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Effect of Ethylenediaminetetraacetic Acid (EDTA) on Some Serum Constituents of *Oncorhynchus mykiss*

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Abstract: Effect of sublethal concentrations of EDTA on some serum biochemical factors of *Oncorhynchus mykiss* were studied in vitro conditions. These experiments were carried out during 96 h. Fish with an average weighing 51±12 g and length 18.5±2.1 cm were distributed in five groups with 800, 1100, 1400, 1700 and 2100 mg L^{-1}. The experiments periods were controlled water physicochemical factors such as: pH, total hardness, dissolved oxygen and temperature. The results showed that the mean LC_{50} values of EDTA at 96 h were 2231 mg L^{-1}, to the rainbow trout. Also, Maximum Allowable Concentration (MAC) value of EDTA was determined, 223.1 mg L^{-1}. Also Lowest Observed Effect Concentration (LOEC) and Non Observed Effect Concentration (NOEC) for EDTA were determined 2000 mg $L^{-1}$and 1200 mg L^{-1}, respectively. Results showed that exposure to EDTA causes a decrease in sodium, calcium and triglyceride and cause an increase in glucose, cholesterol, potassium, chloride, iron, total protein and albumin values of blood serum in *Oncorhynchus mykiss* ($P < 0.05$). The results showed that exposure to EDTA causes changes in some blood serum biochemical factors of *Oncorhynchus mykiss*. The results of this study showed that EDTA has negative effects on rainbow trout health.

Key words: Acute Toxicity · Blood Serum · EDTA · Oncorhynchus Mykiss

INTRODUCTION

Oncorhynchus mykiss is one of the most economically important and valuable fishes in the world. Water ecosystems are constantly faced with problems caused by pollutants from various sources. Industrial wastewaters, mostly agricultural and municipal waste water without treatment, are entered in water ecosystems. The detergents are one of the major pollutants that by sewage entered into coastal waters, rivers and other water resources directly and indirectly. The detergents can inhibit oxygen exchange of surface water, which results in impaired ecosystems. Today, detergents are very important due to consumption of synthetic. Detergents are dangerous for aquatic such as fish. Ethylenediaminetetraacetic acid (EDTA) is a chemical used in detergents structure. Ethylenediametetraacetic acid (EDTA) is a synthetic chelating agent that forms strong complexes with cations and it has been widely used in food systems as a stabilizer and sequestrates [1]. EDTA has also been shown to possess antimicrobial effects since it confines the availability of essential cations for growth. EDTA also destabilizes the cell membrane of bacteria by complexing divalent cations that act as salt bridges between membrane macromolecules, such as lipopolysaccharides (LPS) [2-4]. This has led to the use of EDTA as a preservative in many products. However, studies regarding the influences of EDTA on fish are still limited. Most of researches focused on the use of EDTA as part of an antimicrobial catheter lock solution to prevent catheter-related infections caused by clinical microorganisms, including Staphylococcus, Pseudomonas and Candida [5-10]. EDTA is a widely used acronym for the chemical compound ethylene diaminetetraacetic acid (which has many other names) [11]. Also, this material is used in detergents. Therefore, it can be entered in the water environment and aquatic animals, including rainbow trout that lives in cold water

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river. Since some of Iran's water ecosystems are exposed to domestic and industrial detergents and pollutants, it is necessary to review the impact of pollutants on aquatic organisms. To the best of our knowledge, the effect of lethal concentrations of EDTA on *Oncorhynchus mykiss* has not been reported yet. In addition, toxicological and effects of EDTA on blood serum biochemical factors in *Oncorhynchus mykiss* have not been documented yet. In this study, we report the acute toxicity and effect of EDTA at low concentrations on some blood serum biochemical factors of *Oncorhynchus mykiss*.

