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# Free and nanoencapsulated curcumin prevents scopolamine-induced cognitive impairment in adult zebrafish

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ABSTRACT

Cognitive dysfunctions are the main symptoms associated with Alzheimer's disease (AD). In the last decade, the use of natural substances to prevent or alleviate neurological symptoms have attracted great interest. Curcumin, the main polyphenolic component of turmeric (*Curcuma longa*) rhizomes, has a variety of beneficial properties, including potent antioxidant and anti-inflammatory properties. Despite the therapeutic potential for neurodegenerative diseases including AD, the use of this natural compound as a drug in therapies has huge limitations due to its low water solubility, physicochemical instability, and poor bioavailability. Nanotechnology is an important tool to overcome these drawbacks. Here, the safety of curcumin and its nanoencapsulated form was evaluated using zebrafish embryos. We also investigated the potential protective effect of curcumin and nanoencapsulated curcumin (50 mg/kg, single injection, i.p.) against scopolamine-induced memory deficits in adult zebrafish. The embryotoxicity test revealed that the formulations in any dose tested did not cause embryo mortality or affect the hatching rate. Data showed that both curcumin and nanoencapsulated curcumin are effective in preventing scopolamine-induced cognitive impairments without altering the animals' locomotion. These findings suggest that these treatments are safe and could be useful for enhancing memory function in AD or other age-related disorders.

## 1. Introduction

Alzheimer disease (AD), the most common form of dementia worldwide, is characterized by the progressive decline of memory and cognitive functioning, speech loss, personality changes, and ultimately death [1]. AD is the sixth leading cause of death in the U.S. [2], and its occurrence is increasing due to ageing populations and a lack of disease treatment. The major brain changes associated with AD are the accumulation of beta-amyloid plaques outside neurons. These plaques interfere with neuron-to-neuron communication at synapses, and the accumulation of a tau tangles inside neurons block the transport of nutrients and other essential molecules [3]. Additionally, deficits in cholinergic and glutamatergic neurotransmission have been associated with the symptomatology of Alzheimer's disease [4], as well as oxidative stress which contributes to the aging and progression of neurodegenerative diseases [5,6].

The current therapeutic treatments used in AD include acetylcholinesterase inhibitors and one *N*-methyl-D-aspartate (NMDA) receptor antagonist [1]. These drugs help manage disease symptoms, but they are unable to reverse the disease or to stop the damage and destruction of neurons. Consequently, their effectiveness is limited in duration and eventually declines as brain cell damage progresses [2]. In addition, acetylcholinesterase inhibitors have short half-lives and severe side effects [7]. Therefore, there is a critical need to develop safe and effective pharmacological agents aimed at the prevention and amelioration of AD.

The use of dietary polyphenols has been proposed as a novel, safe, and effective strategy for the prevention and amelioration of neurodegenerative disease symptoms [8]. Curcumin, a dietary compound found in the curry spice turmeric, is a precious natural molecule which exhibits

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an extraordinary list of pharmacological effects. This polyphenol has multiple desirable characteristics for neuronal disorders, including anti-inflammatory and antioxidant activities [9]. Curcumin possesses the ability to modulate different molecular targets involved in neurodegenerative diseases which include transcription factors, growth factors, protein kinases, and enzymes [10]. Previous studies have reported acetylcholinesterase inhibitory activity for curcumin as well as highlighted its potential use in the prevention of learning and memory impairments [11–15]. Moreover, Akinyemi and co-workers [15] reported the mechanisms by which curcumin improves cognition and exerts neuroprotective efficacy in a model of scopolamine-induced memory impairment in rats. This polyphenol showed inhibitory effects on the activity of enzymes of the cholinergic system (acetylcholinesterase and butyrylcholinesterase) and adenosine deaminase. Also, it decreased lipid peroxidation and increased nitric oxide (NO) levels and antioxidant status when compared with scopolamine-treated rats.

The major drawbacks associated with curcumin are its low bioavailability, extensive metabolism, chemical instability, and low solubility in water [16,17]. Even with all of the therapeutic benefits, these limitations challenge the production of suitable formulations and are the main obstacles for potential medical applications of curcumin [18]. Poorly water-soluble compounds are difficult to formulate using conventional approaches and are associated with formulation-related performance issues like low bioavailability, which results in suboptimal drug delivery [19].

Polymeric nanoparticles provide a type of effective drug delivery system for lipophilic compounds such as curcumin [20]. Compared to conventional drug delivery systems, the use of nanoparticles offers advantages such as controlled release, better stability, increased bioavailability, and selective targeting. Additionally, nanotechnology can be used to improve the efficacy and reduce the number and frequency of drug doses [21–23]. In our previous report, we showed that the association of curcumin to polymeric nanocapsules provides its controlled release [24], improves its stability, and increases its *in vitro* antioxidant activity when compared to non-encapsulated curcumin [25]. The nanoencapsulation also enhanced anti-inflammatory activities of curcumin [26] and reduced the dosage required for pharmacological effects [14,24].

