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ORIGINAL ARTICLE

Synthesis of Quercetin-Metal Complexes, In Vitro and In Silico Anticholinesterase and Antioxidant Evaluation, and In Vivo Toxicological and Anxiolitic Activities

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Abstract

The level of acetylcholine, a neurotransmitter essential for processing memory and learning, is lower in the brains of patients with Alzheimer's disease due to the higher concentration of the enzyme acetylcholinesterase. The main compounds used for Alzheimer's treatment are acetylcholinesterase inhibitors. Quercetin coordination complexes with the metal ions Cu^{+2}, Zn^{+2}, Ni^{+2}, Co^{+2}, and Fe^{+2} were synthesized to investigate their potential use against Alzheimer's disease, by evaluating the inhibition of acetylcholinesterase in vitro and in silico, as well as the antioxidant activity, toxicity, and anxiolytic action in the zebrafish (*Danio rerio*) model. The organic complexes were characterized by UV-Vis and FT-IR. The spectral information suggested that coordination of metals occurs with the carbonyl group and OH linked to the C-3 carbon of quercetin. The quercetin-Fe (QFe) complex presented the best antioxidant and antiacetylcholinesterase actions, and these results were confirmed by molecular docking. In the toxicity and locomotor evaluation, the quercetin molecules and the synthesized complexes, mainly QCu and QZn derivatives, showed the highest degree of inhibition of the fish's locomotor activity, suggesting a possible anxiolytic action. Then, quercetin complexes with metals, mainly with Fe^{+2}, represent valuable compounds and deserve more investigation as promising agents against Alzheimer's disease.

Keywords Alzheimer's disease · Coordination compounds · Flavonoids

Introduction

Alzheimer's disease is the most common type of dementia. Evidence exists for both cholinergic and glutamatergic involvement in the disease's etiology. Acetylcholine (ACh), a neurotransmitter essential for processing memory and learning, is decreased in both concentration and function in patients with Alzheimer's disease (Paul and Borah 2017). Acetylcholinesterase (AChE) inhibitors have proven to be the most viable therapeutic target for symptomatic improvement of Alzheimer's disease (AD), because cholinergic deficit is a consistent and early finding in AD (Mehta et al. 2012).

Individuals with high anxiety have a 53% higher risk of AD, demonstrating that anxiety is a potential risk for this disease (Santabarbara et al. 2019). Depression is also related to AD and is one of the most frequent non-cognitive symptoms. The prevalence of depressive symptoms and syndromic depression in AD depends on the severity of dementia and the scales used for its detection (Müller-Thomsen et al. 2005).

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Oxidative stress caused by free radicals has also been shown to be a major contributor to the development of AD (Yar et al. 2015). Oxidative stress, an increased process in the aging brain, is induced by an imbalance in the redox state, involving the generation of excess reactive oxygen species (ROS) or dysfunction of the antioxidant system.

The zebrafish (*Danio rerio*) has long been used as an animal model for biomedical research, particularly in genetic and developmental studies. It is physiologically homologous to humans, allowing researchers to probe the pathways and mechanisms relevant to human pathogenesis and clinical treatments (Egan et al. 2009). Zebrafish is fast becoming a promising model organism for research into anxiety and stress (Collier et al. 2017). Indeed, this species is demonstrating the potential to be an "exceptional" animal to investigate experimental, genetic, and pharmacological models of neurobehavioral disorders, such as depression (Pittman and Piato 2017).

Inhibition of AChE has proven to be the most viable therapeutic treatment for the symptomatic improvement of Alzheimer's disease (Geldmacher 2007), and flavonoids are promising natural compounds for the development of multipotent drugs against AD. These compounds have advantages, such as their high AChE inhibitory and antioxidant activities and low toxicity (Uriarte-Pueyo and Calvo 2011).

In the brains of patients with Alzheimer's disease, the levels of metals like iron and copper decrease in the affected areas (Magaki et al. 2007; Cassetta et al. 2012), leading to the hypothesis that deficiency of these elements contributes to the pathogenesis. Brocardo et al. (2005) showed zinc's inhibitory effect on AChE activity of the cerebral cortex and hippocampus of rats using a quercetin-Zn complex, with increased inhibition of AChE.

Typically, flavonoid-metal complexes are more active compared with free binders, since flavonoids play an important role in the bioavailability of metal ions, which are present at low levels in the human body (Park et al. 2018).

Quercetin is known as a versatile molecule because of its wide clinical effects, such as carcinogenic inhibition, decrease of cardiovascular disease, antiobesity effect, reduced risk of cataracts, and protective effects against neurodegenerative diseases (Kazemipour et al. 2018). Quercetin is one of the most abundant flavonoids and is found in fruits, leaves, and other plant parts. Due to the presence of chelating sites in its structure, quercetin is able to form complexes with a series of cations (de Castilho et al. 2018).

The chelating properties of quercetin (Fig. 1) are determined by their chemical structure, with two aromatic rings (benzoyl ring A and cinnamoyl ring B) having various hydroxyl groups, joined by O-heterocycle. The possible chelating sites are the 3-hydroxychromone, 5-hydroxychromone, and 3′,4′-dihydroxyl groups (Kalinowska et al. 2016).

Studies suggest that quercetin can effectively activate survival neurotransmitters (PKD1/CREB/BDNF) as well as enhance mitochondrial biogenesis in dopaminergic cells (Ay et al. 2017), while prophylactically protecting neuronal cells of neurotoxic action induced by chlorpyrifos, a powerful crystalline insecticide of the organophosphate class (Kaur et al. 2019). Our results suggest that quercetin is a promising neuroprotective agent, especially when combined with metal coordination, as shown below.

