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Histopathological Studies on Kidneys and Gills of *Onchorhynchus mykiss* Exposed to Sublethal Concentration of Ethylenediaminetetraacetic Acid (EDTA)

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Abstract: Study the effects of sublethal concentrations of EDTA on kidney and gill of *Onchorhynchus mykiss* were conducted in vitro conditions. These experiments were carried out based on the standard TRC, 1984 method during 96 h. Fishes with an average weight 51±12 g and length 18.5±2.1 cm were distributed in seven groups with 800, 1000, 1200, 2000, 2200, 2500 and 3500 mg L^{-1}. The physicochemical parameters of the experiments (PH, total hardness, dissolved oxygen and temperature) were controlled. The toxicity results showed that the 96h LC_{50} values of EDTA was 2231 mg L^{-1}. Pathology results showed that, EDTA cause hyperplasia and hypertrophy of cells lining the gills, congestion, secondary lamellar sticking together, degeneration lamella, increase colorid cell and Pilar loss of cells in gills and increase inflammatory cells between tubules and interstitial tissue, acute nephritis, shrinkage and retraction of the kidney corpuscle, increase the glomerular space, reduce the number of severely damaged tubules to interstitial tissue, fibrosis, necrosis kidney corpuscle, cloudy swelling and hyaline degeneration particles in the kidney. The results of this study show that EDTA damages gill and kidney of the *Onchorhynchus mykiss* and endangers their health.

Key words: Acute toxicity · EDTA · Gill · Kidney · *Onchorhynchus mykiss*

INTRODUCTION

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. Ethylenediaminetetraacetic 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 sequestrant [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, 3]. This has led to the use of EDTA as a preservative in many products. However, studies regarding the influence 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* [4, 5]. EDTA is a widely used acronym for the chemical compound ethylenediaminetetraacetic acid (which has many other names) [6]. This material is used in detergents. Therefore, it can be entered in the water

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environment and aquatic animals, including rainbow trout that lives in cold water 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. For these reasons, present study was conducted to determine the toxicological and histopathological effects of EDTA to *Onchorhynchus mykiss* kidney and gill.

Onchorhynchus mykiss, which belongs to *Salmonidae,* is one of the most economically important and valuable fishes in the world.

Histopathological studies have been conducted to help the establishment of causal relationships between contaminant exposure and various biological responses. Histopathological investigations have proved to be a sensitive tool to detect direct effects of chemical compounds within target organs of fish in laboratory experiments [7, 8]. Histopathology provides a fast method to detect effects of irritants in various organs [9]. The exposure of fish to chemical contaminants is likely to expose a number of lesions in different organs [10]. Kidney and gills are appropriate organs for histological examination in order to determine the effect of pollution [11, 12]. To the best of our knowledge, the effect of lethal concentrations of EDTA on *Onchorhynchus mykiss* has not been reported yet. In addition, toxicological and histopathological effects of EDTA in gill and kidney on *Onchorhynchus mykiss* have not been documented yet. In this study, we report the acute toxicity and effect of EDTA at low concentration in gill and kidney of *Onchorhynchus mykiss* for the first time.

MATERIALS AND METHODS

Experimental Design: 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. The experimental design incorporated eight groups (seven test group and control group) of that *Onchorhynchus mykiss* were exposed to 800, 1000, 1200, 2000, 2200, 2500 and 3500 mg L^{-1} of EDTA. Fish were exposed for 96 h. Each treatment group and control consisted of a single fiberglass test tank containing 10 fish. Fish were randomly distributed in each test tank had the correct number of fish. Each test tank had its own water supply line through which water of the appropriate temperature entered; there was no water recirculation or reuse. Fishes were exposed with different concentrations for 96 h. 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 [13] method.

Fish: 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 tank (10 fishes/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 sublethal treatment concentration was calculated from percentage mortalities of fish as described by Veena and Chacko [14].

Test Water: Water chemistry characteristics of temperature, dissolved oxygen and PH were determined in all test tanks every day. Mean dissolved oxygen ranged from 7.5 to 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 $CaCO_3$.

