



## Research paper

## Lithium prevents scopolamine-induced memory impairment in zebrafish



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## ABSTRACT

We examined whether lithium carbonate ( $\text{Li}_2\text{CO}_3$ , 100 mg/L) is able to prevent memory impairment induced by scopolamine. Moreover, we evaluated the effects of lithium on anxiety-like behavior and acetylcholinesterase activity in adult zebrafish. We demonstrated that lithium prevents the memory impairment induced by scopolamine, decreases exploration and increases the activity of acetylcholinesterase in zebrafish. Collectively, this contributes to a better understanding of the pharmacology of lithium, its interaction with cholinergic neurotransmission, and its possible application to therapeutic treatments aimed at improving cognition.

## 1. Introduction

Lithium carbonate ( $\text{Li}_2\text{CO}_3$ ) has been used for decades in the treatment of bipolar disorders [1–4], and recent studies showed that this drug may reduce symptoms of Alzheimer's disease [5–7].  $\text{Li}_2\text{CO}_3$  has been identified as a promising drug in the treatment of neurodegenerative diseases, since it inhibits 1) the enzyme inositol monophosphatase (IMPase) promoting inositol depletion and increase of synaptic connections [8] and 2) glycogen synthase kinase 3 beta (GSK-3 $\beta$ ) activity by altering the phosphorylation of tau proteins, and reducing  $\beta$ -amyloid plaques, leading to neuroplastic changes associated with mood stabilization and neuroprotection [9]. The neuroprotective effects can be attributed to adenosine diphosphate inhibition and adenosine monophosphate hydrolysis, suggesting that it exerts modulatory effects on the activities of ectonucleotidases and consequently adenosine levels [10].

Considering the several mechanisms presented, it is suggested that lithium can be used in the treatment of neurodegenerative diseases or brain lesions [10–13]. The current therapeutic treatments used in Alzheimer's (AD) disease include acetylcholinesterase inhibitors [14]; however, these drugs have limited efficacy and can cause depression and anxiety [15]. Besides, scopolamine, an acetylcholine muscarinic

receptor antagonist, has largely been used in experimental animals to induce impairment in their performance of learning and memory, mimicking a type of dementia observed in AD [16]. Studies on neurodegenerative diseases have been performed using zebrafish (*Danio rerio*) as an animal model, considering the homology of some genes to that of humans [17]. Thereby, we evaluate the potential of lithium in preventing memory impairment, as well as the effects of this drug on behavior and acetylcholinesterase (AChE) activity in adult zebrafish.

## 2. Methods

## 2.1. Animals

A total of 40 six-month old adult zebrafish (~50:50 male:female ratio) of the wild-type short-fin (SF) strain, were housed in three aquariums (30 L each aquarium) equipped with constant aeration and a 14 h light: 10 h dark photoperiod (lights on at 8:00 am). Water temperature was maintained at  $27 \pm 0.5$  °C; pH  $7.0 \pm 0.15$ ; dissolved oxygen at  $6.0 \pm 0.1$  mg/L; total ammonia at  $< 0.01$  mg/L; total hardness at 6 mg/L; and alkalinity at 22 mg/L  $\text{CaCO}_3$ . The fish were fed twice a day with a commercial flake fish food (Alcon<sup>®</sup> Basic, MEP 200 Complex, Brazil). There were no changes in water parameters between

Abbreviations: AChE, acetylcholinesterase; AD, Alzheimer's disease; GSK-3 $\beta$ , glycogen synthase kinase 3 beta; IMPase, inositol monophosphatase;  $\text{Li}_2\text{CO}_3$ , lithium carbonate; NTT, novel tank test; SCH, thiocholine

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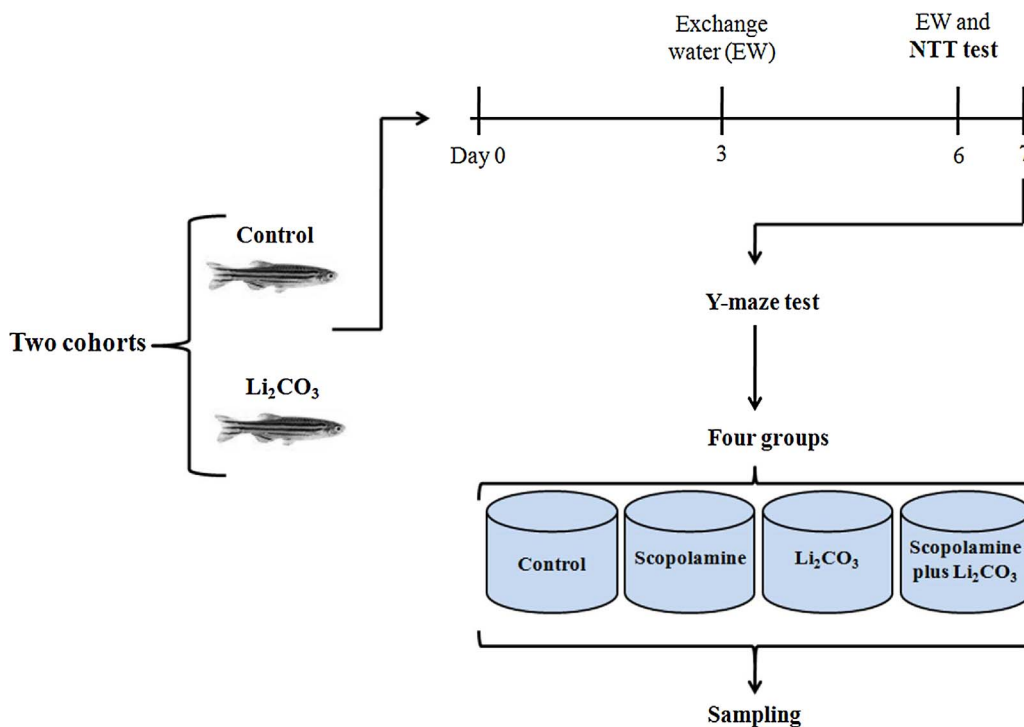