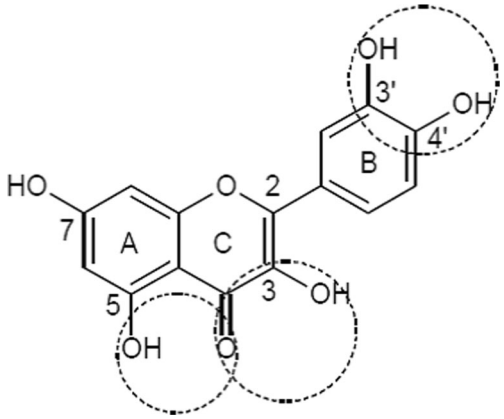

Fig. 1 Representation of the quercetin structure, showing the chelating sites

This article describes efforts to obtain a useful product for the treatment of Alzheimer's disease through the synthesis of quercetin complexes with five metals (Fe^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, and Cu^{2+}) and compares their antioxidant, anxiolytic, and acetylcholinesterase inhibition actions and toxicity in zebrafish.

Materials and Methods

All reagents and solvents employed in the syntheses and analyses were purchased from commercial sources and used without prior purification.

Quercetin Complex Synthesis

Syntheses of the complexes were performed with the metal salts $FeSO_4 \cdot 7H_2O$, $Cu(CH_3COO)_2$, $Zn(CH_2COO)_2$, $NiCl_2$, and $CoCl_2$ with quercetin (Q) as ligand in a stoichiometric ratio of 1:2. The quercetin binder was dissolved in 10 mL of methanol and the metal salt in 10 mL of distilled water. The solutions were added to a 50-mL flask under stirring at room temperature. After 20 min of reaction, 3 drops of triethylamine were added to the solution, which immediately changed the color of the solution. The reacting solution was stirred for 3 h. After this period, the solution was left at low temperature (refrigerator) for 2 days for the total precipitation of the synthesized compound, which was then filtered through a porous plate funnel and stored in a desiccator under vacuum.

Spectroscopic Measurements

Spectrophotometric analyses were performed with a Shimadzu 1800 UV-Vis spectrophotometer. Infrared spectra were obtained using a Nicolet iS5 spectrophotometer from Thermo Scientific. The samples were prepared as KBr pellets in the ratio 1:20 (m/m) (sample: KBr), and the spectra were recorded in the range of 4000 to 400 cm^{-1}, using 32 scans and 4 cm^{-1}.

Antioxidant Activity

The antioxidant activity was measured in 96-well flat bottom plates using a BioTek ELISA reader, model ELX 800, using the Gen5 V2.04.11 software, based on the method described by Wang (2017). In the 96-well plates, the following solutions were used per well: 180 μL of DPPH (2,2-diphenyl-1-picrylhydrazyl) methanolic solution and 20 μL of the extract sample dissolved in methanol and diluted 10 times to obtain the final concentration of 0.2 $mg.mL^{-1}$.

The percent inhibition (PI%) was calculated by comparing the reaction rates of the samples relative to the controls. The standard used as the positive control was the quercetin molecule. The results were expressed as percent inhibition, calculated with the following formula:

$$PI\% = \frac{AC - AS}{AC} \times 100$$

AC absorbance of DPPH control at time 0
AS absorbance of sample containing DPPH after 60 min

Antiacetylcholinesterase Activity

The inhibitory activity of the enzyme acetylcholinesterase (AChE) was measured in 96-well flat bottom plates using the same BioTek ELISA reader and software identified above, based on the method described by Ellman et al. (2002).

The dilutions of the samples and of the positive standards used in the quantitative microplate assays were based on 20 mg/mL standard solution to prepare the other solutions: 200, 100, 50, 25, 12.5, 6.25, 3.12, 1,56, and 0.78 μg mL^{-1}.

The parameters referring to the natural color of the extracts were dropped from the analysis. The percent inhibition of acetylcholinesterase was calculated by comparing the reaction rates (substrate hydrolysis) of the samples against the blank (considered total AChE activity, 100%). The standard used as a positive control was galantamine.

Locomotor Activity of Zebrafish (Open Field Test) and In Vivo Measuring of Acetylcholinesterase Inhibition

The open field test was performed to evaluate the possible alteration of the motor coordination of the animals, through sedation and/or muscle relaxation. Initially, the fish ($n = 6$/group) were treated (v.o.) with a test sample (4.0, 12, or 40 mg/kg) of the vehicle (DMSO 3%, 20 μL) or diazepam (DZP, sedative control; 2.5 mg/kg.). A group of animals without treatment was included (naive). After 1 h of treatment, the animals were placed in glass Petri dishes (10 × 15 cm) containing the same water as the aquarium, marked with four quadrants, and the locomotor activity was analyzed by counting the number of crossing lines. Using the CL value of the naive group as the baseline (100%), the percentage of locomotor activity (%LA) was calculated individually for 0–5 min.

After the zebrafish locomotor activity test, open field test, the fish was anesthetized in cold water and euthanized by decapitation. Shortly afterwards, the brains were dissected and homogenized. The hydrolysis rate 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.

Prior to substrate addition, 10 μg of protein sample was preincubated with the reaction medium described above for 10 min at − 18 °C. Acetylthiocholine hydrolysis was monitored by formation of DTNB dianion thiolate at 412 nm for 3 min at intervals of 30 s (de Abreu et al. 2019).

Sampling and Determination of Oxidative Status

The whole body of the animals was homogenized in pH 7.4 phosphate buffer. Then, the samples were centrifuged (3000*g* at 4 °C for 10 min). The precipitate was discarded and the supernatant was used for biochemical analysis. The oxidative mediator profile of the animals was expressed in relation to the protein concentration obtained in each homogenate. This concentration was determined by the Bradford method.

