Role of fulvic acid on the reduction of cadmium toxicity on tilapia (*Oreochromis niloticus*)

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Abstract

The effect of fulvic acid on cadmium (Cd) toxicity, the impact on fish immunological, and haematological changes in Nile tilapia (*Oreochromius niloticus*) were studied. The fish (100±10g) were exposed to 10 ppm Cd alone or with 0.1, 0.2 and 0.3 ppm for 15 and 45 days. Cd exposure reduced significantly ($P<0.04$), for example the erythrocyte count (RBCs), haemoglobin content (Hb), haematocrit value (Hct), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration. These parameters were improved when fulvic acid was applied with Cd. The values of RBCs, Hb, Hct, MCH and MCHC were increased significantly in the control fish group. The addition of fulvic acid to Cd contaminated medium considerably reduced metal absorption and accumulation in fish tissues, while metals in water and faeces increased. Fish exposed to Cd alone accumulated 2.15 and 5.970 mg Cd/g dry weight in the liver tissue over 15 and 45 days, respectively. Cd reduced significantly to 1.292 and 4.16.; 0.92 and 3.791; and 0.41 and 2.43 mg Cd/g dry weight tissue in fish exposed to 0.1, 0.2 and 0.3g fulvic acid/L over 15 and 45 days, respectively. Similar trends were observed in gills and musculature.

Keywords: Nile tilapia, cadmium, immunological, fulvic acid, haematology, liver, gills, musculature.

1 Introduction

Nile tilapia are considered the most popular widely distributed, cheapest and intensively cultured fish in Egypt. The clinical picture in naturally infested and polluted Tilapia sp was revealed, some were aggregated on the water surface, and accumulated at the water inlet of the pond and air pump of aquaria. Almost all appeared dull with a loss of escape reflex (Eissa et al. [1] and Eaton and

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Stinson [2]). The present study reveals that the fish exposed to Cd alone showed significant reduction in RBCs, Hb and Hct, compared with those exposed to Cd with different levels of fulvic acid. The reduction of these parameters in Nile tilapia, O niloticus, at sublethal levels of cadmium might be due to the destruction of mature RBCs and the inhibition of erythrocyte production due to reduction of haemsynthesis, which is affected by pollutants (James and Sampath [3]). Also, the decrease in the RBC count may be attributed to haematopathology or acute haemolytic crisis that results in severe anaemia in most vertebrates, including fish species exposed to different environmental pollutants (Yamawaki et al. [4]) or it may be that the decrease in RBCs can attributed to the reduction of growth and other food utilization parameters, which results in sever anaemia (Wintrobe [5]). Moussa [6] found a significant decreased in total erythrocyte count, haemoglobin content, haematocrit value and mean corpuscular haemoglobin concentration in air breathing fish, Channa punctatus, after exposure to a sublethal dose of Cd (29 mg Cd/L). The addition of fulvic acid improves the haematological parameters (RBCs, Hb and Hct), which indicates the capability of fulvic acid to chelate Cd from the media. Subsequently, the Cd toxicity was reduced. These results are in agreement with those of Snedecor and Cochran [7], who observed that *Oreochromis mossambicus* exposed to copper along with fulvic acid showed a significant improvement in blood parameters over those of copper alone. The calculated blood indices MCV, MCH and MCHC have a particular importance in anaemia diagnosis in most animals (Coles, 1986). The perturbations in these blood indices (increased MCV, decreased MCH and MCHC) may be attributed to a defence against Cd toxicity through the stimulation of erythropiosis or may be related to the decrease in RBCs, Hb and Hct due to the exaggerated disturbances that occurred in both metabolic and hemopoietic activities of fish exposed to sublethal concentration of pollutants (Huang et al. [8]). The present results indicate that fulvic acid is effective in removing Cd from water, and reducing Cd bioaccumulation in fish. Particulate organic matter can scavenge metal from water and help to reduce metal from fish. These results are in agreement with Shalaby [9], whose study shows that any agent that can remove Cd from water helps to reduce the bioaccumulation of this metal in fish. The present study showed that the addition of fulvic acid to the Cd media reduced significantly ($P<0.05$) the Cd level in water and metal uptake as compared to fish exposed to Cd alone. The Cd concentration in water was 9.31 mg/L and it decreased significantly ($P< 0.05$). The Cd accumulation in the liver, gills and muscle of fish exposed to Cd alone was higher than that of fulvic acid. These results suggest that fulvic acid could chelate Cd ions producing a stable complex, thus reducing the chance for metal uptake by tissues. Besides, the fulvic acid eliminated a greater amount of Cd from the body through faeces. The formation of a Cd-fulvic acid complex in water and the elimination of a greater amount of Cd in faeces evidently reduced the metal burden in tissues and thereby improved the haematological parameters of fish exposed to Cd. Planas-Bohne and Lehman [10] found a low level of cadmium in tissues due to increased excretion of metals through faeces and urine when rats were administered Cd intravenously along with fulvic acid (Table 1).

