RESEARCH ARTICLE

Protective Effects of Quercetin in Zebrafish Model of Alzheimer's Disease

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Abstract

Introduction: Alzheimer's disease (AD) is a neurodegenerative disease which is characterized by deposition of amyloid beta plaques and neurofibrillary tangles. The plaques formation affects the synaptic transmission and increases neuroinflammation in brain. Quercetin is the most potent flavonoid which protects brain tissue from neuroinflammation. Zebrafish is a reliable model for the study of neurological disorders. **Materials and Methods:** Wild adult zebrafishes were used for induction of AD and were done by administrating aluminum chloride intraperitoneally. Behavioral and biochemical estimations were done. Target identification and gene enrichment studies were also performed on probable quercetin receptors. Molecular docking of quercetin in comparison to AD marketed drugs was executed. **Results and Discussion:** The results showed protective effects of quercetin on AD model. Behavioral and biochemical tests confirm the reduction in oxidative stress and increased cognition. Computational analysis of stereoisomers of quercetin and its different binding positions and modes helps in better understanding of this flavonoid as a drug. **Conclusions:** Quercetin has protective effects on brain, and this is mediated by its regulating different protein receptors simultaneously.

Key words: Quercetin, Alzheimer's disease, Zebrafish, cognition

INTRODUCTION

AD is a neurodegenerative disorder characterized by the deposition of amyloid beta (Ab) and neurofibrillary tangles.[1] In AD, the brain contains low level of acetylcholine which is due to accumulation of Ab protein which results in the formation of plaques which eventually affect the synaptic transmission and starts the inflammatory processes.[2] Oxidative stress is showed by DNA oxidation, protein oxidation, 3-nitrotyrosine formation, and lipid peroxidation (LPO).[3] Brain tissue has numerous possible sources of reactive oxygen species (ROS) and huge oxidative capacity, but its capacity to fight oxidative stress is limited.[4] Central nervous system has the high content of polyunsaturated fatty acids that are particularly susceptible to lipid oxidation which also leads to oxidative damage.

Flavonoids are phenolic substances which are isolated from a range of vascular plants. The antioxidant activity of flavonoids comes from their ability to diminish free radical formation and scavenge free radicals. Quercetin, a flavonoid found in apples and onions have antioxidant properties and increases glutathione levels and antioxidant enzyme function.[5] It is one of the most potent flavonoids for protecting the body against ROS. It is produced through the normal oxygen metabolism. A molecule of quercetin contains five hydroxyl groups whose occurrence determines the compound's biological action and the possible number of derivatives. Several findings suggested that the administration of quercetin at early stages with optimal dose may improve brain damage and offer neuroprotection.[6]

Zebrafish has been a reliable model of the cognitive deficits characteristic of many neurobehavioral disorders such as Alzheimer's disease (AD). Rodents have been used to study cognitive phenotypes extensively, but zebrafish (*Danio rerio*) is attaining popularity as a good model to balance current

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translational neuroscience research.[7] They are economical and can give a comparatively quick sign of possible functional efficiency. They have a complex nervous system and elaborate behavioral range. Zebrafish provides a good transitional model between *in vitro* receptor and cell-based assays and classic mammalian models for drug screening.[8]

METHODS

Animals

Animal model used for study is zebrafish. Zebrafish (*Danio rerio*) was procured from a certified fish vendor. Subjects were 3–4 months old, and the same size fish were selected for the study. Proper conditions were maintained, and fishes were supplied with external oxygen using bubble sparger. Zebrafishes were kept in a tank for acclimatization for 3 months. Fishes were fed twice (once in the morning and early evening) a day. Water temperature of 27°C–29°C was maintained. A cycle of 14 h light, 10 h dark was followed.[9,10] pH was maintained at 7.0–8.0. Tanks are made of high-quality glass.

Chemicals

All the chemicals were of analytical grade. Aluminum chloride ($AlCl_3$), 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB), trichloroacetic acid (TCA), thiobarbituric acid (TBA), and acetylthiocholine (ATC) iodide were purchased from Sigma, (India).

