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Research Paper

Brain acetylcholinesterase activity and the protective effect of Gac fruit on scopolamine-induced memory impairment in adult zebrafish

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ABSTRACT

Gac fruit (*Momordica cochinchinensis*) belongs to the Cucurbitaceae family. This study aimed to investigate the anti-memory impairment effect of Gac fruit aril extract and brain acetylcholinesterase activity in adult zebrafish (Danio rerio). The behavioral test was performed using a color-biased appetite conditioning T-maze test and an inhibitory avoidance test to evaluate memory performance. The time spent in the green arm in the T-maze test was recorded, and latency time was recorded in the inhibitory avoidance test. Brain acetylcholinesterase (AChE) enzyme activity was measured using a 96-well microplate reader based on Ellman's method. Zebrafish that received rivastigmine and Gac extract had significantly increased time spent in the green arm and latency time when compared to the SCO group. Zebrafish that received rivastigmine and Gac fruit extract at 200 mg/kg had lower AChE activity than the SCO groups; however, there were no statistically significant differences between the groups. These findings suggest that Gac fruit extract has anti-memory impairment activity and may be beneficial for the development of health products to prevent Alzheimer's disease.

1. Introduction

Zebrafish are vertebrates with a central nervous system that is similar to that of mammals. The central pallium and medial pallium of zebrafish are similar to the amygdala, and the dorsal pallium is similar to the isocortex (Khan et al., 2017; Mueller et al., 2011). The zebrafish encephalon consists of the forebrain, midbrain, and dorsal-brain regions (diencephalon, telencephalon, and cerebellum), which have receptors, such as muscarine cholinergic, gamma aminobutyric acid (GABA), glutamate, serotonin, dopamine, histamine, and acetylcholine neurotransmitters (Santana et al., 2012). Therefore, zebrafish have been used as experimental animals to discover new drugs that can treat neurodegenerative diseases such as Alzheimer's disease.

The spatial learning and memory of zebrafish from the color-biased appetite conditioning T-maze test showed that the colors affecting the learning and memory of zebrafish were red and green (Avdesh et al., 2012). The T-maze test revealed that when a zebrafish swims to the red arm, the fish will be punished. In contrast, if the zebrafish swims to the green arm, the fish will be rewarded (Maddula et al., 2017). Zebrafish exhibit behaviors learned and memorized from the lateral pallium (located in the telencephalon), which is similar to the mammalian hippocampus (Kaur et al., 2015).

The fear memory was evaluated from the inhibitory avoidance test, in which animals learn to avoid environmental hazards caused by stimuli. This method has been used previously to study learning in zebrafish (Kim et al., 2010; Ng et al., 2012; Richetti et al., 2011; Seibt et al., 2011; Truong et al., 2014). The recognition of fearful behavior in laboratory zebrafish is a result of the amygdala, found in vertebrates and mammals (Perathoner et al., 2016).

Gac fruit (*Momordica cochinchinensis*) belongs to the Cucurbitaceae family and is a type of perennial melon used as a food and traditional medicine in Southeast Asia (Tran et al., 2015). Gac fruit contains several phytochemical components, such as phenolics, flavonoids, and carotenoids (Abdulqader et al., 2019), which are widely known to utilize antioxidant activity. Many studies have reported that carotenoids can improve cognitive function and enhance neural functions (Khazdair et al., 2018; Polidori et al., 2021); however, no studies have evaluated the effects of Gac fruit extract on memory impairment. Therefore, the purpose of this study was to investigate the effect of Gac fruit aril extract on scopolamine-induced memory impairment and to measure brain

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acetylcholinesterase (AChE) activity in zebrafish.

2. Materials and methods

2.1. Preparation of the extract

Fresh Gac fruit was purchased from Phare Province, Thailand. The whole of the Gac fruit was scooped out, and the red aril surrounding the seeds was completely separated. For aqueous extract preparation, Gac aril was blended in distilled water at a ratio of 1:5 in a laboratory blender. The resulting juice was filtered twice and lyophilized ((Kha et al., 2011)). Operational conditions were set at a condenser temperature of − 20 °C and pressure of 250 Pa for 48 h. The freeze-dried aril powder samples were packed in aluminum foil and kept at − 20 °C until use.