Histological Methods: After completing 96 h of exposure, fishes from each of group exposed fish and fishes from control groups were anesthetized with 200 ppm of clove oil. Then, to conduct the histopathology studies kidney and gill, were carefully removed and preserved in 10% neutral-buffered formalin (NBF) for 48 h. Organs were rinsed in 2 changes of 50% ethanol (EtOH) and stored in 50% EtOH until further processing. They were dehydrated in isopropanol, cleared in xylene, infiltrated in paraffin and sectioned at a thickness of 5 µm by the machine Shandon 315. Sections were stained with hematoxylin and eosin (E & H) and examined with a light microscope [13, 15].

Statistical Analysis: After obtaining the final results, the information was 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 LC10 in 96 h and NOEC (Non Observed Effect Concentration) [16].

RESULTS

Acute Toxicity: The acute toxicity results showed that LC_{10}, 50, 90 values after 96 h of EDTA were calculated on *Onchorhynchus 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}. In this study, the behaviours and reactions of the fish were evaluated in response to different concentrations of the EDTA. The results revealed that *Onchorhynchus mykiss* immediately reacted to high concentrations of EDTA and they constantly moved fast until they got tired and fell to the bottom of breeding tank. When placed in low concentration, the fish did not react immediately in a significant way. The most important effect of the EDTA was alterations of the nervous and brain systems, which were obvious with lack of equilibrium and spiral swimming pattern of fish; other effects include curvature of spinal column, exophthalmia and bleeding in the gill and abdomen area.

Histopathology

Gill: The results gained from studying the effects of EDTA on the gill of the *Onchorhynchus mykiss* showed that EDTA cause hyperplasia and hypertrophy of cells lining the gills, congestion, secondary lamellar sticking together, degeneration lamella, increase colorid cell and Pilar loss of cells in gills (Figures 1-5). According to these results, tissue damage is more severe when fishes exposed to the highest amount of EDTA and the least effects were seen when were exposed to the lowest amount to EDTA. So, at higher concentrations of EDTA hyperplasia, congestion sever, metaplasia and Pilar loss of cells were observed.

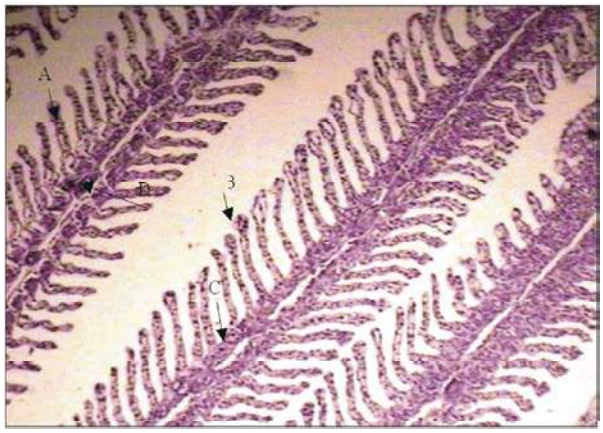

Fig. 1: Rainbow trout gill blades of the primary in Control group (H&E, X 40)

A) Red Blood Cells, B) Secondary gill blades, C) Cells between the blade and D) Cartilage back up

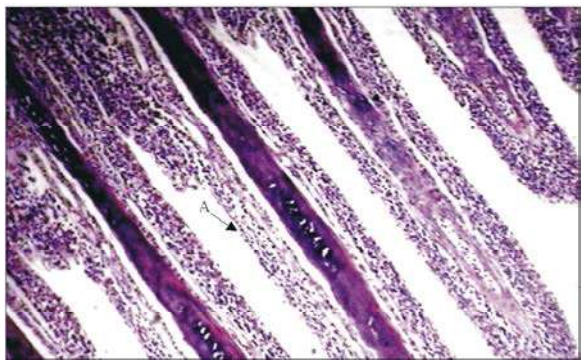

Fig. 2: A) Degeneration lamella in gill at 2000 mg L^{-1} of EDTA (H&E, X 40)

