

The Effect of Mackerel Meat on the Number of Purkinje Cell in the Cerebellum of Congenital Hypothyroid Rat (*Rattus norvegicus*)

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Abstract- Early detection through screening of congenital hypothyroid is not a regular program of the government. so that cases of congenital hypothyroid can not be managed appropriately. Therefore, it needs to be done the research about the benefits of the granting of a bloated fish containing omega-3 on congenital hypothyroid as a food supplement to stimulate nerve growth especially brain development. The sample of this research is the droppings of a white rat (*Rattus norvegicus*). The 30 rats are divided into 6 groups consisting of 5 rats. Four groups was induced with hypothyroid on the day 5 of gestation until neonatus of the day 15, ..and two other groups stayed normally. Thyroxine and mackerel meat were given on the 21st day after the birth until the 8th week. Histology and preparations werw made of cells Purkinje after being observed in the cerebellum. The number of Purkinje cells was analized with One-way Anova continued with multiple comparison test. The result is that the number of Purkinje cells in the normal group, normal + mackerel, hypothyroid, hypothyroid + mackerel, hypothyroid treatment with thyroxine, and thyroxine treatment with hypothyroid + mackerel are as follows: 60±12, 71±16, 40±6, 64±7, 70±5, and 65±20. The average number of Purkinje cells in the group that got mackerel significantly ($p < 0,05$) increases compared to hypothyroid. The conclusion is that the supplementation of mackerel fish increases the number of Purkinje cells in the cerebellum of the rat with congenital hypothyroid.

Keywords: *congenital hypothyroid, mackerel fish, omega-3, purkinje cells.*

I. INTRODUCTION

Hypothyroidism is a condition caused by a lack of production of thyroid hormone or by abnormal thyroid hormone receptor activity occurring before or at birth¹. The most common cause of hypothyroidism worldwide is the lack of iodine. Hypothyroidism is considered to be one of the most

widespread causes that impede the development of the central nervous system. It was estimated that 26 million people suffered from brain damaged among the congenital hypothyroid patients². However, Indonesia does not have national data. In RSUP dr. Cipto Mangunkusumo Jakarta and RS Hasan Sadikin Bandung in 2000 to 2014, there were 85 of 213,669 infants who had hypothyroidism or 1: 2,513 births of this data that exceeded the global ratio of 1: 3000 births³.

The depriving of thyroxine hormones in fetui ... proven in animal experiments causes brain damage and decreases cognitive abilities⁴. Brain damage is mainly found in the prefrontal, hippocampus and cerebellar cortex⁵. This is caused by cell propagation disorders (proliferation), cell migration, cell differentiation, and decreases in synapses formation, myelin formation, neuron growth, neurotransmitter formation, and increased cell death and astrocytic proliferation, and consequently the connectivity between neurons perfect⁶. Connectivity between parts of the brain is an essential requirement for cognitive function⁶. In addition, hypothyroidism can also cause mental retardation, and decreases speech and hearing ability and motor impairment. The brain development disorder experienced is irreversible⁷.

Thyroid hormone deficiency in the developmental period of the brain can lead to motor dysfunction and impaired coordination of movements that disrupt activity⁸. Various abnormalities resulting from this thyroid hormone deficiency have been studied in the cerebellum⁹.

Iodine deficiency and PTU treatment during pregnancy and lactation reduce Purkinje cells¹⁰. Early detection through congenital hypothyroid congenital (SHK) screening has not been a routine in the government program so that the congenital hypothyroid case has not been adequately managed.

Given with the majority of hypothyroid events in Indonesia due to iodine deficiency, the administration of foods containing lots of iodine and nerve growth stimulant substances should be encouraged. The iodine content in mackerel is about 91,005 microgram / 100 gr¹¹. If a person consumes 100 grams of bloated fish in one day, he can meet his iodine need of 50

micrograms per day.¹². According to Institute of Nutrition Department of Health RI, several types of Indonesian marine fish have high content/ level of omega-3 fatty acids (there are up to 10.9 g / 100 g) such as lemuru fish, eel, terubuk, mackerel, bloated, kite, pomfret, seren, slengseng, and tun¹³. Therefore, research on the benefits of giving bloated fish containing lots of iodine and omega-3 for the improvement of brain structure needs to be done.

