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Research Paper

Effect of Folic Acid and Vitamin E Administration on Fetal Growth of Pregnant Rats (*Rattus norvegicus*) Exposed to Cigarette Smoke

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ABSTRACT

Background: Cigarette smoke exposure can cause poor pregnancy outcomes in both mother and fetus. This study was aimed to investigate the effect of folic acid (5 mg/kgBW/day) and/or vitamin E (100 mg/kgBW/day) administration on fetal growth in pregnant rats (Rattus norvegicus) exposed to cigarette smoke (2 cigarettes/day).
Methods: The study was conducted by using post-test-only control group design. Thirty female rats (*Rattus norvegicus*) were divided into 5 groups (6 rats per group), namely the negative control group (without treatment), positive control group (CSE), treatment group 1 (FA+CSE), treatment group 2 (VE+CSE), and treatment group 3 (FA+VE+CSE). Oral administration of folic acid and/or vitamin E, cigarette smoke exposure is given from the first day to 19 days of pregnancy and terminates on the 20th day. Rats' fetuses were weighed, body length was measured, and external malformations were observed using a magnifying glass. Data were analyzed using the non parametric Kruskal Wallis test and continued with the Mann Whitney test. A two-tailed *P*-value of <0.05 was considered statistically significant.
Results: The results of the study known there was a significant effect of folic acid and/or vitamin E administration on fetal body weight (p=0.009), fetal body length (p=0.014), and no significant effect of folic acid and/or vitamin E administration on fetal external malformations (p=0.382).
Conclusion: This analysis confirmed cigarette smoke exposure can inhibit the intrauterine fetal growth, and cause external malformations in the fetuses. Folic acid and/or vitamin E can minimize the teratogenic effects of cigarette smoke exposure.
Keywords: Folic Acid, Vitamin E, Cigarette Smoke, Fetal Growth

INTRODUCTION

Pregnant women who smoke during pregnancy, can be directly related to perinatal morbidity and mortality, and have been associated with several negative outcomes in infants and children, including low birth weight, premature birth, congenital malformations, and are risk factors for intrauterine fetal growth retardation. Pregnant women who smoke, on average give birth to children with lighter birth weight (<2500 g), and shorter (<45 cm) when compared to mothers who do not smoke. [1,2]

Cigarette smoke exposure during the prenatal period, mothers who smoke or use nicotine during pregnancy can induce the occurrence of oxidative stress in the fetus and the inflammatory response that occurs in the tissue and the production of Reactive Oxygen Species (ROS) in blood vessels and pancreas. [3,4] Oxidative stress results from the formation of unstable free radicals and these free radicals cause an increase in ROS. The levels of ROS in cigarette smoke can

exceed the antioxidant levels of the mitochondrial base whose duty is to remove endogenous and exogenous ROS. It has been shown that there is a decrease in serum antioxidant levels in a smoker. [5-7]

There are 4000 chemical compounds in cigarettes, of which 60 of them are carcinogenic. Damage that occurs in the use of tobacco in pregnancy is not only limited to tobacco products that are smoked, but secondhand smoke (SHS) exposure also has the same risk. Although the rate of exposure of pregnant women as passive smokers is 10 percent of active smokers, it still has an adverse effect on neuroherbivoral development. Active smokers or exposure to secondhand smoke during pregnancy can cause fetal defects. [8]

Folic acid and vitamin E administration are very appropriate to be used to protect the fetus from oxidative damage caused by cigarette smoke. In addition to acting as an antioxidant, folic acid and vitamin E play an important role in preventing fetal growth disorders because the need for folic acid and vitamin E always increases with increasing gestational age. [9,10]

The aim of the study was to determine the effect of folic acid and vitamin e administration on fetal growth of pregnant rats (*Rattus novergicus*) exposed by cigarette smoke.

