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The Efficacy of Seluang Fish (*Rasbora argryotaenia*) Oil in Cognitive Function and Expression of *Brain-Derived Neurotrophic Factor* (BDNF) in *Rattus norvegicus* Strain Wistar.

Radiyati Umi Partan[1], Rachmat Hidayat[2]*, and Evi Lusiana[3].

[1]Internal Medicine, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia.
[2]Biomolecular Laboratory, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia.
[3]Pharmacology Department, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia.

ABSTRACT

Rasbora argryotaenia (Seluang fish) is freshwater fish, has oily skin and slim body. Fish oil contain omega-3, EPA and DHA. Omega-3 has a potential to increase expression of BDNF and restoration cognitive function. An experimental research design, eight-week-old male Wistar Rat (n = 28) were randomized into four groups, and each group consisted of 7 rats. Normal control group, group 2,3 and 4 : rats were induced by seluang fish oil 0,1 mL/kgBW, 0,2 mL/kgBW and 0,4 mL/kgBW for 21 days. Cognitive function was assayed by eight-arm maze test. Concentration of BDNF in hippocampus was assayed by ELISA. Eight-arm maze test and concentration of BDNF in group 1 (600 second, 163,65pg/mL); group 2 (459 second, 168,25pg/mL); group 3 (298 second, 234,63pg/mL); group 4 (163 second, 270,45pg/mL). Seluang fish oil at doses 0,2 mL/kgBW and 0,4 mg/kgBW had efficacy to restore cognitive function via increased concentration of BDNF in hippocampus area.

Keywords: Seluang Fish Oil – Omega 3 – Cognitive – Eight Arm Maze Test – BDNF

Corresponding author

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INTRODUCTION

Seluang Fish (Rasbora argryotaenia) is a freshwater, belonging to the family Cyprinidae. Seluang fish spread in Sumatra, Java and Kalimantan (Indonesia), Malaysia and the Philippines. Seluang Fish has a slender body and has a body length of about 180 mm. It has a yellowish-green body on the dorsal and has a silver-colored body in the ventral (abdominal) part. [1] Fish oil is one of the products obtained from fish body tissue. Fish oil is rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which plays a role in decreasing inflammatory response. [2] Fish Oil is also useful in the management of hypertriglyceridemia and is useful in the prevention of cardiovascular disorders, such as heart attack and stroke. [3-5] Fish oil and omega 3 has been widely studied and has many benefits to prevent depression, anxiety, cancer and macular degeneration. [6-8]

Fish oil is generally produced from deep sea predator fish such as sharks, swordfish, tilefish, and albacore tuna. Fish oil from deep sea predator fish is generally rich in omega-3, but because deep sea predator fish is an organism located in the top food chain, causing accumulation of toxic substance and heavy metal. The toxicity of heavy metals is very serious in the initiation of health problems, such as cancer and other degenerative diseases. [9-11]

Omega-3 is believed to have a role in improving intelligence. Omega-3 play a role in the regulation of neurotrophins (growth factors that regulate proliferation, differentiation, and survival of neuronal cells in the brain). Omega-3 can increase the expression of some neurotrophin in the brain such as NGF (nerve growth factor) and BDNF (Brain-Derived Neurotrophic Factor). This increase in neurotrophin expression is believed to be useful in improving intelligence. [12-14]

The objective of study was to determine the efficacy for Seluang Fish Oil in cognitive function via regulated expression of neurotrophins, BDNF, in the brain of Rattus norvegicus.

METHODS

The research design was experimental with pre-post test only with control group, in vivo. Wistar rats (Eight old weeks, weight 150-200 g) were provided by Laboratory Research Unit Sriwijaya University, Palembang, Indonesia, with approval of the Ethics Committee of Bioethics and Humaniora Unit Universitas Sriwijaya (no.156/kptfkrsmh/2017), Palembang, Indonesia.

The study used 32 animals were randomized into four groups. Group 1: 8 rats were injected aquadest intragastric, 21 days. Group 2: 8 rats were injected seluang fish oil 0,1 mL/kgBW intragastric, 21 days. Group 3: 8 rats were were injected seluang fish oil 0,2 mL/kgBW intragastric, 21 days. Group 4: 8 rats were injected seluang fish oil 0,4 mL/kgBW intragastric, 21 days.