II. MATERIAL AND METHOD

This research is an experimental research, with design of posttest only for control group. Posttest is used to analyze changes in the number of Purkinje in the cerebellum layers of mice in various treatment groups. White rats (*Rattus norvegicus*) with strain of Sprague Dawley, specifically rat cubs, required were as many as 30 rats divided into 6 groups containing 5 rats in each group.

White rats (*Rattus norvegicus*) with strain of Sprague Dawley, specifically rat cubs, required were as many as 30 rats divided into 6 groups containing 5 rats in each group. The adult/ parent rats required for pregnancy were 12, divided into 6 groups consisting of 2 rats for each group. There were four (4) hypothyroid-induced groups with propylthiouracil (PTU), and 2 other groups were normal. Rat cubs were grouped according to the treatment as follows:

KI: Normal without treatment. KII: Normal + Bloated fish. KIII: Hypothyroid without treatment, KIV: Hypothyroid + Bloated fish, KV: Hypothyroid with thyroxine treatment. KVI: Hypothyroid with thyroxine treatment + mackerel.

Randomization was done on the parent and the cubs in one parent. It was because the rats continued to suckle for about 20 days. Each treatment group used 5 rats. This figure was obtained from the calculation of the number of samples according to Federer (1963).

This research was carried out at UPHP UMY for maintenance, parent treatment, thyroxine replacement hormone treatment, treatment of baby rat in the form of fish bloated meat supplements, and surgical removal of rat brain organ. The preparation of rat brain preparation was done at Anatomical Pathology Laboratory of Faculty of Medicine UGM. The shooting of preparations was done in the Histology Laboratory of Faculty of Medicine UMY.

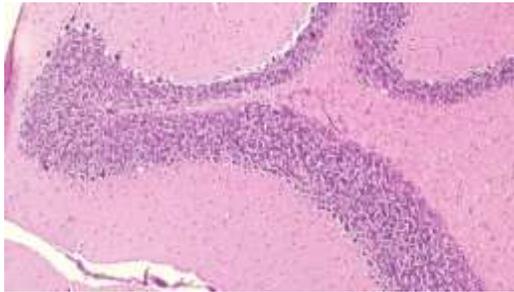
Variables in this study included:

Independent variables were the group of rats based on treatment, namely congenital hypothyroid induction, thyroid hormone replacement therapy, and therapy of bloating 2. The dependent variable was the average number of Purkinje cells with viewing field on the cerebellar ganglionare layer.3. Controlled variables were the feed and cage conditions which were the same in each group. Materials and Instruments of the study included 12 white Sprague Dawley rat babies, mouse cage, rat feed, bloated fish supplement, Propiltiourasil (PTU), thyroxine, used mineral water bottle with lid and hose, sufficient water, dope (chloroform), jars, rat surgery equipment, pots for tissue storage, diluted alcohol, glass preparations, microscopes and cameras, calculators, computers and others. The research

