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Effects of Fluoride Exposure During Pregnancy in Mice Brain Neurogenesis (*Mus musculus*)

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ABSTRACT

Background: Fluoride is a type of micromineral found in teeth and bones. This mineral is also found in nature, such as in soil, water, plants, rocks, and even air. Fluoride can cross the placental barrier and diffuse into the cord blood, with a range from 60% to 91% of maternal fluoride. Maternal fluoride exposure during pregnancy and lactation can affect learning ability, memory, and expression of glutamate receptors. This study aimed to determine the effect of fluoride exposure during pregnancy and lactation on brain neurogenesis in vivo. **Methods:** In vivo, experimental study using mice (*Mus musculus*) C57BL/6 as many as 60 brood mice (20 males and 40 females) and as many as 30 offspring mice. Mice broodstock were first mated to produce offspring. During pregnancy, the female mice were exposed to fluoride (NaF). Exposure of broodstock mice to NaF was divided into 0 mg/L, 50 mg/L, 100 mg/L, and 150 mg/L exposure groups. Expression of PSD-95 protein was carried out by immunohistochemical examination of mouse brain tissue. Data analysis was carried out with the help of SPSS using univariate and bivariate methods. **Results:** There was a decrease in the expression of PSD-95 with increasing exposure to NaF doses during pregnancy and lactation. Giving NaF at a dose of 50 mg/L did not decrease PSD-95 expression because the results were not different from the control. In contrast, the administration of NaF doses that can reduce the expression of PSD-95 are doses of 100 and 150 mg/L. **Conclusion:** Fluoride exposure during pregnancy contributed to a decrease in mice brain neurogenesis, as indicated by the expression of the PSD-95 protein in the hippocampus area.

1. Introduction

Fluoride is a type of micromineral found in teeth and bones. This mineral is also found in nature, such as in soil, water, plants, rocks, and even air. Fluoride is used in dental hygiene. This mineral can help strengthen enamel, which is the outermost layer of teeth. In 2006, the US National Research Council (NRC) evaluated fluoride standards and concluded that excessive fluoride exposure produces a variety of symptoms and pathological changes. In recent years, increasing epidemiological investigations have demonstrated a negative association between high fluoride exposure and low child intelligence. The adverse effects of fluoride on the brain can occur directly and indirectly. Children who live in high fluoride areas have lower intelligence quotient (IQ)

scores than children in low fluoride areas. Furthermore, animal experiments have also confirmed the neurotoxicity of fluoride. Various animal behavior methods support that fluoride can induce behavioral changes and impair learning and memory.[1-4]

The cellular mechanisms underlying fluoride's effects on the brain are still being studied. Fluoride has the potential to influence spatial learning and memory formation triggered by special receptors at synapses. In the mammalian brain, most of the ecstatic synapses are glutamatergic, which act as neurotransmitters. Glutamate concentrations in the hippocampus were reduced in fluoride-exposed rats. Glutamate concentrations occupy more than 30% of the total ecstasy neurotransmitters in the brain. Glutamate in blood serum cannot penetrate the blood-brain barrier, and brain glutamate is only synthesized in the brain.[5-10]

Fluoride can cross the placental barrier and diffuse into the cord blood, with a range from 60% to 91% of maternal fluoride. Exposure to maternal fluoride during pregnancy and lactation can affect learning ability, memory, and expression of glutamate receptors in puppies. The developing human brain is inherently much more susceptible to injury from neurotoxic agents, such as fluoride, than is the adult brain. Several studies have demonstrated increased fluoride exposure and its association with cognitive deficits in children. The higher fluoride content of residential drinking water is associated with poorer IQ performance at school age.[11-14] This study aimed to determine the effect of fluoride exposure during pregnancy and lactation on brain neurogenesis in vivo.

2. Methods

This study was an in vivo experimental study with a post-test-only design with a control group design and used mice (*Mus musculus*) C57BL/6 as subjects weighing 20-25 g. As many as 60 brood mice (20 males and 40 females) and 30 offspring mice became the subject of this study. Mice were obtained from PT BMTI Bogor, Indonesia. This study was approved by the medical and health research ethics committee of the Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia.

After a week of acclimatization, one male and two female mice were placed together in one cage at night, and the sperm plug in the vagina of the female mice was examined the next day in the morning. The day when the sperm plug was present was designated as day 0 of pregnancy. Each pregnant mouse was separated and stored individually in cages and randomly divided into four groups with different concentrations of sodium fluoride (NaF), namely 0, 50, 100, and 150 mg/L. After giving birth, female mice were continued with the same treatment until the end of lactation. Daughter mice were administered intragastrically with NaF for 21 days.

