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The Effect of Mackerel Oil During Pregnancy on Apoptotic Index in the Cerebrum of Newborn *Rattus Norvegicus*

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ABSTRACT
The advancement of a country is determined by the quality of the next generation. Pregnancy must be prepared with adequate nutrition for optimal fetal brain development. Malnutrition during pregnancy affects the development of fetal brain cells. Nutrients needed by the brain, such as omega-3, can be obtained from high levels of mackerel. This study aims to analyze the effect of mackerel oil during pregnancy on the apoptotic index in the cerebrum of newborn *Rattus norvegicus*. This study is a true experiment with a posttest-only control group design. 30 pregnant *Rattus norvegicus* aged 2-3 months were randomly divided into 3 groups, namely the control group (K), the mackerel oil treatment group (P1), and the omega-3 supplements group (P2). The experimental animals were acclimatized and superovulated. The treatment was given at day 1-17 of pregnancy. 3 newborn *Rattus norvegicus* with the criteria of the largest, medium, and lightest body weight were taken from each parent to be made preparations on the 18th day. Examination of preparations carried out by immunohistochemistry. The mean ± SD of the apoptosis index in the cerebrum were 4.93 ± 0.87 (K), 2.53 ± 0.45 (P1), and 3.72 ± 0.54 (P2). The results of statistical tests showed that there was a significant effect on the apoptotic index in the cerebrum of newborn *Rattus norvegicus* between groups with p-value <0.05. Administration of mackerel oil to the mother *Rattus norvegicus* during pregnancy resulted in a lower apoptotic index in the cerebrum of newborn *Rattus norvegicus* than other groups.

Keywords: Mackerel Oil, Apoptotic Index, *Rattus Norvegicus*

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BACKGROUND

Smart children are the dream of every parent and even every country has a goal to prepare a smarter future generation. A healthy and smart nation will support a developed country (Joewono, 2013). The smart generation must be supported by adequate nutritional needs (Marangoni et al., 2016). Malnutrition during pregnancy, especially during the early period of life, affects the development of fetal brain cells (Cusick & Georgieff, 2016). The brain is a part of a complex organ that has many functions. Omega-3 unsaturated fatty acids play an important role in the morphological, biochemical, and molecular development of brain organs. Lack of omega-3 fatty acids caused by insufficient intake or due to diseases that reduce absorption, inhibit brain development, physical health, and environmental interactions have a strong effect on the formation of cognitive development (Nurasmi et al, 2018).

Lack of omega-3 fatty acids causes nervous disorders and can interfere with the development of the nervous system. As a result, there may be disturbances in the memory system (Diana, 2012). Sphingomyelin is formed by omega-3, EPA, and DHA which are used to form brain cells and nerve cells myelin. If the EPA and DHA in the fetal brain are not sufficient, the signal transmitted from the brain to the axons and myelin will be disrupted, the acceleration of the signal transmitted by the brain will be disrupted (Lewkowicz et al., 2019). Damage to the number of neurons can occur in the prenatal period. This is thought to be caused by programmed cell death known as apoptosis. Only half of the neuron cells will survive. In the organizational phase of brain development, nerve cells form a connection between cells which is known as the synaptogenesis process. The apoptosis process occurs a lot in this phase. The more neuron cells on the dendrite site, the more synapses will be formed, so that the number of cells undergoing apoptosis will also decrease (Hapsari et al., 2018).

The messages conveyed by the brain will be transmitted by neurotransmitters under the orders of the brain so that the development of the resulting body motor movements becomes fast and well developed. DHA and EPA supplies are needed, especially during pregnancy, especially in the third trimester, post-birth, and early childhood (Zhang et al., 2018). The brain has a big enough role in the development process of a person. Besides, the level of individual intelligence is formed in the cerebrum. DHA and EPA at the time of fetal development must be fulfilled. DHA and EPA will provide neurotransmitter effects. Changes in the content of DHA and EPA in the nerve membrane cause functional changes in the activity of receptors and other proteins that may be associated with the synaptic function (Tanaka et al., 2012).

Omega 3 is the highest found in fish. There is a lot of fish that contain quite high omega-3 and easy to find, one of which is mackerel. Darmawati et al. (2018) showed that mice given mackerel fish showed a significant increase in the number of pyramid cells in the cerebral cortex compared to the group that was not given mackerel meat. The types of omega-3 fatty acids identified from the fatty acid esters of mackerel are linolenic acid, EPA, and DHA (Zivkovic et al., 2011). Consideration of choosing mackerel in this research based on DHA & EPA content, the number of fish which is quite abundant in Indonesian waters, is economically easy to obtain, relatively cheap price and affordable for the community. It is hoped that mackerel can be a source of meeting the DHA and EPA requirements for fetal brain growth during pregnancy.