2.2. The beta-carotene and lycopene determination of gac fruit extract

The beta-carotene and lycopene in Gac fruit extract were measured by using high performance liquid chromatography (HPLC). In brief, 0.1 g of Gac extract dissolved in 5 ml of distilled water, centrifuged in 3000 rpm at 25 °C for 10 min 5 ml of the supernatant was volume adjusted with 5 ml of 95% n-hexane and was analyzed by HPLC at wavelength 470 nm. The analysis carried out and approved under ISO/IEC 17025 accredited Laboratory Central Lab Thai No.060524224.

2.3. Zebrafish and drug administration

Adult male zebrafish (*Danio rerio*) were cultured in an acrylic tank containing reverse osmosis water and oxygen. Water temperature and pH were maintained at 28 ± 2 °C and 6.5–7.5, respectively, with a 12:12 light:dark cycle. The zebrafish were divided into 6 groups (n = 5 to 7) including the control, scopolamine 200 μM (SCO), scopolamine plus rivastigmine 1.5 mg/kg (SCO+RV), scopolamine plus Gac fruit extract at dose 200, 400 and 800 mg/kg (SCO+GAC200, SCO+GAC400, and SCO+GAC800 groups), respectively. The protocol of feeding and treating substance in zebrafish followed the experimental procedures from (Singsai et al., 2021).

The zebrafish in each group received the substance once daily for 7 days. On day 8, scopolamine was used to induce memory impairment in all groups except the control group before starting the behavioral test. Behavioral tests were performed using a color-biased appetite conditioning T-maze test and an inhibitory avoidance test to evaluate memory performance. All experiments were performed according to the guidelines of the Institute of Animals for Scientific Purposes Development (IAD). Animal ethics no. UP-AE62–01-04–027 was approved by the Laboratory Animal Research Center of the University of Phayao.

2.4. Color-biased appetite conditioning T-maze test

The experimental apparatus for the color-biased appetite conditioning T-maze test followed the method stipulated in (Singsai et al., 2021). The tank for the color-biased appetite conditioning T-maze test was prepared as an acrylic glass T-shaped maze consisting of 1 long arm and 2 short arms (red and green arms), as shown in Fig. 1.

During the experiment, the zebrafish were placed at the terminal of the long arm. One minute later, the sliding door was opened to allow the fish to swim towards the short arms. In the training session, the zebrafish were trained, and they learned that when it swims to the red arm, the fish will be punished. In contrast though, if it swims to the green arm, the fish will be rewarded. Repeat training was conducted for 3 days (Maddula et al., 2017; Vemula et al., 2014). In the test session, the fish entered the test by performing the same test as the training process, without reward or punishment. The time the fish spent in the green arm was recorded at 4 min.

2.5. Inhibitory avoidance test

The inhibitory avoidance test followed the method stipulated in (Singsai et al., 2021). The apparatus was divided into white and dark compartments using a sliding door. The dark compartment was connected to an electrical stimulator via an electrode plate, as shown in Fig. 2.

In the training session, a 3 mA shock current was applied for 2 s when the zebrafish swam into the dark compartment. On the test day, the test was performed using the same procedure, but an electric shock was not applied. The latency time was considered as the time from the start until the fish entered the dark compartment.