Experimental design

The model was set up for 6 weeks, and during this interval, all the behavioral changes were monitored religiously. 50 subjects were divided into two sets each - diseased and treatment. Dosing was performed intraperitoneally, and $AlCl_3$ (18.75 ul each) was administered carefully thrice every week for 3 weeks. The initial behavioral observations for all the subjects were the same, i.e. the subjects showed increased thrashing, increased gill movement, and extreme anxiety. Diseased subjects were dissected after 3 weeks to check the progression of the disease and treatment model was given Quercetin (2 ul each) thrice every week and assessed for another 3 weeks. All fishes were kept in same environmental conditions. Periodic observation was done to observe changes. After 3 weeks treated, subjects were sacrificed.

Behavioral analysis

Native area test

This test setup consists of a chamber with two areas, one which is native to the subject, i.e., conditions in which subject was acclimatized in lab, and the other is new area. Herein, the subject displays memory and learning capabilities.

Tank diving test

This test setup consists of a large tank with upper, middle, and lower markings on it. This test evaluates a subject for anxiety and fear related behavior.

Novel object test

This test setup consists of a chamber. Later a new object is added in front of a subject. This test measures cognition, fear, and mindfulness of the subject.

Light dark test

This test setup has two separate chambers, one open and other covered. Covered chamber is dark. This test is done to assess cognition and anxiety in subjects.

Sample preparation

The zebrafish was sacrificed by cold treatment (cryo-anesthetized) using ice cold water and then placed on wax plate and then fixed using pins without damaging any organs. Then carefully remove brain. Saline solution was used during the process for easy removal and washing. Brains were carefully stored in phosphate buffer and kept at 4°C in separate microfuge tubes.

Biochemical estimations

The biochemical analysis of brain tissue of control, diseased, and quercetin-treated subjects was performed.

Post-mitochondrial supernatant (PMS) preparation

The brain tissues were kept in 0.1 M phosphate buffer saline (pH-7.4). Tissues were properly homogenized and centrifuged at 10,500 g for biochemical analysis.

Measurement of LPO

Estimation of LPO was done using the protocol of Wright *et al*.[11] The sample mixture contains 10% tissue homogenate, 10% of TCA, and 0.67% of TBA. Samples were placed in a boiling water bath for 45 min. Then, they were kept in ice bath and centrifuged at 2500× *g* for 10 min. Malonadialdehyde (MDA) content in samples was analyzed by measuring the absorbance at 532 nm.

Measurement of catalase (CAT) activity

The CAT assay was done by following the method of Claiborne *et al*.[12] with some modifications. The test mixture consists of 0.05M phosphate buffer (pH 7.0), 20 mM hydrogen peroxide (H_2O_2), and 0.05 ml PMS in a total volume of 3.0 ml. The absorbance was measured at 240 nm.

Measurement of acetylcholinesterase (AChE) activity

AChE activity was measured using the protocol of Ellman *et al*.[13] method. For AChE activity analysis, the test sample contains 1.3 ml sodium phosphate buffer, 0.05 ml DTNB, 10 µl ATC, and 0.2 ml supernatant. The absorbance was measured at 412 nm.

Measurement of glucose level

Estimation of glucose level was performed using the Autospan diagnostics glucose kits. For glucose measurement, the reaction volume of 3 ml is contained 20 µl sample, 1500 µl working reagent, and 1500 µl water. The absorbance can be measured at 520 nm.

Statistical analysis

Experiments were performed using tissue samples of control and treated zebrafishes. Representative data are depicted in graphs. Intergroup comparisons were made using one-way analysis of variance, and *P* value was measured using GraphPad software.

Molecular docking

Protein Preparation was done in Maestro 10.3 package of Schrodinger Suite 2015-3.[14,15] Entry "1EVE"was downloaded in Maestro and protein preparation wizard was executed.[16] Ligands (Rivastigmine (PubChem ID: 77991),[17] Donepezil (PubChem ID: 3152),[18] Galantamine (PubChem ID: 9651),[19] and Memantine (PubChem ID: 4054),[20] and Quercetin (PubChem ID: 5280343)[21] were downloaded from PubChem Database[22] and stereoisomers were generated using LigPrep 3.5.[23] The grid was generated in Maestro and Extra Precision (XP) docking was performed using Glide 6.8.[24]

Target identification and gene enrichment analysis

Binding database was used to identify the probable targets for quercetin.[25] Later these target proteins were analyzed with The Database for Annotation, Visualization, and Integrated Discovery v6.8 for gene enrichment.[26] Proposed molecular processes which can be regulated by quercetin were reported.

