Effects of Atrazine (Herbicide) on Blood Biochemical Indices of Grass Carp (*Ctenopharyngodon idella*)

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Abstract

Atrazine is currently the most widely used herbicide in agriculture for the production of corn and other crops in the world. In the present research, the effect of acute and chronic toxicity of atrazine (herbicide) on blood biochemical indices of grass carp (*Ctenopharyngodon idella*) an important species of commercial fish was studied. In acute toxicity, pollutant toxicity to fish was tested by using lethal concentrations and determining the LC50 (lethal concentrations for 50% of fishes). Obtained results from PROBIT analysis showed 96h-LC50 values at 80 mg/L of atrazine. In chronic toxicity, the fish were exposed at different concentrations of atrazine (0, 10, 100, 1000, 10,000 µg/L with three triplicates for each treatment) for a month (30 days). Sampling was carried out in three times (10th, 20th and 30th days). The results showed that plasma total protein, albumin, glucose, cholesterol and triglycerides decreased significantly (P<0.05) with increasing concentrations and days. Results also showed that minimum mortality rate of fish belonged to control (0%) and 10 µg/L and maximum mortality was belonging to 10000 µg/L up to the 10th day and 1000 µg/L up to 20th day and reached 100%. Therefore, these parameters could be used as an index for evaluation of pathological conditions of this fish species.

Keywords: Atrazine, Blood, Biochemical indices, Grass carp, *Ctenopharyngodon idella*

1. Introduction

Use of herbicides is common for fish management in fish farms to control water grass (Wu et al., 1980). Atrazine is one of the most consumed and major herbicides in the world in the past four decades. In Iran, atrazine has been used as herbicide on cotton, sugar cane, corn, etc. farms in Golestan and the southern provinces especially Khuzestan. These are major fish farming regions in the country. Atrazine is also absorbed through leaves but has 0% transference rate. Atrazine has the trade name of Gesaprim in Iran distributor into the market with weight purity 80% formulation.

The half-life of atrazine within the soil is about four days, but normally the volume of the product may remain in the soil up to 385 days in dry and sandy areas. In pure water, the half-life of atrazine is considered to be three days, whereas in seawater, this period is 30 days. The half-life of atrazine on the sea bed is 35 days and in vertebrate animals is 72 days.
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Extensive use of atrazine has brought about focus of studies on the environmental chemical effects. Studies on the toxic effects of atrazine on fish have shown varied responses according to the type of species and dosages, but the deadly concentration of the product has been assigned as a 3 to 45 mg/L (Elia et al., 2002).

Aaronson (1980) has proven that rainbow trout (Onchorhynchus mykiss) was died in pools containing 1000 µg/L of atrazine.

Ramesh et al. (2009) studied the terminal toxicity effects of atrazine on common carp (Cyprinus carpio) blood factors and showed that the studied blood parameter levels were significantly affected by atrazine toxicity. Nwani et al. (2010) studied the toxic effects of the herbicide atrazine on fat oxidation and antioxidant enzyme activity of Channa punctatus. Weignad et al. (2001) studied the changes of the toxicity of atrazine on Zebra fish embryos and concluded that it caused damage and disorder in the growth stages of embryos.


The morphological effects of strong and terminal toxicity of atrazine on rainbow trout (Onchorhynchus mykiss) were carried out by Fisher et al. (1991).

Hossien et al. (1996) carried out a comparison study of herbicidal atrazine on varied species of Oreochromis niloticus and Chrysichthys auratus in the east Egypt. Pugazhendy and Jayachandran (2009) studied the histological changes of fingerlings Labeo rohita exposed to the toxin.

Oulmi et al. (1995) studied the deadly effects of various atrazine concentrations (between 10 to 160 µg/L) and observed effects on the kidney including endoplasmic softening. Vuren van and Du Preez (1992) demonstrated that the environmental clustering of atrazine occurred in the kidney (40 µg/gr. after 72 hours) and in the egg sack (50 µg/gr. after 72 hours) of tilapia fish.

Ortiz et al. (2002) studied the effects of environmental chemicals on the bodies of various fishes and observed that fish had high capacities to bioaccumulation of the toxin in their fatty components which is transferred to the body through the gills and skin.

Warining et al. (2004) studied the effects of the atrazine on Atlantic salmon smolt in fresh water and after transfer to salt water and observed that it caused considerable damage to the gill cells. Considering the above cases and importance of the topic, this study aims to analyze the effects of various concentrations of the toxin on some blood biochemical indices of grass carp (Ctenopharyngodon idella).

2. 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 500 grass carp with an average weight of 33.63 ± 9.6 gr and with a mean length of 14.11 ± 1.1 Cm were purchased from Rastin Pooyan Kar Company and maintained for one week in two-tone fiberglass tanks for adaptation. Then, according to the necessary classifications, the fish were divided into of 20 groups and placed into 15 half-tone fiberglass tanks with the capacity of 200 L of water (completely enclosed and aerated). For Acute toxicity study, a total of 180 fish were exposed to 40, 80 and 120 mg/L of atrazine for 96 hours. PROBIT analysis used for showing 96h-LC50 values. For chronic toxicity study, a total of 300 fish were divided into five triplicate groups, consisting of the control group (zero concentration) and groups with concentrations of 10, 100, 1000, 10,000 µg/L of herbicide, respectively. 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 tanks. In order to calculate the herbicide required the following formula was used: \( C_2V_2 = C_1V_1 \).