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METHODS

Experimental animal

The total samples were 30 rats which were normal, have never been pregnant, have never been used as an animal in other research trials, and healthy condition characterized by active movement, fine hair, and good appetite. The initial weight was120-130 grams. Mother *Rattus norvegicu* was pregnant on day 1-17. Performed superovulation by injecting 10 IU of PMSG hormone. After 48 hours, 10 IU of hCG was injected intraperitoneally. 18 hours later after being married, the diagnosis was carried out. The diagnosis is determined by checking whether there is a copulatory plug.

This research was conducted at the Experimental Animal Cages and Pathology Laboratory, Faculty of Veterinary Medicine, Airlangga University. The experimental animal was divided into 3 groups. Group I was given standard food and drink ad-libitum as the negative control group, group II was given standard food and drink ad-libitum added with mackerel oil at a dose of 3.24 mg / 120 g BW / day, and group III was given food and drink standard ad libitum added with omega-3 supplements at a dose of 3.24 mg / 120 mg BW / day as a positive control group.

Mackrel oil

The examination of DHA and EPA content in mackerel oil from the Madura ocean was carried out at the Testing Service Unit of the Faculty of Pharmacy, Airlangga University, Surabaya using the Gas Chromatography-Mass Spectrometry (GC-MS) method. The results of the examination were obtained from 1 gram per 100 grams of mackerel containing 5.42% DHA and 6.62% EPA and other essential fatty acid compounds.

Apoptotic index examination

On the 18th day, a cesarean section was carried out. Newborn *Rattus norvegicus* were taken for brain tissue to be examined by immunohistochemistry l methods. The procedure for checking the apoptotic index was carried out in each group. This histopathological examination is intended to determine the expression of apoptosis in the brain. The data for each sample is the average observed Immuno Reactive Score (IRS) value at the different ten fields of view at 100x and 400x magnification. Apoptotic expression in the cerebrum is characterized by a chromogen brown color.

Statistical analysis

Statistical analysis using SPSS software tools, with a significance level of 0.05 (95% confident interval). P-values < 0.05 were considered significant. Normality test using the Shapiro-Wilk test. Data analyzed using the Kruskal-Wallis test followed by the Mann Whitney test. The comparison test analysis used an independent sample t-test for data that was proven to be normally distributed and the Mann-Whitney test was used for data that were not proven to be normally distributed.

RESULTS

Figure 1 shows the mean apoptosis index of neuron cells in the cerebrum was highest in the control group (4.93±0.87) while the lowest average was in the group of mackerel oil (2.53±0.45). Figure 2 shows the comparison of fetal brain apoptosis expression in groups (K, P.1, P.2). The red arrow indicates the expression of apoptosis in the cerebrum which is indicated by the presence of a chromogen brown color. Normality test using the Shapiro-Wilk test. All data were normally distributed except in the group of mackerel fish oil (p-value= 0.040). Then analyzed using the Kruskal-Wallis test with p-value= 0.000 (Table 1). The comparative test analysis used an independent sample t-test for data that was proven to be normally distributed and the Mann-Whitney test was used for data that were not proven

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to be normally distributed. The results of the comparison test in Table 2 show that there is a significant difference in the mean apoptosis index of neurons in the cerebrum of newborn *Rattus norvegicus* between K and P1 (p=0.000), K and P2 (p=0.001), and P1 and P2 (p=0.001).

DISCUSSION

Research on the cerebrum of newborn *Rattus norvegicus* found that the mean apoptosis index of the mackerel oil group was shown to be lower than the other group. The treatment of mackerel oil was able to accelerate the decrease in the apoptotic index of neuron cells compared to those given omega-3 supplements. Based on the statistical test, p-value <0.05, which means that there is a significant difference in the apoptosis index of neuron cells in the cerebrum of newborn *Rattus norvegicus*. This following the results of research by Darmawati et al (2018) which provides mackerel meat that has a significant effect on the cerebral cortex. DHA and AA are nutritional elements that are important for neurodevelopment in the brain, help form brain fat tissue (myelination), and maintain the interconnection of brain nerve cells. DHA and AA are the largest components of long-chain polyunsaturated fatty acids (LC-PUFA), which are very important ingredients for the central nervous system organs. DHA is important for the formation of nerve tissue, whereas AA acts as a neurotransmitter and as an essential form of fatty acid, LC-PUFA must be added to food. Source of omega-3, EPA, DHA is naturally found with a high enough level in fish oil (Diana, 2012).

