

RESEARCH PAPER

Studying the Effects of Ethylenediaminetetraacetic Acid and Penicillamine on the Toxicity of TiO₂ Nanoparticles in Nile Tilapia (*Oreochromis Niloticus*)

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ABSTRACT

Metal nanoparticles (NPs), such as TiO₂ NPs, have been extensively researched for their potential use in aquaculture. One of the main uses of TiO₂ NPs in aquaculture is as a natural water purifier. TiO₂ has photocatalytic properties that make it highly effective in breaking down organic pollutants and toxic substances in water. Additionally, TiO₂ NPs have been used to improve the efficiency of aquaculture feed. However, it is important to note that while the use of TiO₂ NPs in aquaculture holds promise, there are also some concerns about their safety and environmental impact. Therefore, in this study, we sought to ascertain whether penicillamine and ethylenediaminetetraacetic acid (EDTA), two compounds used in the treatment of heavy metal poisoning and whose efficacy has been demonstrated in both warm-blooded animals and humans, may lessen the toxicity of TiO₂ NPs in Nile tilapia. In this study, 280 Nile tilapia fries (3.20±0.12 g) were split into four treatments over the course of three replications. Following that, the OECD Guidelines for the Testing of Chemicals was used to evaluate and compare the acute toxicity of TiO₂ NPs. In all stages of toxicity determination, the data demonstrated that the toxicity of TiO₂ NPs in the control treatment was significantly higher than that of the treatments given penicillamine and EDTA. Finally, the efficiency of penicillamine is more than that of EDTA, and both of these drugs can be used orally to prevent and treat seafood poisoning caused by TiO₂ NPs.

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INTRODUCTION

Heavy metals are metallic elements that have high densities and are toxic to living organisms at low concentrations [1,2]. They occur naturally in the

earth's crust, but human activities such as mining, smelting, and industrial activities have greatly increased their concentrations in the environment [3]. Heavy metals can infiltrate aquatic ecosystems

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through various pathways, including direct discharge of industrial wastewater and runoff from agricultural fields. Some of the most common heavy metals that infiltrate aquatic ecosystems include lead, mercury, cadmium, chromium, and arsenic [4,5]. Each of these metals has different toxicological properties and can cause different health effects in humans and other animals. For example, mercury can cause neurological damage, while lead can lead to developmental problems in children [6,7]. To mitigate the infiltration of heavy metals into aquatic ecosystems, it is important to control their sources and prevent their release into the environment. This can be achieved through improved industrial practices, the use of less toxic materials, and the implementation of wastewater treatment facilities. Additionally, monitoring of heavy metal concentrations in water and biota can help to identify potential risks and inform management actions [8].

In recent years, the application of metal nanoparticles (NPs) has grown significantly [9,10]. Metal NPs are tiny particles made of metal that have at least one dimension between 1 and 100 nanometers [11]. They have unique physical and chemical properties that differ from their bulk counterparts, and are used in a variety of applications including electronics, catalysis, and biomedical imaging and therapy. However, there is growing concern over the potential impacts of metal NPs on aquatic ecosystems [12,13]. Metal NPs can infiltrate aquatic environments through various sources such as industrial effluents, consumer products, and nanotechnology-enabled agricultural products [14]. They can interact with different components of the aquatic ecosystem, such as water, sediments, and organisms. When metal NPs enter aquatic ecosystems, they can undergo physical and chemical transformations due to interactions with other environmental components. For example, they can aggregate, dissolve, or be coated with organic or inorganic compounds. These transformations can affect the behavior and toxicity of metal NPs [15].

Titanium dioxide (TiO₂) has been widely studied for its antibacterial properties. When exposed to ultraviolet (UV) light, TiO₂ NPs can produce reactive oxygen species (ROS), which can damage bacterial cell walls and inhibit bacterial growth [16–18]. This property has led to the use of TiO₂ NPs in a variety of applications, including antibacterial coatings on medical devices, water purification systems,

and food packaging [19]. However, there are concerns over the potential toxicity of TiO₂ NPs to human health and the environment. Studies have shown that TiO₂ NPs can enter aquatic ecosystems through various pathways, such as wastewater treatment plants, and can accumulate in aquatic organisms [20]. TiO₂ NPs can have negative impacts on aquatic organisms such as fish, crustaceans, and algae, depending on the concentration, size, and exposure duration [21].