Table 1: Changes in cadmium residue in water (mg Cd/L), liver, gills, musculature and faeces (mg Cd/g dry weigh) of Nile tilapia (O. niloticus) exposed to Cd with or without fulvic acid (FA).

Items	Water	Liver		Gills		Musculureate		Faeces	
Period/days		15	45	15	45	15	45	15	45
Control	0.041	0.048 ^{a}	0.055 ^{a}	0.038 ^{a}	0.039 ^{a}	0.023 ^{a}	0.076 ^{a}	0.003 ^{a}b	0.005 ^{a}b
Cd	± 0.02 9.31	± 0.02 2.15 ^{b}	± 0.004 5.971 ^{b}	± 0.02 1.36 ^{b}	± 0.04 2.56 ^{b}	± 0.002 0.476 ^{b}	± 0.005 1.077 ^{b}	±0.018 0.153 ^{b}	± 0.02 0.189 ^{b}
Cd+0.1g	± 0832 7.15	± 0.253 1.292 ^{c}	± 0.85 4.16 ^{b}	± 0.085 0.65 ^{c}	± 0.276 1.07 ^{c}	± 0.06 0.343 ^{cb}	± 0.15 0.665 ^{c}	± 0.018 0.940 ^{c}	± 006 2.067 ^{c}
FA /l Cd+0.2g	± 0.34 3.78	± 0.056 0.95 ^{d}	± 0.45 3.791 ^{b}	± 0.06 0.394 ^{d}	± 0.11 0.85 ^{c}	± 0.04 0.33 ^{cb}	± 0.021 0.383 ^{d}	± 0.03 2.34 ^{d}	± 0.143 5.443 ^{d}
FA/l Cd+0.3g	± 0.01 1.73	± 0.054 0.42 ^{e}	± 0.29 2.45 ^{c}	± 0.052 0.266 ^{d}	± 0.06 0.71 ^{c}	± 0.08 0.216 ^{c}	± 0.034 0.217 ^{d}	± 0.069 5.282 ^{e}	± 0.345 7.456 ^{e}
FA/l	± 0.02	± 0.034	± 0.23	± 0.073	± 0.42	± 0.03	± 0.025	± 0.32	± 0.528

2 Polluted tilapia….why?

Cadmium is one of the most toxic heavy metals that enters the environment from natural sources and as a result of man's activity, such as recycling of scrap metal, electroplating, industry manufacturing vinyl plastics, electrical contacts, metallic and plastic pipes. Tilapia have the capability of concentrating metals by feeding and metabolic processes, which can lead to the accumulation of high concentrations of metals in their tissues. The reduction of toxic elements, such as cadmium, in aquatic environments is needed by any acceptable method.

2.1 Are there solutions?

The most widely used technique for the removal of toxic elements involves the process of neutralization and metal hydroxide precipitation (Hiemesh and Mahadevaswamy [11]). Chemicals can effectively remove certain toxic elements from industrial wastes or polluted media, but it is usually costly. However, there are some cheap natural products that are also free from undesirable side effects. In recent years, the remobilization of metals by synthetic anthropogenic chelating agents has received much attention. The literature reported a number of chelators that have been used for chelate-induced hyper accumulation (Khangarot and Tripathi [12]). Natural compounds, such as fulvic acid, are known to be effective chelating agents of heavy metals (Karuppasamy [13]).