Catalase

Catalase activity was evaluated as previously described (Li and Schellhorn 2007), where H_2O_2 consumption is measured by reducing absorbance at 240 nm. Fifty microliters of sample was mixed with 2.9 mL phosphate buffer solution (pH 7.4) and 50 μL substrate (0.3 M H_2O_2). Absorbances at time zero and 60 s after substrate addition were measured, and absorbance reduction was used to calculate the concentration of H_2O_2 produced per minute. For conversion, it was considered that 1 mmol of H_2O_2 produced every minute is equivalent to 1 IU/mL of enzymatic activity.

Determination of TBARS Levels

Thiobarbituric acid reactive substances (TBARS), represented mainly by malondialdehyde (MDA), are formed as a byproduct of lipid peroxidation that can be detected by the TBARS assay using thiobarbituric acid as a reagent (Kil et al. 2014). MDA concentration was determined as an indicator of lipid peroxidation as described above using the TBARS method.

To perform the assay, 100 μL of the homogenate was mixed with 100 μL of 40% trichloroacetic acid and 400 μL of 60% thiobarbituric acid. The mixture was incubated at 96 °C for 30 min and then kept in an ice bath. To stop the reaction, 200 μL of glacial acetic acid was added to the centrifuged suspension (1700*g*, 30 min). The obtained supernatant was read on a spectrophotometer (ASYS UV 340, Biochrom, Cambridge, UK) in a 530-nm reader. TBARS concentrations were then calculated using a calibration curve using MDA as standard and expressed in μg MDA/mg protein.

Determination of GSH Levels

For the determination of reduced glutathione (γ-glutamil-L-cisteinilglicin, GSH), 400 μL of cell lysate was added to 800 μL of Tris-HCl buffer (0.4 M, pH 8.9) and 20 μL of the DTNB (5,5′-dithiobis-(2-nitrobenzoic acid)) chromogen, also called Ellman's reagent (Sigma-Aldrich, St. Louis, MO, USA). The absorbance was measured by spectrophotometry at 412 nm (UV ASYS 340, Biochrom, Cambridge, UK), using a GSH standard as a calibration curve. The results were expressed as nmol of GSH/mg of protein (Sharma et al. 1997).

Acute Toxicity

The acute toxicity study was carried out against adult zebrafish (*D. rerio*) according to the methods proposed in the literature (Oecd 1992; Huang et al. 2014). The animals ($n = 8$/each) were treated orally with 20 μL of a test sample (4.0, 12, or 40 mg/kg) or vehicle (control, DMSO 3%, 20 μL) and allowed to stand to analyze the mortality rate. Ninety-six hours after treatment, the number of dead fish in each group was counted and the median lethal dose (LD_{50}, the dose able to kill 50% of the animals) was determined using the trimmed Spearman-Karber method with confidence interval of 95% (Arellano-Aguilar et al. 2015).

Molecular Docking

The structures of quercetin and quercetin-Fe (QFe) ligands were plotted and optimized using Avogadro's code (Curtis et al. 2012), configured to use the classic MMFF94 force field (Molecular et al. 1996) (Molecular Mechanic Force Field 94), with cycles of 500 interactions, and the steepest descent algorithm, having the convergence limit of $10e^{-7}$.

The structure of acetylcholinesterase was obtained from the Protein Data Bank (http://www.rcsb.org/pdb/home/home.do), deposited with the code PDB 4EY6 generated from X-ray diffraction with resolution of 2.3 Å, classified as a hydrolase inhibitor, *Homo sapiens* expression system and organism, complexed with the ligand (galantamine) GNT (Cheung et al. 2012). The proteins for the molecular docking were prepared by removing the residues (H_2O, FUC, EDO, PE8, NO3, NAG, and GNT), then adding the polar (H) hydrogens, after which the structure was saved in PDBQT format. After the preparation of the ligands and the receptor, the molecular docking was performed using the code AutoDock Vina (Trott and Olson 2010) considering the rigid protein and the flexible ligands. Molecular and redocking graphs were plotted using the UCSF Chimera package (Pettersen et al. 2004).

Statistical Analysis

The results were expressed as mean ± standard error of the mean for each group of 6 animals. After confirming the normality of distribution and homogeneity of variance of the data, the differences between the groups were submitted to analysis of variance (one-way ANOVA), followed by the Tukey test. All analyses were performed using GraphPad Prism v. 5.0, with 5% statistical significance ($p < 0.05$).

Results and Discussion

Electronic Spectroscopy in the Ultraviolet and Visible Region

The electronic spectrum of quercetin in methanol has two absorption bands related to transitions $\pi \rightarrow \pi^*$, one at 378 nm, called band I, corresponding to the conjugated system between ring B and carbonyl of ring C (cinnamoyl system), and another at 260 nm, band II, referring to the conjugated system between ring A and carbonyl of ring C (benzoyl system). It is still possible to identify another band in the region of 215 nm in the electronic spectrum in the ultraviolet region of the molecule quercetin, attributed to the electronic transitions of the aromatic ring (Birjees Bukhari et al. 2008). The electron spectra of the complexes QCo, QCu, QFe, QNi, and QZn in dimethyl sulfoxide (DMSO) presented a bathochromic shift occurring in band I (Table 1), which can be explained by the interaction of the metal with the 3-hydroxyl group of quercetin, resulting in an electronic redistribution between the flavonoid molecule and the metal ion, forming an extended π binder system. According to Dehghan and Khoshkam (Cherrak et al. 2016), the 3-hydroxyl group has a more acidic hydrogen, so the 3-OH and 4-oxo groups are the first coordination sites

Table 1 Spectroscopy data in the UV-Vis region of the quercetin (Q) ligand in methanol and the QCo, QCu, QFe, QNi, and QZn complexes in DMSO

Compound	Bands (nm)	Band I (nm)	Band II (nm)
Quercetin (Q)	378, 260, 215	378	260
QCo	426, 255	426	255
QCu	256, 370, 420	420	256
QFe	256, 379, 442	442	256
QNi	256, 348, 449	449	256
QZn	254, 370, 443	443	254

involved in the process of complexation and influence the displacement of the band. The hydroxyl group located in position 5 (Fig. 1) is not involved in the coordination process due to its lower acidity and the spatial impediment caused by the first complexation.

