Toxicological effects of four chemicals used for prophylaxis of Amazonian ornamental fish

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ABSTRACT

The objective of this study was to determine the acute toxicity of chemotherapeutic drugs used to diseases control in ornamental fish cardinal tetra (Paracheirodon axelrodi). The fish were exposed to four chemicals: formalin, oxytetracycline, copper sulfate and malachite green. Four experiments were performed, distributed in a completely randomized design with seven concentrations of product test (formalin, oxytetracycline, copper sulfate and malachite green), a control and three repetitions. A static system with five fish per recipient containing 2 L of water for 96 hours was used. The estimated lethal concentration 50% (LC\(_{50}\)) was 67.94 mg.L\(^{-1}\), 3.83 mg.L\(^{-1}\), 1.65 mg.L\(^{-1}\) and 0.85 mg.L\(^{-1}\) to formalin, oxytetracycline, copper sulfate and malachite green, respectively. Thus the present study considered the formalin in low toxicity for the cardinal tetra and the copper sulfate, oxytetracycline and malachite green are moderately toxic.

Keywords: ornamental fish; fish diseases; chemotherapeutic; lethal concentration.

Introduction

The state of Amazonas is recognized by its ornamental fish industry. This activity generates an incoming of more than US$ 6 million every year to the State of Amazonas (TAVARES-DIAS et al., 2009). The cardinal tetra (Paracheirodon axelrodi; Schultz 1956) is the main specie commercialized, very attractive for its strong glowing blue, represents almost 80% of all the fish caught in this region (CHAO et al., 2003).

However all the fishery processes occur under serious technical restrictions, mainly related to the unorganized capture of the fish, inadequate conditions of transport (SANTOS; SANTOS, 2005) and maintenance, resulting in mortalities rates reaching 50% from the capture to the destiny as America, Europe or Asia (TLUSTY et al., 2005). But in front of this considerable decrease of profits, some substances are used in the attempt to control the diseases outbreaks.

Among the most used chemicals are the malachite green, the formalin, the copper sulfate and the oxytetracycline. The malachite green is a water soluble triphenylmethane dye, presenting a high effectiveness against protozoa and fungal infections (CARNEIRO et al., 2005), however known by its carcinogenic, teratogenic and mutagenic potential (FERNANDES et al., 1991; RAO, 1995; CARNEIRO et al., 2005). The formalin is an aqueous solution of 37-49% formaldehyde gas, usually used as parasiticide, mainly for ectoparasites as monogeneans and monogonons, can be used also as bactericide and algicide (NOGA, 2010), leading the cells to death by cross-linking proteins (SANCHES et al., 2007). The copper sulfate (CuSO\(_4\)) is an efficient algicide and can be used as a therapeutic for protozoan parasites (STRAUS et al., 2009). The oxytetracycline, an antibiotic of the tetracyclines group with wide range of action, is frequently used in some human and animal disease treatments (DOLLERY, 1999; CARRASCHI et al., 2012) and acts inhibiting the bacterial protein synthesis.

Although these chemicals constitute part of the routine in this fish production, there are not studies validating their use for cardinal tetra, what obligates the fishermen to follow the prescription for other fish. Moreover, the application of incorrect doses of therapeutics is the most harmful problem in this activity, resulting in substantial financial and environmental losses as reported by Torres et al. (2008). Hence, the realization of aquatic toxicity assays can measure the degree of fish responses exposure to level of the chemical concentration and it is the first step to characterize optimal doses for disease treatments in this specie.

The objective of the present work was to evaluate the acute toxicity of the chemicals formalin, oxytetracycline, malaquite green and copper sulfate for cardinal tetra.

Material and Methods

Prior to the present study, 1000 cardinal tetra fish (Paracheirodon axelrodi) was acquired from a commercial store (Manaus, Amazonas, Brazil) and placed in 310 L glass fiber tanks, in order to acclimate to the laboratory conditions, all under constant aeration and water recirculation. Afterwards, the animals were submitted to 24 hours of feed restriction and then placed five fish per aquarium (mean weight of 1.73g ± 0.08), each one containing 700 mL of water. After this, another 300 mL, containing the test
The shorter period that cardinal tetra fish was exposed to each substance without any mortality, is presented in table 3. The formalin presented the first mortality after 24h of exposure, at the concentration of 60 mg.L⁻¹. The copper sulfate showed a lethal toxicity after 48 h exposed to a concentration of 10 mg.L⁻¹, and the oxytetracycline after 36 h, at 2 mg.L⁻¹. The malachite green group had its first mortality after two hours of exposure at a concentration of 0.8 mg.L⁻¹.

Table 3. Cardinal tetra exposure (n=15 fish per treatment) time to occur the first mortality in different concentrations of chemotherapeutics. / Tabela 3. Tempo de exposição (n=15 peixes por tratamento) do taro-cardinal para ocorrer a primeira mortalidade em diferentes concentrações de chemotherapeutics.

<table>
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<th>Formalin</th>
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<th>Mortality (%)</th>
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<td>0.3</td>
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Discussion
In the present study toxicity of four chemicals commonly used to disease treatment in fish was tested on cardinal tetra fish, in different periods of exposure.

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None of the water parameters were restrictive to the maintenance of fish to the experimental conditions, being within the range recommended by IBAMA (1987). There was no correlation between the concentrations of the different chemicals used the water parameters from the conducted tests.

Macniven and Little (2001) found that the formalin was less toxic to Nile tilapia (Oreochromis niloticus), where the LC(50-96h) was 429.68 mg.L⁻¹, but, Tister and Koncan (SANTOS et al., 2012) observed a higher sensitivity in rainbow trout (Oncorhynchus mykiss), with a LC(50-96h) of 6.1 mg.L⁻¹. The Coridora melanistius, an ornamental fish from Amazon rivers, presented a LC(50-96h) of 50.75 mg.L⁻¹ to formalin, similarly to the LC found in the present experiment, but morphological alterations as hyperplasia of the gills cells were also found, reaching lamellae fusion (SANTOS et al., 2012).