Evacuation of the Hippocampus area of the mice brain was carried out, and the brain organs were put into a 10% NBF (Neutral Buffer Formalin) solution. Furthermore, the paraffinization process was carried out on the organs by gradually incorporating the organs into the ethanol solution in a gradient from 70% to 96% concentration and Xylene I, II, III, then ending with the manufacture of paraffin blocks. Next, the paraffin block was cut by rotary sectioning with a thickness of 4 um. Furthermore, the deparaffinization process was carried out, and the PSD-95 immunohistochemical staining process was carried out in the hippocampal area of the mouse brain. Quantification of PSD-95 expression was carried out with the help of ImageJ software to obtain the area of the PSD-95 fraction. Data analysis was carried out with the help of SPSS version 18. Univariate analysis was carried out to present the frequency distribution of the data. Bivariate analysis was performed to compare PSD-95 expression between groups, with $p<0.05$.

3. Results

PSD-95 expression was used to prove the effect of NaF administration which was thought to reduce PSD-95 expression in the dentate gyrus of the hippocampus. PSD-95 is the main protein that is expressed at postsynaptic and plays an important role

in the development of synaptic neurons. Figure 1 shows PSD-95 immunoreactive cells distributed in the dentate gyrus with different densities between groups. There was a decrease in the expression of PSD-95 with increasing exposure to NaF doses during pregnancy and lactation. On statistical analysis, one-way ANOVA obtained p value <0.001, so it can be concluded that there is a difference in the average PSD-95 value in each treatment. To see the location of the difference, further Bonferroni tests were carried out. The results showed that the NaF treatment group at a dose of 100 and 150 mg/L gave the most different results, except for the control group and the NaF treatment at a dose of 50 mg/L. Thus, treatment with a NaF dose of 50 mg/L did not decrease PSD-95 expression because the results were not different from the control. In contrast, the administration of NaF doses that can reduce the expression of PSD-95 are doses of 100 and 150 mg/L. Figure 1 below presents a comparison of the average PSD-95 expression between treatment groups.

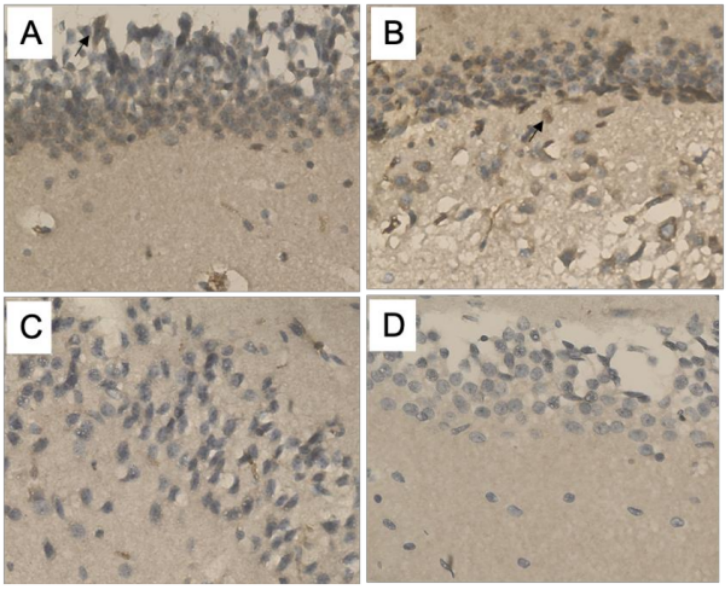

Figure 1. Immunohistochemical staining results of PSD-95 in the control and treatment groups. Information: A. Control group; B. The NaF treatment group at a dose of 50 mg/L; C. The NaF treatment group at a dose of 100 mg/L. D. The NaF treatment group at a dose of 150 mg/L. The black arrows indicate cells expressing PSD-95. 40x magnification of the microscope.

