

Impacts of Atrazine on some blood and biochemical indices in farmed *Acipenser nudiventris*

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Abstract

In this study, the effects of the Atrazine on some blood biochemistry indices in *Acipenser nudiventris* during a 96 hours period was studied. These examinations were made by 4 treatments and each treatment repeated these exams three times with a blank, and these were done on 135 *Acipenser nudiventris* with the average weight of 50 ± 10 g. The temperature, pH and hardness of the water and the dissolved oxygen were $24 \pm 1^\circ\text{C}$, 8, 400 and 7 mg per liter, respectively. The amount of LC50-96h of Atrazine was estimated 32.01 mg per liter. The minimum and maximum level of albumin in treatment 50 mg per liter was observed in the 24th and 48th hour and there was a significant difference ($p < 0.05$). The cholesterol level in all concentrations showed significant decrease in comparison with the blank in time. The triglyceride had its minimum amount in the concentration of 37 mg per liter, which had a significant difference with respect to the blank and some other treatments ($p < 0.05$). The glucose level in all the treatments in 24 hours showed no significant difference with the blank treatment ($p < 0.05$). As time went, the hematocrit increased meaningfully with the increase of the atrazine concentration, and the red blood cells (RBC) decreased meaningfully in different treatments. The amount of the white blood cells (WBC) had decreased meaningfully in the 24th and 48th hour in comparison to the blank. The amount of hemoglobin showed meaningful tolerance in different times and concentrations. Changes in the average cellular volume had a meaningful difference with the increase of the atrazine dose and in different treatments and times. The average changes in the hemoglobin corpuscular showed a meaningful difference in different treatments and different times. The average of corpuscular hemoglobin in 24 hours had a meaningful increase in the most of the treatment in comparison with the blank. In some of the treatments the neutrophils have increased meaningfully with the increase of concentration and with time passing by. Its minimum amount has been observed in 96 hours in the concentration of 50 mg per liter. In some of the treatments, with time going by, the amount of the lymphocytes with the increase of the atrazine concentration has increased meaningfully in comparison to the blank. The amount of neutrophils in the 24th and 48th hour showed meaningful increase with the increase of the atrazine concentration and in time, in some of the treatments in comparison to the blank. The monocytes have increased meaningfully in time and with the increase of the atrazine concentration. According to the table of the toxicity levels, atrazine is considered as 'toxic' for *Acipenser nudiventris* and we can use the changes in the desired indices as tools in analyzing the situation in the pathobiology of fishes.

Keywords: Atrazine, *Acipenser nudiventris*, Blood and biochemical indices, LC50- 96h.

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Introduction

Guilan, Mazandran and Khuzestan provinces are important in Iranian aquaculture that used high amount of plant herbicides. From a total of 35000 tones herbicides using in Iran, 25000 tones used in the north provinces farms by farmers (Mosavi, 1997). In some cases, herbicides had the more disruptive effects on aquatic animals than pests that led to more sensitivity and mortality in aquatic animals. At the south coasts of the Caspian Sea, some rivers such as Sepidrood, Gorganrood, Polrood, Tajan and Shafarood were located adjacent to many agriculture farms and transfer high amount of toxins residue into the Caspian Sea. These toxins changed water quality and led to mortality in fingerlings and even bigger fish (Aslan Parviz, 1991). Atrazine is one of the most consuming and major herbicide in the world during last 40 years that used for controlling many plants. In Iran, this toxin used as an herbicide in farms of Golestan and Khuzestan provinces. These provinces known as the most sites for warm water rearing. Atrazine is a triazine that absorbed rapidly via root and transferred via apoplast and leaves. This toxin is available in Iran markets with commercial name of Gesaprim and with formulation WP80%. Atrazine is a triazine white color crystals synthetic herbicide with chemical formula: Chloro-4-ethylamino-6-isopropylamino-1-3-5-triazinre that used in 80 countries recently for destroying broad leafs grass weeds in agriculture farms. Usage of herbicides had harmful effects on fish and changed plankton food chain (Mason, 1991). Abdali *et al.*, 2012, studied the effects of atrazine on some immunological and blood indices in famed Grass carp (*Ctenopharhyngoden idella*) and observed significant changes in some indices. Moreover, Aaronson (1980), indicated that *Onchorhynchus mykiss* died in ponds polluted with 1000 µg/L atrazine (Elia *et al.*, 2002). Therefore, by notice to releasing millions sturgeon fingerlings into Sepidrood River for restocking and shortage of information about atrazine effects on sturgeon in Iranian waters and also high usage of it in agriculture farms specially cotton and cereals in the