The use of TiO₂ NPs in aquaculture has been increasing in recent years due to their ability to improve water quality and enhance the growth and survival of aquatic organisms [22]. TiO₂ NPs can act as a photocatalyst and break down organic matter and bacteria in water, thereby reducing the risk of disease and improving water quality [23]. In addition, TiO₂ NPs can be used as a feed supplement for fish and shrimp, as they have been shown to improve growth rates and feed conversion efficiency [24]. However, there are concerns over the potential toxicity of TiO₂ NPs in aquaculture [25,26]. Once inside the organism, TiO₂ NPs can cause oxidative stress, inflammation, and DNA damage, which can affect growth, survival, and reproduction. Furthermore, TiO₂ NPs can be ingested by humans who consume fish and shrimp that have been exposed to TiO₂ NPs, raising potential health concerns [27].

Chelation therapy, which involves introducing chelating agents into the bloodstream to remove hazardous chemicals like heavy metals, is one technique used to reduce the toxicity of metals [4]. Chelating agents are organic compounds that have a high affinity for metal ions, such as lead, mercury, and cadmium, and can remove them from the body by forming stable complexes that are excreted in urine [2,28]. These compounds are often flexible molecules with two or more functional groups, such as amine or carboxylic acid groups, that can form covalent bonds with metal ions. Chelating agents play an important role in removing metal toxicity by binding to metal ions and preventing them from binding to cellular proteins and enzymes [2,4,16]. This can help to reduce the harmful effects of metal toxicity on the body, including damage to organs, the nervous system, and the immune system. Some common chelating agents used in medicine include EDTA (ethylenediaminetetraacetic acid), DMSA (2,3-dimercaptosuccinic acid), and DMPS (2,3-dimercapto-1-propanesulfonic acid). These

agents are used to treat heavy metal poisoning, such as lead and mercury poisoning, and can be administered orally or intravenously [29].

Penicillamine is a chelating agent that is used to treat heavy metal toxicity, particularly copper toxicity in patients with Wilson's disease, a genetic disorder that causes the accumulation of copper in the body [30]. Penicillamine works by forming stable complexes with copper ions, which are then excreted in urine. Penicillamine is a sulfur-containing amino acid that can bind to copper ions through its thiol (-SH) group. It is administered orally or intravenously and is rapidly absorbed from the gastrointestinal tract. Once in the bloodstream, penicillamine binds to copper ions and forms a stable complex that is then excreted in urine [2].

Due to the increasing use of TiO₂ NPs in various industries, such as disinfectants, antibacterial agents, and water treatment, there is a growing concern about the potential contamination of water sources with these NPs. TiO₂ NPs have been shown to have potential toxic effects on aquatic organisms, and their accumulation in water sources can lead to adverse ecological effects.

Thus, it was attempted to ascertain in this study whether the two compounds penicillamine and EDTA, which have chelating effects and reduce the toxicity of heavy metals in humans and warm-blooded animals, also reduce the toxicity TiO₂ NPs in Nile tilapia.

MATERIALS AND METHODS

In this study, 280 Nile tilapia fries (3.20±0.12 g) were purchased. The fish were housed in a 500-liter tank for a week to become used to the aquarium's environment and human feeding, and they were fed with special carp fish food during that time [31]. To evaluate the physical and chemical conditions of the water, the temperature was maintained at 27±2°C during the experiment, with a pH of 8.6, an electrical conductivity (EC) of 930 µS/cm, dissolved oxygen levels ranging from 0.9-4.3 mg/l, and low levels of NH₃ (<0.01 mg/l) and NO₂ (<0.01 mg/l) detected. Additionally, NO₃ was found to be less than 0.1 mg/l. Dechlorinated water was used for the experiment. TiO₂ NPs were purchased from Desunnano Co., Ltd, Taiwan. The X-ray diffraction (XRD) pattern, Fourier-transform infrared spectroscopy (FTIR), and scanning

