Original Article

Neurobehavioral assessment of *Danio rerio* intoxicated by sodium arsenate and the use of *Arsenicum Album* to reverse the condition of anxiety

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Abstract

Background: Various studies have indicated that the zebrafish serves as a useful and easily executable experimental model. Environmental pollution caused by heavy metal waste is very relevant due to the widespread use of heavy metals in industrial and agricultural practices, and the fact that many effluents are released into the environment without any treatment. Sodium arsenate changes behavioral parameters and brain ectonucleotidase activities in zebrafish¹.

Aims: The objective of this paper is to assess behavioral changes in zebrafish (*Danio rerio*) induced by sodium arsenate, through an analysis of locomotor activity and anxiety-related parameters, and determine whether ultra-diluted substances are able to mitigate neurobehavioral effects.

Materials and methods: Zebrafish were held in aquariums with controlled temperature and pH, until the time of the toxicological experiment. The animals were exposed for 96 hours to the heavy metal sodium arsenate and divided into four groups with eight fish in each: *Arsenicum Album* 6 cH, *Arsenicum Album* 30 cH and inert aqueous solution (positive control) groups received drugs made in distilled water, according to the Brazilian Homeopathic Pharmacopoeia; the experiment was blinded. The fourth white control group (negative control) did not receive the sodium arsenate or drugs. All the homeopathic drugs that treated the heavy metal toxicity were initiated 5 days before the end of the experiment (therefore, the animals were already taking the medication one day before exposure). Afterwards, the animals underwent behavioral tests (locomotion and anxiety) to assess neurotoxicity. All the animals went through various steps: assessment of neurotoxicity signs where the following was observed: time and frequency on the surface and erratic movements; general activity test in an Open Field, where it was observed: time in seconds of locomotion and immobility; and light/dark test (anxiety-like behavior), where it was observed: the number of times the animals crossed from the light to the dark compartment, how long they spent on each side, number of attempts to enter each compartment and immobility time on the light side. The data obtained was analyzed statistically by ANOVA, followed by Bartlett's Test and the Tukey Test, with $p \leq 0.05$ ². CEUA Protocol No. 07/2016.

Results and Discussion: The heavy metal sodium arsenate produced an anxiogenic effect in the animals that were subjected to it without medication (aqueous solution, positive control group). This effect was observed in the light/dark test through increased time (sec) of the animals on the dark side (245.3 ± 61.82) in relation to the control group (101.1 ± 62.3) and a consequent decrease in time of the animals on the light side (54.3 ± 36.3) compared to the control group (198.9 ± 62.3). There

was also a decrease in locomotion (quadrants) on the light side (96.2 ± 63.7) in relation to the control group (201.8 ± 89.2). In the Open Field, there was a decrease in the total of quadrants crossed (346.2 ± 41.33) compared to the control group (520.5 ± 131.6), as well as an increase in the erratic movements (3.5 ± 0.7) in relation to the control group (1.7 ± 0.9), proving the toxic effect of heavy metals in the animals. The group that was medicated with *Arsenicum Album* 30 cH showed a decrease in the erratic movements (0.66 ± 1.0) in relation to the intoxicated animals, resulting values close to the control group. The group that took *Arsenicum Album* 6 cH reversed all the positive control parameters (aqueous solution) in a statistically significant way, maintaining the values of the dark side time, the light side time and the clear side locomotion (161.6 ± 63.7 ; 138.4 ± 96.3 ; 121.0 ± 49.8 , respectively) close to the values of the control animals. *Arsenicum* 6 cH also increased the total number of quadrants in the Open Field (525.0 ± 142.3) and reduced the erratic movements (2.5 ± 1.1), demonstrating anxiolytic ability. Clear side time for the animals treated with *Arsenicum Album* 6 cH, in relation to the control group, was more similar, and the same occurred in regard to the dark side time. When fish are treated with anxiolytics, they tend to remain more time on the light side, which indicates decreased anxiety (Serra et al. 1999). In relation to locomotion on the light side, there was a greater similarity between the control group and the fish treated with *Arsenicum Album* 6 cH, as well as in the displacement behavior in the Open Field with respect to the total number of quadrants of the fish displacement, which coincides with data from the literature that reports increased locomotor activity, as anxiety decreases. Locomotion or swimming activity is also used as an anxiety index and suppression of this activity is indicative of anxiety³. In relation to erratic movements, it was observed that this behavior decreased in animals treated with a concentration of 6 cH as well as with those treated with a concentration of 30 cH, compared to the positive control.

Conclusion: The animals intoxicated by the heavy metal sodium arsenate showed increased anxiety, which was reversed by *Arsenicum Album* that in a concentration of 6cH proved to be more effective as a possible anxiolytic. Other studies are already being conducted by our group.

Keywords: Homeopathy, High Dilution, Zebrafish, Intoxication.

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