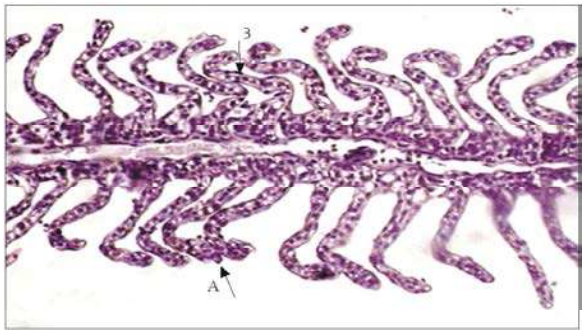

Fig. 3: A) Degeneration secondary lamella and B) Epithelial cell hypertrophy (H&E, X 100)

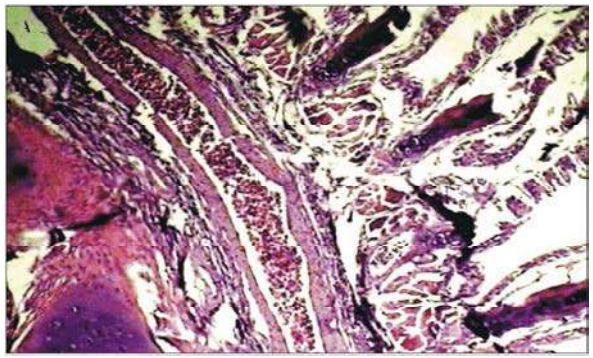

Fig. 4: A) Congestion severe in gill at 2200 mg L^{-1} of EDTA (H&E, X 40)

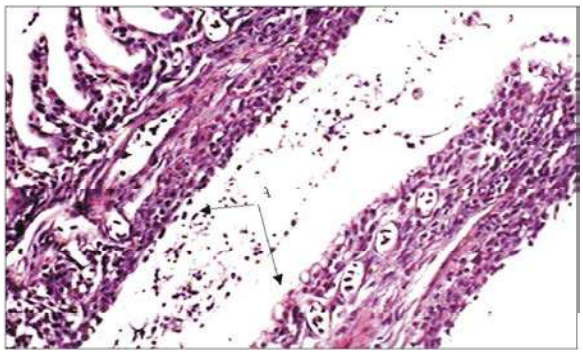

Fig. 5: A) Sever hyperplasia and necrosis (H&E, X 40)

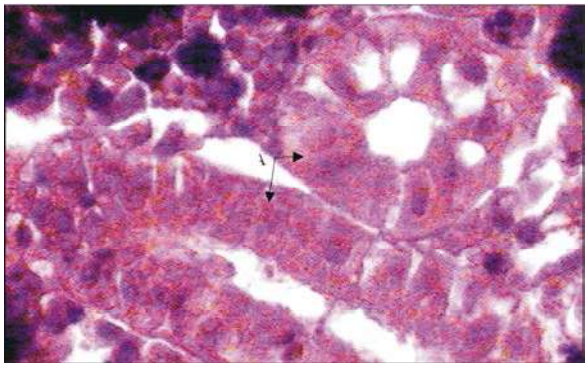

Fig. 6: A) Control kidney tubules (H&E, X 100)

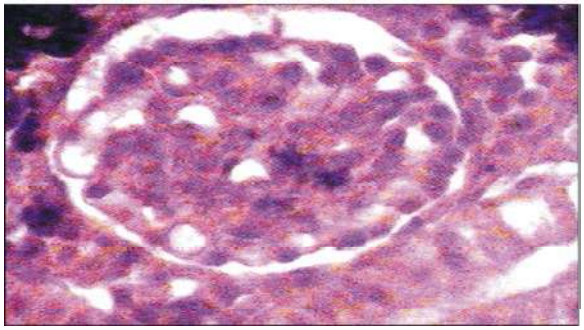

Fig. 7: Control kidney glomeruli (H&E, X 100)

