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Mutagenic and genotoxic effects of the Atrazine herbicide in *Oreochromis niloticus* (Perciformes, Cichlidae) detected by the micronuclei test and the comet assay

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Abstract

Atrazine is the triazinic herbicide most found in the rural aquatic environments due to its extensive use and its stability in such places. The mutagenicity and the genotoxicity of different concentrations of the Atrazine herbicide were determinated by the micronucleus test and the comet assay, using *Oreochromis niloticus* as test-system. The tested concentrations of Atrazine herbicide were 6.25, 12.5 and 25 μg/L, both for the micronuclei test and for the comet assay. The results showed a significant rate of micronuclei and nuclear abnormalities for all the tested concentrations of Atrazine herbicide. For the comet assay, we also observed results significantly different from the control in 6.25, 12.5 and 25 μg/L concentrations. Due to these results, we could infer that such herbicide may be dangerous to the lives of those organisms exposed to it.
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Keywords: Atrazine herbicide; *Oreochromis niloticus*; Micronucleus; Nuclear abnormalities; Micronuclei test; Comet assay; Genotoxicity; Mutagenicity; DNA fragmentation; Triazines

1. Introduction

The significant increase of chemical emissions in the water resources has lead to a serious of deleterious effects for aquatic organisms [1,2], besides direct and indirect hazards to human health [3]. Many of such chemicals can induce, besides death of exposed organisms, other effects, like genetic disorders and physiologic alterations. Some substances, when present in low concentrations, may not cause acute detectable effects in organisms, but may, in the long run, reduce their life span [4].

Pesticides presents in aquatic environments can affect aquatic organisms in different ways. The accumulation rate of such chemicals depends on the kind of associated food chain, on availability and persistence of the contaminant in the water and, most of all, on the physical and chemical characteristics of the agrochemical [5]. Fish and aquatic invertebrates can accumulate pesticides in concentrations much higher than those found in the waters where they live, because such chemicals may go through bioaccumulation or connect to the particulate material in suspension, which can be ingested by organisms present in the environment [6].

A number of chemicals related to the urban and agricultural activities have already been detected in the aquatic environment, confirming their potentiality in the environmental contamination [2,7–11].

Triazines are the oldest herbicides and the most commonly used, representing around 30% of the pesticides market in the world [12]. Atrazine is a substance that belongs to the triazines group, rated as moderately toxic for aquatic species [13], mostly found in the rural aquatic environments, due to its extensive use in agricultural and

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to its high stability in the water [14,15]. However, according to some authors [16], this herbicide has highly variable persistence in the water. This variation occurs due to the nature of the aquatic ecosystem in which the herbicide is introduced and due to the chemical conditions of the exposed environment itself [17]. The higher acidity and the amount of organic matter dissolved in water, the higher is the stability of Atrazine herbicide in the aquatic environment [18–20].

Atrazine reaches aquatic environments due to proximities of the agricultural countrysides to the water places, or directly due to the careless application in such environments [21]. According to some authors [15], residues of Atrazine herbicide were found in water for public supply in different regions of the USA.

In general, Atrazine and other triazinic compounds have showed genotoxic and mutagenic actions in *Drosophila*, yeasts and plants, but not mutagenic actions in bacteria. The tests with Atrazine also evidenced a non genotoxic action for mammalian cells *in vivo* and *in vitro*, although some positive results for tests of chromosomal aberrations and of DNA damage have been found in human lymphocytes, besides of DNA damage and formation of micronuclei in rats and mice [22,23].

The use of fishes as bio-indicators of pollutant effects is being more and more used, since such practice can help detect possible environments problems [24]. Results obtained in assays carried out with fishes can be used for the evaluation of the presence of substances that have potentiality to cause teratogenic and carcinogenic effects in human beings [2,25].

Species of fishes as *Oreochromis niloticus* and *Hoplias malabariscus* are test-system excellent for toxicity assays of water contaminants [26]. *Oreochromis niloticus* (Perciformes, Cichlidae) is a commercially important specie in the southeast of Brazil, particularly in the São Paulo State. This specie is also commonly found in estuaries all over the world, and it is recognized for its sensibility to answer, quickly, to environmental alterations [27].

The micronucleus test in erythrocytes of fishes has been used as one of the first measure in the evaluation of clastogenic potential of one substance or environment. Several studies have shown that erythrocytes of fishes present a high incidence of micronuclei, after exposition to different pollutants, under field and laboratory conditions [2,24,28–30].

The comet assay is a sensitive, fast and economic test, besides requiring just few cells for its execution [31–34], and it has been indicated as a detection method of small changes in the DNA structure, such as repair activities, its packing mode and its entirety [35,36].

According to several authors [2,37,38], the comet assay has been successfully applied in erythrocytes of many fish species exposed to the different genotoxic agents, because that assay allows to evaluate the potentiality of DNA strand breaks of such organisms, due to the action of different xenobionts. In this paper, we evaluated mutagenic and genotoxic effects of Atrazine herbicide, using *O. niloticus* as test-system, by means of micronucleus and nuclear abnormalities test and comet assay.

2. Materials and methods

2.1. Tested substance

Atrazine (CAS no. 1912-24-9; pure 97.7%) is a selective herbicide of the triazines chemical group and its composition is 2-cloro-4-ethylamine-6-isopropylamine-*s*-triazine. This pesticide belongs to the toxicological class III, considered moderately toxic, used as a selective herbicide of pre and post emergency in different cultures. The LC50% of Atrazine herbicide is 9.35 mg/L for the species *O. niloticus* [21].

2.2. Treatment solutions, test-system and bioassays

The Atrazine concentrations used in the experiment were: 25, 12.5 and 6.25 μg/L, and the highest concentration used consisted of solution indicated for agricultural use. The others solutions were obtained through progressive dilutions from the highest concentration in distillate water. All the concentrations used are much lower than the LC50% reference of the Atrazine herbicide for the specie *O. niloticus*.

The species *O. niloticus* (Perciformes, Cichlidae) was used as test-system to detect possible mutagenic and genotoxic damages induced by Atrazine herbicide. The fishes, with an average size of 15 cm, obtained in the fishing growing tanks, without any contamination, kept by São Paulo State University—Rio Claro, were brought to the laboratory and acclimatized in aquaria under controlled conditions.

For the realizations of the bioassays, four aquaria were used: one aquarium for the control test, containing water from an artesian well only and three aquaria containing the different Atrazine concentrations (6.25, 12.5 and 25 μg/L). To each aquarium five specimens of *O. niloticus* were added and left for 72 h. After this exposition time, for the micronucleus and nuclear abnormalities test, *O. niloticus* blood samples were obtained by means of cardiac puncture using heparinized syringes. Smears were prepared on slides, the material was fixed in absolute methanol for 10 min, dried at room temperature for 24 h and stained with 5% Giemsa for 20 min. The slides analyzes were carried out with the use of light microscope, with 40× and 100× objective lens, totalizing five slides from each specimen. The average and standard deviation of the number of erythrocytes with micronuclei and nuclear abnormalities were determined by the analysis of 5,000 blood cells per slide. The Kruskal–Wallis Statistical No-Parametric Test or H-Test [39] was used to compare the results for fish exposed to different concentrations with those for control test exposed to water from an artesian well only.

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