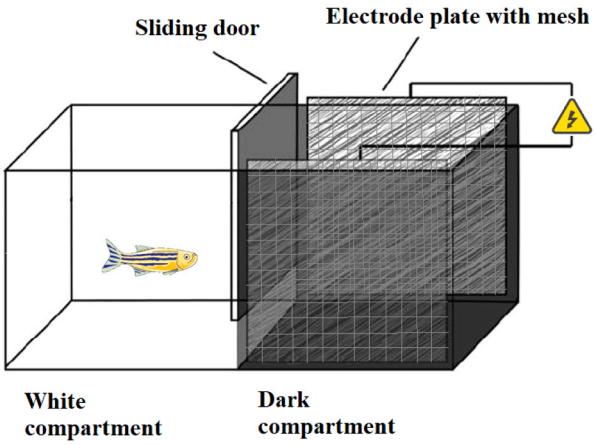

Fig. 2. Experimental apparatus of the Inhibitory avoidance test.

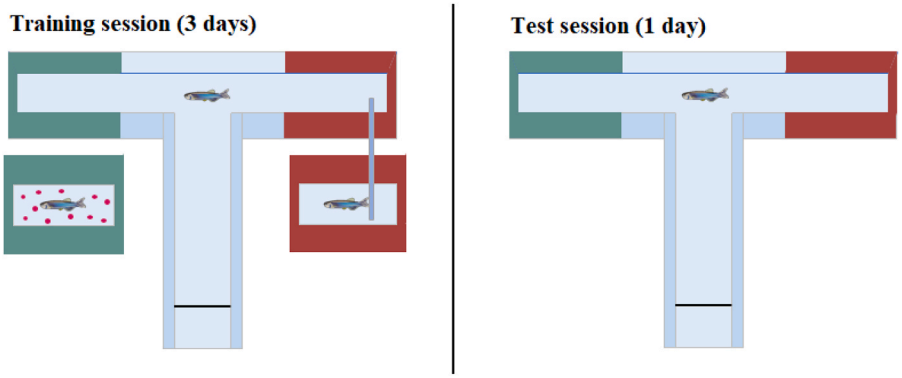

Fig. 1. Experimental apparatus of the color-biased appetite conditioning T-maze test.

2.6. Brain acetylcholinesterase activity

Zebrafish brains were homogenized in 150 μL of phosphate buffer (pH 8.0), and the homogenate was centrifuged at 3000 g for 10 min at 4 °C. The supernatant was used for biochemical assays. Brain AChE activity was measured using the colorimetric assay of Ellman's method. Briefly, in a 96-well plate, 240 μL of 0.1 M phosphate buffer (pH 8.0) was added, before10μL of 0.01 M 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) was added, 40 μL of zebrafish brain supernatant was then added, followed by 10 μL of 0.015 M acetylthiocholine iodide. Immediately after, the solutions were measured with a multimode microplate reader. The yellow color of 5-thio-2-nitrobenzoic acid was detected at 5 min, broken into 1-minute intervals at a wavelength of 405 nm. AChE enzyme activity was calculated using the following formula (Srikumar et al., 2004), and was expressed as μmol/min/g of tissue.

$$R = 5{\cdot}74 \times 10{-}4 \text{ x } A/CO$$

Where, R = Rate in moles of substrate hydrolyzed/min/g tissue.
A = Change in absorbance/min.
CO = Original concentration of the tissue (mg/ml).

2.7. Statistical analysis

Statistical analysis was performed using SigmaPlot software (version 12.0). Data were analyzed using 1-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. The bar graph with individual points was created using GraphPad Prism 10. The criterion for statistical significance was set at $p < 0.05$. All values are expressed as mean ± SEM.

3. Results

3.1. The beta-carotene and lycopene in Gac fruit extract

The contents of beta-carotene and lycopene in Gac fruit extract equal as 111.03 and 334.46 mg/kg, respectively. The test report is shown in supplementary data.

3.2. Effects of the Gac fruit extract on color-biased appetite conditioning T-maze test

The effects of scopolamine, rivastigmine, and Gac extract on the color-biased appetite conditioning T-maze test are expressed as mean ± SEM (Fig. 3). We found that zebrafish that received scopolamine had significantly decreased time spent in the green arm (1.288 ± 0.257 min) compared to the control group (2.564 ± 0.300 min).