The muscarinic antagonist scopolamine is widely used as a model of cognitive deficits to determine the potential of new therapeutic agents for Alzheimer's disease [27]. While rodent models have been traditionally used for this purpose, zebrafish (*Danio rerio*), a small freshwater fish, is gaining popularity [28–30]. The zebrafish is a powerful animal model that is widely used in biomedical research, including neuroscience [31]. It is currently used as an animal model for various human brain disorders like anxiety, depression, cognitive behavior, neurodegeneration, epilepsy, and others [32,33]. The major zebrafish neurobehavioral tests of exploration, anxiety, and locomotion are parallel to those traditionally used in mammals [32]. Its utility for pharmacological research is supported by the ability of these animals to respond to the drugs in a manner similar to humans [34]. Also, zebrafish have been used to evaluate *in vivo* toxicity and the safety of drugs and chemicals as well as nanoparticles [35–37].

The zebrafish embryotoxicity model is used in toxicological trials due to the short time required for analyses, the transparency of embryos, rapid embryonic development (out of the maternal body), high fertility, and genetic similarity. Therefore, this model can be used to predict toxicity effects in humans [38]. The general advantages of zebrafish are their high degree of physiological and genetic similarity to humans and a central nervous system (CNS) with a homologous general structural plan [31,39]. Zebrafish are also very cost-efficient, easy to breed, develop rapidly, and can be housed in large numbers in a relatively small space [32].

Although curcumin has been reported to have beneficial effects for treatment of cognitive impairment, there are no studies reporting these effects using a zebrafish animal model, which may be an alternative model for future studies to elucidate the possible biochemical pathways. There has been an increase in requests to reduce, refine, and replace mammalian models. Therefore, in this study we evaluated the potential of acute administration of both curcumin and its nanoencapsulated form in preventing cognitive impairment caused by scopolamine in adult zebrafish. In addition, the embryotoxicity of these treatments was also assessed.

## 2. Materials and methods

### 2.1. Materials

Curcumin, poly (ε-caprolactone) (PCL), and sorbitan monostearate were obtained from Sigma-Aldrich (São Paulo, Brazil). Grape seed oil and polysorbate 80 were purchased from Dellaware (Porto Alegre, Brazil) and Vetec (Rio de Janeiro, Brazil), respectively.

### 2.2. Preparation and characterization of polymeric nanocapsules

Curcumin-loaded nanocapsules ($NC_{CURC}$) were prepared (3 batches, $n = 3$) by interfacial deposition of preformed polymer method [40]. $NC_{CURC}$ was produced by dissolving 0.01 g of curcumin, 0.1 g of PCL, 165 μL of grape seed oil, and 0.0385 g of sorbitan monoestearate in acetone. This organic phase was injected into an aqueous phase containing 0.077 g of polysorbate 80. The suspension was submitted to reduced pressure to eliminate acetone and adjust the final volume to 10 mL. Nanocapsules without curcumin were prepared by omitting it in the organic phase, and this formulation was called NC. Both formulation ($NC_{CURC}$ and NC) batches were prepared in triplicate and stored protected from light. Photon correlation spectroscopy (Zetasizer Nano ZS, Malvern Instruments, Malvern, UK) was used to measure the particle size (mean particle diameter, Z-average), the polydispersity index (PDI), and the zeta potential value of the nanoparticles. Nanoparticle suspensions were diluted (1:500) with ultrapure water (Z-average and PDI) and 10 mM NaCl (zeta potential), and the measurements were conducted at 25 °C. The pH measurements were carried out directly in the samples using a calibrated potentiometer (VB-10, Denver Instrument, USA). Morphological analyses were carried out by transmission electron microscopy (JEM 1200 Exll, JEOL, Tokyo, Japan) operating at 120 kV (Centro de Microscopia, Federal University of Rio Grande do Sul). $NC_{CURC}$ suspensions were diluted in ultrapure water (1:10 v/v) and placed on a specimen grid (Formvar-Carbon support films). Uranyl acetate solution (2% w/v) was used as a negative staining reagent. The curcumin content of the NC was determined after appropriate dissolution in acetonitrile and later in the mobile phase, and it was assayed by high-performance liquid chromatography (HPLC) using a previously validated method [25]. All analyses were carried out for three different batches.

### 2.3. Animals

Adult females and males wild type zebrafish (*Danio rerio*) (0.2–0.25 g) were obtained from a local specialized supplier (Redfish Agroloja, RS, Brazil). Animals were kept in housing tanks with unchlorinated water, in a proportion of five animals per liter, at a targeted temperature of 26 ± 2 °C and continuously aerated under a 14:10 h light:dark photoperiod. The fish were acclimated to the laboratory environment for at least 20 days, and they were fed three times a day with commercial flake food supplemented with brine shrimp. During fish maintenance, water parameters were monitored daily and maintained in the following ranges: pH at 6.5 to 7.5, conductivity at 400 to 600 μS, and ammonium concentration at < 0.004 ppm. For breeding, females and males (1:2) were placed in breeding tanks (beach style design - Tecniplast, Italy) overnight and separated by a transparent barrier. After the adults spawned, viable embryos were collected and used for toxicity assays. The study followed the guidelines of the National Animal Experimentation Control

Council (CONCEA), and all protocols were approved by the Animal Care Committee from Pontifícia Universidade Católica do Rio Grande do Sul (CEUA 11/00248).

