4
D	59.0±1.6	57.4±1.1 ^{ae}	56.6±1.5	56.6±3.4	53.6±2.3 ^{e}	55.0±1.9	52.6±1.7	53.8±2.8 ^{bdef}	53.6±1.5 ^{de}	53.0±2.3 ^{bdef}
MCHC (%)										
A	25.8±1.9	25.8±2.1	25.9±1.5	26.0±1.6	25.6±1.7	25.3±1.2	25.5±2.8	25.5±1.4	25.5±1.9	25.4±1.1
B	26.4±1.1	26.4±1.8	26.3±2.3	26.3±0.7	26.0±1.2	25.5±1.5	25.0±1.3	24.8±2.2	24.9±1.4	24.8±2.6
C	25.4±1.1	25.6±1.7	24.6±1.1	24.8±2.9	24.4±2.5	24.7±2.3	23.9±3.7	24.1±1.4	23.6±4.0	23.1±2.7
D	25.4±1.5 ^{de}	24.8±1.5	25.4±2.9	25.2±1.6	24.6±2.4	23.8±3.0	23.8±1.6	23.5±2.7	23.2±3.0	23.0±3.2

a= Indicating values are significantly different between A and B.
b= Indicating values are significantly different between A and D.
c= Indicating values are significantly different between C and D.
d= Indicating values are significantly different between B and C.
e= Indicating values are significantly different between B and D.
f= Indicating values are significantly different between A and C.

Biochemistry

Fish exposed to diazinon (group D) showed a higher levels of ALP, ALT and LDH up to 28, 21 and 14 days post-exposure, respectively compare to other groups of A, B and C (Table 2), while the levels of these enzymes were lower in this group for the rest of experiment. Also, fish receiving glucan and diazinon bath (group C) showed insignificantly higher levels of these enzymes up to 21 days post-exposure compared to those fish receiving glucan but without diazinon bath (group B) ($P>0.05$). However, such levels reduced to a lower level for the rest of experiment ($P>0.05$).

No significant difference was observed in the level of total protein between exposed fish to the toxicant (group D) and unexposed group (group A) for two weeks ($P>0.05$), while it decreased significantly in exposed fish (group D) compared to untreated one (group A) for the rest of experiment ($P<0.05$). Also, fish receiving glucan and diazinon bath (group C) showed generally lower levels of total protein concentrations in blood plasma compared to fish receiving glucan without diazinon bath (group B) ($P<0.05$) (Table 2).

The level of glucose was generally significantly higher in fish exposed to the toxicant (group D) than fish receiving glucan and diazinon bath (group C) ($P<0.05$) while, level of total protein was generally significantly lower in exposed fish to diazinon (group D) ($P<0.05$). Also, fish receiving glucan and diazinon bath (group C) showed generally lower and higher levels of total protein and glucose concentrations in blood plasma compared to fish receiving glucan without diazinon bath (group B), respectively ($P<0.05$) (Table 2).

Table 2: The effect of constant sublethal concentration of diazinon at 1.5mg/mg/L on enzyme activities, glucose level and total protein concentrations of blood plasma of *Huso huso* at 22°C. (Mean± SD) A= Fish without diazinon bath, B=Glucan injected fish without diazinon bath, C= Glucan injected fish with diazinon bath, D= Fish with diazinon bath