In addition, the results showed that zebrafish that received rivastigmine spent significantly increased time in the green arm (2.637 ± 0.388 min); Gac extract at doses of 200, 400, and 800 mg/kg showed 2.710 ± 0.299, 3.240 ± 0.174 and 3.338 ± 0.306 min, respectively when compared to the SCO group.

3.3. Effects of the Gac fruit extract on inhibitory avoidance test

The effects of scopolamine, rivastigmine, and Gac extract on inhibitory avoidance test are expressed as mean ± SEM (Fig. 4). The results showed that the latency time in the training session was not significantly different between groups. Zebrafish that received scopolamine (0.328 ± 0.061 min) had a significantly decreased latency time in the test session when compared to the control group (1.658 ± 0.430 min). Moreover, zebrafish that received rivastigmine (2.362 ± 0.861 min) and Gac extract at doses of 200, 400 and 800 mg/kg (1.964 ± 0.221, 1.730 ± 0.260 and 2.102 ± 0.150 min) had significantly increased latency times in the test session when compared to the SCO group.

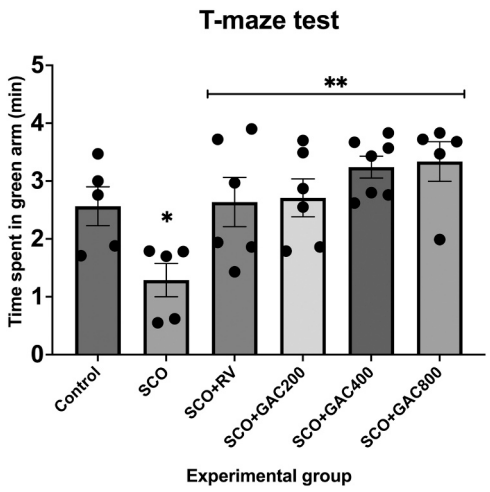

Fig. 3. Color-biased appetite conditioning T-maze test in zebrafish. The effect of scopolamine, rivastigmine, and Gac extract on time spent in the green arm (minutes) expressed as mean ± SEM. Zebrafish that received rivastigmine and Gac extract at all doses had significantly increased time spent in green arm when compared to the SCO group. *p value < 0.05 when compared to the control group, * *p value < 0.05 when compared to the SCO group.

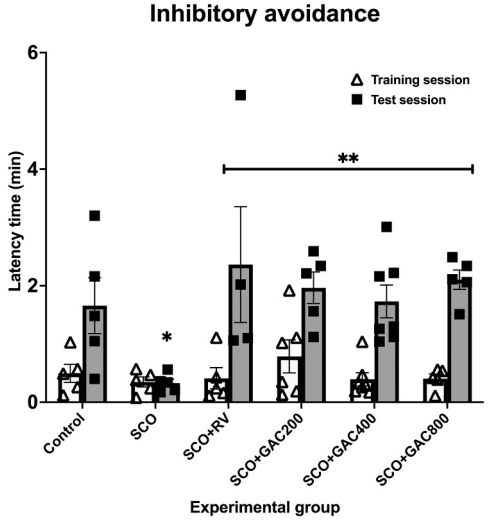

Fig. 4. Inhibitory avoidance test in zebrafish. The effect of scopolamine, rivastigmine, and Gac extract on latency time (minutes) expressed as mean ± SEM. Zebrafish that received rivastigmine and Gac extract at all doses had significantly increased latency time in test session when compared to the SCO group. *p value < 0.05 when compared to the control group in test session, * *p value < 0.05 when compared to the SCO group in test session.

Table 1
Effect of scopolamine, rivastigmine, and Gac extract on Brain acetylcholinesterase activity in zebrafish brains, expressed as μmol/min/g tissue.