Feeding of fish with grass was carried out twice daily. Sampling of the fish was performed every 10 days. At each sampling time, 5 fish were bled repeatedly. For bleeding, the caudal vein was punctured by using 2 mm heparinated 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 et al. 2006). For measurement 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.

3. Results

The results showed that mortality rate of fish at concentrations of 10,000 µg/L reached 100% on the 10th day, and that concentrations of 1000 µg/L on the 20th day where all fishes died. At the same time, at concentrations of 10, 100 µg/L and control group, the fish stayed alive until the end of the experiment.

The total protein level at the 10,000 µg/L dose on the 10th day was less compared with the other groups and showed significant difference (P<0.05). The level in the 1000, 100, 10 µg/L groups were reduced with time and in some concentrations (100 and 10 µg/L) showed significant difference (Fig. 1).

The minimum albumin level in the 1,000 µg/L concentration group was observed on the 20th day which showed significant difference (P<0.05) with other groups. The albumin level in the 10,000 µg/L concentration groups on the 10th day had significant reduction, compared to other groups, while the other groups showed almost constant volumes. In the 10 and 100 µg/L concentration groups the albumin level on the 30th day, compared with the 10th and 20th days showed significant reduction (P<0.05) (Fig. 2).

The minimum cholesterol level in the 1000 dose was observed on the 20th day showing significant difference (P<0.05), compared with the other groups and periods. The level of cholesterol in the 10, 100,
1000 doses reduced significantly (P<0.05) in time (Fig. 3).

The lowest amount of triglyceride on the 10th day was observed in the 10,000 µg/L concentration group, which compared to other groups, showed significant difference (P<0.05). No significant difference was observed, between the 10 µg/L group and control group but there was significant difference with the other groups (P<0.05) (Fig. 4).

The minimum level of glucose was observed in the fish located in the 10,000 µg/L concentration group on the 10th day and was significantly different (P<0.05) compared to the other groups and days. The level of glucose in the other concentrations, except control group, reduced with time (Fig. 5).

4. Discussion

The aim of this study was to analyze the effects of herbicide atrazine on grass carp with emphasis on the blood biochemical indices. According to the results obtained, the minimum of fish mortality rate was observed in the control group (0%) and 10 µg/L concentrations (2%) as well as the maximum mortality was observed in the 10,000 µg/L concentration groups on the 10th day and 1,000 µg/L concentration groups, on the 20th day which reached 100% and all the fish were dead, which indicated the threshold tolerance of the studied fish against the chronic toxicity of the atrazine in the related concentrations of this study.

In all studied groups, the levels of total protein, albumin, cholesterol, tri-glyceride and glucose indices were reduced significantly (P<0.05) with the increase of the atrazine and passing of time in such a way that the lowest levels were observed in the 10,000 and 1000 µg/L concentration groups, respectively.

Changes in the carbohydrate metabolism were measured as glucose plasma which can be the normal stress factor within the fish. The reduction of the glucose serum levels after proximity with the toxin appears to be due to hypoxic conditions which lead
to extra consumption of stored carbohydrates. Hossien et al. (1996) found a significant reduction of blood serum glucose. This reduction might be the result of the effects of the toxin atrazine on the kidney of the fish (Braunbec et al. 1982). In recent studies, the reduction of the glucose plasma level during toxicity might belong to hypoxic conditions resulting from the herbicide atrazine. Also Hossien et al. (1996) reported that the reduction in food intake of the fish in proximity to the toxin atrazine might be another reason for the reduction of the glucose level in plasma.

The concentration of the fish blood protein serum is an index of the general health condition of the fish. Das et al. (2004) have reported that increased energy demand might increase protein consumption, a process where protein is converted into energy, and therefore the protein serum will be reduced. Imbalance in the blood is an important index of kidney damage. Damage of the kidney causes increased kidney protein secretion into the blood stream and may also lead to the reduction of protein serum in finger sized fish. Hossien et al. (1996) have reported that reduction in total protein of Oreochromis niloticus and Chrysichthys auratus was due to globulin reduction which shows that the effect of the toxin atrazine is on the fish immunity system. In the present study, the reduction of fish protein plasma is because of the suspicious effect of the toxin atrazine due to the toxic effects of the toxin on the kidney, spleen and liver.

Ramesh et al. (1985) 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 the species of carp placed in the proximity of 100 µg/L concentration of atrazine for 72 hours showed significant reduction in plasma protein concentrations in their blood 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 are needed (Kazemi et al. 2010). 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).

We should assume that atrazine has strong effect on the blood biochemical indices of the fish tested. Therefore, the use of the herbicide atrazine must be at a minimum level and these parameters can be used as effective guidelines for the toxic level indices for farmed grass carp fish.

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