After the formation of the five complexes, band II shifted by approximately 0–5 nm, while band I was very substantially shifted to the red region by approximately 100 nm, suggesting the metal-QC coordination through ring C. According to studies of the coordination of quercetin with iron ion II (Cherrak et al. 2016), a band at 438 nm in the electronic spectrum indicates the coordination of the metal with the 3-OH group to the metal center of Fe. In this work, the band observed in the electronic spectrum of QFe appeared at 442 nm, so in comparison, it can be stated that the coordination of the iron ion II also occurred with the 3-OH group. The data obtained in Table 1 are in agreement with those reported by Zhou et al. (2001) for the complexes under study. The bands of charge transfer transitions of the metal-binder type are also in the region observed for band I, which also corroborates the large shift.

Infrared Spectrum of Quercetin and Metal Complexes

Table 2 presents the electronic spectroscopy data in the infrared region of quercetin and the complexes under study, in KBr tablets. The comparative analysis of the infrared spectra of the quercetin molecule and the complexes under study indicated the presence of the main bands, with some changes due to the complexation process. The main alteration in the complexes synthesized is relative to the band assigned to the carbonyl group (νC=O). The absorption band is shifted to a lower frequency due to a decrease in the absorption of C=O bonding (1661 cm^{-1} in free quercetin), while in the complexes, the bands appear from 1647 to 1606 cm^{-1}. The absorbance for C–OH ranged from 1361 to 1380 cm^{-1} in relation to quercetin at 1375 cm^{-1}. Note also a variation of the order of binding of the band concerning the deformation of the C–OH phenol bond (1090 cm^{-1} in the ligand). The bands observed in the IV spectra in the range of 1508–1448 cm^{-1} in the QM complex are attributed to the asymmetric stretching of the C–O–metal group at the chelating site of the quercetin molecule, and in accordance with (Birjees Bukhari et al. 2008), the coordination occurs with the 3-OH group.

The absorption bands related to νM–O appear in the range of 410–590 cm^{-1}, as also reported by Bukhari (Arellano-Aguilar et al. 2015). The values of νM–O in the complexes under study followed Hooke's law, whereby the larger the mass of the metal, the lower the vibration frequency of the O–M bond, where $M = Fe^{II}$, Cu^{II}, Ni^{II}, Zn^{II}, or Co^{II}.

Figure 2 shows the proposed structure of the synthesized quercetin complexes with the metals, indicating the coordination of two quercetin molecules with one metal molecule due to the proportion used in the reaction.

Antiacetylcholinesterase and Antioxidant In Vitro Activities

The compounds QCo, QCu, QFe, and QZn presented relevant acetylcholinesterase inhibition (AChEI) potential at the concentrations tested. The best anti-AChE activity observed was for compound QFe, which showed IC_{50} values of 18 ± 0.29 μg/mL against the standard substances eserine and galantamine (Table 3).

In relation to the antioxidant activity, we found that IC_{50}Q = QFe > QCo > QNi = QZn > QCu, being closely related to the standard redox potentials of the metals, for Fe (− 0.44), Co (− 0, 28), Ni (− 0.25), Zn (− 0.76), and Cu (0.15) (Masterton et al. 1990).

Copper is an active redox metal that is used to catalyze a large number of biochemical reactions, such as the conversion

Table 2 Infrared spectroscopy data of quercetin and metal complexes in KBr pellets

Compound	ν(O–H) cm^{-1}	ν(C=O) cm^{-1}	ν(C=C) cm^{-1}	ν(C–OH) cm^{-1}	ν(C–O–C) cm^{-1}	δ(C–OH) cm^{-1}	νM–O
Quercetin	3250	1661	1602	1375	1239	1090	–
QFe	3417	1635	1595	1360	1273	1036	522
QCu	3403	1610	1560	1380	1263	1093	412
QNi	3403	1639	1606	1367	1268	1089	487
QZn	3421	1647	1599	1361	1268	1114	419
QCo	3407	1642	1611	1367	1273	1088	590

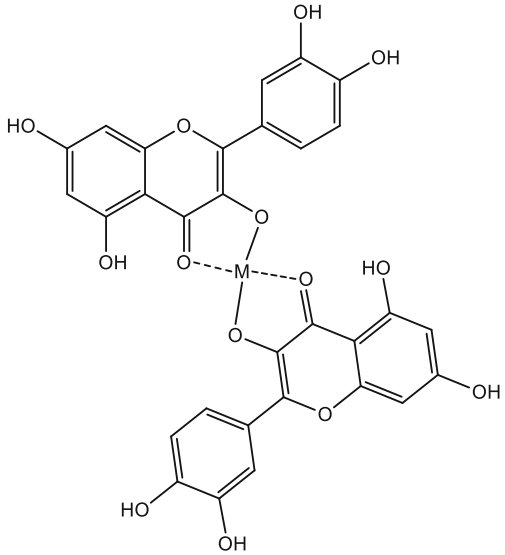

Fig. 2 Representation of chemical structure of the complexes with the quercetin molecule and $M = Fe^{II}$, Cu^{II}, Ni^{II}, Zn^{II}, or Co^{II}

of superoxide free radical into hydrogen peroxide, facilitating the export of iron from cells. It also plays a key role in the electron transport chain in mitochondria, as well as other important cell functions (Ridge et al. 2008; Robinson and Winge 2010; Horn and Barrientos 2008).