### 2.4. Zebrafish embryo toxicity assay

Embryos were collected from breeding tanks and transferred to sterile 24-well cell culture plates (6 embryos per well, n = 3) kept in incubators at 28.5 °C and a controlled 14-10 h light-dark cycle. Eighteen embryos were used for each treatment. Embryos were treated from 1 h post-fertilization (hpf) with system water (RO water equilibrated with Instant Ocean salts - $H_2O$) or DMSO, Tween 80®, curcumin aqueous solution containing 5% DMSO and 1.5% Tween 80® (0.05, 0.5, 5 or 10 μM), NC and $NC_{CURC}$ (0.05, 0.5, 5 or 10 μM) diluted in system water (3 mL). The treatments were renewed at 24 h intervals. The survival and hatching rates of zebrafish embryos were monitored until 3 days post-fertilization (dpf) under an inverted stereomicroscope (Nikon).

### 2.5. Behavioral analyses

#### 2.5.1. Locomotion

Locomotor analysis was performed to verify if the treatment with curcumin, in the free and nanoencapsulated forms, would be able to alter the locomotion of the animals *per se*. Adult animals were anesthetized with tricaine (1 g/L) and were injected intraperitoneally (i.p) with saline, vehicle (5% DMSO and 1.5% Tween), curcumin aqueous solution containing 5% DMSO and 1.5% Tween (25 and 50 mg/kg), NC, and $NC_{CURC}$ (25 and 50 mg/kg) at 2 h before the behavioral analysis. The locomotion of each animal was measured between 9:00 h and 13:00 h. Animals were placed individually in the experimental tanks (30 cm length x 15 cm height x 10 cm width) and habituated for 60 s, after which their behavior was recorded on video for 5 min [41,42]. The videos were analyzed using the ANY-Maze software (Stoelting Co. Wood Dale, IL, USA) with the experimental tank divided into equal parts by three digital vertical lines and one horizontal line. The behavioral parameters analyzed were distance travelled (m), mean speed (m/s), line crossings, and time spent in the upper zone (s).

#### 2.5.2. Inhibitory avoidance task

To assess whether curcumin could prevent the cognitive deficit caused by scopolamine, the animals anesthetized with tricaine were injected (i.p.) with saline, vehicle (5% DMSO and 1.5% Tween), curcumin aqueous solution containing 5% DMSO and 1.5% Tween (50 mg/kg), NC and $NC_{CURC}$ (50 mg/kg) 2 h before the beginning of the experiment. Immediately after injection, the animals were placed in a separate tank with highly aerated unchlorinated tap water (25 ± 2 °C) to facilitate the animal's recovery from the anesthesia. One hour before the beginning of the training session, the animals were transferred to another tank to receive the second treatment which consisted of the scopolamine treatment (200 μM dissolved in the water for 1 h) (Fig. 1). The animals that did not receive scopolamine were also transferred to another tank filled with water to ensure the homogeneity of stress presented to the fish. The experiment consists of two training sessions and the test, with a 24 h interval between them. In each session, the animals were placed individually in an experimental aquarium (18 cm long × 9 cm wide × 7 cm high) divided by a guillotine door (9 cm high × 7 cm wide) into two compartments of equal size, one black and one white. During the training session, the animal was placed in the white compartment with the door closed for 1 min for habituation and environment recognition. After this period, the division was lifted so that the fish could cross over to the dark side of the tank. Once the animal passed into the dark side, the guillotine door was again closed and an electric shock pulse of 3 ± 0.2 V was applied for 5 s. The animal was removed from the apparatus and returned to the aquarium housing with only water. Twenty-four hours later, the animals were subjected to the test session, which consisted of the same training protocol but without the shock. The latency to enter the dark compartment during the two sessions was determined, and the expected increase in the test session was used as an index of memory retention [30,43].

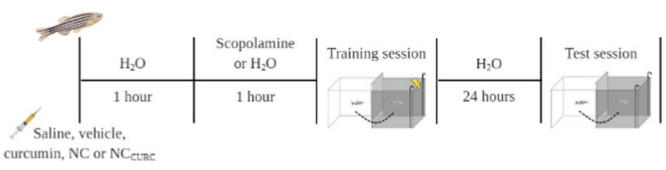


**Fig. 1.** Timeline of the experimental procedure of the inhibitory avoidance task. (Created with BioRender.com).

### 2.6. Statistical analysis

Survival data was analyzed by the Kaplan-Meier test. Inhibitory avoidance memory data are presented as mean ± S.E.M. Training and test latencies for each group were compared by the Wilcoxon matched pairs test. Latencies of multiple groups were compared using Kruskal–Wallis and Mann–Whitney U tests. Locomotion assessment was analyzed via one-way analysis of variance (ANOVA) followed by post hoc comparisons using Tukey's test, and it was expressed as the mean ± S.E.M. For all comparisons, $p < 0.05$ was considered significant.