Experimental group	Brain acetylcholinesterase activity (μmol/min/g tissue)
Control	5.824 ± 1.381
SCO	8.390 ± 1.935
SCO + RV	7.938 ± 1.962
SCO + GAC200	8.143 ± 1.368
SCO + GAC400	9.038 ± 2.874
SCO + GAC800	8.772 ± 2.038

3.4. Effects of the Gac fruit extract on brain acetylcholinesterase activity

The effects of Gac fruit extract on AChE activity in zebrafish brains are shown in Table 1. Zebrafish that received rivastigmine and Gac fruit extract at 200 mg/kg had lower AChE activity than those in the SCO groups. However, there were no statistically significant differences between the groups.

4. Discussion

Acetylcholine (ACh) is an important neurotransmitter in the central and peripheral nervous systems. The cholinergic system plays an important role in learning and memory processes and is predicted to be affected by Alzheimer's disease. Alzheimer's disease is associated with disorders such as the loss of cholinergic neurons in the basal forebrain and hippocampus (Ferreira-Vieira et al., 2016). The cholinergic system in the zebrafish brain can be examined using cholinergic methods, such as histological and biochemical assays (Mans et al., 2019). In addition, zebrafish neurons contain several types of AChE in the central nervous system, including the olfactory bulb, telencephalon, cerebellum, medulla oblongata, and spinal cord. However, AChE in the zebrafish CNS has been poorly analyzed in adults (Clemente et al., 2004). These factors are the reason why enzyme differences were not observed in this study.

Scopolamine is a muscarinic antagonist that causes memory impairment in fish, based on the ability of scopolamine to induce such learning and memory impairments in zebra fish, there is established and widespread evidence. It was previously reported that physostigmine (an AChE inhibitor, inhibits Ach breakdown at the synaptic site) rescues the amnesic effects of scopolamine in zebrafish ((Kim et al., 2021)), which is related to the present result that rivastigmine can ameliorate the memory impairment caused by scopolamine. Previous studies have shown that quercetin and rutin, both flavonoids, which the Gac fruit contains (Do et al., 2019), can prevent the induction of memory impairment with scopolamine (Richetti et al., 2011).

Previous studies have shown that long-term oxidative damage is associated with decreased cognitive ability and neurodegenerative diseases (Cobley et al., 2018; Singh et al., 2019). This finding suggests that antioxidant therapy may improve these conditions (Halliwell, 2001). Due to their recently revealed activities, particularly their neuroprotective qualities, the interest in carotenoids has expanded significantly during the past ten years. Carotenoids have antioxidant, anti-inflammatory, and anti-apoptotic properties in addition to having the potential to enhance brain plasticity. These properties operate as neuroprotective mechanisms (Su et al., 2023). Therefore, the proposed mechanism of action of Gac fruit might reduce oxidative stress in the brain. Moreover, recent evidence has reported that lycopene reduces the inflammatory response caused by beta-amyloid and inhibits NF-κB signaling at the choroid plexus in the early stages of Alzheimer's disease (Ratto et al., 2022). These carotenoids exhibit anti-neurodegenerative effects. Therefore, natural products containing these phytochemicals may have benefits for neurodegenerative disorders, including Alzheimer's disease.

5. Conclusion

The present study indicated that Gac fruit extract had anti-memory impairment activities, including spatial and fear memory. However, the absence of changes in enzyme levels in the zebrafish brain was a limitation of this research. Therefore, more molecular analysis is needed. The value of this research is to create a new body of knowledge on the neuropharmacological activity of Gac extract as an idea for further investigation. These findings suggest that Gac fruit extract might be beneficial for the development of health products to prevent Alzheimer's or related diseases.

CRediT authorship contribution statement

Kanathip Singsai: Conceptualization, Methodology, Investigation, Project administration, Funding acquisition. **Niwat Saksit**: Investigation, Writing – original draft, Writing – review & editing. **Puwich Chaikhumwang**: Investigation, Writing – original draft.

Declaration of Competing Interest

The authors report no conflicts of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ibneur.2024.02.004.

References

Abdulqader, A., Ali, F., Ismail, A., et al., 2019. Antioxidant compounds and capacities of Gac (*Momordica cochinchinensis* Spreng) fruits. Asian Pac. J. Trop. Biomed. 9, 158–167.