The same ability of copper to participate in these biological pathways also allows it to participate in various activities related to neurodegenerative mechanisms, such as in Menkes', Wilson's, and Alzheimer's diseases (Kodama et al. 1999; Bull et al. 1993; Deibel et al. 1996). In the brains of patients with Alzheimer's disease, copper levels decrease in the affected areas (Magaki et al. 2007; Cassetta et al. 2012), leading to the hypothesis that this copper deficiency contributes to the pathogenesis. Studies have shown that this metal, when coordinated with flavonoids, has biological activities such as antitumor (Tan et al. 2009), anti-inflammatory (Pereira et al. 2007), and anticoagulant (Pereira et al. 2007), among others. In this work, copper presented antioxidant activity correlated with antiacetylcholinesterase activity and statistically similar to that of QCo.

Table 3 Data from the biological assays of inhibition of acetylcholinesterase and the antioxidant activity of quercetin and its derivatives

Compounds	DPPH (μg/mL)	Antiache (μg/mL)
Copper (II) sulfate	44.92 ± 0.17^{f}	36.65 ± 0.02^{h}
Zinc acetate	52.89 ± 0.46^{g}	57.52 ± 0.04^{i}
Cobalt chloride	37.53 ± 0.17^{e}	57.30 ± 0.06^{i}
Nickel chloride	59.23 ± 0.13^{h}	28.18 ± 0.02^{g}
Iron (II) sulfate	58.65 ± 0.45^{h}	70.80 ± 0.59^{j}
Quercetin (Q)	1.05 ± 0.55^{a}	11.53 ± 0.4^{e}
Quercetin-cobalt (QCo)	3.08 ± 0.05^{b}	5.99 ± 0.14^{c}
Quercetin-copper (QCu)	5.65 ± 0.03^{d}	5.94 ± 0.12^{c}
Quercetin-iron (QFe)	1.08 ± 0.04^{a}	2.18 ± 0.29^{b}
Quercetin-nickel (QNi)	4.86 ± 0.02^{c}	12.36 ± 0.22^{f}
Quercetin-zinc (QZn)	4.34 ± 0.02^{c}	8.92 ± 0.07^{d}
Eserine	np	1.15 ± 0.05^{a}
Galantamine	np	1.07 ± 0.30^{a}

Similar lower case letters indicate similarities between rows for each assay ($p < 0.0001$, ANOVA followed by Tukey's test)

np not performed

According to Raszka et al. (2010), the exposure of snails to nickel was maintained for 2 weeks, evidencing an increase in AChE activity. Our results corroborate those, where the QNi compound caused a slight improvement in AChE inhibition activity in relation to the free ligand, whereas the antioxidant activity shown was statistically similar to that of QZn. Brocardo et al. (2005) reported a zinc inhibitory effect on AChE activity of the cerebral cortex and hippocampus of rats, and in the present study, QZn increased inhibition of AChE. It is known that some metals when in contact with cells induce oxidative stress leading to neurodegeneration. In previous studies, it was found that Zn demonstrates neuroprotective effect to DNA damage by Al-induced oxidative damage in rat brains, corroborating the results obtained in the present study (Singla and Dhawan 2013).

Iron is a very important transition metal in biological systems. It is present in the Fe^{2+} and Fe^{3+} oxidation states and is used in several essential reactions. The entropy of its distribution in the brain leads to several pathologies (Madsen and Gitlin 2007). Iron deficiency in patients with Alzheimer's disease may be associated with myelin degradation, indicating that the entropy of iron distribution is an early event in Alzheimer's and its correlated diseases (Magaki et al. 2007) implying that medicinal substances with an abundance of iron may act to prevent iron deficiency. There are reports of coordination of Fe^{2+} with antioxidant activity (Souza and Giovani 2004; Sungur and Uzar 2008). In this study, it was observed that the antioxidant activity of compound QFe and the ligand quercetin were statistically similar. As for antiacetylcholinesterase activity, the compound QFe showed that the coordination of iron with the quercetin molecule markedly increased the degree of enzymatic inhibition, and this activity was correlated with the antioxidant activity, as shown in Table 3.

In Vivo Antiacetylcholinesterase Activity Using Zebrafish

The best anticholinesterasic compound found in vitro experiments was QFe. The cholinergic system is important for the regulation of various behavioral and cognitive processes, acetylcholine is a signaling molecule of the cholinergic system that acts on both the CNS and neuromuscular synapses through nicotinic and muscarinic receptors (Picciotto et al. 2012). AChE cleaves extracellular acetylcholine into choline

