Applications of bioactive material from snakehead fish *(Channa striata)* for repairing of learning-memory capability and motoric activity: a case study of physiological aging and aging-caused oxidative stress in rats

Sunarno Sunarno¹, Siti Muflichatun Mardiati¹, Rully Rahadian¹

¹Department of Biology, Faculty of Sciences and Mathematics, Diponegoro University, Semarang, Indonesia, Email: sunzen07@gmail.com, +6285693142989, Phone/Fax (024) 76480923
Jl. Prof. Soedarto, SH Kampus Undip Tembalang Semarang, 50275

**Abstract.** Physiological aging and aging due to oxidative stress are a major factor cause accelerated brain aging. Aging is characterized by a decrease of brain function of the hippocampus which is linked to the decline in the capability of learning-memory and motoric activity. The objective of this research is to obtain the important information about the mechanisms of brain antiaging associated with the improvement of hippocampus function, which includes aspects of learning-memory capability and motoric activity as well as mitochondrial ultrastructure profile of hippocampus cornu ammonis cells after treated by fish snakehead fish extract. Snakehead fish in Rawa Pening Semarang District allegedly holds the potential of endemic, which contains bioactive antiaging material that can prevent aging or improve the function of the hippocampus. This research has been conducted using a completely randomized design consisting of four treatments with five replications. The treatments were including rats with physiological aging or aging due to oxidative stress which was treated and without treated with meat extract of snakehead fish. The research was divided into two stages, i.e., determining of learning-memory capability, and determining motoric activity. The measured-parameters are time response to find feed, distance travel, time stereotypes, ambulatory time, and resting time. The result showed that the snakehead fish meat extract might improve function hippocampus, both in physiological aging or aging due to oxidative stress. The capability of learning and memory showed that the rats in both conditions of aging after getting treatment of meat extract of snakehead fish could get a feed in the fourth arm maze faster than rats untreated snakehead fish meat extract. Similarly, the measurement of the distance traveled, time stereotypes, ambulatory time, and resting time showed that rats which received treatment of meat extract of snakehead fish were better than the untreated rats. To conclude, the meat extract of snakehead fish can be used as antiaging material to improve the function of the hippocampus, to improve the capability of learning and memory, to improve motoric activity, and to prevent aging. These findings are expected to provide comprehensive information for the development of antiaging research as an effort to improve public health and to improve learning-memory capability and motoric activity.

**Keywords:** Channia striata, antiaging, hippocampus, mitochondria, the capability to learn and remember, motoric activity
1. Introduction
The aging hippocampus is a type of aging that occurs due to increasing age or due to the induction of oxidative stress. Symptoms of the type of aging of the brain are characterized by a decreased ability to learn and remember, changes in motoric activity, and mitochondrial inefficiency [10]. The hippocampus is the medial-lateral region of the brain located next to the ventral cerebrum. This brain region contains many horn-shaped cells (cornu ammonis) which have an important role in the process of learning and memory and regulation of emotional balance. Many indicators are found along with the decline in the function of the hippocampus, one of which is the depletion of glutathione levels below the normal threshold. Depletion of the hippocampal glutathione level may lead to histomorphological disorders and changes in the mitochondrial structure of neurons. The disorder is characterized by high neuronal death and axon structure disorder in neurons. Both types of disorders correlate with changes in the structure of mitochondria, especially pyramidal neurons in the hippocampal cornuccas [15]. It was reported that some of these disorders could lead to decreased learning-memory and motoric behavior.

The aging of the brain characterized by the decline in hippocampus function has become a health problem for society these days. In addition to lowering performance, decreased function of the hippocampus can decrease the function of the body's defense system, causing disturbance of the balance of coordination and regulatory system, and increase the vulnerability of other body systems to the environment. Decreased hippocampus function is always present in humans and animals as chronological age increases or because of oxidative stress, but the rate of decline can be slowed. One way of decreasing the function of the hippocampus is to use exogenous antiaging agents that can induce the synthesis of glutathione in the body. Glutathione is an endogenous metabolite that has both antioxidative and antiaging capabilities. Based on the criteria and potential of the material, this study used the ingredients of the meat of snakehead fish (Channia striata) derived from Rawa Pening of Semarang Regency.

Snakehead fish from Rawa Pening as an endemic fish also has potency as antiaging which have not been studied. As a food source of animal protein, the meat of snakehead fish contains many glutamines and other amino acids that are thought to be glutathione antioxidant precursors. Application of snakehead fish extract in the diet can significantly increase blood glutamine levels, improve liver function, and speed up the wound healing process after surgery. Glutamine, cysteine, and glycine are related to glutathione synthesis. Several studies have reported that glutamine, cysteine, and glycine serve as glutathione precursors in the body ([3]; [4]; [15]). Glutamine can be converted to glutamic acid and together with cysteine and glycine are used for the synthesis of glutathione in the hippocampus [13]. Glutamine, cysteine and glycine are stable during the process in the body, faster in hydrolysis process, able to cross the blood-brain barrier, can be utilized by neurons directly, and can increase glutathione levels in the hippocampus.

