Potential of Glutathione Antioxidant in the Hippocampus Repair: Preliminary Study on Bioactive Materials Antiaging of Snakehead Fish (Channa striata) in Animal Models of Aging

by Sunarno Sunarno

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Sunarno

Biology Laboratory Structure and Function of Animals, Department of Biology, Faculty of Science and Mathematics, Diponegoro University, Jl. Prof. Soedarto, SH, Tembalang, Semarang, Central Java 50275
Email: sunzen07@gmail.com

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I. Introduction

Aging of the brain is one of decline in organ function as a result of an increase in chronological age or oxidative stress. One of the marked clinical symptoms of brain aging is a decrease in the function of the hippocampus (Reddy 2009). The hippocampus is a brain region that is located on the mid-lateral in the ventral side of the cerebrum. This brain region is component of the limbic system that plays a role in the regulation of emotional balance.

Decreased function of the hippocampus is characterized by depletion of glutathione levels to below the normal threshold. Depletion of glutathione levels can cause interference hippocampus histo-morphological and structural changes in neuronal mitochondria. The disorder is characterized by high mortality and impaired neuronal axons in neuronal structure. Both types of these disorders are strongly correlated with changes in mitochondrial structure, especially in the pyramidal neurons of the hippocampus cornu ammonis (Sunarno *et al*., 2009; Sunarno *et al*. 2013). It was further reported that some of these disorders can lead to a decrease in the ability of learning-memory and motor behavior.

Aging of the brain that is characterized by a decrease in hippocampal function has become a public health problem lately. In addition to lower performance, decreased hippocampal function can reduce the function of the immune system, impaired balance a system of coordination and regulation, as well as increasing the vulnerability of other body systems on the environment. Decreased hippocampal function will always occur in humans and animals with increases in chronological age or due to oxidative stress triggered, but the rate of decline in function that occurs can be slowed. One way of handling decreased function of the hippocampus is to use antiaging exogenous materials that can induce the synthesis of glutathione in the body. Glutathione is an endogenous metabolite that has antioxidative capabilities as well as antiaging. Based on the criteria and the potential of these materials, this study will use material from snakehead fish meat (*C. striata*) obtained from Rawa Pening, Semarang regency, Central Java. Snakehead fish in Rawa Pening, besides endemic and natural, which allegedly holds the potential antiaging not been explored in depth. As food sources of animal protein, cork fish meat contains many glutamine and other amino acids are thought to be the precursor of the antioxidant glutathione. Application extracts the cork fish in the diet can significantly increase levels of glutamine in the blood, improving liver function, and accelerate the wound healing process after surgery. Glutamine, cysteine, and glycine is linked to the synthesis of glutathione. Several studies have reported that glutamine, cysteine, and glycine serve as a precursor of glutathione in the body (Daren *et al*., 2007; Fernandes *et al*., 2010; Sunarno *et al.* 2013). Glutamine can be converted into glutamic acid and together with cysteine and glycine is used for the synthesis of glutathione in the hippocampus (Sunarno, 2009). Glutamine, cysteine, and glycine have stable properties during the process experienced in the body, more rapid hydrolysis process, is able to cross the blood-brain barrier, can be utilized by neurons directly, and is able to increase the levels of glutathione in the hippocampus.

II. MATERIALS AND METHODS

Preparation of Extracts and Analysis of Nutrient Components in Cork Fish Extract

Snakehead fish used for research was taken from the Rawa Pening, Semarang regency. Test animals used were *Sprague dawley* rats. Preparation of snakehead fish extract performed using snakehead fish meat. The meat is cleaned and cut into pieces with a thickness of 1 cm. Pieces of meat then inserted into the extraction tool. Extraction was carried out at 70^{0}C until the extract produced a clear drop. Extract pasteurized, then stored in the refrigerator, and ready to be used as a treatment.

Analysis of the composition of snakehead fish, including glutamine, cysteine, and glycine was conducted using ever done by previous researchers. Analysis glutamine snakehead fish extract were calculated using Wang *et al*. (2010) with a spectrophotometer at a wavelength of 630 nm. Analysis of cysteine and glycine extract cork fishing is done by using a spectrophotometer at a wavelength of 560 consecutive and 525 nm.