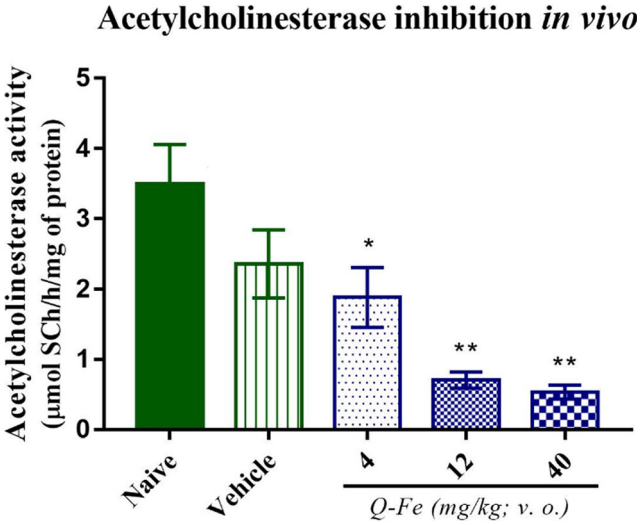

Fig. 3 Effect of QFe on cerebral AChE inhibition in zebrafish (*Danio rerio*). Oral treatment; naive—untreated animals; vehicle—DMSO 3% (2.5 mg/kg, v.o.). Values represent the mean ± standard error of the mean (SEM) for µmol SCh/h/mg of protein produced by 6 animals/group; ANOVA followed by Tukey (**$p < 0.001$; *$p < 0.05$ vs. naive or vehicle)

and acetate, potently regulating cholinergic neurotransmission (Gold et al. 2011). In this study, it was observed that QFe interacts with the cholinergic system, significantly reducing acetylcholine-evoked neurosignalizing currents in *Danio rerio* CNS cells and directly inhibiting local enzymatic activity. No data were found in the literature of other metal-coordinated natural compounds capable of modulating this activity as described, which is the first prospective study in this area so far (Fig. 3).

Evaluation of Locomotor Activity (Open Field Test)

The open field test in Petri plates, proposed by Ahmad and Ahmad and Richardson (2013), is employed to evaluate locomotor activity of adult zebrafish (ZF) under analgesic drug action (Batista et al. 2017). In this context, the same method was used with the samples (quercetin, QZn, QFe, QNi, QCu, and QCo) to evaluate their actions on the ZF locomotor system. The results are described in Figs. 4 and 5.

Free quercetin did not present an effect on zebrafish locomotion at any doses tested, similar to its cobalt derivative, and only had a slight effect on QFe. In the experiments related to the other derivatives, the effects on the locomotor activity of zebrafish were evidenced in the different doses. QZn was effective at the lowest dose, 4 mg/kg, compared with free quercetin similarly to other studies (Doboszewska et al. 2014), using zinc hydro aspartate, where it was observed an anxiolytic effect in rodents, indicating zinc is an agent for anxiolytic therapy. QCu, among all the quercetin derivatives, showed strong action on the locomotor system of zebrafish at doses of 4 mg/kg and 40 mg/kg, demonstrating possible anxiolytic activity. There are reports in the literature with studies with lithium metal, which demonstrate its effects on the modulation of the hippocampal antioxidant system and reduction of oxidative stress by normalized conduct with the metal in animals with high anxiety index. Reduced SO was followed by changes in protein levels that modulate neurotransmitter activity in metal-treated chronic stress animals (Popović et al. 2019). According to Hasan et al. (2016), the action of coordination compounds derived from naproxen had good anxiolytic results compared with free naproxen, as well as antinociceptive, CNS-depressant, and hypoglycemic properties.

Activity of QFe on Zebrafish Enzymes

Glutathione (GSH) plays an important role in a multitude of cellular processes, including differentiation, proliferation, and apoptosis, and as a result disorders in GSH homeostasis, which are implicated in the etiology and/or progression of

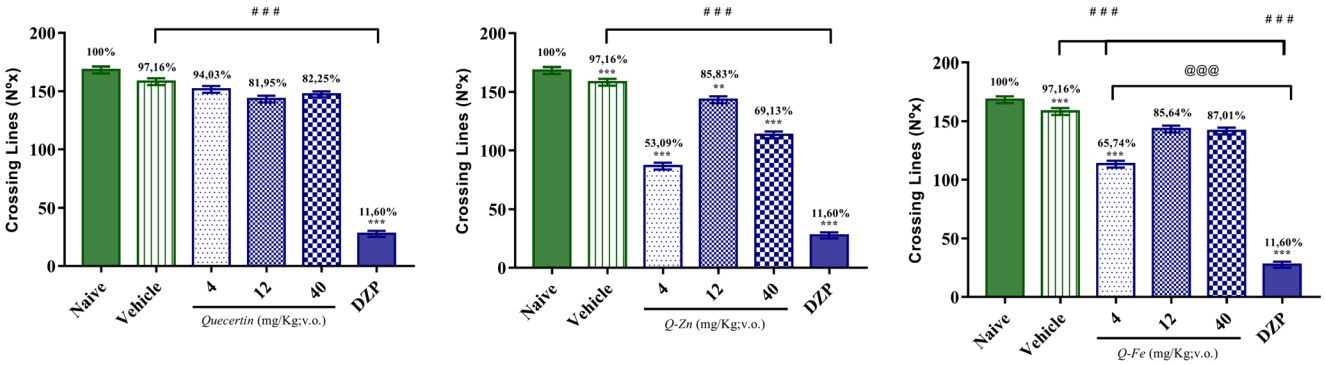

Fig. 4 Effect of quercetin on the locomotor activity of adult zebrafish (*Danio rerio*) in the open field test (0–5 min). Oral treatment; naive—untreated animals; vehicle—3% DMSO; DZP, diazepam (2.5 mg/kg, v.o.). Values represent the mean ± standard error of the mean (SEM) for 6 animals/group; ANOVA followed by Tukey test. Quercetin (***$p < 0.001$ vs. naive or vehicle; $^{###}p < 0.001$ vs. DZP). QZn (**$p < 0.01$; ***$p < 0.001$ vs. naive or vehicle; $^{###}p < 0.001$ vs. DZP). QFe (***$p < 0.001$ vs. naive; $^{###}p < 0.001$ vs. vehicle; $^{@@@}p < 0.001$ vs. DZP). The numbers above each column indicate the percentage of locomotor activity (%LA)