The content of glutathione in the hippocampus is an important indicator of improvement of hippocampus function, either on physiological/chronological aging or aging that is triggered by oxidative stress. Therefore, this study was conducted to obtain data on mitochondrial ultrastructure images, the ability to learn and remember rats in both aging conditions, and improvement of motoric activity. Thus, we hope that this research will be able to explain comprehensively about the mechanism of the active ingredients contained in the meat extract of snakehead fish and its mechanism in enhancing the antioxidant glutathione in the body that serves to repair the body tissues, especially the declining hippocampus or aging.

The objective of this study is to analyze the improvement mechanism of learning-memory or motoric activity. Both parameters are important indicators to determine the improvement of hippocampus function in aging conditions after treatment using meat extract of snakehead fish.

2. Materials and Methods
This research has been conducted using a completely randomized design consisting of four treatments with five replications. The treatments were rats with physiological aging or aging due to oxidative
stress-treated meat extract snakehead fish, and rats with physiological aging or aging due to oxidative stress without the treated extract snakehead fish meat.

Snakehead fish used for this study has reached sexually mature and healthy. Snakehead fish was taken from Rawa Pening, Semarang. The extract of snakehead fish was prepared from the meat of snakehead fish. The meat was cleaned and cut into pieces with a thickness of 1 cm. Pieces of meat then inserted into the extractor. Extraction was carried out at 70°C until the extract produced a clear drop. The extract was pasteurized, then stored in the refrigerator, and ready to be used as a treatment.

Four months Male Wistar rats were used as experimental animals. The acclimation of the strain of Sprague Dawley male rats was performed for one week. During acclimation, rats fed with commercial pellets and water ad libitum. Chronological aging of animal models was prepared by treating the rats without oxidative stress. Animal models of aging which induced by oxidative stress was prepared by not feeding the rats for seven days. The rats were given only drinking water ad libitum. Every day the rats were treated swimming in water in a closed bucket for 15 minutes.

The procedure of learning-memory test of rats using the fourth arm maze refers to the previous study by Sunarno et al. [15]. The parameters measured were the time required by the rats to find the feed in the fourth arm maze space. The speed of rats in finding their feed showed their learning-memory skills. This ability correlates with improved hippocampus function.

Clear drops of extracted snakehead fish meat administered orally every day for two weeks 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) was prepared for treatment and stored at 4°C in the refrigerator.

All motoric activity of rats fed with snakehead fish extract was monitored using Optovarimex which was equipped with AutoTrack software, i.e., travel distance, time stereotypes, ambulatory time, and resting time. The procedure used refers to a study conducted at [19], [2], and [14]. Improvements in motoric activity correlate with improved hippocampal function.

Parameters related to learning-memory capability and motoric activity in animal testing were analyzed by ANOVA followed by Duncan's test on 5% significance. Improved parameters associated with the test using the fourth arm maze and motoric activity after the treatment of snakehead fish extract showed improvement in hippocampal function.

3. Results
The treatment of snakehead fish meat extract can improve the ability of learning and remembering and motoric activity in rats, as shown in Table 1 and Table 2.

Table 1. The response time of rats, that experienced physiological aging and aging due to oxidative stress, in finding the food on the fourth arm maze based with or without giving the meat extract of snakehead fish.

| Treatments | Response time to find the feed (seconds) |
|------------|-----------------------------------------|
| Physiological aging that has been treated meat extract of snakehead fish | 55±1.09 |
| Aging due to oxidative stress that has been treated by meat extract of snakehead fish | 88±2.30 |
| Physiological aging of untreated meat extract of snakehead fish | 83±1.80 |
| Aging due to oxidative stress untreated meat extract of snakehead fish | 122±3.6 |

Note: Data shown is the means ± standard deviation. The different superscript letter means it is statistically significantly different (P < 0.05).
Table 2. Responses of motoric activity in rats with physiological aging and aging due to oxidative stress with or without treatment of meat extract of snakehead fish

| Treatments                                         | Distance travel (cm) | Time stereotypes (seconds) | Ambulatory time (seconds) | Resting time (seconds) |
|----------------------------------------------------|----------------------|----------------------------|---------------------------|------------------------|
| Physiological aging treated with meat extract of snakehead fish | 25±1.08              | 27±2.30                    | 9±0.5                     | 546±14.20              |
| Aging due to oxidative stress treated with meat extract of snakehead fish | 145±4.60             | 45±3.40                    | 55±2.30                   | 486±6.70               |
| Untreated physiological aging                      | 136±5.60             | 38±3.00                    | 125±6.60                  | 478±7.80               |
| Untreated aging due to oxidative stress            | 225±7.70             | 78±4.00                    | 155±7.60                  | 226±8.40               |

Note: Data shown is the means ± standard deviation. The different superscript letter means it is statistically significantly different (P < 0.05).