## 3. Results

### 3.1. Physicochemical characterization of polymeric nanocapsules

The physicochemical characterization data of the nanocapsules in aqueous suspension are summarized in Table 1. Formulations presented a macroscopic homogeneous aspect. The PCS analysis showed nanometric size around 200 nm with narrow size distribution (PDI ≤ 0.10). Nanocapsule suspensions presented negative zeta potential with values between −14 and -7mV and pH values around 6.0. $NC_{CURC}$ presented a drug content of (0.98 mg/mL), which was close to the theoretical value (1.00 mg/mL). TEM images are shown in Fig. 2.

### 3.2. Zebrafish embryo toxicity assay

The toxicity of free and nanoencapsulated curcumin to zebrafish embryos was studied by observing survival and the hatching rates during the 72-hpf period. No significant difference in survival rate was observed when all groups were compared (Fig. 3). After 72-hpf, all of the embryos hatched successfully and no delayed hatching time was observed in any treatment.

### 3.3. Behavioral analyses

Zebrafish exploratory activity was evaluated 2 h after i.p. injections of saline, vehicle (5% DMSO and 1.5% Tween), curcumin aqueous solution (25 and 50 mg/kg), NC, and $NC_{CURC}$ (25 and 50 mg/kg) to evaluate if these compounds would be able to alter the locomotion of the adult animals. No significant differences in the distance travelled

**Table 1**
Physicochemical characteristics of polymeric nanocapsules (NC) and curcumin-loaded polymeric nanocapsules ($NC_{CURC}$) after preparation (Mean Value ± S.D, n = 3).

| Formulations | NC | $NC_{CURC}$ |
|---|---|---|
| Particle size (nm) | 193 ± 4 | 195 ± 1 |
| Polydispersity index | 0.10 ± 0.02 | 0.08 ± 0.00 |
| Zeta potential (mV) | −14.5 ± 2.3 | −7.0 ± 0.4 |
| pH | 6.5 ± 0.3 | 6.0 ± 0.1 |
| Drug loaded (mg/mL) | – | 0.98 ± 0.01 |

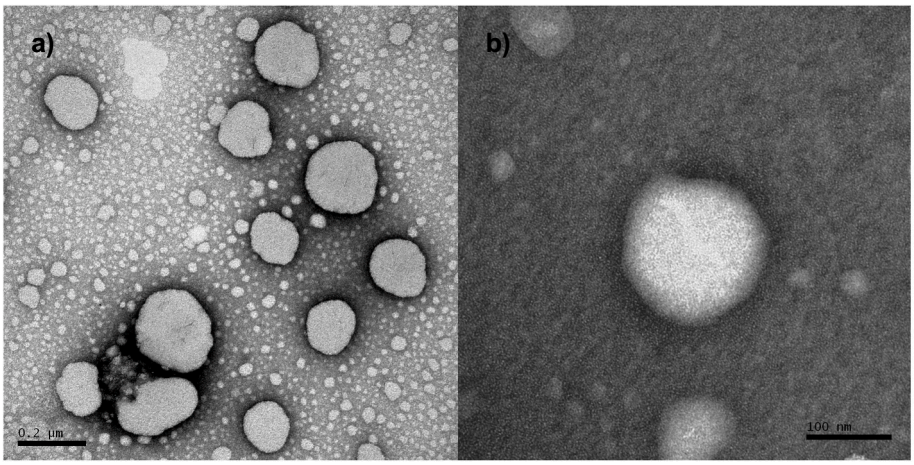


**Fig. 2.** Transmission electron microscopy images of $NC_{CURC}$. (a) bar = 200 nm (100,000x) and (b) bar = 100 nm (250,000x).

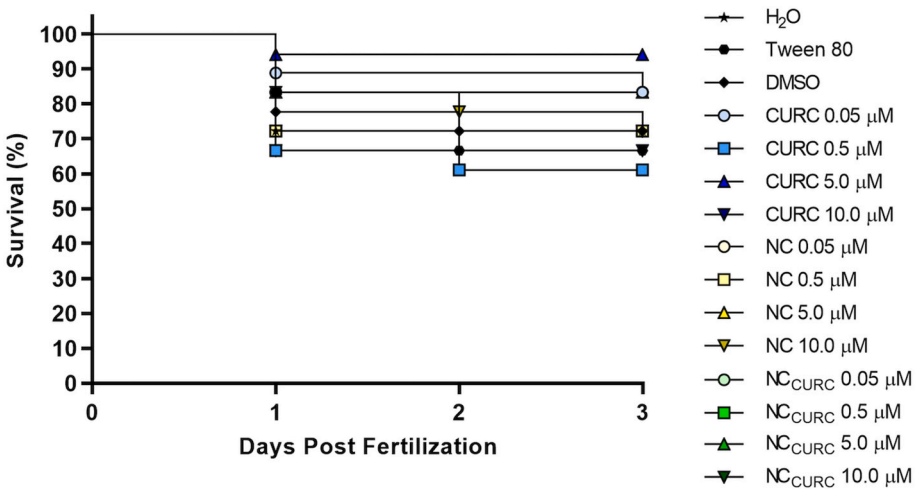


**Fig. 3.** Kaplan-Meier survival comparison for all groups throughout the experiment showed no significant effects (Log-rank (Mantel-Cox) test, p = 0.8440, N = 18 in triplicates).