This indicates that, despite the formaldehyde to be considered, according to ZUCKER (1985) classification, just "slightly toxic", the exposure of the cardinal tetra to this chemical may cause pathophysiological problems.

Paixão et al. (2013) submitted the Hemigrammus brachycephalus, an Amazonian species inhabiting the same environment as the Cardinal tetra at three concentrations of formalin: 250mg.L⁻¹ and 100mg.L⁻¹ submitted to one hour bath and 25mg.L⁻¹ at 24 hours baths for three days.

The cardinal tetra show mortality at concentration of 140 mg.L⁻¹ after 2 hours of exposure to formaldehyde, indicating that for this species, the recommendation of short baths (up to 60 min, 150 mg.L⁻¹) can be performed without resulting in mortality (Martins, 2004). But analyzing the results obtained by Paixão et al. (2013) and the present work is possible to identify a large variability in the physiological response of the species, even inhabiting the same environment, highlighting the need for such tests.

Regarding recommended by Martins (2004) for long duration bath (up to 24 h, 10 to 15 mg.L⁻¹) became totally suitable to the cardinal tetra since the dose and duration of treatment are compatible the LC₅₀ presented here. Considering the results obtained by Santos et al. (2012) from the toxicological and histological results and Paixão et al. (2013) from the assessment of mortality and changes in hematological parameters, this concentration can be used to control parasites.
The copper sulfate toxicity is also influenced by some water parameter (MAZON et al., 2002), being highly dependent of alkalinity and pH, which, when is low, turns the heavy metals more available in the environment. Because of this, the treatment with this substance must be carefully monitored with kits that identify and quantify total, chelated or free copper.

In the present study, the toxicity of the copper sulfate to cardinal tetra was classified as moderate (1 mg.L⁻¹ > LC₅₀ > 10 mg.L⁻¹) but, since the LC₅₀ was found very close to the threshold of “very toxic” interval (1.65 mg.L⁻¹), this chemical must considered very carefully to use in this specie.

The toxicity in channel catfish (Ictalurus punctatus) presenting a LC₅₀ range of 1.5 to 19 mg.L⁻¹ (STRAUS; TUCKER, 1993). This fluctuation values are due to alkalinity (76 to 240 mg.L⁻¹), however the concentration of 0.1 to 0.2 mg.L⁻¹ of the chemical.

However Païão et al. (2013) exposed the Hemigramus sp. concentration of 0.3 mg.L⁻¹ for 24 hours showed increased mortality and decreased lymphocytes and neutrophils, monocytes and eosinophils, behavior significantly similar for all variables in the control group but significantly different for the treatment that caused the lowest mortality rate during the experiment (25 mg.L⁻¹ formalin). Indicating that the treatment itself can be as stressful as the parasitic infestation. Thus, the concentration of 0.5 mg.L⁻¹ used in this trial with cardinal tetra probably resulted in hematologic changes and the applicability of this concentration on the realization of the control of parasites may not be applicable due to the stress associated with parasitic infestation, may result in higher mortalities.

In the present study, the oxytetracycline was classified as moderately toxic for the cardinal tetra with a LC(1)₅₀ of 3.83 mg.L⁻¹. Similar sensibility was found by Cruz et al. (2006) in guaru (Phallocerus caudimaculatus), with a LC(1)₅₀ of 3.4 mg.L⁻¹ of oxytetracycline. According to Bassler (2009), the best dose of oxytetracycline in the control of bacteria is 150 to 200 mg.L⁻¹ for short duration baths, and 2 to 20 mg.L⁻¹ for long duration bath.

Yang (2010) recommend a concentration of 10 mg.L⁻¹ of oxytetracycline in baths of 6 to 12 h, during a period of 10 days. According to our data, the cardinal tetra can be submitted to the treatments of Bassler (2009) and Yang (2010) without any risk of mortality, however it is important the study of antibiotic efficacy for each of the pathogenic bacteria.

In the commercial chain of cardinal tetra in Brazil, the use of antibiotics as a prophylactic strategy is a common practice adopted by Amazonic exporters (WAICHMAN et al., 2001), being already reported by Mendonça and Camargo (2008) to cardinal tetra.

On the other hand, the appearance of bacterial strains resistant to one or more drugs, including the oxytetracycline, have already been reported to be a severe menace to public health (LEVY; CRUZ, 2011; STRAUSS; TUCKER, 1993). The copper sulfate toxicity is also influenced by some water parameters, and fungicides (BASSLEER, 2009; YANOG, 2010) to control parasitic parasites. However, some fungicides in the commercial chain of cardinal tetra in Brazil, the use of antibiotics as a prophylactic strategy is a common practice adopted by Amazonic exporters (WAICHMAN et al., 2001), being already reported by Mendonça and Camargo (2008) to cardinal tetra.

According to the present study of the use of antibiotics, oxytetracycline cannot be used as stress reducer for water parameter, being highly dependent of alkalinity and pH, which, when is low, turns the heavy metals more available in the environment. Because of this, the treatment with this substance must be carefully monitored with kits that identify and quantify total, chelated or free copper.

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Comparing the data obtained by Declerq et al. (2013) and those obtained in this work, the highest concentration of oxytetracycline (2 mg.L⁻¹) causing no mortality in cardinal tetra is not effective in inhibiting bacterial infection at least on strain of Flavobacterium columnaris isolated from neon tetra or cardinal tetra.

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