north of Iran and considering to subject important, present study was carried out with the aim at evaluating the effects of atrazine herbicide on biochemical and hematological parameters in *Acipenser nudiventris* fingerlings and determining LC50- 96h.

Materials and methods

The required herbicide atrazine was purchased in one kilogram bags and provided by the Delta Sabz Jonoob Company. The herbicide atrazine had 80% purity (WP=80) in powder form, dilutive in water.

A total of 135 *Acipenser nudiventris* with an average weight of 50 ± 10 g were purchased from the International Sturgeon Research Institute and maintained for 96 hours in 13 aquariums with volume of 200 L for adaptation. According to the necessary classifications, the fish were divided into of 5 groups including 3 replicates and placed into 15 aquariums with the capacity of 200 L of water (completely enclosed and aerated). For Acute toxicity study, a total of 135 fish were exposed to 25, 37.5, 50 and 100 mg/L of atrazine for 96 hours. PROBIT analysis method used for showing 96h-LC50 values. In order to provide the required concentrations of herbicide, the Germ/volume approach was used. At first, the total required herbicide was calculated and dissolved in a specific volume of consumption water (river water) and provided the solution of stock. Then, according to the required concentrations, specific volumes of the stock were poured into the aquariums. In order to calculate the herbicide required the following formula was used: $C_2V_2=C_1V_1$. No feeding was done for fish during experiment. Sampling was performed every 24 hours. At each sampling time, 3 fish were bled repeatedly. For bleeding, the caudal vein was punctured by using 2 mm heparinized syringes. After bleeding, the samples were transferred to the physiology and biochemistry Dept. of the hematology laboratory of International Sturgeon Research Institute of Dr. Dadman, where plasma was separated by utilizing a centrifuge set at 3000 rpm for 10 min. (Yousefi Jourdehi, 2006). Differential count of

leukocytes was done by providing of a smear from blood and studied with light microscope. Hematocrit (Hct) levels were measured by microhematocrit centrifuge set at 7000 rpm for 5 min. For measurement hemoglobin (Hb) of biochemical parameters including cholesterol, triglyceride, total protein, albumin and glucose of the blood plasma, the spectrophotometer set was used (Kazemi *et al.*, 2011). The data was analyzed as Mean±S.E.M. at reliability 95% and significant level of $p<0.05$. In order to test the significant of the test, t-test method was used in SPSS 16 software. Excel software used for drawing figures.

Results

A- Biochemical indices results

Total protein levels were decreased significantly with the increase of atrazine concentration at the same times ($p<0.05$) (Fig. 1).

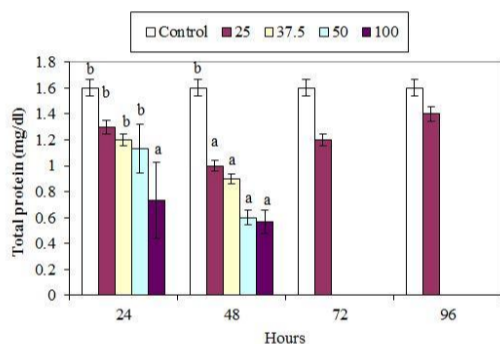


Figure 1: Total protein levels changes at different concentration of atrazine.

*, fish were survived at concentration 25 mf/L for 96 hours. None similar letters indicated significant differences.

Minimum and maximum level of albumin observed at concentration 50 mg/L in 24 and 48 hours, respectively. At all times albumin levels showed significant difference at various concentrations than control depended on time ($p<0.05$) (Fig. 2).