RESULTS

Behavioral analysis

During dark/light tests, it was seen that control zebrafish prefers dark region, but diseased subjects were unable to differentiate between dark and light regions. These subjects displayed reduced interest and cognition [Figure 1a]. Novel

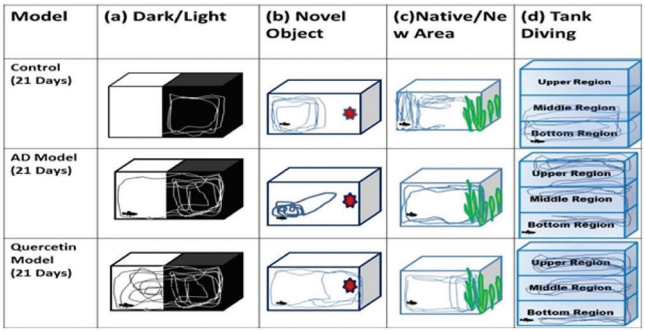

Figure 1: Behavioral assessment of zebrafishes. (a) Setup for dark/light test, (b) setup for novel object test, (c) setup for native/new area test, and (d) setup for tank diving test

object test was performed to assess the fear and anxiety-related behavior. Control fishes displayed extreme anxiety and did not come closer to the object, but diseased fishes did not show any sign of anxiety and were freely swimming near the object [Figure 1b]. Native/new test was performed to understand the differences between the memory and learning differences between the diseased and treated subjects. Control subjects did not go to new area at all while diseased subjects were seen swimming in both areas [Figure 1c]. Tank diving test was performed to assess fear and anxiety. Control subjects were observed to be swimming in the lower region of the tank (measurement of anxiety) while diseased subjects were freely swimming in all regions and were mostly observed in the upper region [Figure 1d].

Biochemical analysis

AChE level was measured in the brain tissue of control, diseased, and treated subjects. $AlCl_3$ administered group showed significant changes in AChE activity. In control subjects, AChE activity was observed to be as 0.0066 µmoles/min/mg, in diseased subjects, the AChE levels increased to a significant amount of 0.0082 µmoles/min/mg till 21 days treatment with $AlCl_3$ ($P < 0.001$***). However, after treatment with quercetin, there was sudden deceased in AChE activity to 0.0068 µmoles/min/mg ($P < 0.01$**) which was comparable to control [Figure 2a].

The CAT activity (nmole H_2O_2 decomposed/min/mg protein) exhibited decreased activity of CAT enzyme in the diseased brain with respect to control subjects. In control subjects, CAT activity was observed as 63.5 nmole/min/mg. In diseased subjects, this parameter decreased significantly to 27.46 nmole/min/mg ($P < 0.01$**). After treatment with quercetin, the CAT activity was observed as 51.46 nmole/min/mg ($P < 0.001$***) [Figure 2b].

The glucose level of control subjects was 95.23 mg/dl. After $AlCl_3$ administration, glucose level in zebrafish brain was found to be 124.29 mg/dl ($P < 0.05$*) which was significantly higher than control subjects. However, quercetin treatment reduces the level of glucose in brain

tissue, i.e., 110.29 mg/dl ($P < 0.01$**) which was comparable to control subjects [Figure 2c].

The MDA levels were measured in brain tissue of control, diseased, and in treated subjects. The MDA level of control subjects was 0.964 nmoles/mg. The MDA level in brain tissue of diseased subjects was 1.57 nmoles/mg, respectively. The MDA level significant decreases after treatment with quercetin and its MDA level was found to be 0.99 nmoles/mg [Figure 2d].

Molecular docking

Molecular docking was performed between ligands (quercetin, rivastigmine, donepezil, memantine, and galantamine) and Receptor (1EVE|Acteylcholinesterase). This was done to assess the fitness of quercetin as a ligand for AChE. Ligprep 3.5 was used to compute all possible stereoisomers of ligands. This produced a subset of 20 ligands. Protein preparation wizard was used to pre-process protein structure before docking analysis. In this, bond orders were assigned, and incomplete valences were corrected by adding hydrogens to the structure. Water molecules beyond 5 Angstrom were deleted. Water molecules were also deleted from the active site. Missing side chains were also filled using Prime 4.1, which models the side chain by employing homology modeling algorithm [Figure 3a-d].

The final refined structure was taken forward for prediction of nearby allosteric binding sites possible in the receptor. This was done using SiteMap 3.6 of Maestro 10.3 module. It predicted 4 binding sites near to the native active site present in 1EVE [Table 1]. The detailed list of predicted sites is given in supplementary Table 1. Binding site 2 overlapped with the native active site, and since its volume was highest, it was taken forward for docking.