2.2 Why fulvic acid?

Fulvic acid is the most commonly used chelator because of its small molecular weight and strong chelating ability for different heavy metals (Litchfield and

Wileoxon [14] and Donor and William [15]). Metal bioaccumulation can occur via complication, coordination, chelation, ion exchange and other processes of greater or lesser specificity. Bioaccumulation processes are sometimes due to active (metabolism dependent) metal accumulation by living cells. In spite of the amount of data published on the effect of water borne exposure of cadmium and fulvic acid singly, information on the effects of a Cd/fulvic acid mixture on aquatic organisms are limited and not uniform. Therefore, fulvic acid appears to be a promising tool to control cadmium pollution in aquaculture. The present study, short- and long-term bioassays were designed to evaluate the influence of fulvic acid on the retention of cadmium in water. It was carried out to investigate the effect of fulvic acid on the reduction of the toxicity of cadmium, to enhance the change of blood parameters and enzymes and to assess its impact on some physiological parameters of Nile tilapia *(Oreochromis niloticus)*.

3 Practical procedures

The present study showed that the addition of fulvic acid to Cd contaminated media reduced significantly the Cd level in water and helped to eliminate Cd from the fish body, which in turn improved the clinical signs and the haematological parameters as compared to fish exposed to cadmium alone.

3.1 Collecting sample tilapia

A healthy 75 fish of Nile tilapia *Oreochromis niloticus* weighing (100±10g)/fish were collected from the ponds of Kafr Eel Sheikh governorate fish farms, Egypt. Fish were acclimated in cement fish ponds for two weeks. Acclimated fish were exposed to different concentrations of cadmium and mortality was observed for 96-h. A static renewable bioassay method (Duncan [16]) was adopted for the determination of 96-h median lethal probity analysis; Santschi [17] was followed for the calculation of 96 hr LC_{50}. A control group was maintained in metal-free tap water. The 96 hr LC_{50} of cadmium for *Oreochromis niloticus* was 40 ppm. A stock solution of cadmium was prepared by dissolving 10.686 g of annular grad cadmium sulphate ($CdSO_4$– 8/3H_2O) in 1/L of distilled water and diluted with water to obtain the desired concentration (10 ppm) for this experiment. The fish were distributed randomly in five cement ponds at a rate of 15 fish/aquarium, which containing aerated tap water. These aquaria were divided into five groups with three replicates each per group. Fish were fed frequently on a diet containing 25% crude protein (CP) at a rate of 3% of live body weight twice daily for 15 and 45 days. Siphoning of three quarters of the aquariums was done every day for waste removal and it was replaced by an equal volume of water containing the same concentration of Cd and fulvic acid. Dead fish were removed and recorded daily.

3.2 Sample classification

The first group was free of Cd and fulvic acid and maintained as a control. The second group was exposed to 10 ppm of Cd SO_4 only. The third, fourth and fifth groups were exposed to 10 mg Cd/L and 0.1, 0.2 and 0.3 g fulvic acid/L, respectively. Each aquarium was supplied with compressed air via air-stones from air pumps. A well-aerated water supply was provided from a storage fibreglass tank. The temperature was adjusted at 27±1^{o}C by means of thermostats (Table 2).

Table 2: Field experimental groups and their notation.

S. No.	Groups in field ponds	Nation
1	Control (metal free water)	C
2	Cadmium (10 ppm) alone	Cd
3	Cadmium (10 ppm) +0.1g fulvic acid /l	Cd fulvic acid 1
4	Cadmium (10 ppm) +0.2 fulvic acid /l	Cd fulvic acid 2
5	Cadmium (10 ppm) +0.3g fulvic acid /l	Cd fulvic acid 3

3.3 Cd residue

Cadmium sulphate and fulvic acid was obtained from the El-Nasr chemical and Grotech companies (Egypt), respectively, and prepared in aquatic solution to provide the required concentrations of cadmium and fulvic acid. Cadmium was measured in the water, liver, gills, musculature and faeces according to method of Norvell [18].

3.4 Statistical analysis

The obtained data were subjected to analysis of variance between means and were done at the 5% probability level, using Duncan's new multiple range test by Spraggue [19].

4 Results

The present study showed that addition of fulvic acid to Cd contaminated media reduced significantly the Cd level in the water and helped to eliminate metal from the fish body (liver, gills, musculature and faeces) and in turn improved the biochemical parameters as compared to fish exposed to Cd alone (see Table 2).

4.1 Clinical examination

The clinical examination of most examined fish showed asphyxia, some aggregated on the surface, accumulated at the water inlet of the pond and the air pump of aquaria. Others appeared dull with loss of escape reflex.