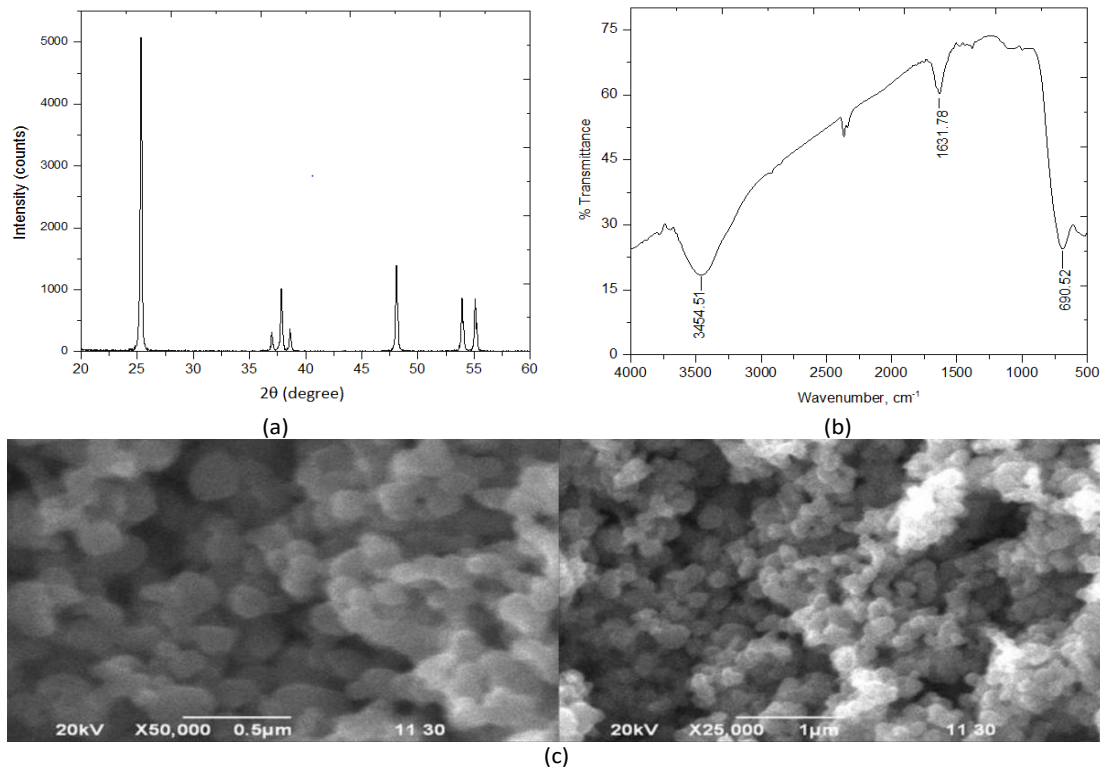


Fig. 1. Analysis results of TiO₂ NPs: (a) XRD measurement, (b) FTIR spectroscopy, and (c) SEM image.

electron microscope (SEM) image of the TiO₂ NPs employed in this investigation are all displayed in Fig. 1.

To investigate the potential of oral penicillamine and EDTA in reducing the toxicity of TiO₂ NPs, four treatment groups (each with three replicates) were established in separate 150-liter aquariums. Given that penicillamine was reported at two concentrations of 100 and 150 mg/kg of feed, one treatment was taken into account for each concentration.

- The 1st treatment group was fed with feed containing penicillamine at a rate of 100 mg/kg (with three replicates, each with 20 fish).
- The 2nd treatment group was fed with feed containing penicillamine at a rate of 150 mg/kg (with three replicates, each with 20 fish).
- The 3rd treatment group was fed with feed containing EDTA at a rate of 150 mg/kg (with three replicates, each with 20 fish).
- The 4th treatment was fed with basic feed (common carp standard feed) without any additives.