MATERIALS AND METHODS

This study has been carried out in laboratory of department of fishery, Bandar Gaz branch, Islamic Azad University (Bandar Gaz, Golestan, Iran) in 2011 fall. The first experiment primarily aimed to determine the effects of acute toxicity (96h LC_{50}) of EDTA. Individuals with a body weight of 51±12 g and length 18.5±2.1 cm were selected by gravimetric measurements and then they were acclimated five days to laboratory conditions, removing the suspected unhealthy subjects. Fish were housed in a 50 L capacity fiberglass test tanks (10 fish/aquarium) provided with aeration system. Fishes were fed twice a day with commercial dry pellets containing 38% protein before experiments and not feeding during tests. Water circulation was supported by two air pumps. The mortality records were taken every 24 h (24, 48, 72, 96 h). Movements and behaviors of the fishes were investigated at the time of experiments. Experiments were carried out under static conditions based on the standard TRC, method [12]. After obtaining the final results, the information were analysed statistically with Probit Analysis. Then determined the LC_{10}, LC_{50} and LC_{90} values at 24, 48, 72 and 96 h; the maximum allowable concentration (MAC) value (96h LC_{50} divided by 10), LOEC (Lowest Observed Effect Concentration) which is called LC_{10} in 96 h and NOEC (Non Observed Effect Concentration) (TRC, 1984). The final experimental design incorporated six groups (five test group and control group) of that *Oncorhynchus mykiss* were exposed to 800, 1100, 1400, 1700 and 2100 mg L^{-1} of EDTA. Fish were exposed for 96 h. Water chemistry characteristics of temperature, dissolved oxygen and pH were determined in all test tanks every day. Mean dissolved oxygen ranged from 7.5-7.8 mg L^{-1} during the tests. Mean water temperature ranged from 15 to 16°C. The mean pH during experiments ranged from 7.8 to 8.1. Total hardness was determined during experiments. Total hardness in all tests ranged from 370 to 378 mg L^{-1} as $CaCO_3$. After completing 96 h of exposure, fishes from each treated and untreated (control) groups were anesthetized with 200 mg L^{-1} of clove oil. Then, blood samples were obtained through tail vein puncture and the non-heparinized blood samples were centrifuged for 5 minutes at 3000 *g* and separated serum were used to determine with an automatic analyser machine (Persige 24 I) the levels of the following factors: glucose (BS), cholesterol, triglyceride (TG), calcium (Ca), sodium (Na), potassium (P), chloride (Cl), iron (Fe), total protein (TP) and albumin (Alb). Biochemical data were analyzed with SPSS 13 for Windows using one-way analyses of variance (ANOVA) and significant means were subjected to a multiple comparison test (Duncan) at $P<0.05$. When the normality of data did not present, the nonparametric test Kruskal-Wallis to compare treatments and test Mann - Whitney for paired comparison between treatments were used.

RESULTS AND DISCUSSION

The $LC_{10,\ 50,\ 90}$ values after 96 h of EDTA were calculated on *Oncorhynchus mykiss* as 1778 mg L^{-1}, 2231 mg L^{-1} and 2684 mg L^{-1}, respectively. Therefore, the MAC value of EDTA is 223.1 mg L^{-1}. The results showed that the mean LC_{50} values of EDTA at 96 h were 2231 mg L^{-1}, to the rainbow trout. Also, MAC value of EDTA was determined, 223.1 mg L^{-1}. Also, LOEC and NOEC for EDTA were determined 2000 mg L^{-1} and 1200 mg L^{-1}, respectively. The results showed that how EDTA tested concentration increases, fish died in less time. In fact, for 24 hours the mortality of rainbow trout in the high amount of EDTA is needed more than 96 hours.

So far, studies that specifically studied the effect of the EDTA have been done on rainbow trout. Some other studies investigated the concentrations of EDTA that killed 50% of the fishes in 96 h in some aquatic animals. In other studies, the LC_{50} 96h values for synthetic detergents such as NTA ($(CH_2COONa)3N\text{-}H_2O$) were determined for the *Palaemonetes vulgaris*, 1800 mg L^{-1}, for *Pagurus longicarpus* is 1875 mg L^{-1} and the LC_{50} value at 96 hours in *Stenotomus chrysops* is 2200 mg L^{-1}, in *Homarus americanus* is 3150 mg L^{-1}, *Mytlus edulis* is 3400 mg L^{-1}, in *Nassarius obsoletus* is 5100 mg L^{-1},*Nereis virens,* 5500 mg L^{-1}, *Fundulus heteroclitus,* 5500 mg L^{-1} and *Roccus saxatilis*, 5500 mg L^{-1}. Also, the results of this study and its comparison to the literature finally showed that the range of sensitivity to EDTA is more than that to the NTA.

