

**DOCOSAHEXAENOIC ACID ADMINISTRATION AMELIORATES SCOPOLAMINE-INDUCED MEMORY IMPAIRMENT IN MICE**

KOTHARI SAROJ, SINGHAL TULIKA\*

Department of Pharmacology, Gajra Raja Medical College, Gwalior, Madhya Pradesh, India. Email: sdrtulika02@gmail.com

Received: 07 February 2018, Revised and Accepted: 22 March 2018

**ABSTRACT**

**Objective:** The main objective of this work was to study the memory-enhancing activity of docosahexaenoic acid (DHA) supplementation in normal memory function and scopolamine-induced impaired memory in mice.

**Methods:** The gum acacia suspension of DHA was administered by gavage at the dose of 200 and 300 mg/kg in mice for 30 days to evaluate memory-enhancing potential on normal and scopolamine-induced impaired memory in albino mice. Escape latency in Morris water maze (MWM) and transfer latency (TL) in elevated plus maze (EPM) were recorded, respectively. Mice were given four trial sessions per day to locate the platform for 4 days in MWM model. Scopolamine 1 mg/kg was injected i.p. to produce memory impairment in mice.

**Result:** DHA suspension at the dose of 300 mg/kg showed significant reduction of escape latency and TL as compared to control group, and the effect was comparable to that of standard nootropic agent piracetam at the dose of 100 mg/kg in normal and scopolamine-treated mice. However, DHA at the dose of 200 mg/kg showed significant memory-enhancing effect in only scopolamine-induced impaired memory model.

**Conclusion:** The study revealed that the chronic administration of DHA exhibited significant memory-enhancing activity against both normal as well as scopolamine-treated impaired memory mice groups, however, this effect was more marked on scopolamine-induced memory impairment as compared to normal memory function.

**Keywords:** Docosahexaenoic acid, Scopolamine, Morris water maze, Elevated plus maze, Piracetam.

© 2018 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2018.v11i6.25187>

**INTRODUCTION**

Learning is defined as the acquisition of information and skills and subsequent retention of that information is called memory. Learning and memory are fundamental higher brain functions that allow the individual to adapt to the environment. Learning is acquired when a stimulus or a sequence of stimuli are transmitted to the brain which is encoded into memory trace, possibly by formation of new synaptic connections in the brain areas involved in forming and storing the acquired information including hippocampus, cortex, and cerebellum [1].

The cognition refers to the process of acquiring, storing, and utilizing intellectual knowledge. Cognitive enhancers such as piracetam (PCT), oxiracetam, and aniracetam are used primarily to treat cognitive or motor function in disorders such as Alzheimer's disease, mental retardation in children, and memory impairment due to brain injury [2]. These drugs work by increasing the brain's supply of acetylcholine, increase perfusion of brain's oxygen or by activating nerve growth [3]. The main concern with these drugs is their efficacy in such clinical conditions and adverse effects [4].

Docosahexaenoic acid (DHA) (DHA, C22:6  $\omega$ -3) is a long-chain polyunsaturated omega-3 fatty acid and is an integral part of neural membrane phospholipids [5]. DHA has been shown to accumulate in areas of the brain involved in memory and attention such as the cerebral cortex and hippocampus [6]. Advanced cognitive function is uniquely human and impairment of cognitive abilities affects the quality of life [7]. The use of cognition enhancers by healthy individuals in the absence of a medical indication is one of the most debated topics among neuroscientists and psychiatrists. DHA additions to various foods such as dairy products, juices, beverages, and bakery products are being tried and are available in some countries. Nonetheless, the

international sales of cognition-enhancing supplements exceeded US\$1 billion in 2015, and the global demand for these compounds is still growing rapidly [8].

The effect of DHA supplementation on cognition during childhood is controversial [9]. Some studies reporting no effect [10], whereas some suggesting improvement in verbal learning and memory [11]. Therefore, the present study is planned to clearly define the role of chronic DHA supplementation in specific aspects of normal memory function and scopolamine impaired memory function in mice.