Fish oil contains omega-3 fatty acids which play a role in preventing apoptosis of brain cell neurons by increasing phosphatidylserine in cell membranes which will activate Akt / PI3K phosphorylation. PI3K will activate Akt which plays an important role in cell survival, dendrite, and axon growth in nerve cells. Research shows that PI3K / Akt is a central node that integrates developmental signals necessary for brain development (Sánchez-Alegría et al., 2018). This process will inhibit caspase-3 which has a role to reduce inflammation as well as an antioxidant that has the potential to be protective against neuronal cells (Zulissetiana et al, 2019). Caspase-3 can activate other caspases, such as procaspase-6 and procaspase-7 which will provide amplification against cellular damage. The presence of cellular stress can increase the expression of the p53 protein resulting in GI arrest or apoptosis (Rastogi et al., 2009).

Nutrients play a vital role in the process of growth and development of brain neuron cells. Omega-3 fatty acids are derivatives of their precursors, namely linoleic and linolenic essential fatty acids. Essential fatty acids cannot be formed in the body so they are needed from food. Then the precursors enter the elongate and desaturate processes that produce three forms of omega-3 fatty acids: LNA (C18: 3, n-3), EPA (C20: 5, n-3), and DHA (C22: 6, n- 3) (Diana, 2012). The parent of omega-3 fatty acids is ALA. ALA with the help of delta-6-desaturase enzymes can be converted into stearidonic acid, then the body converts delta-5-desaturase into EPA and by delta-4-desaturase enzymes converted into DHA. DHA and AA are facilitated by desaturase and elongase enzymes (Utami et al, 2018).

Fat will produce fatty acids which are needed to form membrane cells in all organs. Important organs such as the central nervous system are mainly composed of fat. Fatty acids are needed by body tissues, especially essential fatty acids. Essential fatty acids are fatty acids that cannot be made in the body so they must be obtained from food, consisting of linoleic, linolenic, and arachidonic acids (Kaur et al., 2012). Fatty acids stimulate gene expression and nerve activity, promote synaptogenesis and neurogenesis, and prevent nerve inflammation and apoptosis. Omega-3 fatty acid content acts directly at the apoptotic rate in

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mitochondria by restoring the balance of the Bcl-2 protein family. The Bcl-2 protein family, together with the pro-apoptotic member Bax, regulates the regulation of mitochondrial integrity during the apoptotic process (Sinha et al., 2019). Creb is known to activate Bcl-2 transcription, which can stimulate cell survival directly (Wang *et al.,* 2018).

Rees & Walker (2011) stated that the number of cells that experience apoptosis depends on the synapse, the more synapses the less apoptosis occurs. The underlying mechanism, which affects neuron cells, is related to nutrition and the availability of neurotrophic factors (Hidayati et al, 2020). The richest dietary sources of fatty acids come from marine sources and fish oil supplements. In this study, mackerel oil was used which contains more benefits than omega-3 supplements. Omega-3 supplementation also can significantly restore cellular and cognitive damage and can prevent stunted dendrite forms and apoptosis (Pal et al., 2013).

Fish oil is a major source of long-chain fatty acids known as EPA and DHA, which are omega-3 fatty acids. Omega-3 is a potential prophylactic agent to prevent brain damage (Sinha et al., 2009). The recommendations for the omega-3 fatty acid diet should be applied at the onset of pregnancy. Pregnant women need to consume omega-3 fatty acids from sea fish and omega-3 fatty acid supplements that contain EPA and DHA. Fatty acids stimulate gene expression and nerve activity, increase synaptogenesis and neurogenesis, and prevent nerve inflammation and apoptosis (Hussain et al., 2013).

CONCLUSION

This study showed that the administration of mackerel oil had a significant effect on the apoptotic index with a p-value= 0.026. The lowest mean of the apoptotic index is shown by giving the mackerel oil. This situation can be supported by the content of mackerel oil which is rich in EPA and DHA so that it supports brain development.