Quercetin was observed to be binding equally efficiently as marketed drugs for the AD. Its binding energy was calculated as −11.784 kcal/mol. It means that if quercetin gains better access to brain then it can be utilized as a drug.

Target identification for quercetin was performed using Binding database. Around 66 targets were predicted and were ranked according to their scores. The detailed list is provided in supplementary Figure 1. Gene Enrichment Analysis gave four groups i.e. all the probable protein receptors predicted for quercetin were grouped in these four different groups [Figure 4a-d].

Group 1 consists of proteins from carbonic anhydrase family. Group 2 consists of proteins from cytochrome P450 family 1 subfamily A and B. Group 3 consists of proteins such as nicotinamide adenine dinucleotide phosphate oxidase, and ATP binding cassette family. Group 4 consists of different proteins such as insulin and epidermal growth factor receptor.

DISCUSSION

Brain is vulnerable to oxidative damages which are due to the production of ROS, the presence of polyunsaturated fatty acids in cell membrane and comparatively less antioxidant defense system.[27,28] In the existing literature, information on the experimental analysis of the protective effect of quercetin on the brain of zebrafish is less. Therefore, in this study, we have analyzed the effects of quercetin and its binding sites on AChE. Behavioral analysis concludes that the effect of $AlCl_3$ was more pronounced and the introduction of quercetin did modulate the behavior of animal model gradually. In ideal tank diving test, zebrafish tends to go to the bottom of the tank, stayed there and then eventually came back at upper levels over the period of analysis, this pattern is similar to thigmotaxis which is phenomenon of time spent toward the walls of container, same as used for rodents.[29] In this study, it

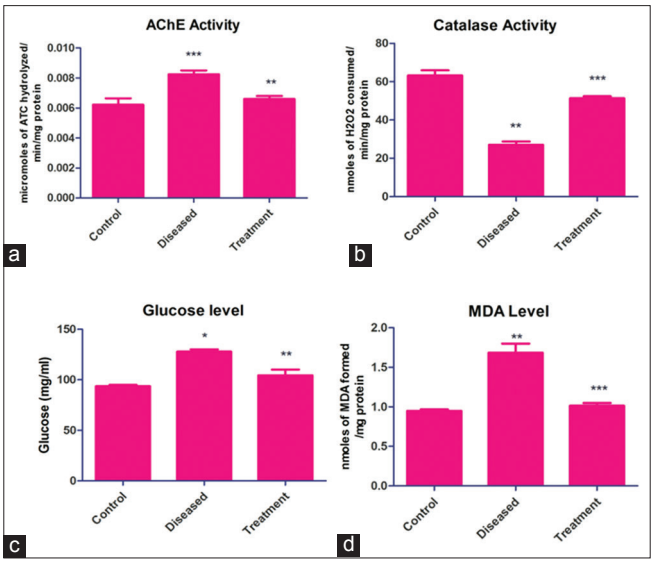

Figure 2: Biochemical analysis of control, diseased, and treated brain. Different biochemical assays were performed for zebrafish subjects (a) acetylcholinesterase activity, (b) catalase activity, (c) glucose activity, and (d) lipid peroxidation level (*<0.05; **<0.01; ***<0.001)

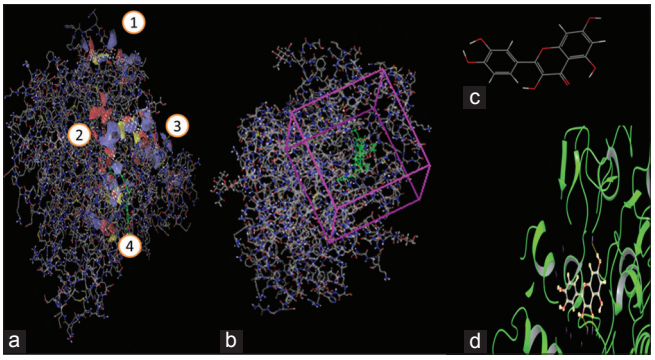

Figure 3: Quercetin docked with acetylcholinesterase **(1EVE).** (a) Predicted binding sites in 1EVE, (b) grid generation, (c) quercetin structure, and (d) docked quercetin in 1EVE active site