Acclimation of Test Animals and Animal Model of Aging

The study begins with acclimation strain *Sprague dawley* male rats for one week. During acclimation, rats fed with commercial pellets and water ad libitum. Preparation of animal models of chronological aging is done by treating the rats with the treatment without oxidative stress. The manufacture of animal models of oxidative stress-induced aging is done in a way not to feed on rats for 7 days, rats were given drinking water ad libitum, and every day the rats activity swim in the water in a bucket covered for 15 minutes.

Clear drops of extracted snakehead fish meat administered orally every day for 2 months using a syringe injection (gavage) at a dose of 30 ml/kg bw/day, both in rats with chronological aging or aging-induced oxidative stress and control treatments. Stock solution (clear drops) prepared each treatment and stored at 4°C in the refrigerator. Tests conducted snakehead fish extract in animal models of aging using a design comparison population. Each treatment group consisted of 10 rats.

Parameter Measurement and Analysis of Brain Hippocampus

At the end of the treatment the rats were sacrificed and followed by blood sampling and hippocampus. Determination of levels of glutamine, cysteine, and glycine blood and hippocampus performed by using a spectrophotometer at a wavelength of 630 nm according to the procedure that has been performed by Wang *et al.* (2010) with some modifications. To get the levels of glutathione in the hippocampus is the determination of glutathione levels using a spectrophotometer at a wavelength of 520 nm. Preparation procedure and the determination of the level of glutathione in hippocampal refers to the method that has been done by Feoli *et al.* (2010).

III. RESULTS AND DISCUSSIONS

Analysis of glutamine, cysteine, and glycine in snakehad fish extracts follow the procedures Wang *et al.* (2010) using a spectrophotometer at a wavelength of 630, 560, and 525 nm obtained the results as shown in Table 1.

Table 1. The composition of amino acids in 100 grams of snakehead fish meat

No	Amino acids	Clear drops of snakehead fish (%)
1	Glutamine	32,39
2	Cysteine	6,61
3	Glycine	9,69

Based on Table 1 can be explained that the highest amino acid content in snakehead fish meat, consecutive glutamine, glycine, and cysteine. The third amino acid is a non essential amino acid that can not be synthesized in the body and obtained only from outside the body. All three of these amino acids play an important role as a precursor of the antioxidant glutathione as a potential scavenging the free radicals. Thus, there is a correlation between the level of availability of these three types of amino acids to increase glutathione levels which affect the repair of body tissues, both due chronological aging or aging-induced oxidative stress. Mechanism of biosynthesis of glutathione has been linked with the availability of the three types of essential amino acids.

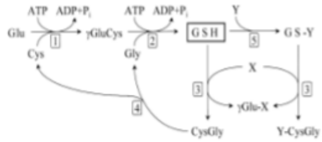

Figure 1. Mechanism of glutathione biosynthesis

Glutathione synthesis occurs through a sequential process involving two main enzymes, namely γ-synthetase and glutathione synthetase glutamycysteine, each requiring ATP as kosubstrat. The enzyme γ-glutamycysteine synthetase requires glutamic acid and cysteine as a substrate to produce a dipeptide glutamyl-cysteine (γ-GluCys). Through continued reaction, the resulting dipeptide synthesized by glycine into glutathione (GSH) by glutathione synthetase. Glutathione was subsequently used as a substrate for γ-glutamyltransferase ektoenzim (γ-GT). The reaction between glutathione, γ-glutamyl group acceptor (X), and γ-GT generates dipeptide cysteinyl-glycine (CysGly). Through different reaction pathways, intracellular glutathione conjugated by glutathione-S-transferase by xenobiotics or endogenous compounds (Y) produces glutathione-xenobiotics conjugate or endogenous compounds-glutathione conjugate (GS-Y). Glutathione biosynthesis as shown in Figure 1.

Three levels of the amino acid precursors of glutathione in the blood and the hippocampus in animal models of aging after oral treatment every day for 2 months with a dose of 30 ml/ kg bw/day are shown in Table 2 Furthermore, hippocampal glutathione content test results are shown in Table 3.

Table 2. The results of the test content of glutamine, glycine and cysteine in the blood and the hippocampus in animal models of aging between control and treatment after snakehead fish meat extract.