($F_{(6,133)} = 2.402$, $p > 0.05$; Fig. 4a), mean speed ($F_{(6,133)} = 1.692$, $p > 0.05$; Fig. 4b) or line crossings ($F_{(6,133)} = 1.351$, $p > 0.05$; Fig. 4c) were observed in animals treated with curcumin or $NC_{CURC}$ when compared to the control group (saline) or specific vehicle (aqueous solution containing 5% DMSO and 1.5% Tween or NC, respectively). Moreover, no significant differences were found for time spent in the upper zone of the tank measure; this parameter was used to evaluate anxiety ($F_{(6,133)} = 0.7796$, $p > 0.05$; Fig. 4d).

Since no alterations were observed in the locomotor analyses, and this behavior would not influence the memory task, the effect of curcumin and nanoencapsulated curcumin at the highest dose (50 mg/kg) was studied on scopolamine-induced memory impairment using an inhibitory avoidance task. The effects of pretreatment with non-encapsulated curcumin (50 mg/kg) and $NC_{CURC}$ (50 mg/kg) were independently evaluated after the pre-training with scopolamine (200 µM) exposure (Fig. 5). Saline was used as naive control, while an aqueous vehicle containing DMSO (5% w/v) and Tween 80 (1.5% w/v) was used as the vehicle for non-encapsulated curcumin, whereas NC was used as the vehicle for nanoencapsulated curcumin. The use of these different controls for each treatment (curcumin in solution and nanoencapsulated curcumin) was necessary due to the need to use DMSO and Tween 80 as aqueous solubility enhancers for curcumin, a low water soluble molecule, whereas the nanoencapsulated curcumin had already been formulated as an organic solvent-free aqueous dispersion. Moreover, this strategy is important to avoid any misinterpretation of data due to the presence of the nanosized particles in the treatment. Fig. 5a shows that animals treated with saline, vehicle, or curcumin demonstrated a robust memory retention during the test session performed 24 h after the training session ($p < 0.001$, $p < 0.0001$ and $p < 0.0001$, respectively). Similarly, Fig. 5b demonstrates the same behavior when the animals were treated with saline, NC, and $NC_{CURC}$ following exposure to water ($p < 0.001$, $p < 0.01$ and $p < 0.01$, respectively). Exposure to scopolamine after treatment with saline ($p = 0.8438$, Fig. 5a; and $p = 0.0985$, Fig. 5b), vehicle ($p = 0.0472$), and NC ($p = 0.5304$) showed that animals did not exhibit memory retention during the test session performed 24 h after training. The treatment with curcumin (Fig. 5a) and $NC_{CURC}$ (Fig. 5b) prevented the memory impairment caused by scopolamine exposure, as observed by the difference in latencies between training and test sessions for each treatment ($p < 0.0001$ and $p < 0.001$, respectively).

## 4. Discussion

Cognitive dysfunctions are the main symptoms associated with AD. Scopolamine, a nonselective muscarinic antagonist, has been used in the field of neuropsychopharmacology as a standard drug for inducing age- and dementia-related cognitive deficits in animals, and it is widely used for screening of anti-dementia drugs [27]. Although rodents are the most