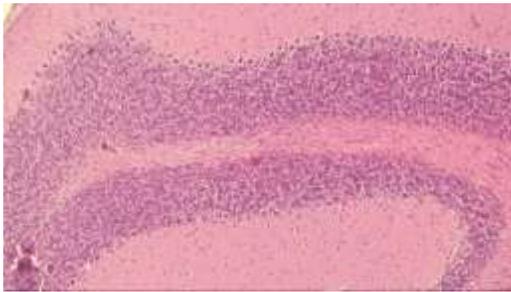
procedure was as follows: 1.) Procurement of rats. Rats were grouped into 6 groups with 5 rats per group. 2.) Rats were adapted for 3-6 days. 3.) Rats mated to males and pregnancy detection with vaginal swabs. If there is a rat sperm cell, it is recorded as the first day of pregnancy. 4.) White rat was induced PTU 0.015%. How to induce PTU: induction of PTU was starting from pregnancy day 5 post- natal day 15 with the dose as follows: dose 15 ppm mixed with boiled water and divided according to the needs of drinking water dam per day. 5.) Measurement of serum with FT4 levels T4 levels at the age of 3-week-old rats with Elisa method. 6.) The white rat was given with thyroxine. This is how to administer thyroxine: administration of thyroxine in rat mother starting from 21st day post- natal to 2 months with the following dose: dose of 1.8 mg / 200 g BW / day. In this study, the average of initial weight of rats when treated was 50 g, so the dose of thyroxine given was 0.45-0.5 mg / day, and dosing will be adjusted to the body weight of the rats. Thyroxine was dissolved in aquadest (according to the amount of water consumed by rats per day). 7.) Rats were fed with bloated fish. The method of administration of bloated fish supplements was as follows: Provision of mackerel in rat cubs from weaning until the end of the study. Fish supplements were given 20% of the feed (Khomsan A, 2004). The prosedure of processing bloated fish as a mouse food was as follows: a.) Steamed the bloated fish b.) Released his flesh from bone. c.) Roasted to dry d.) Mixed and placed at the top of rat food with a percentage of 20% of rat food e.) Given by starting from day 21 to end of study or week 8. 8.) Performed rat surgery at the end of the study. Surgery began with euthanasia using chloroform anesthesia to kill the rat. Then, his brain organ was taken using tweezers and surgical scissors. The brain was placed in a pot that has been filled with 10% formalin before it was sent to UGM Anatomy Pathology Laboratory. 9.) Rat brain organ was made for histology preparations. For preparing the histopathologic preparations, the main ingredient was in the form of brain tissue, which is fixed in 10% formalin (BNF) solution. The tissue was cut and arranged in a tissue cassette dehydrated automatically by dehydration machine, dried by vacuum machine, and blocked with paraffin liquid, and and then the block is cut 3-5 μ m by microtome machine and the piece was attached to the object glass. After that, the object glass was colored manually with eosin hematoxylin. The coloration will provide a clear blue and red color balance to the tissue so that the cell components can be clearly identified. 10.) Histologic preparations were then observed and photographed using a microscope and digital camera with microscopy software with ocular lens of 10 and an objective of 4 to obtain a total magnification of 40x in the 52x39 = 2,067 micrometer viewing field. The assessment was carried out on the number of Purkinje cells that can be calculated on the ganglionare layer of the animal's caulex of the test animal with a cell uni per view. Analyzed the resulting data in the resulting average value. The normal distribution of the data used One Way Anova statistical analysis continued with multiple comparison test.

III. RESULTS AND DISCUSSION

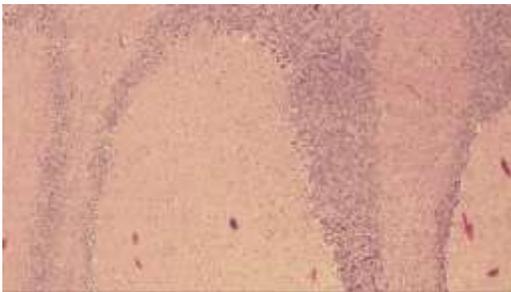
This study aims to determine the effect of giving bloated fish supplements to the number of Purkinje cells in the cerebellum ganglionare layer of congenital hypothyroid rat. The observations made were the counting of Purkinje cells in the cerebellar ganglionare layer. Referring to the difference in all treatment groups to ascertain and to demonstrate that the droppings of white rat actually succeeded in becoming hypothyroid rat from the PTU-induced parent, the rat was drawn at 3 weeks of age to retrieve the FT4 data. It means that FT4 Levels of Mouse and Rat Tumus The 3-week age can be seen in Table 1 and in Pic. 1 – Pic. 6.



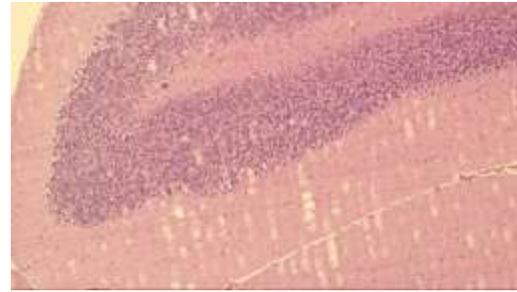
Pic.1= KI
(Normal group without treatment).



Pic2 = KII
(Normal Group + Bloated fish.)



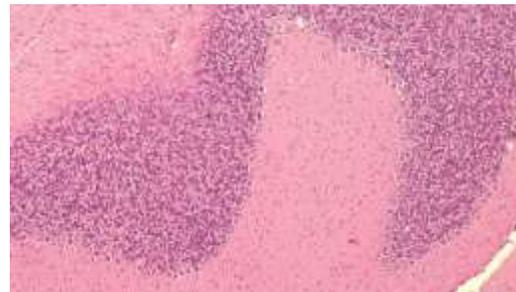
Pic 3 = KIII
(Hypothyroid group without treatment.)