To incorporate penicillamine and EDTA into the feed, the desired dose was first diluted with distilled water and then sprayed onto the pellet feed, ensuring that both substances were thoroughly and evenly mixed with the feed. After preparing the food for an hour at 45°C, it was sealed in plastic bags and kept at 5°C in the refrigerator. The fish were acclimated for a period of three weeks in 150-liter aquariums and fed with species-specific diets according to their respective treatment groups. Following this

period, the toxicity of TiO₂ NPs was assessed in the experimental fish. The toxicity of TiO₂ NPs was assessed using the standardized method outlined in the OECD (Organization for Economic Cooperation and Development) Guide No. 203 for Static-Constant Test Conditions [32]. To achieve this goal, fish in each of the four treatment groups were subjected to increasing concentrations of TiO₂ NPs to evaluate their level of resistance against the NPs' toxicity. Initially, a pilot study was conducted to determine the range of toxic concentrations of TiO₂ NPs in Nile tilapia (*Oreochromis niloticus*). Three concentrations of TiO₂ NPs, with a significant range between them (0.1, 1, and 5 mg/L), were administered to the fish to establish the effective toxic dose. Then, between six and eight consecutive concentrations of TiO₂ NPs were then taken into consideration. Three replicates were prepared for each concentration, with each replicate being housed in a 50-liter tank. Ten fish were introduced into each tank for each replication. Mortalities were recorded every 20 hours (at 20, 40, 60, and 80 hours), and lethal concentrations (LC10, LC15, LC50, and LC90) were determined using the Probit method in SPSS software version 23.0.

RESULTS AND DISCUSSION

Table 1 demonstrates that the 80-hour LC50, a crucial metric for assessing the acute toxicity of poisons, varied significantly across different treatments involving TiO₂ NPs (p<0.05). The treatment group supplemented with penicillamine at concentrations of 100 and 150 mg/kg feed

Table 1. The lethal concentrations (LC10, LC20, LC50, and LC90) of TiO₂ NPs in Nile tilapia fed with basic feed, penicillamine, and EDTA were determined over a period of 20, 40, 60, and 80 hours of exposure.

Lethal concentrations (mg/l)	Adjacency duration of treatment	20 hrs.	40 hrs.	60 hrs.	80 hrs.
LC10	Penicillamine 100	2.996	2.958	2.484	2.420
	Penicillamine 150	3.036	2.996	2.958	2.500
	EDTA 150	2.394	2.297	2.388	2.318
	Control	1.533	1.533	1.447	1.405
LC20	Penicillamine 100	3.183	3.143	2.669	2.562
	Penicillamine 150	3.226	3.183	3.143	2.648
	EDTA 150	2.537	2.431	2.480	2.412
	Control	1.637	1.637	1.526	1.483
LC50	Penicillamine 100	3.575	3.528	3.060	2.855
	Penicillamine 150	3.624	3.575	3.528	2.956
	EDTA 150	2.834	2.708	2.668	2.604
	Control	1.854	1.854	1.689	1.641
LC90	Penicillamine 100	4.266	4.208	3.770	3.367
	Penicillamine 150	4.327	4.266	4.208	3.494
	EDTA 150	3.351	2.708	2.668	2.604
	Control	2.243	2.243	1.973	1.918