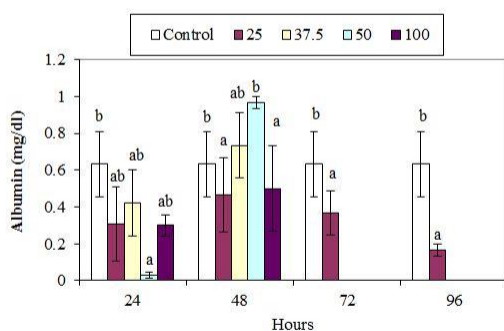


Figure 2: Albumin level changes at different concentrations of atrazine in *Acipenser nudiventris*.

*, fish were survived at concentration 25 mf/L for 96 hours. None similar letters indicated significant differences.

Cholesterol levels decreased significantly at all concentrations of atrazine depended on time ($p<0.05$) (Fig.3).

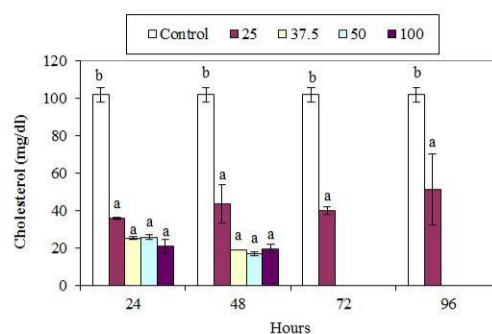


Figure 3: Cholesterol level changes at different concentrations of atrazine in *Acipenser nudiventris*.

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Minimum levels of triglyceride was observed at concentration 37.5 mg/L of atrazine and showed significant different than control (Fig.4) ($p<0.05$).

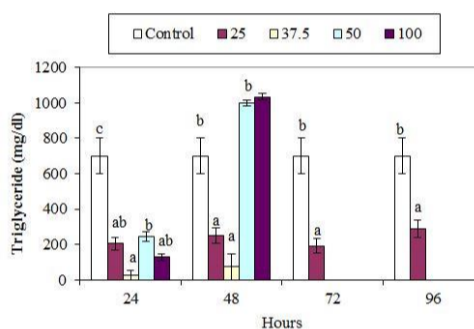


Figure 4: Triglyceride level changes at different concentrations of atrazine in *Acipenser nudiventris*.

*, fish were survived at concentration 25 mf/L for 96 hours. None similar letters indicated significant differences.

Glucose levels showed no significant difference at all concentrations of atrazine in compared to control in 24 hours, while decreased significantly at higher concentrations in 48 hours (Fig.5) ($p<0.05$).

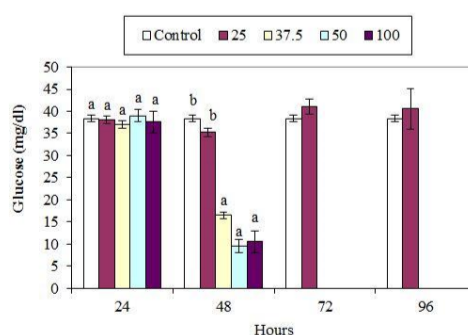


Figure 4: Glucose level changes at different concentrations of atrazine in *Acipenser nudiventris*.

*, fish were survived at concentration 25 mf/L for 96 hours. None similar letters indicated significant differences.

B- Cytological indices results

Hematocrit level increased significantly with increase of atrazine concentration and time (Fig. 6) ($p < 0.05$).

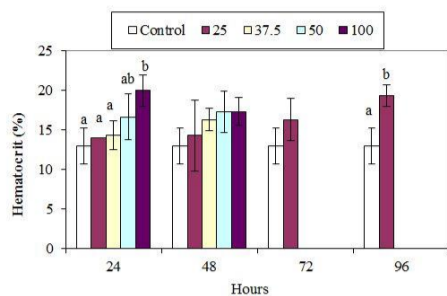


Figure 6: Hematocrit level changes at different concentrations of atrazine in *Acipenser nudiventris*.

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Red blood cells (RBC) numbers decreased significantly ($p < 0.05$) at different concentrations of atrazine and increase of time (Fig. 7).