**MATERIALS AND METHODS****Materials***Drugs and chemicals*

DHA was purchased from Green Heaven India (A Herbal Manufacturing Unit), Nagpur, Maharashtra. Scopolamine hydrobromide (Injection Buscogast - Sovereign Pharma Pvt., Ltd.) and PCT (Tablet Nootropil Dr. Reddy's Laboratories Ltd.) were purchased from the medical store. All the drugs were administered as 2% gum acacia (GA) suspension.

*Animals*

Randomly bred 6–8-week-old albino male mice weighing 30–40 g, raised in the animal house of the Department of Pharmacology, Gajra Raja Medical College, Gwalior, were used for the study. These were maintained at 24±2°C, humidity 50±5% with 12 h light and dark cycle and kept on standard pellet diet (Pranav Agro Industries, Delhi, India) and water *ad libitum*. The care and maintenance of animals was as per the approved guidelines of the Committee for the purpose of Control and Supervision of Experiments on Animals in India. The Institutional Animal Ethics Committee approved the protocol. (Registration number 846/GO/Ere/S/04/CPCSEA).

## Methods

### Morris water maze (MWM)

The MWM is a white, circular pool with an inner diameter of 110 cm and walls 20 cm high. It was filled with normal tap water to a depth of 13 cm. The water was at room temperature ( $\pm 22^{\circ}\text{C}$ ) and made opaque by adding a small amount of milk powder with no noticeable side effects to the animals. The entire pool was divided into four quadrants of equal size by two diagonal lines running through the center of the pool. A removable circular escape platform (diameter: 10 cm) positioned at the middle of a quadrant. The pool was placed at the far end of a rectangular room dimly lit by white light. The walls of the room were equipped with a variety of spatial cues which remained unchanged during the whole experiment [12].

On 26<sup>th</sup> day of drug administration, training of mice was started. Mice were brought from their housing facility to the behavior room and were kept in an area where they cannot see the pool or spatial cues to adjust to the new environment for at least 30 min before testing. During the 4 subsequent days, the mice were given four trial sessions per day with the platform in place. When mice located the platform, it was permitted to remain on it for 10 s. The escape latencies (s) to reach the platform were recorded. If the mice did not locate the platform within 120 s, it was placed on the platform for 10 s. The time interval between trial sessions was 30 min. After 24 h learning period, mice were evaluated for retention of learning (memory). EL was recorded after 45 min of administration of the last dose of DHA, on 30<sup>th</sup> day and again after 24 h, that is, on 31<sup>st</sup> day in normal mice. To study the effect on impaired memory, scopolamine (1 mg/kg) was injected i.p. after 45 min of administration of DHA or standard drugs or vehicle on the 30<sup>th</sup> day, and EL was recorded after 45 min of injection of scopolamine on the 30<sup>th</sup> day and after 24 h.

### Elevated plus maze (EPM)

The plus maze was in the shape of a cross or plus with two closed arms each with roof open measuring 30 cm  $\times$  5 cm  $\times$  20 cm, extending from the central region (5 cm  $\times$  5 cm) running along a North-South axis and two open arms each measuring 30 cm  $\times$  5 cm running East-West. The wooden apparatus was elevated to a height of 50 cm from the floor in a dimly illuminated room. Animals were placed individually at the end of either of the open arms facing away from the central platform. The time taken by each animal to move from open arm to either of the closed arms was recorded. This duration of time was called transfer latency (TL). If the animal does not enter into any of the enclosed arms within 120 s, it was gently pushed into any of the enclosed arms and TL was considered as 120 s. Later, the animal was allowed to explore the plus maze for 5 min and send back to the home cage [13,14]. TL measured On 30<sup>th</sup> day serves as a parameter of acquisition (learning), while TL on 31<sup>st</sup> day indicates retention of learning (memory). An arm entry was defined as all four feet in the arm. The apparatus was cleaned with alcohol after each mouse was tested to remove any residue or odor.

TL was recorded after 45 min of administration of the last dose of DHA on 30<sup>th</sup> day and again after 24 h, that is, on 31<sup>st</sup> day in normal mice. To study the effect on impaired memory, scopolamine (1 mg/kg) was injected i.p. after 45 min of administration of DHA or standard drugs or vehicle on 30<sup>th</sup> day and TL was recorded after 45 min of injection of scopolamine on 30<sup>th</sup> day and after 24 h.