4. Discussion

Hippocampus consists of neurons that play a role in tasks related to cognitive functions, including learning and memory, navigation, and motoric activity. Physiological aging and aging due to oxidative stress caused a decrease in the function of the hippocampus.

Table 1 indicates that physiological aging and oxidative stress have a significant effect on the decrease in learning-memory ability. Decreased learning-memory ability was seen significantly in rats aging due to oxidative stress without treatment of snakehead fish extract. This pattern can be seen from the time of the mouse to find the feed in the fourth arm maze space which shows the slowest time, which is 122 seconds. This response is slower than rats that have physiological aging without treatment of snakehead fish extract, and rats’ aging due to oxidative stress with meat extract of snakehead fish, 83 and 88 seconds respectively.

The rats with physiological aging treated with snakehead fish extract had the fastest response in finding the feed (55 seconds). This result is significantly different from the other treatments. This response means rats with oxidative stress or rats with physiological aging without the treatment of snakehead fish extract take the longer time to find the feed in the fourth arm maze. This suggests that the treatment of snakehead fish extract can improve the ability of learning and memory in rats that experience oxidative stress in better condition (normal). This treatment can also improve the ability of learning and memory is much better in rats with the condition of physiological aging.

Table 2 shows that rats with aging physiology treated with meat extract of snakehead fish had a better motoric activity response than rats treated using oxidative stress with or without the extract of snakehead fish meat. This result is demonstrated by shorter distance responses, shorter stereotypes, and shorter ambulances, and longer rest periods than other treatments, 25 cm, 27 seconds, 9 seconds, and 546 seconds, respectively. As for rats with oxidative stress with or without the meat extract of snakehead fish and rats with physiological aging without snakehead fish extract has longer travel distance, longer stereotype time, and longer ambulatory, and shorter rest periods. The response of motoric activity, which includes mileage, stereotypes, and rest periods between rats with physiological aging without snakehead fish extract differed from rats aging due to oxidative stress with snakehead meat, while ambulatory time was significantly different. In general, this evidence suggests that the treatment of snakehead fish extract may improve motoric activity in rats undergoing oxidative stress to better or normal conditions and can even increase motoric activity in rats with physiological aging conditions.
The results of this study describe that aging due to oxidative stress can have a serious impact on the decreased ability to learn-memory and motoric activity. Decreased cognitive function and motoric activity have a very close association with decreased function of neuronal cells in the hippocampus ammonium section. Decreased function of the hippocampus ammonium cornucae cells affects the disturbance of cellular substrates and neurophysiological changes, such as synaptic plasticity, synaptic linkage, electrophysiological changes, as well as changes in the proportion of neurochemistry in the hippocampus. Cellular substrate disturbances can disrupt metabolic processes, neuron integrity, and decreased neuron function, whereas neurophysiological changes can interfere with signals related to motoric performance, navigation, object recognition, and information processing [5]. The results showed that rats with oxidative stress conditions in the fourth arm maze and optovarimex examination chamber looked aggressive, anxious, uneasy, anxious, and irregular movements. In contrast, the treatment of snakehead fish extract, whether under physiological or aging conditions due to oxidative stress can improve both learning-memory and motoric activity. This improvement demonstrated the ability to find faster the feed, shorter distance, shorter stereotypes and ambulatory times, and longer rest periods.

Moyer and Brown [8] suggested that physiological aging and oxidative stress could lead to a decrease in neurophysiological conditions that result in decreased hippocampus function, especially neuronal cells in the cornu ammonis. Decreased function in this section may interfere with various tasks related to the function of the hippocampus, such as navigation in arm radial space maze ([17]; [21]). It was further reported that physiological aging and oxidative stress could lead to behavioral aberrations and decreased synaptic plasticity. Decreased synaptic plasticity begins with hyperpolarization and increased activity of calcium ion-dependent neurons [18]. The decrease in synaptic plasticity leads to a decrease in synaptic strength. This trend is resulting in disruption of processes associated with motoric performance, decreased the ability to respond to changes in the microenvironment in the hippocampus, and disruption of information storage processes ([17]; [20]; [1]; [10], [11]). Shukitt [12] suggested that the presence of synapses in the hippocampus determined both good and bad learning-memory and motoric activity.

5. Conclusions and Recommendation
This study proves that snakehead fish extract (Channa striata) could improve the ability of learning-memory and motoric activity of rats, either in rats with chronological aging or with aging due to oxidative stress. Hence, the use of meat of snakehead fish is highly recommended and important as consumption, especially to improve the function of the hippocampus of the brain, improvement of learning-memory capability and motoric activity, and prevention of aging.

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