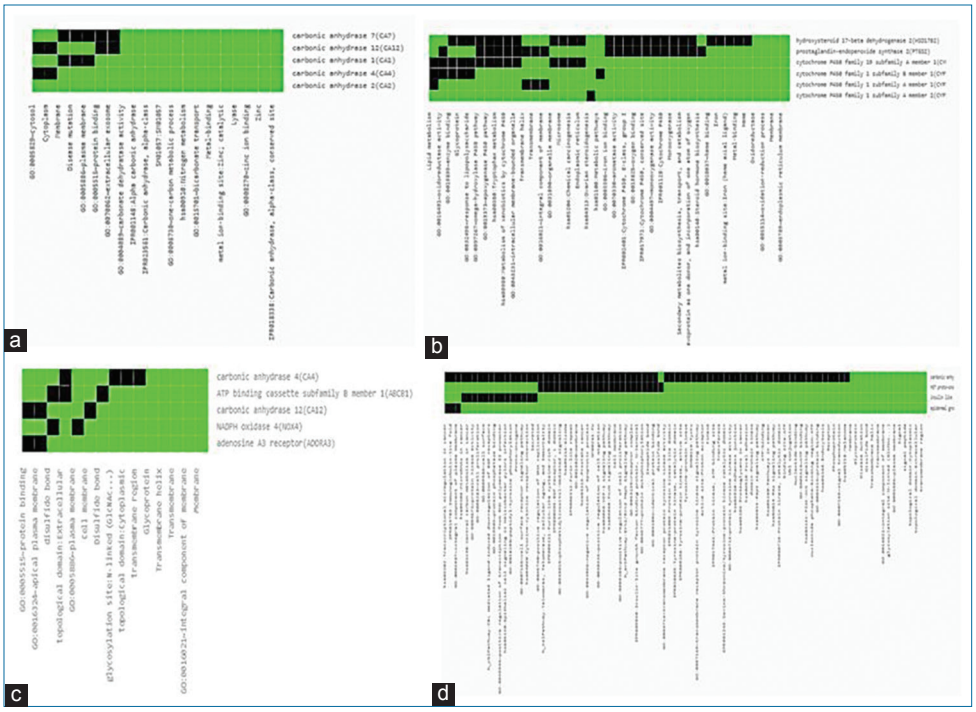

Figure 4: Gene enrichment analysis. Enrichment analysis produced two clusters. (a) Group 1: Enrichment score: 4.54 (b) Group 2: Enrichment score: 3.17 (c) Group 3: Enrichment score: 1.35 (d) Group 4: Enrichment score: 1.28

Table 1: Comparison of docking energies

Title	Docking score	Glide gscore	Glide evdw	Glide ecoul	Glide energy	Glide einternal	Glide emodel	XP HBond
Donepezil	−11.989	−12.002	−39.152	−8.383	−47.535	8.583	−75.352	−0.266
Donepezil	−11.989	−12.002	−39.152	−8.383	−47.535	8.583	−75.352	−0.266
Quercetin	−11.989	−11.784	−37.132	−8.934	−45.755	6.563	−73.552	−0.186
Quercetin	−11.989	−11.784	−37.132	−8.934	−45.755	6.563	−73.552	−0.186
Galantamine	−11.135	−11.157	−32.239	−10.68	−42.919	0.533	−59.32	−1
Galantamine	−11.135	−11.157	−32.239	−10.68	−42.919	0.533	−59.32	−1
Galantamine	−11.135	−11.157	−32.239	−10.68	−42.919	0.533	−59.32	−1
Galantamine	−11.135	−11.157	−32.239	−10.68	−42.919	0.533	−59.32	−1
Rivastigmine	−9.957	−9.997	−29.677	−6.799	−36.477	1.41	−53.473	−0.437
Quercetin	−9.482	−9.482	−34.312	−20.08	−54.392	0	−73.714	−3.172
Quercetin	−9.482	−9.482	−34.312	−20.08	−54.392	0	−73.714	−3.172
Memantine	−8.361	−8.361	−20.323	−6.016	−26.34	0	−35.803	0
Donepezil	−7.11	−9.344	−39.272	−5.959	−45.231	7.984	−−68.986	−0.7
Donepezil	−7.11	−9.344	−39.272	−5.959	−45.231	7.984	−68.986	−0.7
Donepezil	−7.11	−9.344	−39.272	−5.959	−45.231	7.984	−68.986	−0.7
Donepezil	−7.11	−9.344	−39.272	−5.959	−45.231	7.984	−68.986	−0.7
Galantamine	−5.756	−7.72	−22.908	−9.33	−32.238	0.115	−52.571	−1.062
Galantamine	−5.756	−7.72	−22.908	−9.33	−32.238	0.115	−52.571	−1.062
Galantamine	−5.756	−7.72	−22.908	−9.33	−32.238	0.115	−52.571	−1.062
Galantamine	−5.756	−7.72	−22.908	−9.33	−32.238	0.115	−52.571	−1.062
Rivastigmine	−4.941	−6.553	−31.265	−2.813	−34.078	8.067	−44.278	−0.713