### Study design

To ensure consistency of experience before the test session, animals were brought to the testing room 1 h before the start of behavior testing. Test room lighting, temperature, and noise level were kept constant for all mice used in the study. DHA or standard drugs were given for 30 days. Dose of DHA was chosen on the basis of the previous study done to see effect of DHA.

To study the effect on normal memory using MWM and EPM model mice were divided into four groups having six animals in each group as follows:

- Group 1: Control received 2% GA at the dose of 10 ml/kg
- Groups 2 and 3: Test drug-treated group received DHA 200 mg/kg and DHA 300 mg/kg respectively
- Group 4: Standard drug-treated group received PCT 100 mg/kg.

To study the effect on impaired memory using MWM and EPM model mice were divided into five groups having six animals in each group.

- Group 1: Normal control received 2% GA at the dose of 10 ml/kg
- Group 2: Negative control received 2% scopolamine 1 mg/kg
- Groups 3 and 4: Drug-treated group received scopolamine 1 mg/kg + DHA 200 mg/kg and scopolamine 1 mg/kg + DHA 300 mg/kg, respectively
- Group 5: Standard drug-treated group received scopolamine 1 mg/kg + PCT 100 mg/kg.

### Statistical analysis

Statistical evaluation was done using one-way ANOVA followed by Tukey's multiple comparison tests.  $p < 0.05$  were considered statistically significant. Data were presented as mean + standard error of the mean. All statistical analysis was performed by Sigma Stat software version 2.0, Jandel Scientific Inc. USA.

## RESULTS

### Effect of DHA on normal memory

EL and TL of the 30<sup>th</sup> day of drugs treatment reflected learning behavior of animals, whereas TL and EL on the 31<sup>st</sup> day reflected retention of learned task as memory. Administration of DHA at the dose of 200 mg/kg decreased escape latency by 16% and TL by 6% in MWM, respectively, and was statistically not significant ( $p > 0.05$ ) as compared to control. DHA at the dose of 300 mg/kg decreased escape latency by 39%, TL by 45% in MWM and EPM, respectively, and was statistically significant ( $p < 0.05$ ) as compared to control and DHA 200 mg/kg suggesting memory-enhancing effect. PCT decreased escape latency by 51% and TL by 54% in MWM and EPM, respectively, and was significant ( $p < 0.05$ ) as compared to control and DHA 200 mg/kg. Effect of DHA 300 mg/kg was comparable to PCT (Tables 1 and 2).

### Effect of DHA on impaired memory

Administration of scopolamine 1 mg/kg increased escape latency by 46% in MWM and TL by 94% in EPM as compared to normal control group and was statistically significant ( $p < 0.05$ ) demonstrating memory impairment. Administration of DHA at the dose of 200 mg/kg in scopolamine-treated animals decreased escape latency by 30% and TL by 23% in MWM and EPM, respectively, and was statistically significant ( $p < 0.05$ ) as compared to negative control suggesting cognitive-enhancing effect. DHA at the dose of 300 mg/kg in scopolamine-treated group decreased escape latency by 39%, TL by 42% in MWM and EPM, respectively, and was statistically significant ( $p < 0.05$ ) as compared to negative control suggesting improvement in memory. Administration of PCT in scopolamine-treated animals decreased escape latency by 57% and TL by 51% in MWM and EPM, respectively, and was significant ( $p < 0.05$ ) as compared to negative control and DHA 200 mg/kg. Effect of DHA 300 mg/kg was comparable to PCT (Figs. 1 and 2).

## DISCUSSION

Memory is divided into two general types, declarative and non-declarative. Declarative memory relates to the conscious recollection of facts and events and can be further subdivided into episodic and semantic. Episodic memory is memory for personally experienced events that occur at a specific place and time and is measured by memory of stories, word lists, or figures [15]. In the present investigation, effect of chronic administration of DHA was studied on learning and memory of normal and scopolamine-treated mice.