4.1.1 Haematological parameters

The results of erythrocyte count (RBCs), haemoglobin content (Hb) and haematocrit value (Hct) obtained from the fish exposed to a sublethal dose of Cd

Table 3: Changes in mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) in the blood of Nile tilapia (O. niloticus) exposed to Cd with or without fulvic acid (FA).

Items	MCV		MCH		MCHC	
Period	15 days	45 days	15 days	45 days	15 days	45 days
Control	95.32^{ad}	100.02^{a}	34.35^{a}	43.21^{a}	34.77^{a}	43.32^{a}
	± 1.86	± 2.243	± 0.342	± 1.432	± 1.121	± 0.928
Cd	106.93^{b}	106.75^{b}	33.02^{b}	36.56^{b}	31.15^{b}	37.27^{b}
	± 2.23	± 0.874	± 0.177	± 0.846	± 0.909	± 1.85
Cd+0.1 g	93.45^{a}	95.71^{a}	34.52^{a}	32.45^{b}	37.21^{a}	33..77^{b}
FA/l	± 2.05	± 4.26	± 1.23	± 1.17	± 1.26	± 1.49
Cd+0.2 g	96.24^{a}	98.77^{a}	33.23^{a}	36.76^{bc}	34.06^{ac}	37.40^{b}
FA/l	± 2.64	± 0.909	± 1.72	± 1.49	± 1.76	± 1.20
Cd+0.3 g	101.1^{db}	107.95^{b}	32.85^{a}	37.02^{ac}	33.34^{cb}	34.87^{b}
FA	± 2.512	± 2.241	± 1.702	± 1.576	± 0.941	± 1.68

Table 4: Changes in erythrocyte (count x 106/mm3), haemoglobin content (g/100ml) and haematocrit value (%) in the blood of Nile tilapia (O. niloticus) exposed to Cd with and without fulvic acid (FA).

Items	Erythrocyte count (RBCs)		Haemoglobin (HB)		Haematocrit value (Hct)	
Period	15 days	45 days	15 days	45 days	15 days	45 days
Control	1.58^{a}	1.714^{a}	5.48^{a}	7.315^{a}	15..31^{a}	17..32^{a}
	± 0.073	± 0.051	± 0.353	± 0.133	± 0.308	± 1.665
Cd	1.268^{b}	1.06^{b}	4.21^{b}	4.12^{c}	13..5^{b}	12.01^{b}
	± 0.064	± 0.073	± 0.235	± 0.354	± 0.47	± 0.576
Cd+0.1g	1.572^{a}	1.57^{d}	4.54^{ab}	5.12^{b}	14.66^{a}	15.05^{a}
FA/l	± 0.064	± 0.023	± 0.395	± 0.136	± 1.454	± 0.76
Cd+0.2g	1.56^{a}	1.786^{ac}	5.17^{ab}	6.605^{b}	15.02^{a}	17.65^{a}
EDTA/l	± 0.086	± 0.032	± 0.458	± 0.305	± 1.72	± 0.916
Cd+0.3g	1.956^{c}	2.01^{c}	6.464^{c}	7.68^{a}	20.0^{c}	22.02^{c}
FA/l	± 0.086	± 0.063	± 0.277	± 0.133	± 0.365	± 1.471

(10 mg/l) alone or with different doses of fulvic acid are given in Table 4. Table 3 shows that the RBCs, HB and HCt were reduced in fish exposed to Cd over both periods and they were less than that of the control ($P<0.05$) The RBCs count decreased significantly in fish exposed to Cd at 15 and 45 days. On the other hand, these parameters returned to the normal values and increased significantly in fish exposed to Cd with 0.2 and 0.3 g of fulvic acid/L for 15 and 45 days. These values increased significantly in fish exposed to Cd with 0.3 g fulvic acid/L. Blood parameter were improved in fish exposed to Cd with different levels of fulvic acid. The blood indices calculated from the mean values of blood parameters for the aforementioned treatments are given in Table 3.

Data shows that the MCV increased significantly in fish exposed to Cd alone, while the MCH and MCHC decreased significantly in fish exposed to Cd only when compared with the control. These parameters increased with increasing of exposure time of fish to Cd. The addition of fulvic acid to Cd-polluted media maintained the MCV, MCH and MCHC at levels close to those of the control.

5 Recommendation

From the present study, it is recommended that an optimum dosage of 0.3 g fulvic acid/L can effectively chelate Cd from contaminated water. Hence, a scientific method of detoxification is essential to improve the health of fish in any stressed environmental conditions.

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