On statistical analysis, one-way ANOVA obtained a p-value <0.05, so it can be concluded that there is a difference in the average PSD-95 value in each treatment. To see the location of the difference, further Bonferroni tests were carried out. The results showed that the NaF treatment group at doses of 100 and 150 mg/L gave different results, except for the control group and the NaF treatment group at 50 mg/L doses. Thus, treatment with NaF dose of 50 mg/L did not decrease PSD-95 expression because the results were not different from the control. In contrast, the administration of NaF doses that can reduce the expression of PSD-95 are doses of 100 and 140 mg/L. Figure 2 below presents a comparison of the average PSD-95 expression between treatment groups.

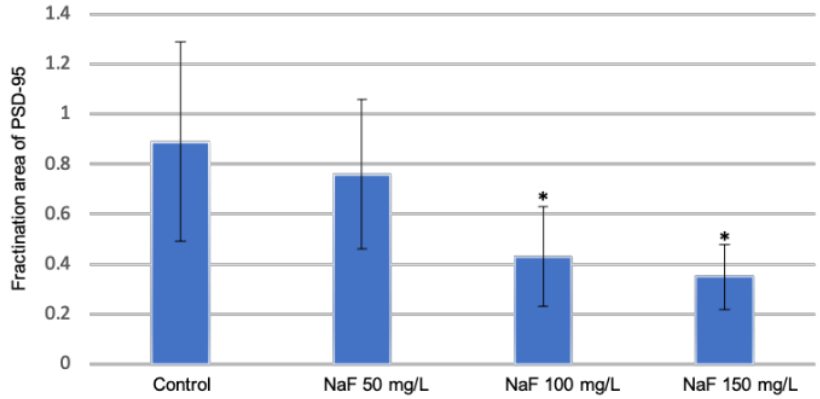

Figure 2. Percentage area of the PSD-95 fractionation in the control group and the treatment group. Information: A. Control group; B. The NaF treatment group at a dose of 50 mg/L; C. The NaF treatment group at a dose of 100 mg/L. C. The NaF treatment group at a dose of 150 mg/L. One-way ANOVA test showed that there was a significant difference in the effect of NaF administration on PSD-95 expression [F(3,27) = 7,22; P < 0.05]. Test post hoc Bonferroni showed a significant difference between the 100 and 140 mg/L*: P < 0.05 vs control groups. There was no significant difference between the control and the group given 50 mg/L NaF.

4. Discussion

Fluoride accumulation in brain tissue and its manifestations in the form of learning and memory disorders have been described in previous studies. In this study, fluoride exposure to the offspring was carried out from the embryonic period through the sucked milk of the offspring mice. Neurons in the hippocampus are responsible for spatial learning. Therefore, an analysis of PSD-95 expression in the hippocampus was carried out in this study. PSD-95 has been shown to underlie synaptic formation, regulating receptors and signal transduction molecules in the synaptic area. In contrast, memory formation and consolidation involve functional plasticity and excitatory synapse structures. PSD-95 is very abundant in the postsynaptic network and includes proteins that regulate excitatory synapses. In addition, PSD-95 also regulates synaptic transmission, synaptic plasticity, structure, and long-term synapse stability. The PSD-95-dependent protein complex interacts well with AMPA and NMDA receptors that also play a role in learning and memory processes. Neuronal plasticity is fundamental to learning and memory processes and can occur as a result of neurogenesis and reorganization of neural networks.[15-17]

In this study, there was a decrease in the expression of PSD-95 in mice exposed to NaF. The expression level of PSD-95 was significantly inhibited in the hippocampus of the offspring of mice that were given 100 and 150 mg/L NaF. On the other hand, there was no significant change in the expression of PSD-95 in the progeny group of mice whose parents were given NaF 50 mg/L. Thus, fluoride has the potential to reduce the ability of synaptogenesis primarily through reduced expression levels of PSD-95. This research shows that sodium fluoride has the potential to cross the placental barrier and affect the offspring. Previous studies are in line with the results of this study. Previous studies have found that

children who live in areas with high-fluoride drinking water have lower IQs than children who live in low-fluoride drinking water areas. In similar studies, the adverse effects of fluoride on brain development have also demonstrated learning disorders in children, in addition to other manifestations that have often occurred, namely dental fluorosis, skeletal fluorosis, increased rates of bone fractures, and decreased birth rates. High doses of fluoride ingested during pregnancy have been shown to cross the placenta and affect the human fetus. Studies in experimental animals also show a decrease in the number of fetuses born as a result of fluoride administration.[18-20]

5. Conclusion

Fluoride exposure during pregnancy decreased in mice brain neurogenesis, as indicated by the expression of the PSD-95 protein in the hippocampus area.

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