The MWM was described 30 years ago as a device to investigate spatial learning and memory in laboratory rats. Spatial learning in general and MWM performance, in particular appear to depend on the coordinated action of different brain regions and neurotransmitter

**Table 1: Effect of DHA on escape latency using MWM in normal mice**

Treatment	Mean escape latency (s)	
	On 30 <sup>th</sup> day	On 31 <sup>st</sup> day
GA10	66.83±2.47	65.17±2.75
DHA200	59.50±9.23	55.17±4.38
DHA300	54.33±2.91	39.67±1.26**
PCT100	50.33±5.19	32.00±2.46**

GA10: Gum acacia 10 ml/kg, DHA200: Docosahexaenoic acid 200 mg/kg, DHA300: Docosahexaenoic acid 300 mg/kg, PCT100: Piracetam 100 mg/kg n=6 animals in each group. Values are expressed as mean±SEM, \*p<0.05 as compared to GA10, #p<0.01 as compared to DHA200. SEM: Standard error of the mean

**Table 2: Effect of DHA on TL using elevated plus-maze in normal mice**

Treatment	Mean TL (s)	
	On 30 <sup>th</sup> day	On 31 <sup>st</sup> day
GA10	29.50±3.28	26.33±3.77
DHA200	27.50±3.92	24.83±1.67
DHA300	22.83±3.72	14.50±1.40*#
PCT100	19.33±3.68	12.33±1.02*#

GA10: Gum acacia 10 ml/kg, DHA200: Docosahexaenoic acid 200 mg/kg, DHA300: Docosahexaenoic acid 300 mg/kg, PCT100: Piracetam 100 mg/kg n=6 in each group. Values are expressed as mean±SEM, \*p<0.01 as compared to GA10, #p<0.05 as compared to DHA200 group. SEM: Standard error of the mean

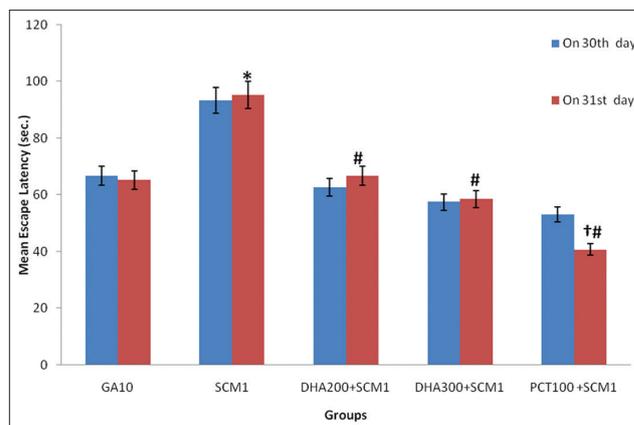
systems constituting a functionally integrated neural network. MWM task has often been used in the validation of rodent models for neurocognitive disorders and the evaluation of possible neurocognitive treatments [16].

EPM served as the exteroceptive behavioral model to evaluate memory in rats. The spontaneous alteration in behavior in the EPM is considered to reflect working memory. Based on the natural aversion of mice of high and open spaces reported that TL (the time in which mouse moves from the open arms to the enclosed arm) on the 2<sup>nd</sup> day onward was shortened than on the 1<sup>st</sup> day and suggested that this shortened TL can be utilized as a parameter for learning and memory [17].

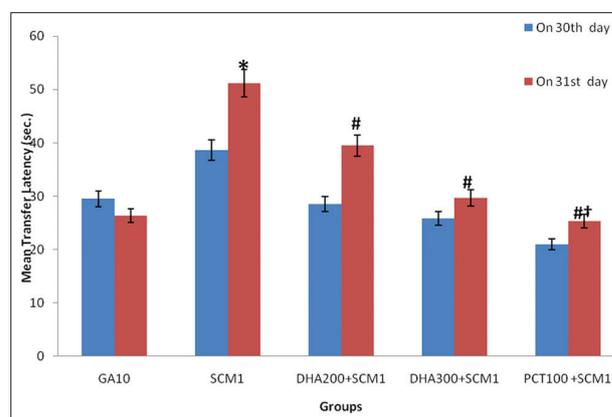
In the present study, chronic administration of DHA in normal mice significantly decreased escape latency in MWM and TL in EPM suggesting enhancement in memory. Improved spatial learning performance due to DHA may be associated with enhanced neurogenesis as reported in earlier studies [18,19]. The hippocampus, in particular, is essential for memory function. DHA promotes hippocampal neuronal development and synaptic function [20] contributing to improvements in the performance of memory-related tasks such as the MWM [21]. On the other hand, dietary deficiency of n3 fatty acid demonstrated moderate impairment of Barnes maze performance compared with n3 fatty acid adequate rats [22].