Table 1: The effect of EDTA on serum biochemistry of *Oncorhynchus mykiss*

Factor	Units	800 mgl^{-1}	1100 mgl^{-1}	1400 mgl^{-1}	1700 mgl^{-1}	2100 mgl^{-1}	Control
BS	mg/dl	59.4±2.41 ^{b}	66.83±2.41 ^{c}	73.8±5.98 ^{d}	79±1 ^{d}	84.33±0.58 ^{e}	54.2±1.92 ^{a}
Ch.	mg/dl	326.2±34.95^{b}	329±25.61^{b}	315.8±19.77 ^{b}	333.67±12.06 ^{b}	277.67±24.42 ^{a}	278.4±18.92 ^{a}
TG	mg/dl	254±63.38^{a}	271±44.46^{ab}	342.2±47.93^{b}	203±66.78^{a}	237±34.22^{a}	261.6±99.21^{ab}
Ca	mg/dl	18.4±0.37^{c}	17.93±0.67^{bc}	17.34±0.21 ^{b}	16.27±0.21 ^{a}	15.73±0.72 ^{a}	19.6±0.32^{d}
Na	meq/l	118±2.34^{b}	116.33±1.63^{a}	116±1.22 ^{a}	115.33±1.53 ^{a}	116±1.73 ^{a}	123.8±0.84^{b}
P	meq/l	6.62±0.27^{b}	6.85±0.23^{bcd}	6.8±0.25 ^{bc}	7.2±0.26 ^{d}	7.17±0.35 ^{cd}	6.24±0.21^{a}
Cl	meq/l	194.6±9.63^{b}	219.83±4.83^{c}	222±30^{c}	271±5.57 ^{d}	282.33±2.52^{d}	119.6±6.11^{a}
Fe	μg/dl	169.4±7.02^{b}	185.5±5.61^{c}	228.4±10.31^{d}	235.67±13.65^{d}	274.33±14.64^{e}	120.8±4.44^{a}
TP	g/dl	4.4±0.12^{b}	4.88±0.2^{c}	5.23±0.19^{d}	5.53±0.12^{de}	5.83±0.21^{e}	3.72±023^{a}
Alb	g/dl	1.9±0.25^{a}	2.4±0.14^{b}	2.9±0.16^{c}	3.17±0.06^{d}	3.33±0.12^{d}	1.78±0.11^{a}

* The similar small Latin alphabetical letters in the same row show that there are significant differences among different concentrations

In this study, the behaviours and reactions of fish were considered in response to different concentrations of the EDTA during the experiment. Our results revealed that most important effect of toxicant was disorder of the nervous and brain systems, which were obvious based on the lack of equilibrium and the spiral swimming patterns. Other apparent toxicity effects include curvature of the spinal column of spinal column, exophthalmia and bleeding in the gill and abdomen area. Similar results have been reported of other pollutants in other fish [13-17].

The effects of EDTA on serum biochemistry are shown in Table 1. Results showed that there were significant differences between the various concentrations at glucose, cholesterol, triglyceride, calcium, sodium, potassium, chloride, iron, total protein and albumin of *Oncorhynchus mykiss* ($P<0.05$).

The effects of EDTA in blood serum biochemical factors on *Oncorhynchus mykiss* have not been documented yet. In this study, we report the acute toxicity and effect of EDTA at low concentration in some blood serum biochemical factors of *Oncorhynchus mykiss* for the first time. The present study showed that exposure to EDTA causes a decrease in sodium, calcium and triglyceride and causes an increase in glucose, cholesterol, potassium, chloride, iron, total protein and albumin values of *Oncorhynchus mykiss* ($P <0.05$). In other studies about pollutants effect on rainbow trout, Bannee *et al.* determined the acute toxicity and evaluated the effect of sub-lethal concentrations of diazinon on some biochemical parameters of blood in rainbow trout *(Oncorhynchus mykiss)* after 7, 14 and 28 days. Their study showed that levels of total protein and albumin were significantly reduced [18]. Similar results were not observed in the present study. Glucose levels in diazinon treated groups were significantly higher than the controlled group at experimental periods. Similar results were observed in the present study.

In conclusion, exposure to EDTA at sublethal concentrations induced biochemical alterations in rainbow trout and offers a simple tool to evaluate toxicity-derived alterations. Finally, the results of this study show that exposure to low concentrations of EDTA changes some biochemical parameters of *Oncorhynchus mykiss*. Finally, the results of this research show that the EDTA, which is one of the most common materials used in the detergents can changes some blood serum biochemical factors of *Oncorhynchus mykiss* even in very low concentration.

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