Seluang Fish Oil Preparation

Seluang fishes were collected from The Musi River Palembang, South Sumatera, Indonesia. Seluang fishes had been determined by Biology Laboratory, Faculty of Mathematics and Science, Sriwijaya University, South Sumatera, Indonesia. Seluang Fishes were washed and removed the feces from the gut. Boiling fish with wet randering method, where the milled fish is mixed with water at a ratio of 1:10. Next, the fish is cooked with a doubel jacket at temperature 90^{o}C for 60 minutes. Fish oil located on the surface of the decoction is separated. Subsequently, the filtered fish oil was subjected to cooling by storing in the coolant temperature 4^{O}C for 12 hours. The cooled oil will form three layers, the most basic layer is water, the second layer is free fatty acid and phosphatide and the top layer is fish oil.

Radial Arm Maze Test

Radial Arm Maze test was used to evaluation spatial learning and memory. Rats were placed in the center of an eight-arm radial maze. Four randomly chosen arms were baited with pellets in opaque containers. Every rat was given the opportunity to visit all the arms and collected the available pellets. The time required by rats to collected pellets in the radial arm-maze was measured and compared between groups.[11]

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BDNF Assay

Samples (hippocampus) were washed by PBS until the samples cleaned enough. Every sample was homogenized and centrifuge 10.000 rpm for 30 minutes at 4^0C. Supernatan was collected. Solid phase sandwich ELISA (Rat BDNF ELISA kit, Sigma) were used to analysis concentration of BDNF.

Fatty Acids Analysis

The fish oil sample was converted to FAMEs according to the AOAC method. The top layer (1 uL) was injected into the Chromatoghraphy Gas.

Statistics

The results were presented as mean ± SD. The mean concentartion of BDNF and the result of Eight arm maze test were compared among groups by ANOVA followed by pos hoc analysis with bonferroni test . A value of $p<0.05$ was considered statistically significant.

RESULTS

Eight-Arm Maze Test

Group 3 (rat induced by seluang fish 0,2 mL/kgBW) and group 4 (rat induced by seluang fish 0,4 mL/kgBW) had lower time to recognize food in eight-arm maze test than group 1 and 2 (table 1).

Table 1: Result of Eight-Arm Maze Test

No.	Group	Mean Time to Recognize Food in Eight Arm Test (Seconds) ± SD
1.	Normal	600±10,24
2.	Seluang Fish Oil 0,1 mL/kgBW	459±40,54
3.	Seluang Fish Oil 0,2 mL/kgBW	298±12,87
4.	Seluang Fish Oil 0,4 mL/kgBW	163±11,12

Seluang fish oil doses 0,2 mL/kgBW and 0,4 mL/kgBW had efficacy to restore cognitive function (table 2).

Table 2: Efficacy Seluang Fish Oil in Eight-Arm Maze Test

Group	Group	p value	
		Unadjusted*	Adjusted**
Normal	Seluang Fish Oil 0,1mL/kgBW	0,012	0,389
	Seluang Fish Oil 0,2 mL/kgBW	0,001	0,006
	Seluang Fish Oil 0,4 mL/kgBW	0,001	0,001
Seluang Fish Oil 0,1 mL/kgBW	Normal	0,012	0,389
	Seluang Fish Oil 0,2 mL/kgBW	0,048	0,237
	Seluang Fish Oil 0,4 mL/kgBW	0,001	0,007
Seluang Fish Oil 0,2 mL/kgBW	Normal	0,001	0,006
	Seluang Fish Oil 0,1 mL/kgBW	0,048	0,237
	Seluang Fish Oil 0,4 mL/kgBW	0,001	0,001
Seluang Fish Oil 0,4 mL/kgBW	Normal	0.001	0.001
	Seluang Fish Oil 0,1 mL/kgBW	0.001	0.007
	Seluang Fish Oil 0,2 mL/kgBW	0.001	0,001

*Independent-T test, **Pos Hoc Bonferroni, p=0,05

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Concentration of BDNF

Group 3 (rat induced by seluang fish 0,2 mL/kgBW) and group 4 (rat induced by seluang fish 0,4 mL/kgBW) had higher concentration of BDNF than group 1 and 2 (table 3).