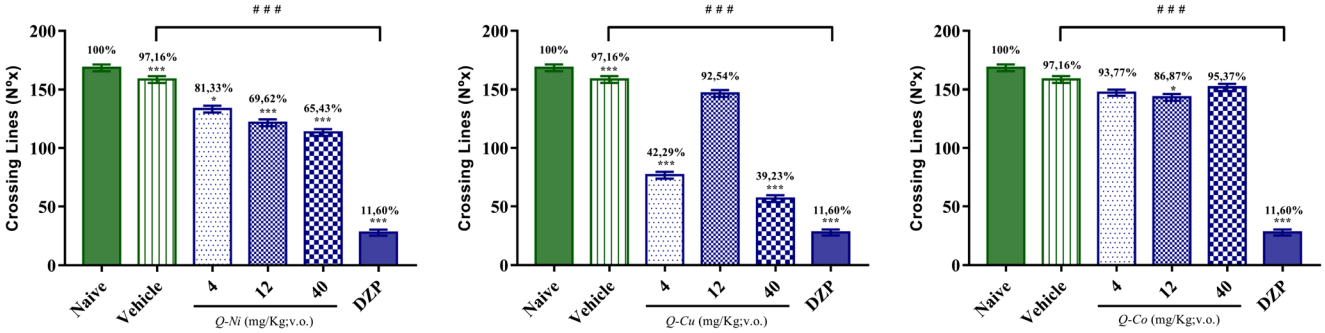

Fig. 5 Effect of quercetin derivatives on the locomotor activity of zebrafish (*Danio rerio*) adult in the open field test (0–5 min). Oral treatment; naive—untreated animals; vehicle—DMSO 3%; DZP, diazepam (2.5 mg/kg, v.o.). Values represent the mean ± standard error of the mean (SEM) for 6 animals/group; ANOVA followed by Tukey. Q-Ni (***$p < 0.001$; *$p < 0.05$ vs. naive or vehicle; $^{###}p < 0.001$ vs. DZP). QCu (***$p < 0.001$ vs. naive or vehicle; $^{###}p < 0.001$ vs. DZP). QCo (***$p < 0.001$; *$p < 0.05$ vs. naive and vehicle; $^{###}p < 0.001$ vs. DZP). The numbers above each column indicate the percentage of locomotor activity (%LA)

various human diseases, including cancer, and aging, cystic fibrosis, and cardiovascular, inflammatory, immune, metabolic, and neurodegenerative diseases (Giustarini et al. 2011).

Aging and many diseases have been linked to oxidative stress, DNA, and protein damage that result in several negative effects on cells. As a marker of oxidative stress, lipid peroxidation can be correctly assessed by the malondialdehyde (MDA) assay (Kil et al. 2014). GSH deficiency or decreased GSH/glutathione disulfide (GSSG) manifests largely through increased oxidative stress, and the damage is believed to be involved in neurodegenerative disorders such Parkinson's disease and Alzheimer's disease (Ballatori et al. 2009).

Central nervous system dysfunction was observed in all diseases related to errors in MDA and GSH metabolism, suggesting that the brain is particularly susceptible to changes in these compounds. When stress-induced animals were maintained with GSH and MDA levels after treatment, it was possible to demonstrate the importance for maintaining homeostasis of these compounds. The increase in catalase activity, an enzyme acting directly on the maintenance of oxidative levels with the reduction of hydrogen peroxide due to cellular metabolism, was reinforced by its affinity with the iron ions coordinated in the quercetin molecule. When H_2O_2 enters the active center of catalase, it interacts with two amino acids in the enzyme polypeptide chain: a histidine and an asparagine (Li and Schellhorn 2007) (Fig. 6).

Toxicity

The data obtained in the present study revealed that the quercetin molecule and its derivatives did not present ZF toxicity until 96 h of analysis. However, QZn (4.0 or 12 or 40 mg/kg, v.o.), QFe (4 mg/kg, v.o.), QNi (4.0 or 12 or 40 mg/kg), QCu (4.0 or 40 mg/kg), and Qo (12 mg/kg) caused a possible sedative effect, as they decreased ZF locomotor activity.

Molecular Docking

After the molecular docking, the following RMSDs (root mean square deviations) of atomic positions were generated between the galantamine (redocking), quercetin, QFe, and protein, with RMSD value of 1932 Å, with affinity energy of − 7.9 kcal mol^{-1} and 2 active galantamine twists (GNT); RMSD of 1824 Å, with affinity energy of − 7.3 kcal mol^{-1} and 6 kinks active in the quercetin docking; and RMSD of