Treatment	Time post-sampling (day)									
	1	7	14	21	28	35	42	49	56	63
ALP (u/l)										
A	1387.2±68.2	1491.8±161.3	1413.2±125.3	1451.0±87.3	1374.0±84.3	1449.6±126.0^{b}	1391.4±67.3	1413.4±52.2	1365.4±70.0	1442.6±181.8
B	1424.0±139.6	1378.8±117.3	1390.8±78.1	1354.6±132.7	1419.8±100.3	1397.6±120.9	1385.6±68.3	1390.6±162.8	1438.0±125.2	1369.6±133.6
C	1262.0±416.5	1476.8±106.6	1583.2±105.8	1558.0±87.1	1383.4±83.1	1292.0±149.4	1208.8±76.1	1161.0±140.8	1069.6±68.6	976.8±124.8
D	1483.8±30.0	1601.6±330.1^{e}	1609.8±12.4^{bdef}	1599.6±163.3^{de}	1511.0±154.5	1259.4±137.3	1139.2±215.7^{bdef}	904.4±114.9^{bcdef}	741.2±138.7^{bcdef}	538.0±134.7^{bcdef}
LDH (u/l)										
A	1879.2±203.9	1923.2±105.8	1886.4±169.0	1918.2±232.5	1868.8±176.7	1916.8±130.9	1870.4±158.3	1831.6±167.7	1889.2±78.2	1906.2±365.6
B	1935.0±121.2	1891.0±42.0	1874.0±272.7	1916.0±197.4	1897.4±97.7	1879.4±97.2	1925.8±54.9	1881.8±125.0	1988.2±162.0	1934.8±91.8
C	1952.2±146.0	1999.0±109.5^{d}	1990.4±128.5	1852.4±266.9	1774.4±189.1	1680.0±141.5	1659.4±128.9	1595.0±88.5	1550.6±176.3	1536.2±183.8
D	1970.8±61.8	1984.4±138.0	1938.4±143.7	1845.0±66.5	1751.2±212.8	1624.8±220.3^{bef}	1498.4±213.3^{bdef}	1456.6±217.8^{bdef}	1444.6±236.9^{bdef}	1453.2±334.0^{bdef}
ALT (u/l)										
A	209.8±11.0	212.8±14.5	210.0±19.1	208.8±20.6	206.4±13.1	209.2±18.5	210.8±15.2	207.8±10.2	209.6±17.1	204.4±23.2
B	209.4±24.4	206.0±16.3	210.2±10.5	207.8±25.5	208.8±17.1	208.8±18.3	210.6±19.6	205.8±16.1	208.6±17.4	205.8±19.9
C	210.8±15.7	216.8±13.5	206.8±27.1	202.6±12.5	200.4±17.1	200.0±15.1	195.8±13.1	187.2±11.5	186.0±14.3	186.6±11.7
D	212.6±13.1	216.6±11.7	200.8±19.8	195.8±13.3	191.0±15.4	191.8±15.7	187.8±9.7^{be}	185.8±15.5^{bdef}	185.0±6.0^{bdef}	186.8±16.5

Table 2 continued:

Treatment	Time post-sampling (day)									
	1	7	14	21	28	35	42	49	56	63
AST (u/l)										
A	5.2±1.3	5.2±1.3	5.0±2.0	5.2±1.3	5.6±1.1	5.2±1.8	5.2±0.8	5.2±1.3	5.2±1.3	5.4±1.8
B	5.2±1.3	5.8±1.3	5.2±0.8	5.4±1.1	5.8±0.8	6.0±1.0	5.6±1.1	5.6±1.5	5.6±1.7	5.8±1.3
C	5.8±0.8	6.2±1.6	5.6±1.8	4.8±1.3	5.2±1.3	4.6±1.3	4.4±1.7	4.6±1.5	4.4±1.8	4.2±0.8
D	5.4±1.5	5.8±1.3	5.0±1.6	4.6±1.3	4.4±1.1	4.0 ± 1.2^{e}	3.8 ± 1.5^{e}	4.0±1.2	3.8±1.3	4.0 ± 0.7^{e}
Total protein (g/dl)										
A	2.5±0.4	2.4±0.1	2.4±0.3	2.3±0.2	2.3±0.3	2.5±0.3	2.5±0.3	2.3±0.2	2.4±0.3	2.3±0.3
B	2.5±0.04	2.5±0.4	2.6±0.5	2.6±0.3	2.4±0.2	2.4±0.2	2.4±0.3	2.4±0.2	2.6±0.3	2.7±0.2
C	2.4±0.1	2.5±0.3	2.2±0.01	2.1±0.2	2.0±0.2	2.1±0.4	1.9±0.1	1.7±0.2	1.9±0.4	1.9±0.3
D	2.3±0.2	2.3±0.2	1.9 ± 0.2^{bde}	1.9 ± 0.3^{bde}	1.7 ± 0.2^{bdef}	1.8 ± 0.3^{be}	1.7 ± 0.2^{bdef}	1.6 ± 0.2^{bdef}	1.7 ± 0.2^{bde}	1.7 ± 0.1^{bdef}
Glucose (mg/dl)										
A	130.0±14.7	126.4±6.6	126.8±6.8	127.6±9.9	121.2±9.7	126.2±8.8	121.6±5.9	125.2±16.5	126.8±7.9	122.2±10.2
B	133.8±13.5	130.6±13.4	129.2±10.9	124.0±6.0	127.6±7.3	129.6±8.2	126.6±8.3	124.4±11.1	129.0±7.9	124.0±11.9
C	133.2±15.0	132.6±10.9	135.8±11.2	137.0±6.7	133.2±15.7	134.4±11.1	136.4±12.2	137.2±7.2	140.4±7.3	138.0±9.9
D	136.8±13.3	137.6±13.4	137.2±14.5	140.4 ± 8.4^{bde}	142.0 ± 10.5^{bdef}	138.8 ± 10.1^{e}	140.8 ± 8.8^{bef}	143.2 ± 6.3^{be}	144.6 ± 6.0^{bdef}	144.4 ± 10.7^{bef}

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e= Indicating values are significantly different between B and D.
f= Indicating values are significantly different between A and C.