Table 3: Concentration of BDNF

No.	Group	Mean Concentration of BDNF (pg/mL) ± SD
1.	Normal	163,65±12,24
2.	Seluang Fish Oil 0,1 mL/kgBW	168,25±11,54
3.	Seluang Fish Oil 0,2 mL/kgBW	234,63±12,87
4.	Seluang Fish Oil 0,4 mL/kgBW	270,45±11,12

Seluang fish oil doses 0,2 mL/kgBW and 0,4 mL/kgBW had efficacy to increase concentration of BDNF (table 4).

Table 4: Efficacy Seluang Fish Oil in Concentration of BDNF

Group	Group	p value	
		Unadjusted*	Adjusted**
Normal	Seluang Fish Oil 0,1mL/kgBW	0,054	0,606
	Seluang Fish Oil 0,2 mL/kgBW	0,001	0,001
	Seluang Fish Oil 0,4 mL/kgBW	0,001	0,001
Seluang Fish Oil 0,1 mL/kgBW	Normal	0,054	0,606
	Seluang Fish Oil 0,2 mL/kgBW	0,001	0,001
	Seluang Fish Oil 0,4 mL/kgBW	0,001	0,001
Seluang Fish Oil 0,2 mL/kgBW	Normal	0,001	0,001
	Seluang Fish Oil 0,1 mL/kgBW	0,001	0,001
	Seluang Fish Oil 0,4 mL/kgBW	0,001	0,001
Seluang Fish Oil 0,4 mL/kgBW	Normal	0.001	0.001
	Seluang Fish Oil 0,1 mL/kgBW	0.001	0.001
	Seluang Fish Oil 0,2 mL/kgBW	0.001	0,001

*Independent-T test, **Pos Hoc Bonferroni, p=0,05

Fatty Acids Analysis

Based on fatty acid analysis, seluang fish oil contains omega 3 around 5,38% and omega 6 around 10,41%.

Table 5: Fatty Acids Analysis

No.		Parameter	Result
1.		**Omega 3**	**5,38 %**
	a.	Linolenat Acid	2,26 %
	b.	Eicosatrienoat Acid	<0,1 %
	c.	Arachidonat Acid	<0,1 %
	d.	EPA	1,00 %
	e.	DHA	2,12 %
2.		**Omega 6**	**10,41%**
	a.	Linoleat Acid	10,14%
	b.	Eicosadienoat Acid	0,27%

DISCUSSION

Working and reference memory are commonly assessed using eight-arm maze test. It needs pretraining, food deprivation, and introducing scent cue confounds. [15-17] The time required by rats to

recognize food in the maze-arm was decreased in treatment group with seluang fish oil at doses 0,2 mL/kgBW and doses 0,4 mL/kgBW. The result of eight-arm maze test showed the efficacy of Seluang Fish oil from clinical aspect of cognitive restoration.

BDNF increases the function and viability of neuronal cell, and it has a povital role for maintaining molecular processes underlying cognitive function. BDNF induces neuronal excitability and facilitates synaptic transmission. Neurons located in hippocampus synthesizes BDNF. Hippocampus is a region in brain that regulated cognitive function. BDNF affects synapsin I (nerve terminal phospho-protein). Synapsin I will induce axonal elongation, neurotransmitter release, and maintenance of synaptic. BDNF induces activation of CREB, a transcription factor involved in learning and memory. [13-15] Concentration BDNF significantly increased in treatment group with seluang fish oil at doses 0,2 mL/kgBW and doses 0,4 mL/kgBW.

Seluang fish oil contains eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). EPA and DHA precursor of certain eicosanoids that have function to reduce inflammation proccess, and have other health benefits, such as treating hypercholesterolemia, preventing heart attacks and strokes. [14-16] Omega-3 fatty acids have been benefit in some clinical condition such as anxiety, cancer and depression. Omega-3 fatty acids promotes the expression of BDNF level and affected the improvement of cognitive function.

CONCLUSION

Seluang fish oil at doses 0,2 mL/kgBW and 0,4 mg/kgBW had efficacy to restore cognitive function via increased concentration of BDNF in hippocampus area.

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