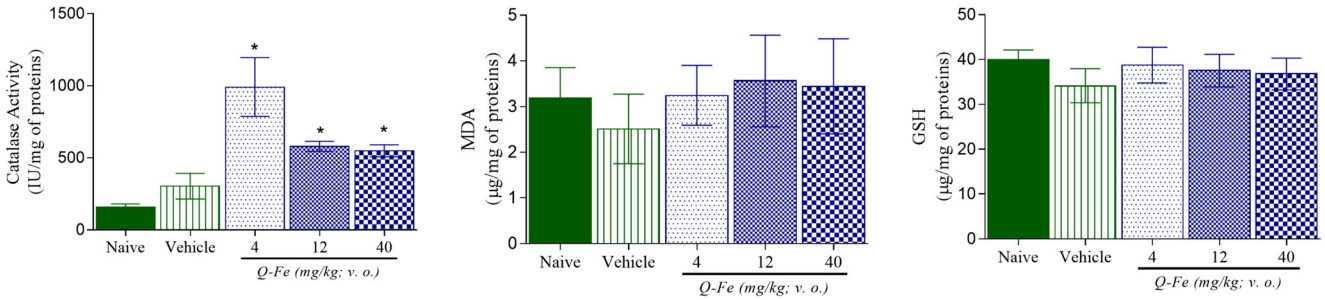

Fig. 6 Effect of QFe antioxidant activity on zebrafish (*Danio rerio*). Oral treatment; naive—untreated animals; vehicle—DMSO 3% (2.5 mg/kg, v.o.). Values represent the mean ± standard error of the mean (SEM) for 6 animals/group; ANOVA followed by Tukey (*$p < 0.001$ vs. naive or vehicle)

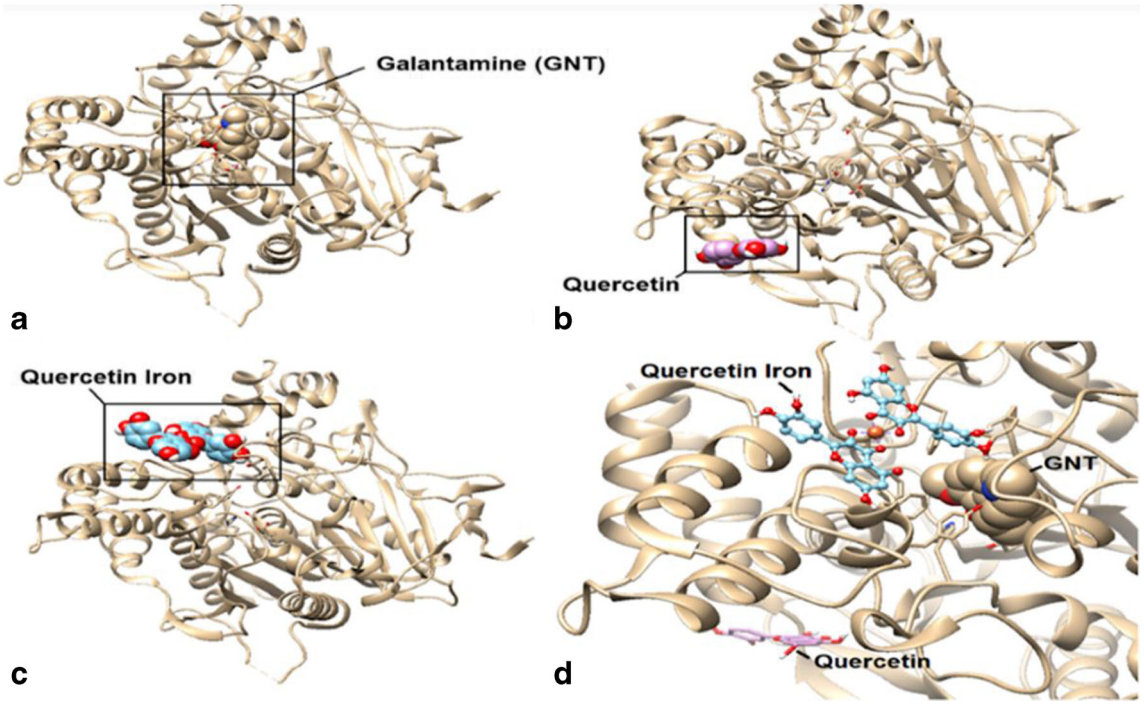

Fig. 7 Three-dimensional visualization of acetylcholinesterase complexed with galantamine (**a**), quercetin (**b**), QFe (**c**), and galantamine, quercetin, and QFe (**d**)

0.973 Å, with affinity of − 8.8 kcal mol^{-1} and 10 active twists in the docking of QFe (Fig. 7).

Figure 7 also shows that after docking simulations, the complex QFe was in the same place as galantamine in relation to the quercetin molecule, in addition to presenting better affinity value (8.8 kcal mol^{-1}). Regarding the docking with the active site of acetylcholinesterase (Cheung et al. 2012), the QFe complex showed better interaction with respect to the free quercetin coordination. Table 4 shows a smaller distance between them, thus a better interaction with the residues of amino acids involved in the active site, explaining the difference in biological activity between the quercetin molecule and the QFe complex.

Table 4 Distance between the binders and active site of acetylcholinesterase

Ligand/residue	Docking		Redocking
	Quercetin	Quercetin-Fe	Galantamine
Y337	13.6 Å	5.2 Å	3.3 Å
F295	14.1 Å	9.8 Å	6.7 Å
F297	19.6 Å	6.8 Å	5.0 Å
H447	12.6 Å	11.6 Å	3.8 Å
W86	17.3 Å	8.5 Å	3.3 Å
G120	20.2 Å	11.9 Å	3.2 Å
G121	21.3 Å	8.8 Å	3.3 Å
G122	20.8 Å	10.2 Å	3.6 Å
S203	15.9 Å	12.6 Å	4.5 Å

Conclusion

The QFe complex presented the best antioxidant and antiacetylcholinesterase actions, and these results were confirmed by molecular docking. In the toxicity and locomotor evaluation, the quercetin molecules and the synthesized complexes, mainly QCu and QZn derivatives, showed the highest degree of inhibition of the fish's locomotor activity, suggesting a possible anxiolytic action. Thus, Fe^{+2}, Cu^{+2}, and Zn^{+2} are suitable metals for complexation of quercetin to produce potential agents against Alzheimer's disease.

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