Discussion

Significant decrease occurred in levels of RBC, PCV, Hb and MCH of *Huso huso* from 7 to 63 days post-exposure to diazinon at the chronic dose of 1.5 mg/l indicating development of an anemia in affected fish. Also, fish received glucan prior to exposure to the toxicant showed a significant decrease in level of RBC compared to fish receiving glucan without diazinon bath. In previous study (Khoshbavar-Rostami *et al.* 2004) significant reduction occurred in levels of RBC, PCV, Hb, MCH, MCHC occurred in *Huso huso* when exposed to acute toxicity of 96-hour LC_{50} diazinon compared to control fish ($P<0.05$). Similar findings on RBC, Hb and PCV were obtained by Svoboda *et al.* (2001), while values of MCV and MCHC of common carp exposed to acute level of diazinon at 19-21°C were identical to control group ($P>0.05$). Therefore, long-term exposure of *Huso huso* to sublethal concentration of diazinon can cause an anemia in fish. Such observations have been also reported by other workers using other organophosphorus pesticides in some species (eg. Anees, 1978; Benarji & Rajendranath, 1990; Khattak & Hafeez, 1996; Tavares *et al.*, 1999).

Fish exposed to diazinon showed higher levels of ALP, ALT and LDH during initial periods post-exposure compared to other three groups indicating the effect of chronic concentration of diazinon in *Huso huso* blood enzymes at the initial stages. Also, treated glucan fish plus diazinon bath showed insignificantly higher levels of these enzymes for up to 14-21 days post-exposure compared to fish received glucan but without diazinon bath ($P>0.05$). Similar results were found on levels of LDH, AST and ALP following exposure to *Channa punctata* and common carp to acute level of diazinon 96-hour (Sastry & Sharma, 1980; Luskova *et al.*, 2002). Significant reduction in LDH activity in the blood plasma of experimental *Huso huso*, compared to the control group, indicates a decrease in the glycolytic process due to the lower metabolic rate as a result of the effect of diazinon as described by Luskova *et al.* (2002) who demonstrated similar results in the exposed common carp to the acute level of diazinon. However, other authors reported an increase in plasma LDH activity in various fish species (e.g. Gill *et al.*, 1990; Ceron *et al.*, 1997; Sancho *et al.*, 1997).

No significant difference was observed in the level of total protein between the exposed fish to the toxicant and unexposed group for two weeks ($P>0.05$), while it significantly decreased in the exposed fish compared to untreated one for the rest of experiment ($P<0.05$). Also, fish received glucan and diazinon bath showed generally lower levels of total protein concentrations in blood plasma compared to fish received glucan without diazinon bath ($P<0.05$). Such reduction in protein level could be due to the negative effect of diazinon in fish cellular functions because RNA plays a vital role in protein synthesis, so decreased protein content is to some extent explained. At the same time the amino acid contents increased as mentioned by Ansari and Kumar (1988). The increased enzymes contents during the initial stages of exposure suggests that the tissue first attempt to repair the damage caused by diazinon. In reparative regeneration of tissue the surviving cells are stimulated to divide faster to compensate for the damage. During mitosis the DNA strand is first increased in DNA indicating the majority of surviving cells are engaged in active mitosis. However, this does not last long due to continuous stress from the pesticide, and finally the DNA content falls.

The level of glucose was generally significantly higher in fish exposed to the toxicant than fish received glucan and diaznion bath ($P<0.05$). Also, fish received glucan and diazinon bath showed generally higher levels of glucose concentrations in blood plasma compared to fish received glucan without diazinon bath ($P<0.05$). An increase in glucose concentration in blood plasma together with a decrease in LDH level indicates a decrease in the glycolytic process due to the lower metabolic rate as a result of the toxicant effect as mentioned by Luskova *et al*. (2002).

In conclusion, long-term exposure of *Huso huos* to diazinon at a chronic concentration disturbs the cell constituents of fish causing erythropenia, hyperglycemia, reduction in total protein, a hepatic enzymes of ALP, ALT, AST and LDH. Also, use of some immuostimulators such as glucan can compensate such negative effects by the toxicant, because no significant change was observed in glucan received fish exposed to long term chronic toxicant.

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