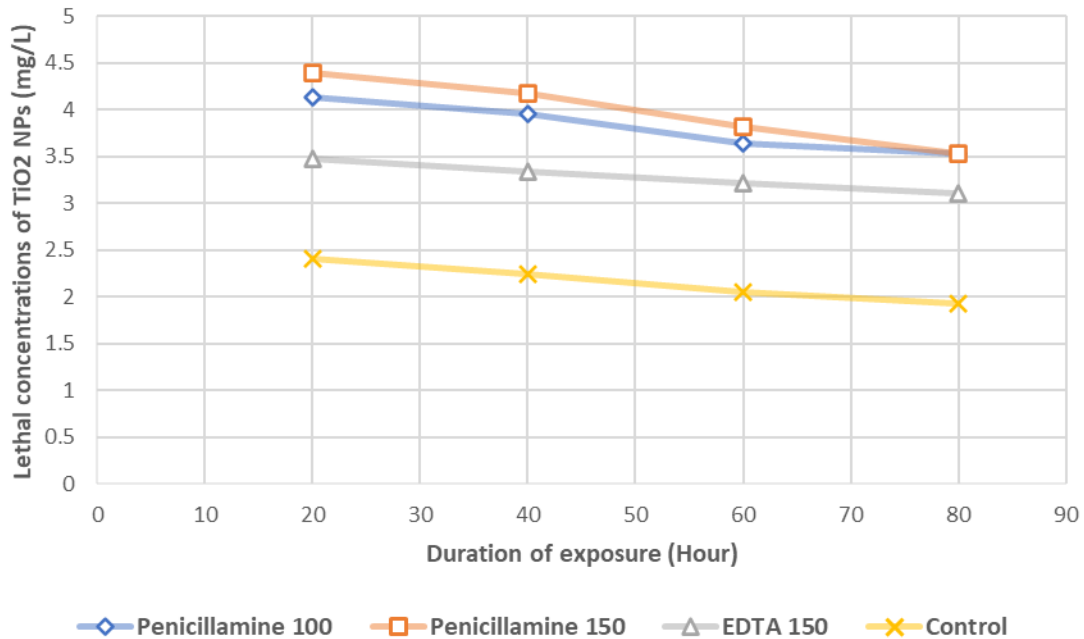


Fig. 2. The trend of the lethal concentration of TiO₂ NPs in four distinct treatments.

exhibited the highest 80-hour LC50 value among the experimental groups. Furthermore, all three treatment groups that were supplemented with penicillamine and EDTA demonstrated significantly higher 80-hour LC50 values compared to the control group. These findings suggest that the inclusion of penicillamine and EDTA in the diet may have a protective effect against the toxicity of TiO₂ NPs in aquatic organisms.

The alterations in the lethal concentration across all treatments during various stages of the experiment are depicted in Fig. 2.

The toxicity of TiO₂ NPs in the control group was found to be significantly higher than the treatment groups administered penicillamine and EDTA in all four stages of toxicity determination (20, 40, 60, and 80 hours). The group administered with penicillamine (150 mg/kg feed for three weeks) demonstrated the greatest resilience to TiO₂ NPs toxicity across all four stages of toxicity testing. Fish that were fed a diet containing 100 mg/kg of penicillamine demonstrated greater resistance to the toxic effects of NPs when compared to those fed a diet containing EDTA.

In all experimental treatments, an increase in the duration of exposure to TiO₂ NPs (from 20 to 80 hours) resulted in a proportional increase in toxicity, as evidenced by a decrease in the LC

of TiO₂ NPs. These results suggest that the toxic properties of TiO₂ NPs were not mitigated by the addition of two chelating agents, penicillamine and EDTA.

In the present study, the toxicity of TiO₂ NPs was evaluated in Nile tilapia by determining the 80-hour LC50 value, which was found to be 1.641 mg/l, indicating a high level of toxicity of this substance for this species. This finding is consistent with a previous study conducted by [33], which reported the 80-hour LC50 values of TiO₂ NPs in Caspian trout juveniles to be 1.16, 0.81, 6.2, and 6.81 mg/L, respectively.

CONCLUSION

According to the results of the study, it can be concluded that the administration of penicillamine and EDTA can reduce the toxicity of TiO₂ NPs in fish. The group administered with penicillamine (150 mg/kg feed for three weeks) demonstrated the greatest resilience to TiO₂ NPs toxicity across all four stages of toxicity testing. However, it was observed that the toxic effects of TiO₂ NPs increased with an increase in exposure duration, indicating that TiO₂ NPs have the potential to accumulate and cause harm over time. Additionally, the results suggest that the chelating agents penicillamine and EDTA were not effective in fully mitigating

the toxic properties of TiO₂ NPs. Further studies are required to fully understand the mechanisms behind TiO₂ NPs toxicity and potential mitigation strategies.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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