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Abstract. In order to define eco-toxicity effect of Ampicillin wastewater on zebrafish, the indoor exposure method was used to study the impact of Ampicillin wastewater on zebrafish. In this study, zebrafish was exposed to ampicillin wastewater of 0.5%, 1.0%, 1.5% and 2.0% groups for 15 days to study the effect of T-AOC（Total Antioxidative Capacity） and LDH（Lactate Dehydrogenase）activities. According to the experimental data, the T-AOC and LDH activities in zebrafish muscle tissue had changed significantly during the period of exposure. The experimental results showed that the T-AOC value of zebrafish in each exposed group showed a "Λ" type change trend. On the 6th day, the 2.0% exposed group reached the maximum value of the whole test period (6.5399U•mg^{-1}). The change of LDH activity was similar to that of T-AOC activity, showing a trend of "sharp increase--decline". Among them, on the 12th day, the 1% exposed group reached the maximum (792.28 U•mg^{-1}). The research shows ampicillin wastewater can cause some degree of oxidative damage to zebrafish.

1. Introduction

Antibiotics are compounds that can prevent and treat human and animal diseases. They are often added to animal feed to promote animals growth and development[1]. At present, China's antibiotic production accounts for 20%-30% of the world's total production[2]. The utilization rate of antibiotic production materials is low, the composition of antibiotic wastewater is complex, and the displacement is large. The reference displacement of antibiotics is between 500 and 6500 cubic meters[3]. According to reports, in the effluent of antibiotic wastewater, the concentration of antibiotics is still as high as tens of milliliters per gram[4]. When antibiotic wastewater is discharged into the environment, it will cause serious pollution to surface water[5], groundwater[6] and soil[7]. Due to the "pseudo-persistence" of antibiotics, antibiotics often have potential hazards to the environment[8].

Ampicillin is a broad-spectrum and semi-synthetic penicillin. It is widely used in clinical treatment because of its broad antibacterial spectrum, strong antibacterial activity and low toxicity [9]. In recent years, the emergence of drug-resistant strains has reduced their antibacterial activity, so they are usually used in combination with other drugs to achieve therapeutic effects[10].

Zebrafish is a model organism which widely used in environmental ecotoxicology research and chemical toxicity evaluation[11]. Total antioxidative capacity (T-AOC) is a comprehensive indicator for measuring the functional status of the body's antioxidant system. It can represent and reflect the compensatory capacity of the body's antioxidant enzyme system and non-enzymatic system for

external stimulation. It aslo can express the state of free radical metabolism in the body[12]. Lactate dehydrogenase (LDH) is present in various tissues and organs of the body, mainly distributed in cells. It is an important enzyme involved in glycolysis in energy metabolism of the body. When the tissues and organs of the body are damaged, its tissues and organs LDH changes, and it can also cause changes in LDH in the blood[13].

In this study, ampicillin wastewater used as the research object, its determination in cell level on zebrafish muscle tissue T-AOC and LDH activities, determine the ecological effects of ampicillin wastewater sodium on aquatic organisms, and provide the scientific basis for ecological risk assessment.

2. Materials and Methods

2.1. Experimental Material

2.1.1. Experimental Equipment and Instrument UV-visible light spectrophotometer (UV-2550); analytical balance (EL204); Pipette (Pipet-Lite; TopPette Pipettor); hypothermia refrigerator (BBC-226STV)；whirlpool mixer (XW-80A); high speed centrifuge (TG16-WS); water bath kettle (DK-S26); thermometer;measuring cylinder; manual glass homogenizer 5L glass aquarium;aeration device; heating rod.

The kit of T-AOC and LDH were purchased from Nanjing biological engineering research institute.

2.1.2. Test Organisms Zebrafish were obtained from Hebei Medical University. Mean length of zebrafish used was 30±2mm. Mean weight of zebrafish was 0.3g±0.05g. Zebrafish was stop fed before entering the laboratory, put the fish in the 5% salt water to disinfection. Feed the fish with fully aerated dechlorinated tap water in the laboratory for 7d. The experimental water meets the “fishery water quality standard (GB11607-1989)”.

2.2. Experimental Method

The concentration of ampicillin wastewater on the subacute toxicity test of zebrafish was set according to the equivalence series, four volume concentration groups and one blank control group.The experimental concentrations of the four groups were set as 0.5%, 1%,1.5% and 2.0%, respectively. The experiment period was 15 days. Each concentration group had 3 parallel groups. The experimental liquid was added to 5 L glass fish tank. 25 zebrafish were put into every fish tank at random and measured zebrafish muscle tissue T-AOC and LDH indicator at 3d, 6d, 9d, 12d, 15d. Keep the experimention solution pH at 7-8, the temperature of water at 22-24℃. During the experiment, change the experimental solution every day.