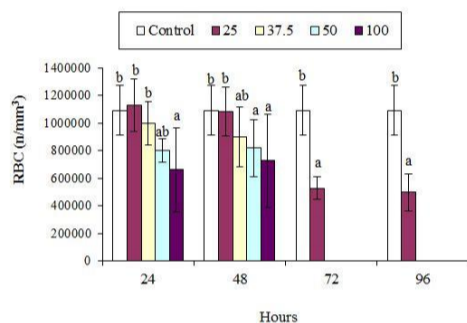


Figure 7: RBC level changes at different concentrations of atrazine in *Acipenser nudiventris*.

*, fish were survived at concentration 25 mf/L for 96 hours. None similar letters indicated significant differences.

White blood cells (WBC) decreased significantly in 24 and 48 hours than control (Fig. 8) ($p < 0.05$).

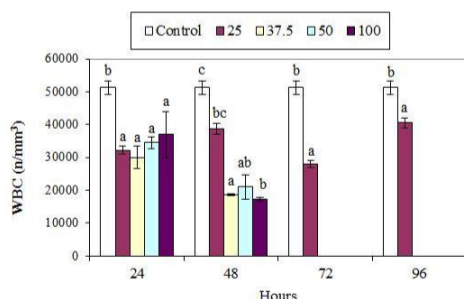


Figure 8: WBC level changes at different concentrations of atrazine in *Acipenser nudiventris*.

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Hemoglobin (Hb) level changes were various at different concentration of atrazine in different times (Fig. 9).

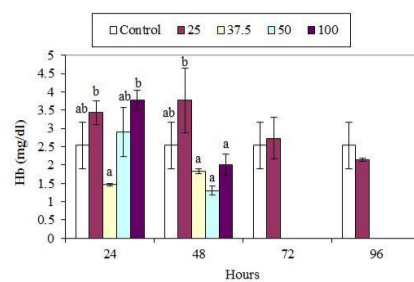


Figure 9: Hemoglobin (Hb) level changes at different concentrations of atrazine in *Acipenser nudiventris*.

*, fish were survived at concentration 25 mf/L for 96 hours. None similar letters indicated significant differences.

Mean corpuscular volume (MCV) changes showed significant different with increase of atrazine concentration in some times at different treatments (Fig.10) ($p < 0.05$).

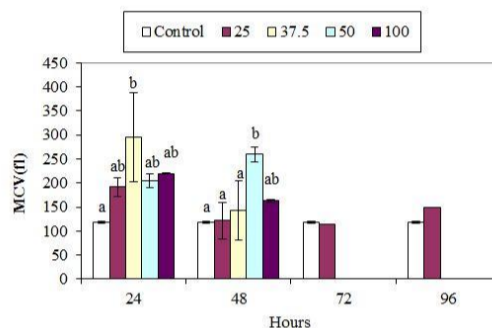


Figure 10: Changes of MCV at different concentrations of atrazine in *Acipenser nudiventris*.

*, fish were survived at concentration 25 mf/L for 96 hours. None similar letters indicated significant differences.

Mean corpuscular hemoglobin concentration (MCHC) levels showed significant fluctuations ($p < 0.05$) in different times (Fig. 11).

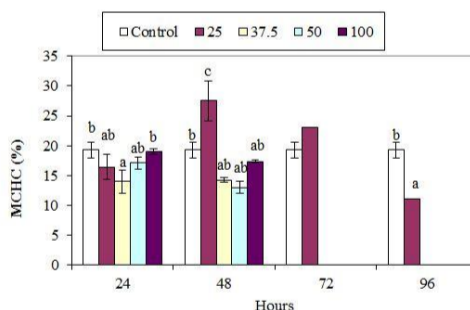


Figure 11: Mean MCHC level changes at different concentrations of Atrazine in *Acipenser nudiventris*.

*, fish were survived at concentration 25 mf/L for 96 hours. None similar letters indicated significant differences.

Mean corpuscular hemoglobin (MCH) showed significant difference between all treatments than control ($p < 0.05$). While decreased significantly at concentration 25 mg/L in 96 hours (Fig. 12).