was observed that control subjects prefer bottom region of the tank, but diseased subjects stayed in the upper region and also showed abnormal swimming pattern. However, after treating them with quercetin, they showed little preference for bottom

just as control subjects, although the condition improved with time. In the light-dark test, the natural preference of fish is staying in dark environment,[30] and our study clearly depicted the same. Due to the effect of neurotoxin $AlCl_3$, the zebrafish did not prefer any area in specific but stayed equal time in dark and light, but on quercetin administration, it showed a preference for dark which is its ideal behavior.

Our results showed an increase in CAT activities in the diseased brain of zebrafish but decrease in CAT activity of quercetin treated in brain. Our data indicate that flavonoids taken in diet have effects on antioxidant enzymes level. In zebrafish, it was seen that quercetin has a protective effect against scopolamine-induced memory dysfunctions.[31] In our results, it was observed that 3-week doses of quercetin have most effects. A similar study by Ansari *et al*. examined a dose-response pattern of quercetin which showed protective effects against Aβ (1–42) toxicity by lowering oxidative stress at lower doses,[5] but higher doses showed toxic effect. Animal model which perfectly repeat complex human central nervous system diseases is rare. The closer an animal model can mimic important aspects of disease, the better it is. This is crucial, and zebrafish has been used successfully to analyze different aspects of the disease.[32]

Bioinformatics approach was utilized for predicting binding sites in AChE. One of the binding site was utilized for molecular docking of quercetin with AChE. Quercetin was highly competitive to other marketed drugs present for inhibiting AChE. It showed impressive docking energy of −11.784 kcal/mol and was as efficient as other AChE inhibitors.

Furthermore, gene enrichment analysis was performed on proposed protein receptors for quercetin, and top results included carbonic anhydrase, cytochrome P450, and insulin receptors. These genes play major role in our body system, and hence it is concluded that quercetin is a multi-target ligand which regulates a variety of receptors at the same time. All these findings give the motivation to prove the hypothesis that quercetin can provide a promising approach for AD treatment and other neurodegenerative diseases related to oxidative stress.