Pic. 4 = KIV
(Groups of hypothyroidism + mackerel.)



Pic. 5 = KV
(The hypothyroid group with thyroxine treatment).



Pic. 6 = K VI
(Hypothyroid group with thyroxine treatment + mackerel)

Before continuing the different statistical test, normality test was done by using descriptive method and Shapiro- Wilk Test analytical method because the sample number was 30. The result of all data test showed that $p = 0.704$ ($p > 0.05$), which means the distribution of normal Purkinje cell number, and it could be continued using the One Way Anova test. One Way Anova test results showed

that there was a significant difference in average number of Purkinje cells among the six groups, with $p = 0.001$ ($p < 0.05$). After it was found that there were significant differences between the six groups, LSD (Least Significant Difference) was tested in post hoc test to know which group was different. LSD test results showed significant differences in K I with K III, K II with K III, K III with K IV, K III with K V and K III with K VI. A non-significant difference was seen in the average number of

Purkinje cells among treatments. Significant values among treatments can be seen in Table 1 and 2.

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Table 1. Significant values between treatments The mean difference is significant at the 0.05 level

Groups	K I	K II	K III	K IV	K V	K VI
K I		0.531	0.048*	0.989	0.644	0.970
K II	0.531		0.001*	0.874	1.000	0.928
K III	0.048*	0.001*		0.012*	0.001*	0.008*
K IV	0.989	0.874	0.012*		0.937	1.000
K V	0.644	1.000	0.001*	0.937		0.970
K VI	0.970	0.928	0.008*	1.000	0.970	

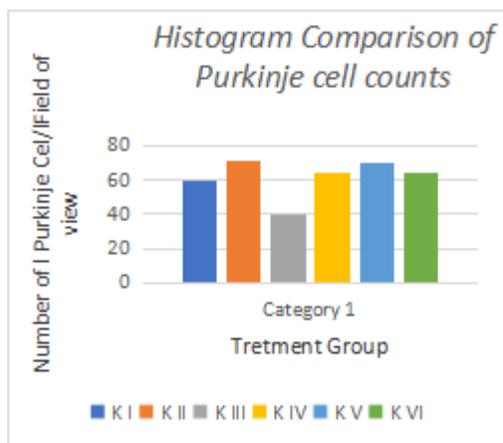
Discription :

K I : Normal group without treatment.
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 K IV : Groups of hypothyroidism + mackerel.
 K V : The hypothyroid group with thyroxine treatment.
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Table 2. Significant values between treatments

Group	Average± SD
1 Normal without treatment	60 ± 8
2 Normal Group + Bloated fish.	71 ± 11
3 Hypothyroid group without treatment.	40 ± 4
4 hypothyroidism + mackerel.	64 ± 5
5 The hypothyroid group with thyroxine treatment	NNo
6 Hypothyroid group with thyroxine treatment + mackerel.	65 ± 12

Referring to other groups (controls), since this group did not get any treatment, there is nothing that can affect the amount of Purkinje cells in the ganglionare layer of the cerebellum cortex. The normal group (control) has a significant difference compared to the hypothyroid group. Thyroid hormones are needed to develop the central nervous system and regulate neuronal differentiation and migration, synaptogenesis, and myelination. This thyroid hormone deficiency will reduce motor skills through abnormal growth and poor cell differentiation of the cerebellum¹⁴. This hypothyroidism will cause irreversible brain damage. The hypothyroid brain exhibits a compacted intracellular space and a decrease in brain weight. The compaction of intracellular space is characterized by a decrease in axonal growth and dendritic arbor. In the cerebellum, the development of axodendritic connections between Purkinje cells and granular neurons is regulated by TH. Hypothyroidism will result in abnormal Purkinje cell development¹⁵. Thyroid hormone deficiency in the cerebellum will delay the proliferation and migration of external granule lining cells to the internal granular layer. In addition, thyroid hormone deficiency reduces axonal growth and dendritic arborization in the cerebral cortex, vision and auditory cortex, hippocampus, and cerebellum. Brain-derived neurotrophic factor (BDNF) is a neurotrophin seen in cerebellar development. Thyroid hormone will regulate the increase of BDNF in postnatal cerebellum of rat¹⁷.