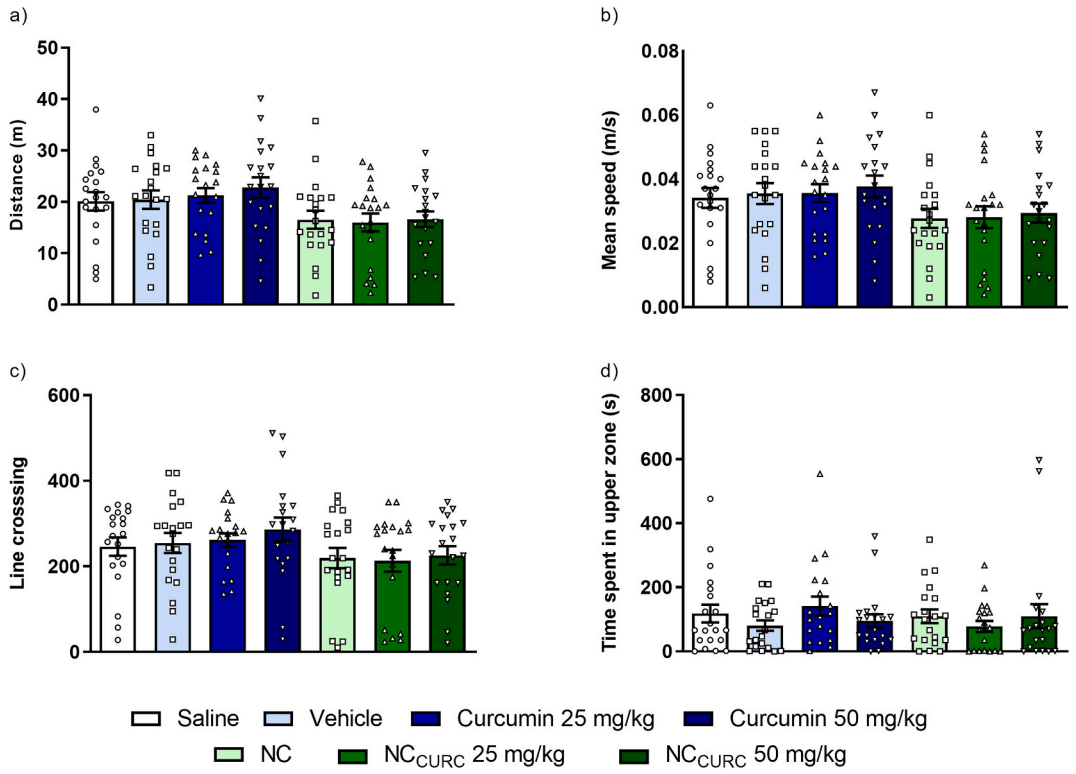


**Fig. 4.** Effects of curcumin and curcumin-loaded nanocapsules on locomotor activity of adult zebrafish. Distance travelled (a), mean speed (b), line crossing (c), and time spent in the tank upper zone (d) were evaluated 2 h after treatment. Data are expressed as the mean ± S.E.M. of 20 animals analyzed individually for each group using one-way ANOVA followed by Tukey's post-hoc test.

frequently employed animals for studies of brain function, in the last years, there has been a growing interest in the zebrafish as an animal model for neurodegenerative diseases [34]. Although some previous studies reported the *in vivo* effects of curcumin in neuroprotection [11–15], there is still a lack of studies regarding the means to reduce, refine, and replace mammalian models to evaluate these curcumin effects. Therefore, the neuroprotective potential of curcumin in solution (non-encapsulated curcumin) and nanoencapsulated was assessed using this animal model (zebrafish).

New therapeutic modalities with efficacy and better safety profiles for the prevention and amelioration of AD have long been desired. Curcumin has been the subject of intense investigation in recent years for its therapeutic potential in several diseases [44]. Pharmacological studies have shown that this polyphenol exerts neuroprotective- and cognitive-enhancing effects in animal models of AD [45]. Akinyemi and co-workers [15] showed that curcumin improves learning and memory associated with inhibitory effect on acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), and adenosine deaminase (ADA) activities as compared to controls. In addition, curcumin pretreatment decreased lipid peroxidation and increased NO levels and antioxidant status when compared with scopolamine-treated rats [15]. However, curcumin is difficult to formulate due to its low aqueous solubility, and it has limited pharmaceutical applications due its poor bioavailability [46]. In this scenario, aqueous dispersions composed of curcumin-loaded nanocapsules were produced as previously reported by our group [24,25,47]. The batches produced here had mean particle sizes in the range of 200 nm, in agreement with our previous report [24]. The formulations (NC and $NC_{CURC}$) showed homogeneous size distribution with PDI values smaller than 0.2. This is an ideal result because values lower than 0.3 indicate low variations in particle size classes [48]. TEM analyses showed spherical particles with particle sizes in agreement with those obtained by photon correlation spectroscopy. The zeta potential, which reflects the charge on the particle surface, was another parameter evaluated. As expected, NCs exhibited a slightly negative zeta potential due to the steric effect caused by polysorbate 80, which is coating the nanocapsules [40]. Moreover, polysorbate 80, a non-ionic surfactant that provides a hydrophilic surface to nanoparticles, has been used as strategy to enhance the brain delivery of biodegradable nanoparticles [49].

The search for more effective treatments is one of the main stimuli for new research studies in the pharmaceutical area, supporting the use of nanotechnology. However, despite the specific properties of nanoparticles that make them extremely attractive, it is also necessary to know their potential toxicity to human and environment health. In recent years, zebrafish have been successfully investigated as model systems in nanotoxicology. The zebrafish embryotoxicity test has been used in the majority of tests applied to study the toxicity of inorganic nanomaterials (mainly Ag NP, $SiO_2$ NPs, $TiO_2$ and ZnO NPs) when compared to organic nanomaterials [36]. On the other hand, some recent reports have investigated the toxicity of some nanocapsule formulations in zebrafish [50,51].

To assess the toxicity of curcumin, $NC_{CURC}$, and NC, the zebrafish embryos were exposed to different concentrations of these formulations, and the survival and hatching rates were observed during 72 hpf. In the present study, no significant difference in these events was observed among all groups. In contrast, Wu and co-workers [52] observed significantly decreasing survival and hatching rates of embryos after curcumin treatments at doses of 7.5 μM or higher. Also, in the presence of 15 μM of curcumin, all embryos died within 48 h of incubation. These different data in comparison with our study may be explained by the differences in the *in vitro* protocols between them, such as the post-fertilization time for the beginning of the treatment, environmental factors, treatment renewal after a period of time, and the composition of the diluent to obtain the curcumin solution. Zebrafish hatching is an important event to understand nanotoxicity. Several studies showed that the inorganic NPs reduced or accelerated the hatching rate of zebrafish