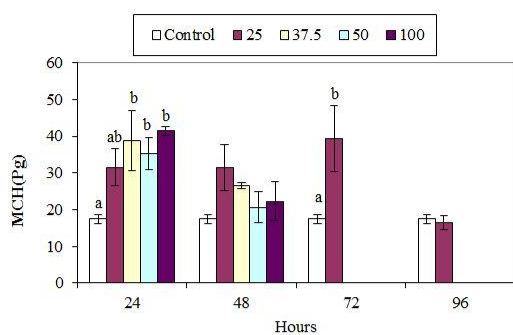


Figure 12: MCH changes at different concentrations of atrazine in *Acipenser nudiventris*.

*, fish were survived at concentration 25 mf/L for 96 hours. None similar letters indicated significant differences.

C- Differential count of leukocytes

Neutrophile numbers increase significantly at some concentrations a times in compared to control ($p < 0.05$). The mean level of it was observed at concentration 50 mg/L in 96 hours (Fig 13).

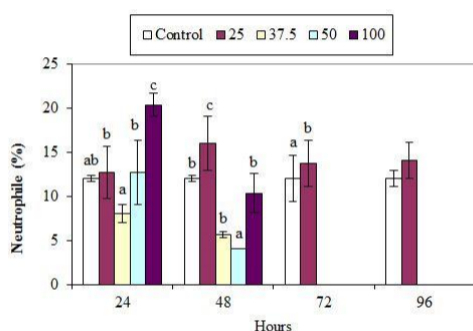


Figure 13: Neutrophile changes at different concentrations of atrazine in *Acipenser nudiventris*.

*, fish were survived at concentration 25 mf/L for 96 hours. None similar letters indicated significant differences.

Lymphocyte number increased significantly with increase of atrazine concentration and times in compared to control ($p < 0.05$) (Fig. 14).

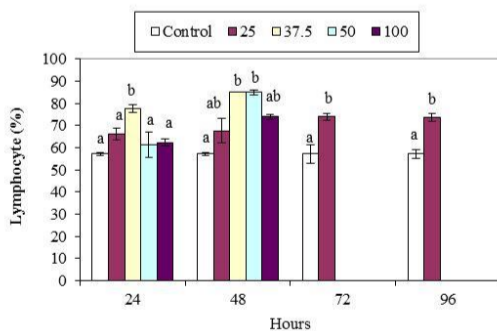


Figure 14: Lymphocyte changes at different concentrations of atrazine in *Acipenser nudiventris*.

*, fish were survived at concentration 25 mf/L for 96 hours. None similar letters indicated significant differences.

Eozynophile numbers showed significant differences in 24 and 48 hours with increase of atrazine concentration ad times in compared to control ($p < 0.05$) (Fig. 15).

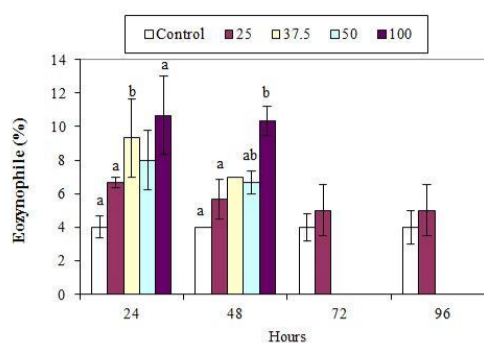


Figure 15: Eozynophile changes at different concentrations of atrazine in *Acipenser nudiventris*.

*, fish were survived at concentration 25 mf/L for 96 hours. None similar letters indicated significant differences.

Monocyte numbers increased significantly with increase of atrazine concentration and time ($p < 0.05$) (Fig. 16).

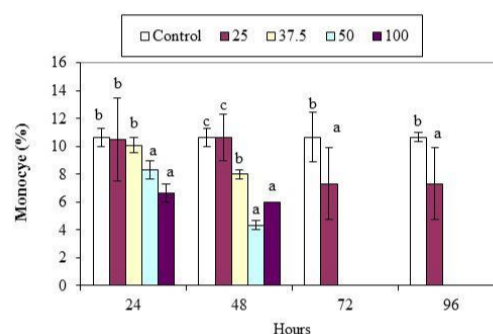


Figure 16: Monocyte changes at different concentrations of atrazine in *Acipenser nudiventris*.

*, fish were survived at concentration 25 mf/L for 96 hours. None similar letters indicated significant differences.