Fig. 1. Experimental design of the study.

treatments.

## 2.2. Drugs

We used lithium carbonate (Li<sub>2</sub>CO<sub>3</sub> CAS 554-13-2, Fagron do Brasil Farmacêutica Ltda, Brazil) and scopolamine hydrobromide trihydrate (C<sub>17</sub>H<sub>21</sub>NO<sub>4</sub>·HBr·3H<sub>2</sub>O, CAS 6533-68-2, Sigma-Aldrich Corporation, St. Louis, MO, USA).

## 2.3. Experimental design

To verify the effect of lithium on memory and anxiety-like behavior in zebrafish, the following two cohorts of fish were used: control and lithium-treated groups at a concentration of 100 mg/L for 7 days (n = 20), as summarized in Fig. 1. For maintenance of lithium concentration, in the third and sixth day, the water of aquariums was exchanged [10] (Fig. 1). Fish from experimental cohorts were then individually tested in the novel tank test (NTT) to evaluate their exploratory behavior and the Y-maze task to verify the spatial memory (Fig. 1).

For memory study, the fish were distributed in four groups: (1) control, (2) scopolamine, (3) Li<sub>2</sub>CO<sub>3</sub> and (4) Li<sub>2</sub>CO<sub>3</sub> plus scopolamine (Fig. 1). Before the training session, fish were transferred into a glass (500 mL) with scopolamine (100 μM) dissolved in water and remained for 1 h. The fish that did not receive scopolamine were also transferred to a glass with water to be exposed to the same procedures [18]. After the memory test, the fish were collected for brain dissection and analysis of acetylcholinesterase activity. This study was approved by the Ethics Commission for Animal Use of the University of Passo Fundo, Brazil (Protocol#17/2016), and fully complied with the guidelines of Conselho Nacional de Controle de Experimentação Animal of Brazil.

## 2.4. Behavioral tasks

In all studies, fish behaviors were recorded by a Logitech HD Webcam C525 camera and the videos analyzed using ANY-maze® software (Stoelting CO, USA).

### 2.4.1. Anxiety test (novel tank test (NTT))

The position (bottom × upper levels) was considered an index of anxiety, similar to the position near the wall versus the center of an open field with rodents [19,20]. Fish were transferred individually to a test aquarium (24 × 8 × 20 cm; width × depth × height) and filmed for 6 min. The following parameters were analyzed: total distance traveled (mm); entries in the upper zone of the tank; and time in the upper zone of the tank (s).

### 2.4.2. Spatial memory test (Y-maze task)

The position in the Y-maze task was considered an index of memory [18]. Fish were tested in a tank with three arms measuring 25 × 8 × 15 cm (length × width × height). Different geometric shapes (squares, circles, and triangles) were used as visual stimuli and placed on the outer wall of each arm, and the remaining area was covered with black plastic. The Y-maze arms were randomly assigned: start arm, in which the fish starts the test, new arm (locked during the initial test, but open during the second test), and the permanently open arm. The Y-maze center was considered a neutral area, and therefore, it was not counted in the analysis. The task consisted of two phases with a 1-h interval between them. In the first phase (5 min training), the fish could explore the start and the open arms with the new arm closed. In the second phase, fish were placed in the start arm and were allowed to freely access the three arms. The following parameter was analyzed: time spent into the novel arm.

## 2.5. Determination of acetylcholinesterase activity

Immediately after Y-maze, zebrafish were cryoanesthetized, euthanized by decapitation and whole zebrafish brains were homogenized on ice in the Tris-citrate buffer in a homogenizer (Tecnal®, Brazil). The rate of hydrolysis of 0.8 mM acetylthiocholine was determined in a final volume of 2 mL with 100 mM phosphate buffer, pH 7.5, and 1.0 mM DTNB using a previously described method [21]. Before the addition of substrate, 10 μg of the protein sample was preincubated with the reaction medium described above for 10 min at 25 °C. Acetylthiocholine hydrolysis was monitored by the formation of the thiolate dianion of DTNB at 412 nm for 2–3 min at 30-s intervals. Controls without the

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