MATERIALS & METHODS

Study Design and Research Sample

The study was conducted using an experimental study with post test only control group design. The sample in this study were thirty female rats (*Rattus norvegicus*) were divided into 5 groups (6 rats per group), namely the negative control group (without treatment), positive control group (CSE), treatment group 1 (FA+CSE), treatment group 2 (VE+CSE), and treatment group 3 (FA+VE+CSE). Oral administration of folic acid and/or vitamin E, cigarette smoke exposure is given from the first day to 19 days of pregnancy and terminates on the 20th day. Rats' fetuses were weighed, body length was measured, and external malformations were observed using a magnifying glass.

Operational Definitions

The variables of this study included independent variables i.e folic acid at a dose of 5 mg/day. Vitamin E at a dose of 100 mg/kg body weight/day. Confounding variable is exposure to cigarette smoke as much as 2 cigarettes per day. Dependent variable i.e fetal body weight, fetal body length and fetal external malformation.

Data Collection Technique

This study was approved by the Ethical Committee of Medical Faculty, Universitas Andalas with registration number 171/KEP/FK/2019.

Data Analysis

The quantitative variables were recorded as mean and standard deviation. Data were analyzed using the non parametric Kruskal Wallis test and continued with the Mann Whitney test. A two-tailed *P*-value of <0.05 was considered statistically significant. Data were analyzed using the SPSS version 21.0.

RESULTS

Average of weight between groups after folic acid and vitamin E administration (Table 1).

Table 1: Average weight between groups after folic acid and vitamin E administration

Groups	n	Weight (g) Mean ± SD	p value
Control (-)	5	3.10 ± 0.42	0.009
Control (+)	5	0.08 ± 0.18	
Intervention I	5	0.24 ± 0.34	
Intervention II	5	0.94 ± 1.48	
Intervention III	5	0.90 ± 9.16	

Table 1 known that the mean fetal body weight of the negative control group was 3.10 ± 0.42, positive control was 0.08 ± 0.18, intervention I was 0.24 ± 0.34, intervention II was 0.94 ± 1.48, intervention III was 0.90 ± 9.16. There was a significant effect of folic acid and vitamin E administration on rat fetal body weight of cigarette smoke exposure (p<0.05).

The group analysis between different group, proceed with the Mann-Whitney test (Table 2).

Table 2: Mann-Whitney test results on Rats fetal weight in each group

Groups	p-value				
	Control (-)	Control (+)	Intervention I	Intervention II	Intervention III
Control (-)	-	0.01*	0.01*	0.08	0.01*
Control (+)	0.01*	-	0.37	0.20	0.04*
Intervention I	0.01*	0.37	-	0.44	0.16
Intervention II	0.08	0.20	0.44	-	0.70
Intervention III	0.01*	0.04*	0.15	0.70	-

Table 2 known there was a significant effect of fetal weight on the negative control group with a positive control group, Intervention group I and intervention group III ($p<0.05$). Other groups did not show a significant effect ($p>0.05$).

Average of fetal body length between groups after folic acid and vitamin E administration (Table 3).

Table 3: Average of fetal body length between groups after folic acid and vitamin E administration

Groups	n	Weight (g) Mean ± SD	p value
Control (-)	5	3.50 ± 2.31	0.014
Control (+)	5	0.30 ± 0.67	
Intervention I	5	0.72 ± 1.00	
Intervention II	5	1.60 ± 1.90	
Intervention III	5	1.62 ± 1.11	

Table 3 known the mean fetal body length of the negative control group was 3.50 ± 2.31, positive control was 0.30 ± 0.67, Intervention I was 0.72 ± 1.00, intervention II was 1.60 ± 1.90, and intervention III was 1.62 ± 1.11. There was a significant effect in the administration of folic acid, vitamin E, as well as the combination of folic acid and vitamin E to the fetal body length of rats exposed to cigarette smoke.

The group analysis between different group, proceed with the Mann-Whitney test (Table 4).