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REFERENCES

1. Butterfield DA, Lange ML. Multifunctional roles of enolase in alzheimer's disease brain: Beyond altered glucose metabolism. J Neurochem 2009;111:915-33.
2. Wollen KA. Alzheimer's disease: The pros and cons of pharmaceutical, nutritional, botanical, and stimulatory therapies, with a discussion of treatment strategies from the perspective of patients and practitioners. Altern Med Rev 2010;15:223-44.
3. Butterfield DA. Amyloid beta-peptide (1-42)-induced oxidative stress and neurotoxicity: Implications for neurodegeneration in alzheimer's disease brain. A review. Free Radic Res 2002;36:1307-13.
4. Mantha AK, Moorthy K, Cowsik SM, Baquer NZ. Neuroprotective role of neurokinin B (NKB) on beta-amyloid (25-35) induced toxicity in aging rat brain synaptosomes: Involvement in oxidative stress and excitotoxicity. Biogerontology 2006;7:1-7.
5. Ansari MA, Abdul HM, Joshi G, Opii WO, Butterfield DA. Protective effect of quercetin in primary neurons against abeta(1-42): Relevance to alzheimer's disease. J Nutr Biochem 2009;20:269-75.
6. Dong J, Zhang X, Zhang L, Bian HX, Xu N, Bao B, *et al*. Quercetin reduces obesity-associated ATM infiltration and inflammation in mice: A mechanism including AMPKα1/SIRT1. J Lipid Res 2014;55:363-74.
7. Stewart AM, Kalueff AV. The Developing utility of zebrafish models for cognitive enhancers research. Curr Neuropharmacol 2012;10:263-71.
8. Levin ED. Zebrafish assessment of cognitive improvement and anxiolysis: Filling the gap between *in vitro* and rodent models for drug development. Rev Neurosci 2011;22:75-84.
9. Matthews M, Trevarrow B, Matthews J. A virtual tour of the guide for zebrafish users. Lab Anim (NY) 2002;31:34-40.
10. Brand M, Granato M, Nüsslein-Volhard C. 'Keeping And Raising Zebrafish'. In: Nüsslein-Volhard and Dahm (2002) Zebrafish - A Practical Approach. Oxford, UK: Oxford University Press,
11. Wright JR, Colby HD, Miles PR. Cytosolic factors which affect microsomal lipid peroxidation in lung and liver. Arch Biochem Biophys 1981;206:296-304.
12. Claiborne AL. Catalase activity. CRC Handbook of Methods for Oxygen Radical Research. Vol. 1. Boca Raton: CRC Press; 1985. p. 283-4.
13. Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961;7:88IN191-9095.
14. Release S. 3: Maestro, Version 10.3. New York: Schrödinger, LLC; 2015.
15. Release S. 3: Schrödinger Suite 2015-3 Protein Preparation Wizard. New York: Schrödinger, LLC; 2015.
16. Kryger G, Silman I, Sussman JL. Structure of acetylcholinesterase complexed with E2020 (Aricept®): Implications for the design of new anti-Alzheimer drugs. Structure 1999;7:297-307.
17. Arntzenius AC, van Gent CM, van der Voort H, Stegerhoek CI, Styblo K. Reduced high-density lipoprotein in women aged 40-41 using oral

contraceptives. Consultation bureau heart project. Lancet 1978;1:1221-3.
18. Uehleke H, Werner T. A comparative study on the irreversible binding of labeled halothane trichlorofluoromethane, chloroform, and carbon tetrachloride to hepatic protein and lipids *in vitro* and *in vivo*. Arch Toxicol 1975;34:289-308.
19. Vesper J, Dietze RA, Schulzki G. Problems of prevention, registration and evaluation of post-vaccination neurogenic lesions in children from clinical and legal viewpoints. II. Problems in the evaluation of neurogenic post-vaccination lesions in childhood. Psychiatr Neurol Med Psychol (Leipz) 1976;28:181-9.
20. Mohler SR. Physiological index as an aid in developing airline pilot scheduling patterns. Aviat Space Environ Med 1976;47:238-47.
21. Manley RS, Jonker JW, Cooper D, Pound TC. Initiation of cellulose biosynthesis. Nat New Biol 1971;229:88-9.
22. Kim S, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A, *et al*. PubChem substance and compound databases. Nucleic Acids Res 2015;44:D1202-13.
23. Release S. 3: LigPrep, Version 3.5. New York: Schrödinger, LLC; 2015.
24. Schrodinger, LLC. 37. Glide, Version 6.8. New York: Schrodinger, LLC; 2015.
25. Liu T, Lin Y, Wen X, Jorissen RN, Gilson MK. BindingDB: A web-accessible database of experimentally determined protein–ligand binding affinities. Nucleic Acids Res 2006;35 Suppl_1:D198-201.
26. Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009;4:44-57.
27. Mates JM. Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. Toxicology 2000;153:83-104.
28. Verstraeten SV, Aimo L, Oteiza PI. Aluminium and lead: Molecular mechanisms of brain toxicity. Arch Toxicol 2008;82:789-802.
29. Levin ED, Cerutti DT. Behavioral Neuroscience of Zebrafish. Boca Raton (FL): CRC Press/Taylor & Francis; 2009.
30. Stewart A, Kadri F, DiLeo J, Chung K, Cachat J, Goodspeed J, *et al*. The developing utility of zebrafish in modeling neurobehavioral disorders. Int J Comparative Psychol 2010;23:104-20.
31. Richetti SK, Blank M, Capiotti KM, Piato AL, Bogo MR, Vianna MR, *et al*. Quercetin and rutin prevent scopolamine-induced memory impairment in zebrafish. Behav Brain Res 2011;217:10-5.
32. Eliceiri BP, Gonzalez AM, Baird A. Zebrafish model of the blood-brain barrier: Morphological and permeability studies. In: The Blood-Brain and Other Neural Barriers. Totowa, NJ: Humana Press; 2011. p. 371-8.

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