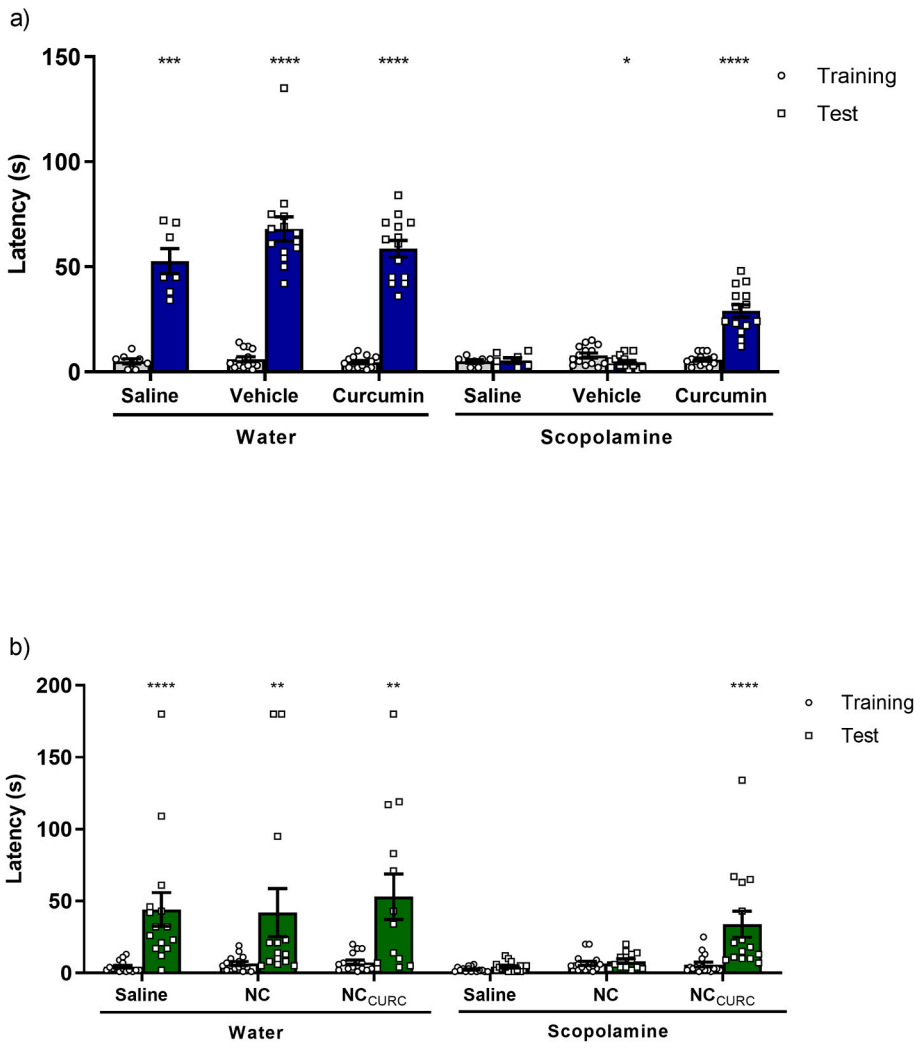


**Fig. 5.** Pretreatment with curcumin (a) or curcumin-loaded nanocapsules (b) prevented scopolamine-induced memory impairment during the inhibitory avoidance task. (a) Animals received a single i.p. injection of saline, vehicle (5% DMSO and 1.5% Tween in water) or curcumin (50 mg/kg). (b) Animals received a single i.p. injection of saline, NC or $NC_{CURC}$ (50 mg/kg). The i.p. treatment, administered 2 h before the training sessions was followed by a 1 h exposure of either water or scopolamine prior to testing. The effects of scopolamine and water on the latency to enter the dark compartment in training and test sessions in the inhibitory avoidance task were evaluated. Data are presented as mean ± S.E.M. (n = 14 per group); symbols indicate the differences between training and test sessions for each group compared via Mann Whitney test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$).

embryos [36]. The results observed were similar to those for chitosan nanoparticles and their Tween 80 modified counterparts. Both biodegradable nanoparticles showed a dose-dependent increase in the developmental toxicity of zebrafish embryos (decreased hatching rate, increased mortality, and incidences of malformation) [53].

Regarding the adult zebrafish experiments, to discard the influence of peripheral locomotor effects in the alteration in behavior, a general analysis of zebrafish locomotor behavior was performed prior to testing. The results showed that no changes in the locomotion parameters occurred after treatments (Fig. 4), confirming that the memory deficits observed in the animals treated with scopolamine were due to this drug effect on the cholinergic system and not due to altered locomotor behavior. Furthermore, the acute intraperitoneal administration of curcumin and nanoparticles (NC and $NC_{CURC}$) did not change exploratory activity in adult zebrafish. Also, we showed that fish exposed to curcumin and nanoparticles (NC and $NC_{CURC}$) did not demonstrate an anxiogenic effect. When compared with the control, no significant differences were found in the time spent in the upper zone of the tank. The time spent in upper zone indicated an anxiolytic-like behavior index because the normal exploratory behavior of the zebrafish, when introduced into a new environment, is to spend more time at the tank bottom and to gradually move to the upper zone after a few minutes [54]. Behavioral toxicity has been commonly noted for some types of nanoparticles. For example, locomotion, novel tank exploration, aggression, shoaling, and color preference activities were significantly reduced for the carbon 60 nanoparticle-treated adult zebrafish [35].