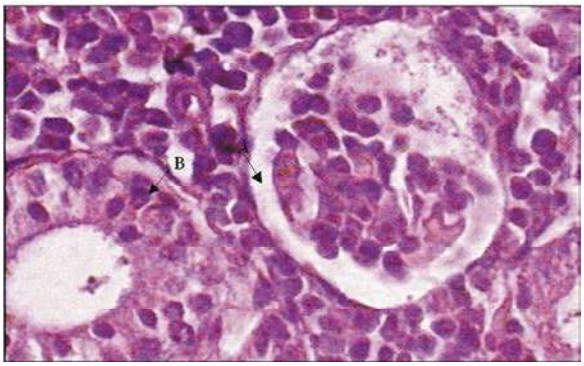

Fig. 8: A) Increase in interstitial spaces due to destruction by inflammatory cells and B) inflammatory cells in kidney (H&E, X 40)

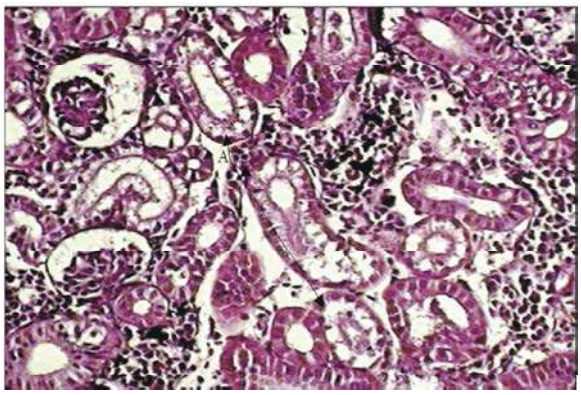

Fig. 9: A) Cloudy swelling (H&E, X 40)

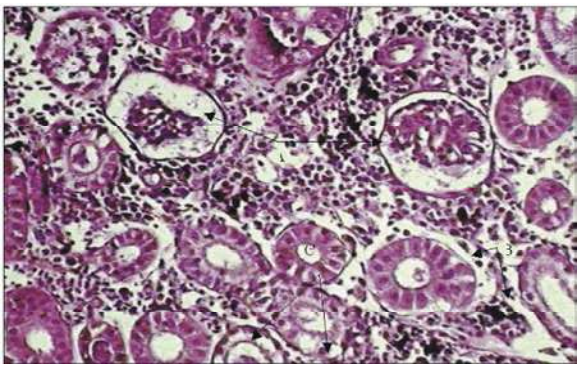

Fig. 10: A) Destruction of the glomeruli, B) Increase in interstitial tissue due to acute inflammation and C) Destruction of tubules in kidney (H&E, X 40)

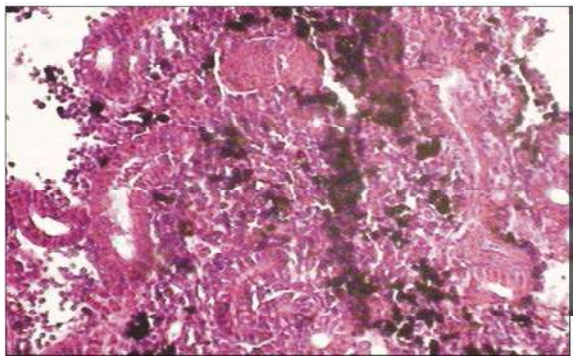

Fig. 11: A) Necrosis (H&E, X 10)

Kidney: The results with regard to the effects of EDTA on the kidney of *Onchorhynchus mykiss* showed that EDTA made changes in tissue. However, compared with control, some changes such as increase inflammatory cells between tubules and interstitial tissue, acute nephritis, shrinkage and retraction of the kidney corpuscle, increase the glomerular space, reduce the number of severely damaged tubules to interstitial tissue, fibrosis, necrosis kidney corpuscle, cloudy swelling and hyaline degeneration particles were observed in the kidney (Figures 6-11). As can be clearly seen in Figures 6-11 the higher the amount of the EDTA, the more severe the above mentioned effects will be in the kidney of the fish.