2.3. Assay Method

Take two or three zebrafish from the exposure concentration group, dissect them, then take the muscle tissue 0.18±0.02g. Rinsed in normal saline and placed in a glass homogenizer. Using a pipetting， take 9 times pre-cooled 0.9% saline at a volume ratio of 1:9 (W/V), pour the muscle tissue of the fish into a glass homogenizer, homogenize in an ice bath, and then pour into a common centrifuge tube to prepare for centrifugation. The ground 10% zebrafish muscle tissue homogenate was centrifuged for 10 min (3000 r•min^{-1}) in a centrifuge, and stored in a refrigerator at 4 ℃ with the remaining 10% supernatant. T-AOC and LDH Activities in samples were estimated by the method of T-AOC test kit (UV spectrophotometry) and LDH test kit.

2.4. Statistical Analysis

Statistical results are expressed as mean ± SD of three sets of parallel data. The experimental data were analyzed by one-way ANOVA using SPSS19.0 statistical software. All figures are drawn using the software Origin 9.0. Significant difference analysis was made between the two groups on the same

day by the least significant difference (LSD) method. 0.01<P<0.05 means significant difference; P<0.01 indicates that the difference is significant.

3. Results and Discussions

3.1. Effect of ampicillin on the T-AOC activity in muscle of zebrafish

Effect of ampicillin on the T-AOC activity in zebrafish muscle tissue was shown in Figure 1.

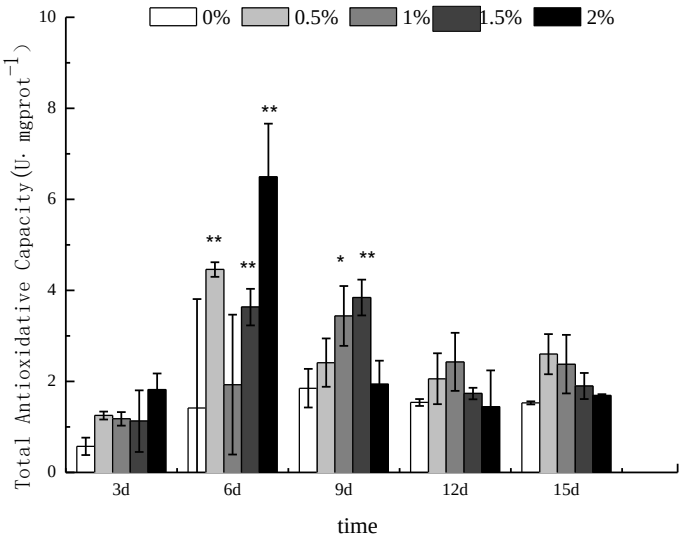

Figure 1. Effect of ampicillin on the T-AOC activity in zebrafish muscle tissue
The same day all the data are compared with control group, *p <0.05, ** p<0.01.

As shown in Figure 1, with the prolongation of exposure time, the T-AOC activities of each exposed group showed different trends. On the 3rd day, the T-AOC activity of each exposed group was higher than that of the control group, and the 2% exposed group was significantly induced (0.01<p<0.05). This may be due to the high concentration of ampicillin wastewater causing oxidative stress in zebrafish, which greatly improves the total antioxidant capacity. On the 6th day, each exposed group was induced to different degrees, among which 0.5%, 1.5%, 2% exposed group was highly significantly induced (p<0.01), and 2.0% exposed group reached the maximum value of the whole test period (6.5399U•mg^{-1}), indicating that the body has cleared a large amount of free radicals through its own antioxidant system, Total antioxidant capacity of zebrafish reaches its peak. On the 9th day, compared with the control group, the 1% exposed group was significantly induced (0.01<p<0.05), the 1.5% exposed group was significantly induced T-AOC activity (p<0.01). It is consistent with the experimental results of the effect of Streptomycin wastewater on T-AOC activity in zebrafish, in this experiment, Wang et al. find high concentration of Streptomycin wastewater significantly effect the activity of T-AOC at 9th day[14]. There was no significant difference in the 0.5% and 2% exposed group (p>0.05). It shows that with the increase of time, the antioxidant system of zebrafish in the 0.5% exposed group and the 2.0% exposed group exerts its effect and reaches equilibrium. From the 12th to the 15th day, there was no significant difference in T-AOC activities in each exposed group compared with the control group (p>0.05). This may be because zebrafish gradually adapt to ampicillin wastewater, zebrafish body's antioxidant system in all test groups returned to dynamic equilibrium.

Overall, the T-AOC values of zebrafish in each exposed group showed an "Λ" shape. Under wastewater stress, the fish body produces a large amount of antioxidant substances to resist damage to the body by foreign substances. As time increasing, T-AOC activity drops to normal levels, indicating that the antioxidant system plays its role and reaches equilibrium.

3.2. Effect of ampicillin on the LDH activity in muscle of zebrafish

Effect of ampicillin on the LDH activity in zebrafish muscle tissue was shown in Figure 2.