In the second part of the present study, chronic administration of DHA significantly reversed scopolamine-induced increased escape latency to reach the platform in MWM and TL in EPM, respectively, suggesting spatial memory-enhancing effect.

Memory impairment in the scopolamine-induced animal model is associated with increased oxidative stress [23,24]. Oxidative stress is well known to impair learning and memory leading to cognitive dysfunction [25]. Cognitive-enhancing effect of DHA in scopolamine-induced memory impairment in the present study could be due to the free-radical scavenging property of DHA [26]. Oral administration



**Fig. 1: Bar diagram showing increased escape latency following scopolamine and ameliorative action of docosahexaenoic acid (DHA) (200 and 300 mg/kg) and piracetam on escape latency in scopolamine-treated mice using Morris water maze. Each column represents mean ± standard error of the mean, n=6 mice in each group. \*p<0.01 as compared to gum acacia 10 (normal control), #p<0.01 as compared to SCM1 (negative control), †p<0.01 as compared to DHA200+SCM1 group**



**Fig. 2: Bar diagram showing increased transfer latency (TL) following scopolamine and ameliorative action of docosahexaenoic acid (DHA) (200 and 300 mg/kg) and piracetam on TL in scopolamine-treated mice using elevated plus maze. Each column represents mean ± standard error of the mean, n=6 mice in each group. \*p<0.01 as compared to gum acacia 10 (normal control), #p<0.05 as compared to SCM1 (negative control), †p<0.05 as compared to DHA200+SCM1 group**

of DHA is accompanied with an increased activity of catalase and glutathione peroxidase enzymes [27].

## CONCLUSION

Thus, the data of the present study support cognitive-enhancing effect of chronic DHA intake in normal and impaired memory mice, however, the memory-enhancing effect of DHA is more marked in scopolamine-induced memory impairment as compared to normal memory function. Long-term interventional studies are required to know the exact mechanism of cognitive enhancing effect of DHA.

## ACKNOWLEDGMENTS

The authors are thankful to Dr. S.N. Iyenger, Dean Gajra Raja Medical College, Gwalior, for his encouragement to do research work.

## AUTHORS CONTRIBUTIONS

Equally contributed.

## CONFLICTS OF INTERESTS

Declared none.