Polymeric nanocapsules have been reported as an important strategy to improve the pharmacological activity of curcumin [14,24,47]. Hoppe and co-workers [14] demonstrated that administration of both non-encapsulated and nanoencapsulated curcumin was effective in preventing behavioral impairments, neuroinflammation, and tau hyperphosphorylation as well as cell signaling disturbances triggered by β-amyloid *in vivo*. Also, both formulations (free and nanoencapsulated) prevented the memory impairment, the redox imbalance, and the alterations observed in the ATPase activities on rats exposed to cigarette smoke [47]. In these studies, nanoencapsulated curcumin in a dose 20-fold lower and 12.5-fold lower, respectively, presented similar neuroprotective results when compared to the effective dose of free curcumin. Data obtained herein showed that both the free form and nanoencapsulated curcumin induces an improvement in scopolamine-induced memory impairment (Fig. 5a and b). In addition, it is important to highlight that nanoencapsulated curcumin was formulated as an aqueous suspension to overcome its low aqueous solubility.

Up to now, there are no studies showing the neuroprotective effects of curcumin or nanoencapsulated curcumin against cognitive impairments induced by scopolamine in a zebrafish animal model. Here, we demonstrated that one single intraperitoneal administration of 50 mg/kg of free or nanoencapsulated curcumin was effective in preventing behavioral impairments. In addition, there was a difference in the latencies of training and test sessions in animals exposed to the vehicle (5% DMSO and Tween 80), followed by scopolamine exposure. These findings suggest that the vehicle exacerbated the memory impairment

induced by scopolamine. Despite this effect, curcumin (dissolved in this vehicle) was able to prevent the memory impairment induced by scopolamine, reinforcing the preventive effect of curcumin. Our results agree with previous studies that revealed similar beneficial effects of curcumin [13,15] or nanoencapsulated curcumin in a rodent animal model [14,47]. However, it should be highlighted that curcumin encapsulated in nanocapsules showed a slower drug release profile compared with its organic solution, as previously reported by our group [55]. Therefore, this point must be kept in mind in the interpretation of these data. The low amount of curcumin released from the nanocapsules after 2 h, as previously reported [55], may explain this lack of differences in the effect on cognitive impairment between nanoencapsulated and non-encapsulated curcumin according to the protocol used in our study.

Therefore, curcumin targeting specific tissues by its nanoencapsulation may be occurring in zebrafish, paving the way for these future studies. The mechanisms involved in the neuroprotective effect of both free and nanoencapsulated curcumin in cognitive deficits induced by scopolamine in adult zebrafish should be also deeply investigated in future studies. However, we can hypothesize that the effects are linked with the effects of curcumin on the cholinergic system and adenosine signaling as well as its antioxidant potential [15,30,56]. Scopolamine effects on learning and memory are characterized by a decrease in central cholinergic functions and adenosine levels in the brain, as well as by lipid peroxidation [15]. In adult zebrafish specifically, the memory deficits caused by scopolamine were previously demonstrated in a variety of learning paradigms [28–30,56].

The cholinergic system is widely known to play a critical role in cognitive processes. AChE is a target for emerging therapeutic strategies to treat AD. Kim and co-workers [28] showed that the amnesic effect of scopolamine on adult zebrafish appeared to be related to the cholinergic system because physostigmine, an AChE inhibitor, reversed the learning deficits. Furthermore, adenosine, a purine ribonucleoside, has been reported as an important neuromodulator in synaptic plasticity, and its depletion can disrupt memory formation [57]. Bortolotto and co-workers [30] showed that the antagonism of adenosine receptors or a possible increase of adenosine levels prevented cognitive impairment induced by scopolamine in adult zebrafish, corroborating with the hypothesis that adenosine signaling is involved in memory processing in zebrafish and may be a target for the development of preventive strategies against cognitive impairment.

## 5. Conclusion

In summary, this study showed that one single administration of 50 mg/kg of both non-encapsulated and nanoencapsulated curcumin was effective in preventing scopolamine-induced memory deficits in a zebrafish model. Both formulations did not affect adult zebrafish locomotor behavior. Also, they did not induce mortality or affect the hatching rate of zebrafish embryos, suggesting the potential safety of this formulation. Although there are previous reports of this curcumin activity in rodent animal models, this is the first study showing the effects of free and nanoencapsulated curcumin on cognitive deficit in a zebrafish model. Further studies should be carried out to evaluate the effects of nanoencapsulated curcumin in this model for a longer time as well as curcumin biodistribution. On the other hand, the data show the neuroprotective action of curcumin and provide a new approach to elucidate the biochemical targets involved in their protective effects using an alternative model organism.

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## Declaration of competing interest

The author reports no conflicts of interest.

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