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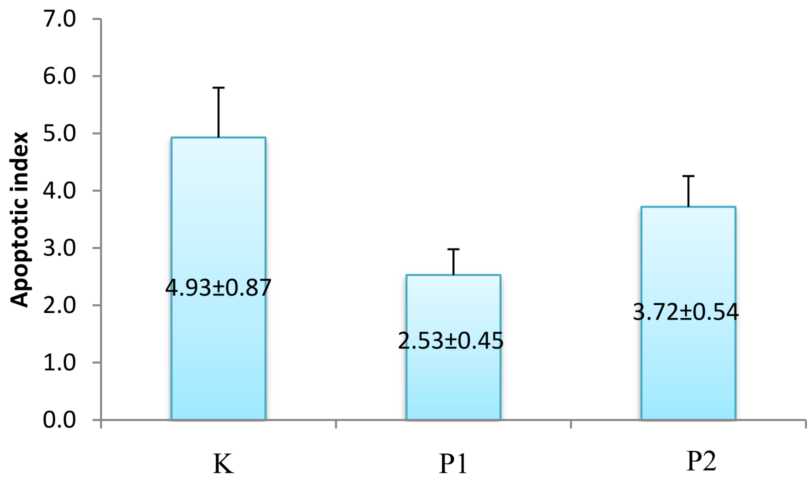


Figure 1 The mean of neuron cell apoptosis index in cerebrum

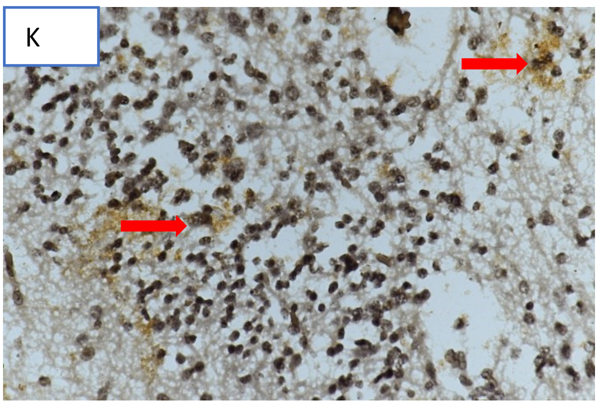
Table 1 Statistical test results on the apoptotic index

Group	Normality test	Kruskal-Wallis
	Sig.	Sig.
K	0.123	
P1	0.040	0.000
P2	0.442	

Table 2 The results of the apoptosis index comparison test between the control group and the treatment group

Group	Apoptotic Index	
	P1	**P2**
K	0.000*	0.001**
P1	-	0.001*

Note: The p-value * is the result of comparison with the Mann-Whitney test.
The p-value ** the comparison results with independet sample t test.



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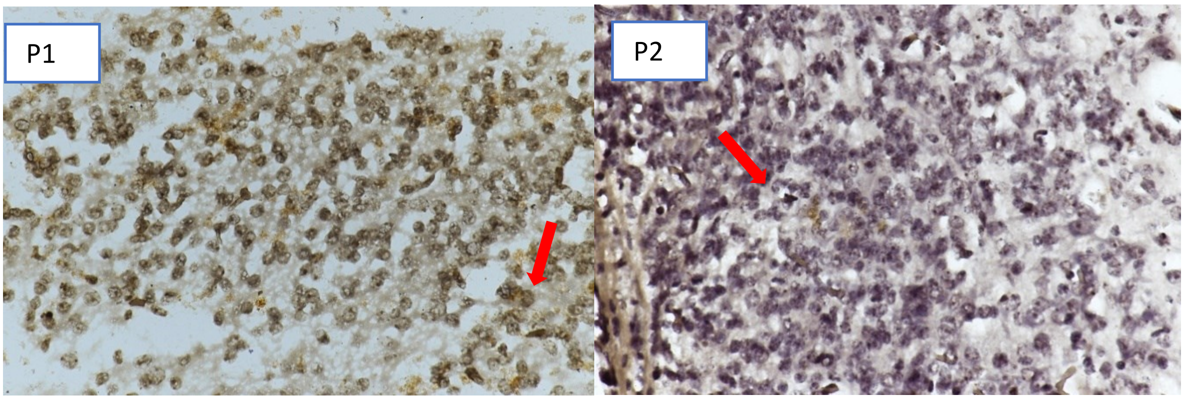

Figure 2 Comparison of fetal brain apoptosis expression in groups (K, P1, P2). The red arrow indicates the expression of apoptosis in the cerebrum which is indicated by the presence of a chromogen brown color. IHC. 400x magnification.