Discussion

Fish are exposed to environment directly that each change in it changes fish responses to environmental and physical process. Fish are one of the most expensive organisms in water and are sensitive to environmental pollution that it may be lead to responses to biological pollutions in waters. It is possible to detect toxic symptom of herbicides by studying cytological and serological indices in fish. Sensitivity of different fish species to toxic materials is different and for this reason toxic experiments were done in different fish. In this study, effects of atrazine acute toxicity on clinical symptoms in *Acipenser nudiventris* fingerlings were measured during 24, 48, 72 and 96 hours. Some symptoms such as mucosa secretion as a none -specific immunity system were increased for protection of fish against to pollutants. Darkness color observed in abdominal surface because of motivation and melanocyte deposition. Sclerosis and neural paralyze were observed in fish exposed

to atrazine toxicity. Semi circle swimming and body surface darkness also observed in examined fish. Anal of fish was swollen and their breathe was intensified. In a study, *Xenopus* and *Hypophthalmichthys nobilis* embryos were exposed to 0, 20 and 250 µg/L atrazine from fertilization time to 180 hours after growth. At all concentrations, Atrazine showed no considerable effects on growth, muscle contraction, embryos length, gut diameter and mortality of fish and frogs. Fish and frogs showed morphological changes exposed to atrazine. Fish exposed to ethynyl estradiol (EE₂) had more thickness may be because of entering toxin into yolk sac (Roberts, 2001). Atrazine toxicity in fish depended on toxin concentration and fish species was between 3 and 45 mg/L (Elia *et al.*, 2002). Acute toxicity effect of 100 g/L atrazine in *Cyprinus carpio* increased glucose and cortisol levels and decreased protein, cholesterol and glycogen in liver and muscles (Gluth and Hanke, 1985), and changed alkaline phosphatase activity, heart, liver and kidney in exposed to 1.5 to 6 mg/L during 14 days (Neskovic *et al.*, 1993). In *Oncorhynchus mykiss*, toxicity concentration of atrazine between 10-160 g/L induced different effects on kidney tubules, propagation, endoplasmic reticulum, mitochondria, lysozyme and Golgi apparatus disasters (Oulmi *et al.*, 1995). Bioaccumulation of atrazine in liver of tilapia (*Tilapia sparrmanii*) at concentration was 50g/g exposed to 16 mg/L after 72 hours (Du preez and van Vuren, 1992). Atrazine exists in drinking water of some people at higher than acceptable dose (MCL) that cause to many problems in cardio - vascular and reproductive system at long term time. Long - term exposure to atrazine increased breast and ovary cancer risk in human (Donna *et al.*, 1989). Expositing to 100 g/L atrazine after 10 days decreased food consumption in Nile tilapia (*Tilapia niloticus*). Increasing of lassitude and reducing food consumption were observed in cat fish exposed to 3 and 6 mg/L atrazine (Hossein *et al.*, 1996). Atrazine increased hematocrit in fish after exposing to saline water and led to inducing stress (Pierson *et al.*, 2004)

and increase more usage of red blood cells (RBC) in spleen (Jensen, 1987) and swelling of them (Wang *et al.*, 1994) or increasing blood concentration because of plasma volume decrease (Wilson and Taylor, 1993). Prasad *et al.* (1991) indicated decreasing work of breathing because of operculum damage in *Tilapia mosambica* exposed to 1.1mg/L atrazine. Basic changes in gill operculum led to hypertrophy and propagation in cells and finally blood contamination and breathing in surface waters (Mallatt, 1985; Cengiz and Unlu, 2003; Oropesa – Jimenez *et al.*, 2005). Acute toxicity of atrazine with concentration 18.5 ppm on common carp (*Cyprinus carpio*) showed that RBCs, hemoglobin, glucose and protein levels decreased 63.17, 27.35, 6.78 and 18.73 %, respectively. While WBCs increased 3.73%. Atrazine toxicity effect symptom on central neural system (CNS) and cardio - vascular system observed in abnormal behavior in fish (Antychowicz *et al.*, 1979). Puigdoller *et al.* (2007), reported that food consumption decreased because of decreasing in acetyl cholinesterase activity in Salmon exposed to 100 g/L atrazine after 10 - 15 days (Hussein *et al.*, 1996). Changes in carbohydrate metabolism were measured as plasma glucose that used as stress indices in fish. Reduction in glucose level after exposure to toxins was because of hypoxia conditions that led to carbohydrate deposition. Hossein *et al.* (1996), observed significant decrease in blood serum glucose because of atrazine toxic effects on fish liver (Braunbeck, 1995). At present study, decrease in plasma glucose in fish exposed to atrazine can be because of hypoxic conditions. Hossein *et al.* (1996), indicated that decrease in food absorption of fish exposed atrazine toxicity can a reason for plasma glucose level reduction. Blood protein level in fish used as indices for popular health condition. Das *et al.* (2004), reported that more requirement to energy may be increased protein consumption, the process that converted protein to energy and then serum protein level decreased. Imbalance of blood equilibrium is important indices of kidney damage that led to blood protein kidney