Histogram Comparison of Purkinje Cell Counts

Discription :

- K I : Normal group without treatment.
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Koibuchi et al. report that Hormone Thyroid regulates BDNF expression according to the promoter in different ways at the stage of brain development 9. In the cerebellum, BDNF expression of mRNA 1 has been suppressed by hormone thyroid on postnatal 2, whereas mRNAs II, III and IV are regulated by the thyroid hormone at postnatal 15 and 30. The functional effect of thyroid hormone may activate BDNF during the development of the brain which has been tested in-vivo. BDNF can be seen and shows delayed migration of granular cells and deficiency of Purkinje dendritic arborization of the cerebellum of hypothyroid rats. Other studies used the BDNF overexpressing cell line and these cells were implanted into the cerebellum of the rat at postnatal 3. Such planting resulted in excessive expression of BDNF in the cerebellum of the hypothyroid that saves internal granular cells from cell death due to hypothyroidism¹⁷. The thyroxine and hypothyroid thyroxine groups of bloated fish have significant differences. The substitute of thyroxine (T4) hormone will be de-iodinated to 3,3', 5-triiodo-L-thyronine (T3) performed by type 2 deiodinase (D2) 7. T3 has a role as Purkinje cell dendritogenesis in the cerebellum. T3 has a target gene that has a genetic program for cell migration, cell proliferation and cell maturation. Granular cell precursors GCPs require T3-regulated neurotrophic factors (neurotrophin 3, sonic hedgehogs, and brain-derived neurotrophic factors) that serve to migrate and proliferate these cells into Purkinje cells. The normal group of mackerel and hypothyroid bloated fish also has a significant difference when compared to the hypothyroid group. This is because the bloated fish has a high content of omega 3, which is 2.6 grams per 100 grams of bloated fish. The content of this bloated fish is higher than that of tuna, shrimp,

and salmon. Omega-3 fatty acids are essential for brain growth, especially in anatomy, histology, and brain biochemical aspect²¹. The endogenous ω 3-PUFA effects of BDNF are associated with autophagy that has recently been linked to brain-derived neurotrophic factor (BDNF) which is one of the neurotrophic mammals that has been shown to be a useful growth factor for nerve function. BDNF gives effect by binding to the receptor tyrosine kinase B (TrkB) and p75. While BDNF levels are found to be higher in pureblue cerebellum cells, then BDNF will monitor the regulation of TrkB signals using Phospho-akt. Thr308 phosphorylation of Akt increases in rat fat compared to control group. Activation of CREB in Neurons may stimulate expression of neuroprotective molecules, such as anti-apoptotic proteins and Bcl-2, which have benefits in cell survival after ischemic or neurotoxic substances. Ser133 phosphorylation of CREB increases in rat cerebellum compared to controls. These data support that giving ω 3 can stimulate BDNF activity by activating Akt and CREB by increasing Thr308 phosphorylation of Akt and Thr 133 phosphorylation from CREB²⁸. Normal groups have no significant differences compared to normal bloated fish, hypothyroid bloated fish, hypothyroid thyroxine and hypothyroid thyroxine bloated fish. Normal group of mackerel has no significant difference compared to hypothyroidism of bloated fish, hypothyroid thyroxine and hypothyroid thyroxine bloated fish. The hypothyroid group of mackerel has no significant difference compared to hypothyroid thyroxine and hypothyroid thyroxine. The thyroxine hypothyroid group has no significant difference compared to the mackerel fish thyroxine hypothyroid.

Someone needs to add supplements that can increase the potential of intelligence and can prevent brain damage. Bloated fish has a high omega-3 content that can be used by everyone for supplements. This supplement can be used by normal people and those who suffer from hypothyroidism. In normal people, this supplement will increase the potential of intelligence and maintain the health of brain cells. In hypothyroid patients, this supplement will improve brain cells, so it can minimize the negative impact caused by hypothyroidism. This study is in accordance with previous literature review and research, where high levels of iodized and omega-3 bloated can improve the number of Purkinje cells in the congenital hypothyroid rat.

IV. CONCLUSION

Provision of mackerel meat supplements (*Restrellinger sp.*) may increase the number of Purkinje cells in the cerebellar ganglionare layer of the congenital hypothyroid rat (*Rattus norvegicus*).

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