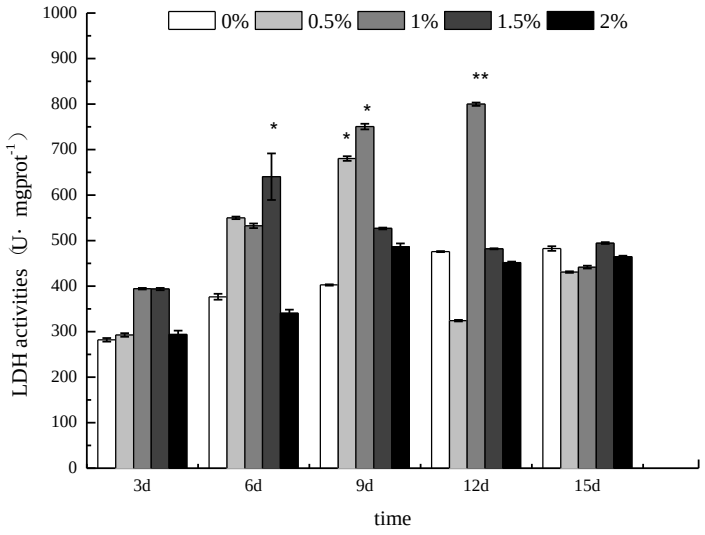

Figure 2. Effect of ampicillin on the LDH activity in zebrafish muscle tissue

According to Figure 2, at the early stage of exposure (3d), the LDH activity of each exposed group was higher than that of the control group, but there was no significant difference ($p>0.05$). In the middle of exposure(6～12d), the LDH activity of each exposed group was induced to different degrees compared with the control group, except that the 0.5% exposed group was lower than the obvious control group ($0.01<p<0.05$) at 12d. The 1% exposed group reached its maximum value at 12d (792.28 U•mg^{-1}), This indicates that the body is under oxidative stress and produces an oxidative stress reaction, which increases the energy metabolism of the body by increasing the activity of LDH. At the end of the exposure period (15d), the LDH activity in each exposed group returned to the dynamic equilibrium level, which was not significantly different from the control group ($p>0.05$), indicating that the fish body was adapted to the ampicillin wastewater environment.

From the exposed group concentration, in low-concentration wastewater (0.5% exposed group), zebrafish muscle LDH activity was induced and increased continuously during the middle exposure period (3~9d), indicating that the body needs to enhance LDH activity to increase energy metabolism due to wastewater stress. With the increase of exposure time, LDH activity was significantly inhibited at 12d ($0.01<p<0.05$). At 15d, LDH activity increased again. Compared with the control group, there was no significant difference ($p>0.05$), indicating the body. In this concentration of wastewater has been adapted to restore the level of dynamic equilibrium. Zebrafish were exposed to medium-concentration wastewater (1.0% and 1.5% exposure groups) in the early and middle stages of exposure (3-12d), compared with the control group, induced by varying degrees, LDH activity continued to increase. At 12d, the 1.5% exposed group reached the peak, indicating that with the extension of exposure time, the body was damaged by the wastewater stress, local hypoxia, free radicals increased, LDH penetrated the cell membrane, its activity increased and its concentration increased. This is the automatic compensation protection compensation mechanism of the body. At 15d, it dropped to the level of the control group, indicating that the zebrafish had adapted at this concentration.

When zebrafish were exposed to 2.0% wastewater, the LDH activity of each exposed group continued to increase with the prolongation of exposure time, but there was no significant difference compared with the control group ($p>0.05$), indicating that the ampicillin wastewater was not cause excessive damage in zebrafish muscle tissue in the exposed group.

4. Conclusion

Through subacute toxicity tests, studies have shown that different concentrations of ampicillin wastewater have different effects on the content of T-AOC and LDH activities in zebrafish muscle tissue. In the early stage of the experiment, the T-AOC activity in the high concentration group was significantly induced, which may be because the high concentration wastewater stimulated the zebrafish to cause oxidative stress. In the middle of the experiment, the activity of T-AOC in the low concentration group was significantly induced, indicating that the long-term exposure to the wastewater environment affected the zebrafish oxidation system. With the extension of exposure time, the zebrafish gradually adapt to the wastewater environment through the coordination of the fish antioxidant system. In different concentrations of ampicillin wastewater, the LDH activity of zebrafish muscle tissue in all exposed groups was in dynamic change, and the 0.5% exposed group showed a trend of increasing first, then decreasing and then increasing the "N" type, 1.0%, The change trend of the 1.5% and 2% exposure groups was similar, and all of them were "Λ" type changes. According to the experimental data, the T-AOC and GSH activities in zebrafish muscle tissue had changed significantly during the period of exposured, but ampicillin did not cause irreversible oxidative damage to zebrafish muscle tissue.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (41373096), National Environmental Protection Project (201509041-05), Hebei Province Natural Science Fundation (B2014208068), Hebei Province Pharmaceutical Molecular Chemistry Laboratory Open Foundation, Hebei Province Environmental Protection Public Interest Project, and Hebei Province Key Disciplines Construction Fund Project.

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