secretion and decrease of serum protein in fingerlings fish. Hossein *et al.* (1996) reported that total protein level decreased in Nile tilapia (*Oreochromis niloticus* and *Chrysichthyes auratus*) because of atrazine toxicity effect on immune system goblin protein reduction. In present study, plasma protein level reduction was due to chronic atrazine toxicity effect on spleen, liver and kidney. Ramesh *et al.* (2009), studied the terminal effects of the toxin atrazine on common carp (*Cyprinus carpio*) blood indices and understood that the studied blood parameter levels were affected significantly by the toxic effects of the toxin atrazine. Hanke and Gluth (1985), indicated that Common Carp was placed in the proximity of 100 µg/L concentration of atrazine for 72 hours, showed significant reduction in plasma protein concentrations in their blood serum which is due to the dilution effect in the blood of the fish group. Cholesterol is the base material for all steroid hormones. When it increases due to cortisol synthesis, then a large amount of cholesterol is needed (Kazemi *et al.*, 2011). Therefore, the reduction in the amount of cholesterol may be related to its utilization in the manufacture of Cortisol arising from stress created by the toxin atrazine. Tri-glyceride is the storage form of fats and major resources of oils and fat which are flowing into the blood. The reduction of Tri-glyceride volumes in blood plasma at high concentrations of the toxin atrazine could be due to the imbalance created by the higher concentrations of the toxin, affecting the digestive system, liver and related enzymes as well as hormonal and natural metabolic imbalance in fish studied (Rabinson, 1990). Hematocrit increased significantly ($p < 0.05$) with the increase of atrazine concentration and days because of swelling and increase in red blood volume induced by stress and catecholamine release from spleen (Beyea *et al.*, 2005). Hemoglobin concentration and high number of red blood cells is a response to high requirement to body metabolism. Increase in RBC was due to high requirement to oxygen for

higher metabolism (Stathiskumar *et al.*, 2011; Zhou *et al.*, 2011). Hematocrit and hemoglobin level was more in blood of *Onchorhynchus mykiss* at stress conditions because of increasing in red blood cell volume and led to hematocrit increase (Gabriel *et al.*, 2007). Spleen releases RBC in to blood and lead to increase in RBC number in blood, hematocrit and hemoglobin concentration for increase of dissolved oxygen transport (Ajani, 2008). RBC number increased significantly with increase of atrazine concentration due to induced stress (Ajani, 2008). Leukocytes regulated immunological functions and increased in response to stress because of increase in their production in lymphoid tissues (Johansson-Sjoberck and Larsson, 1978). At present study, lymphocyte number decreased significantly with increase of atrazine concentration and time ($p < 0.05$). Atrazine decreased lymphocytes type B in fish. Luiz *et al.* (2010), indicated that atrazine reduced natural immunological responses in silver cat fish and induced none - specific body immunity and then irregularity in immune system and some diseases. Other studies showed that atrazine led to disruption in normal body immune system and increase of infection in higher concentrations (Maria *et al.*, 1986). Based on results of this study, *Acipenser nudiventris* fingerlings were exposed to atrazine at concentration 25 ppm survived after 96 hours showed significant effects on many hematological and biochemical parameters. Therefore, atrazine are toxic for *Acipenser nudiventris* fingerlings even at low concentrations and we must desist from using high concentration of atrazine in agriculture.

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