DISCUSSION

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 as 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 *Sternotomies chrysops* is 2200 mg L^{-1}, in *Homarus americanus* is 3150 mg L^{-1}, *Mytilus 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.

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 by Barak [4], Mance [17], Zamini, [18], Alinezhad, [19] and Mirzaie, [12] in other fish.

Histopathological study is a valuable method for assessment of effects of environmental contaminants on fish. In vitro, different pollutants are causing tissue damage in fish organs. They can determine this type of damage as a biomarker to evaluate the presence of pollutants in natural ecosystems [20]. Histological analysis is sensitive and powerful tool to determine cellular change that may occur in vital organs [7]. Kidney [21] and gills [22] are appropriate organs for histological examination in order to determine the effect of pollution [23]. The exposure of fish to lethal and sublethal concentration of pesticides or other chemical contaminants in their environment may cause various histological changes in tissues [24, 25]. Fish gill histopathology is an indicator of chemical toxicity. It is a helpful way to study the effects of exposure of aquatic animals to toxins present in the aquatic environment [8].

The gill of fish is most sensitive and very vulnerable organs because of transportation of respiratory gases and regulation of osmotic and ionic balance. The gills are suitable organ for the primary lesions and systemic infections [26]. Gills damage due to toxic agents causes a chain of destructive events, which may cause respiratory distress [27]. In the present study, some important histological lesions were observed on gills of the rainbow trout which were exposed to EDTA. Our results show that exposure of rainbow trout to EDTA results in structural alterations of the gill lamellae including hyperplasia and hypertrophy of cells lining the gills, congestion, secondary lamellar sticking together, degeneration lamella, increase colorid cell and Pilar loss of cells. Histologically results were observed in rainbow trout when exposed to captan, such as hypertrophy and necrosis of epithelium, separation of epithelium from lamellae (epithelial lifting), lamellar fusion, hyperplasia of lamella and the space under the epithelium filled with eosinophilic material in gills [23]. Similar findings were also observed by Boran *et al.* [23] and Sunitha and Sahai [28]. The results of these studies clearly indicate that sublethal concentrations of pollutants have diverse effects on fish gills. Gills were found to be the most seriously affected organs compared to trunk kidney, perhaps because of the direct contact with the EDTA [23].

Kidney is one of the major organs. It is important to maintain proper water balance and organization of the ions. Kidney not only is responsible for waste removal in the blood but also with the materials necessary to adjust the blood volume, pH of blood and body fluids helped produce the enzyme rennin in the blood pressure is also effective. Kidney of fish is one of the first organs affected are the face of infection quickly. Gills are a target organ for many chemicals, heavy metals and toxins. Contact the chemicals cause damage to or necrosis of renal tubular epithelial cells, especially in fish that have been placed in long-term contact with chemical. In the present study, histological lesions including, increase inflammatory cells between tubules and interstitial tissue, acute nephritis, shrinkage and retraction of the kidney corpuscle, increase the glomerular space, reduce the number of severely damaged tubules to interstitial tissue, fibrosis, necrosis kidney corpuscle, cloudy swelling and hyaline degeneration particles (A significant and irreversible changes in the tissue) were observed in the kidney of *Onchorhynchus mykiss*. Histologically results were observed in rainbow trout when exposed to captan, such as tubular necrosis, shrinkage of glomeruli, renal tubular degeneration and hyaline droplets degeneration in trunk kidney [23]. Similar findings were also observed by Dalela *et al.* [6]. Altinok and Capkin [24] found the same result when rainbow trout was exposed to methiocarb and

endosulfan. Also, Sharifpoor *et al.* [29] found the same result in kidney when *Ctenopharyngodon idella* was exposed to Diazinon. Also, same results were found in kidney when *Cyprinus carpio* was exposed to endosulfan [30].

The results of this research showed that EDTA even in low concentration damages various tissues in the body of the *Onchorhychus mykiss* and threatens their health. Finally, the results of this research show that the EDTA, which is one of the most common materials used in the detergents can damage different parts of the body of *Onchorhynchus mykiss* even in very low concentration.

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