## REFERENCES

- Chen KH, Chuah LY, Sim SK, Chee MW. Hippocampal region-specific contributions to memory performance in normal elderly. *Brain Cogn* 2010;72:400-7.
- Schever K, Rostock A, Bartsch P, Muller WK. Piracetam improved cognitive performance by restoring neurochemical deficits of the aged rat brain. *Pharmacopsychiatry* 1999;32:10-60.
- Kamila S, Madhav NS, Sarkar C. Safety and efficacy study of herbal polyphyto formulations: For its learning and memory enhancing properties. *Int J Pharm Pharm Sci* 2016;8:119-23.
- Colucci L, Bosco M, Ziello AR, Rea R, Amenta F, Fasanaro AM. Effectiveness of nootropic drugs with cholinergic activity in treatment of cognitive deficit: a review. *J Exp Pharmacol* 2012;4:163.
- Anderson GJ, Connor WE, Corliss JD. Docosahexaenoic acid is the preferred dietary n-3 fatty acid for the development of the brain and retina. *Pediatr Res* 1990;27:89-97.
- Guesnet P, Alessandri JM. Docosahexaenoic acid (DHA) and the developing central nervous system (CNS)-Implications for dietary recommendations. *Biochimie* 2011;93:7-12.
- Weiser MJ, Butt CM, Mohajeri MH. Docosahexaenoic acid and cognition throughout the lifespan. *Nutrients* 2016;8:99.
- Tarfarosh SF, Tromboo U, Bhat F. Search for a perfect Nootropic supplement combination-Can we increase human intelligence by nutritional supplements. *J Pharmacogn Phytochem* 2017;6:1020-4.
- Harmita H, Mansur U, Stephanie S. Determination of docosahexaenoic acid in infant formulas with gas chromatography. *Asian J Pharm Clin Res* 2018;11:247-250.
- Osendarp SJ, Baghurst KI, Bryan J, Calvaresi E, Hughes D, Hussaini MK, *et al.* Effect of a 12-mo micronutrient intervention on learning and memory in well-nourished and marginally nourished school-aged children: 2 parallel, randomized, placebo-controlled studies in Australia and Indonesia. *Am J Clin Nutr* 2007;86:1082-93.
- Dalton A, Wolmarans P, Witthuhn RC, Stuijvenberg ME, Swanevelder SA, Smuts CM. A randomised control trial in schoolchildren showed improvement in cognitive function after consuming a bread spread, containing fish flour from a marine source. *Prostaglandins Leukot Essent Fat Acids* 2009;80:143-9.
- D'Hooge R, De Deyn PP. Applications of the Morris water maze in the study of learning and memory. *Brain Res Rev* 2001;36:60-90.
- De Bruin JP, Sanchez-Santed F, Heinsbroek RP, Donker A, Postmes P. A behavioural analysis of rats with damage to the medial prefrontal cortex using the Morris water maze: Evidence for behavioural flexibility, but not for impaired spatial navigation. *Brain Res* 1994;652:323-33.
- Holguin S, Huang Y, Liu J, Wurtman R. Chronic administration of DHA and UMP improves the impaired memory of environmentally impoverished rats. *Behav Brain Res* 2008;191:11-6.
- Budson AE, Price BH. Memory: Clinical Disorders. *Encyclopedia of Life Sciences*. London: Nature Publishing Group; 2001. p. 1-8.
- Bromley-Brits K, Deng Y, Song W. Morris water maze test for learning and memory deficits in Alzheimer's disease model mice. *J Vis Exp* 2011;53:2920.
- Nade VS, Kawale LA, Valte KD, Shendye NV. Cognitive enhancing effect of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers on learning and memory. *Ind J Pharmacol* 2015;47:263.
- He C, Qu X, Cui L, Wang J, Kang JX. Improved spatial learning performance of fat-1 mice is associated with enhanced neurogenesis and neurogenesis by docosahexaenoic acid. *Proc Natl Acad Sci U. S. A* 2009;106:11370-5.
- Titova OE, Sjögren P, Brooks SJ, Kullberg J, Ax E, Kilander L, *et al.* Dietary intake of eicosapentaenoic and docosahexaenoic acids is linked to gray matter volume and cognitive function in elderly. *Age* 2013;35:1495-505.
- Cao D, Kevala K, Kim J, Moon HS, Jun SB, Lovinger D, *et al.* Docosahexaenoic acid promotes hippocampal neuronal development and synaptic function. *J Neuro Chem* 2009;111:510-21.
- Awakita E, Hashimoto M, Shido O. Docosahexaenoic acid promotes neurogenesis in vitro and in vivo. *Neuroscience* 2006;139:991-7.
- Fedorova I, Hussein N, Baumann MH, Di Martino C, Salem NA. n-3 fatty acid deficiency impairs rat spatial learning in the Barnes maze. *J Behav Neurosci* 2009;123:196-205.
- El-Sherbiny DA, Khalifa AE, Attia AS, Eldenshary Eel-D. Hypericum perforatum extract demonstrates antioxidant properties against elevated rat brain oxidative status induced by amnestic dose of scopolamine. *Pharmacol Biochem Behav* 2003;76:525-33.
- Kulkarni KS, Kasture SB, Mengi SA. Efficacy study of *Prunus amygdalus* (almond) nuts in scopolamine-induced amnesia in rats. *Ind J Pharmacol* 2010;42:168.
- Deka P, Kumar A. Pharmacological evaluation of Triphala churna instreptozotocin (i. C. V.) Induced dementia in rats. *Int J Pharm Pharm Sci* 2018;10:97-105.
- Kim YJ, Chung HY. Antioxidative and anti-inflammatory actions of docosahexaenoic acid and eicosapentaenoic acid in renal epithelial cells and macrophages. *J Med Food* 2007;10:225-31.
- Casanas-Sánchez V, Perez JA, Fabelo N, Alemany DQ, Diaz ML. Docosahexaenoic (DHA) modulates phospholipid-hydroperoxide glutathione peroxidase (Gpx4) gene expression to ensure self-protection from oxidative damage in hippocampal cells. *Front Physiol* 2015;6:203.