Avdesh, A., Martin-Iverson, M.T., Mondal, A., et al., 2012. Evaluation of color preference in zebrafish for learning and memory. J. Alzheimer'S. Dis. 28 (2), 459–469. https://doi.org/10.3233/JAD-2011-110704.

Clemente, D., Porteros, A., Weruaga, E., et al., 2004. Cholinergic elements in the zebrafish central nervous system: Histochemical and immunohistochemical analysis. J. Comp. Neurol. 474 (1), 75–107. https://doi.org/10.1002/cne.20111.

Cobley, J.N., Fiorello, M.L., Bailey, D.M., 2018. 13 reasons why the brain is susceptible to oxidative stress. Redox Biol. 15, 490–503. https://doi.org/10.1016/j.redox.2018.01.008.

Do, T.V.T., Fan, L., Suhartini, W., et al., 2019. Gac (*Momordica cochinchinensis* Spreng) fruit: a functional food and medicinal resource. J. Funct. Foods 62, 103512.

Ferreira-Vieira, T.H., Guimaraes, I.M., Silva, F.R., et al., 2016. Alzheimer's disease: targeting the cholinergic system. Curr. Neuropharmacol. 14 (1), 101–115. https://doi.org/10.2174/1570159×13666150716165726.

Halliwell, B., 2001. Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment. Drugs Aging 18 (9), 685–716. https://doi.org/10.2165/00002512-200118090-00004.

Kaur, R., Mehan, S., Khanna, D., et al., 2015. Ameliorative treatment with ellagic acid in scopolamine induced Alzheimer's type memory and cognitive dysfunctions in rats. Austin J. Clin. Neurol. 2 (6), 1053.

Kha, T.C., Nguyen, M.H., Roach, P.D., 2011. Effects of pre-treatments and air drying temperatures on colour and antioxidant properties of gac fruit powder. Int. J. Food Eng. 7 (3), 7.

Khan, K.M., Collier, A.D., Meshalkina, D.A., et al., 2017. Zebrafish models in neuropsychopharmacology and CNS drug discovery. Br. J. Pharmacol. 174 (13), 1925–1944. https://doi.org/10.1111/bph.13754.

Khazdair, M.R., Anaeigoudari, A., Hashemzehi, M., et al., 2018. Neuroprotective potency of some spice herbs, a literature review. J. Tradit. Complement. Med. 9 (2), 98–105. https://doi.org/10.1016/j.jtcme.2018.01.002.

Kim, J., Hong, S.C., Lee, E.H., et al., 2021. Preventive effect of *M. cochinchinensis* on melanogenesis via tyrosinase activity inhibition and p-PKC signaling in melan-A cell. Nutrients 13 (11), 3894. https://doi.org/10.3390/nu13113894.

Kim, Y.H., Lee, Y., Kim, D., et al., 2010. Scopolamine-induced learning impairment reversed by physostigmine in zebrafish. Neurosci. Res. 67 (2), 156–161. https://doi.org/10.1016/j.neures.2010.03.003.

Maddula, K., Kumar, V.P., Anusha, J., 2017. Assessment of aqueous extract of *Ocimum sanctum* leaves in memory enhancement and preventing memory impairment activities in zebra fish model. Int. J. Clin. Pharm. 8 (3), 185–192.

Mans, R.A., Hinton, K.D., Payne, C.H., et al., 2019. Cholinergic stimulation of the adult zebrafish brain induces phosphorylation of glycogen synthase kinase-3 β and extracellular signal-regulated kinase in the telencephalon. Frontiers Mol. Neurosci. 12, 91. https://doi.org/10.3389/fnmol.2019.00091.

Mueller, T., Dong, Z., Berberoglu, M.A., et al., 2011. The dorsal pallium in zebrafish, Danio rerio (Cyprinidae, Teleostei). Brain Res. 1381, 95–105. https://doi.org/10.1016/j.brainres.2010.12.089.