No	Animal Model	Blood (mmol/L)			Hippocampus (μmol/g bw tissue)		
		Glu	Gly	Cys	Glu	Gly	Cys
	Chronological aging						
1	Treatment	0,99^{b}	0,49^{b}	0,34^{b}	12,84^{b}	5,25^{b}	3,47^{b}
2	control	0,61^{a}	0,27^{a}	0,93^{a}	4,23^{a}	2,88^{a}	1,43^{a}
	Aging-induced oxidative stress						
1	Treatment	0,37^{b}	0,21^{b}	0,19^{b}	5,65^{b}	3,39^{b}	1,96^{b}
2	control	0,15^{a}	0,07^{a}	0,01^{a}	1,77^{a}	0,09^{a}	0,05^{a}

Table 3. Glutathione content of the hippocampus in animal models of aging between control and after treatment

No	Animal Model	Hippocampus Glutathione (μmol/g bw tissue)	
		Control	Treatment
1	Chronological aging	0,065^{b}	0,887^{b}
2	Aging-induced oxidative stress	0,025^{a}	0,384^{a}

Table 2 shows that treatment of the extracted snakehead fish meat (clear drops) at a dose of 30 ml/kg bw per day was significantly different influence on levels of glutamine, cysteine, and glycine, both in blood plasma and hippocampus. Highest amino acid content, both in blood and hippocampus, respectively glutamine, glycine, and cysteine. Differences were also found in the levels of glutathione in both animal models of aging (Table 3). Glutathione hippocampus in animal models with the chronological aging an increase of 0.822 mol/g bw tissue compared to control, while in animal models of aging due to oxidative stress with an increase of 0.359 mol/g bw tissue. Improvement of the levels of glutathione in both animal models showed that glutathione biosynthesis capacity is strongly influenced by the level of disruption caused by free radicals, cellular component reliability, and availability of raw materials glutathione precursor. In general, the results of this study provide evidence that cork fish meat extract contains three types of essential amino acids is high and when given the proper dosage can influence the increase in the content of some of the amino acids in the blood. The blood carries the amino acids, either through the pulmonary or systemic circulation system will flow up to the brain and ultimately to ensure the availability of raw materials to support the metabolism of the antioxidant glutathione biosynthesis. Thus, increasing the three types of amino acids in the blood have a direct impact to increase glutathione levels in the brain, particularly the hippocampus. Hippocampus as one part of the brain that have high levels of oxygen consumption and susceptible to free radicals, desperately need all three types of these precursors to maintain and sustain concentration gutation. Glutathione in the hippocampus have scavening action is used to prevent the adverse effects of free radicals or free radical chain reactions against cell biological materials. Scavenging process involving glutathione against free radical is shown in Figure 2.

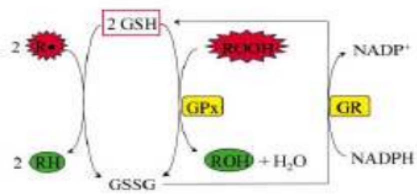

Figure 2. The process of free radical scavenging by glutathione

The process of scavenging the free radicals by glutathione occurs directly through the two different types of reactions, namely the non-enzymatic and enzymatic. Non-enzymatic reaction produces free radicals that are stable and prevent further free radical reactions, whereas the enzymatic reactions involving electron donor in the peroxide reduction reaction catalyzed by glutathione peroxidase into glutathione disulfide (GSSG), a compound that has a hydroxyl group (ROH), and H_2O. GSH reconstituted from GSSG via enzymatic reactions and is catalyzed by glutathione reductase flavoenzim (GR) using NADPH as a cofactor. Through enzymatic reactions involving glutathione peroxidase and glutathione reductase, glutathione is continuously generated repeatedly (Dringen, 2000).

IV. CONCLUSIONS

Snakehead fish meat is a source of amino acids glutamine, cysteine, and glycine that can be used as a precursor to the antioxidant glutathione increase, both in body and in the hippocampus. Snakehead fish meat extract at a dose of 30 ml/kg/day can increase the glutathione levels in the blood and the hippocampus, as well as preventing damage and repairing the aging hippocampus, both in aging and chronological aging due to oxidative stress.

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