Table 4: Mann-Whitney test results on Rats fetal body length in each group

Groups	p-value				
	Control (-)	Control (+)	Intervention I	Intervention II	Intervention III
Control (-)	-	0.01*	0.01*	0.12	0.01*
Control (+)	0.01*	-	0.44	0.20	0.06
Intervention I	0.01*	0.44	-	0.60	0.30
Intervention II	0.12	0.20	0.60	-	0.70
Intervention III	0.01*	0.06	0.30	0.70	-

Table 4 known there were a significant effect on fetal body length in the negative control group with a positive control group, intervention I, and intervention III ($p<0.05$). Other groups did not show a significant effect ($p>0.05$).

Average of fetal external malformation between groups after folic acid and vitamin E administration (Table 5).

Table 5: Average of fetal external malformation between groups after folic acid and vitamin E administration

Groups	n	Weight (g) Mean ± SD	p value
Control (-)	5	0.00 ± 0.00	0.382
Control (+)	5	1.00 ± 2.24	
Intervention I	5	1.80 ± 3.03	
Intervention II	5	2.00 ± 3.40	
Intervention III	5	2.60 ± 3.60	

Table 5 known the mean of fetal external malformations in the negative control group was 0.00 ± 0.00, the positive control group was 1.00 ± 2.24, intervention I was 1.80 ± 3.03, intervention II was 2.00 ± 3.40 and intervention III was 2.60 ± 3.60. There was an increase in the average number of fetuses undergoing external malformations in each group. There was no significant effect of administration of folic acid, vitamin E, and the combination of folic acid and vitamin E on fetal external malformations ($p>0.05$).

DISCUSSION

The results of this study This analysis confirmed cigarette smoke exposure can inhibit the intrauterine fetal growth, and cause external malformations in

the fetuses. Folic acid and/or vitamin E can minimize the teratogenic effects of cigarette smoke exposure.

Folic acid dose of 5 mg/day given from the preconception has a close relationship with tobacco use during pregnancy. [11] Another study found that administration of folic acid in the pre-conception period (three months before pregnancy) and during pregnancy in women exposed to cigarette smoke reduces the incidence of low birth weight. [12]

Previous study found that pregnant mice exposed to chronic oxidative stress cause a decrease in the number of fetuses and increase the frequency of fetal resorption. So, for this reason, red clots occur because the fetus is dead. This happens because the cell is no longer able to produce totipotential properties during embryogenesis, so the cell cannot repair itself from damage and cannot continue the process of development. [13]

Vitamin E intake during pregnancy can increase the concentration of metabolites in the fetal circulation. This shows an increase in vitamin E metabolism in the liver of the mother and fetus. The effect of storing vitamin E reserves in the fetus depends on the ability to transfer vitamin E to the placenta. The ability of taking and deposition of tocopherol tissue by the fetus is important for the growth and development of fetus. [14,15]

Previous study found that bone development in the fetus after exposure to nicotine was delayed. This is caused by some of the effects controlled by nicotine on osteoblast cells by suppressing the process of osteoclastogenesis and endochondral ossification. [16] Another study showed that the administration of a combination of vitamin E supplements and wheat oil containing folate in pregnant mice exposed to cadmium shows the presence of antioxidant activity and protective effects against the adverse effects of cadmium exposure. Fetal body length increased in the group of rats exposed to cadmium and given a combination of vitamin E and wheat oil compared with the group only exposed to cadmium. [7]

Based on previous study known that consumption of vitamin E and combination with other supplements during pregnancy cannot improve any outcome for infants and pregnant women. There are no data to show that regular consumption of vitamin E or a combination of vitamin E with other supplements during pregnancy can prevent stillbirth, neonatal death, premature birth, premature rupture of membranes, or poor fetal growth. [17]

In this study, it is recommended for pregnant women to stop smoking and avoid exposure to cigarette smoke because cigarette smoke adversely affects the health of mothers and fetuses, and pregnant women are advised to consume antioxidant supplements (folic acid and vitamin E) to minimize the adverse effects of exposure cigarette smoke for mother and fetus.

CONCLUSIONS

This study confirmed cigarette smoke exposure can inhibit the intrauterine fetal growth, and cause external malformations in the fetuses. Folic acid and/or vitamin E can minimize the teratogenic effects of cigarette smoke exposure.

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