Ng, M.C., Hsu, C.P., Wu, Y.J., et al., 2012. Effect of MK-801-induced impairment of inhibitory avoidance learning in zebrafish via inactivation of extracellular signal-regulated kinase (ERK) in telencephalon. Fish. Physiol. Biochem. 38 (4), 1099–1106. https://doi.org/10.1007/s10695-011-9595-8.

Perathoner, S., Cordero-Maldonado, M.L., Crawford, A.D., 2016. Potential of zebrafish as a model for exploring the role of the amygdala in emotional memory and motivational behavior. J. Neurosci. Res. 94 (6), 445–462. https://doi.org/10.1002/jnr.23712.

Polidori, M.C., Stahl, W., Griffiths, H.R., 2021. Nutritional cognitive neuroscience of aging: Focus on carotenoids and cognitive frailty. Redox Biol. 44, 101996 https://doi.org/10.1016/j.redox.2021.101996.

Ratto, F., Franchini, F., Musicco, M., et al., 2022. A narrative review on the potential of tomato and lycopene for the prevention of Alzheimer's disease and other dementias. Crit. Rev. Food Sci. Nutr. 62 (18), 4970–4981. https://doi.org/10.1080/10408398.2021.1880363.

Richetti, S.K., Blank, M., Capiotti, K.M., et al., 2011. Quercetin and rutin prevent scopolamine-induced memory impairment in zebrafish. Behav. Brain Res. 217 (1), 10–15. https://doi.org/10.1016/j.bbr.2010.09.027.

Santana, S., Rico, E.P., Burgos, J.S., 2012. Can zebrafish be used as animal model to study Alzheimer's disease. Am. J. Neurodegener. Dis. 1 (1), 32–48.

Seibt, K.J., Piato, A.L., da Luz Oliveira, R., et al., 2011. Antipsychotic drugs reverse MK-801-induced cognitive and social interaction deficits in zebrafish (*Danio rerio*). Behav. Brain Res. 224 (1), 135–139. https://doi.org/10.1016/j.bbr.2011.05.034.

Singh, A., Kukreti, R., Saso, L., et al., 2019. Oxidative stress: a key modulator in neurodegenerative diseases. Molecules 24 (8), 1583. https://doi.org/10.3390/molecules24081583.

Singsai, K., Ladpala, N., Dangja, N., et al., 2021. Effect of *Streblus asper* leaf extract on scopolamine-induced memory deficits in zebrafish: the model of Alzheimer's disease. Adv. Pharmacol. Pharm. Sci. 2021, 6666726 https://doi.org/10.1155/2021/6666726.

Srikumar, B., Ramkumar, K., Raju, T., et al., 2004. Assay of acetylcholinesterase activity in the brain. In: Raju, T.R., Kutty, B.M., Sathyaprabha, T.N., Shanakranarayana Rao, B.S. (Eds.), Brain and Behavior. National institute of mental health and neurosciences, India, pp. 142–144.

Su, W., Xu, W., Liu, E., et al., 2023. Improving the treatment effect of carotenoids on Alzheimer's disease through various nano-delivery systems. Int. J. Mol. Sci. 24 (8), 7652. https://doi.org/10.3390/ijms24087652.

Tran, X.T., Parks, S.E., Roach, P.D., et al., 2015. Effects of maturity on physicochemical properties of Gac fruit (*Momordica cochinchinensis Spreng*. Food Sci. Nutr. 4 (2), 305–314. https://doi.org/10.1002/fsn3.291.

Truong, L., Mandrell, D., Mandrell, R., et al., 2014. A rapid throughput approach identifies cognitive deficits in adult zebrafish from developmental exposure to polybrominated flame retardants. Neurotoxicology 43, 134–142. https://doi.org/10.1016/j.neuro.2014.03.005.

Vemula, P., Kumar, B., Battula, R., et al., 2014. Design and development of a novel color bias avoided and appetite conditioning t-maze model for evaluating the memory enhancing drugs in zebra fish. Int. Res. J. Pharm. 5, 434–437.