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The Effect of Antioxidant Administration on Pregnant Mice Exposed to Cigarette Smoke on the Birth Weight of Mouse Offspring

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ABSTRACT

Exposure to environmental tobacco smoke (ETS) in pregnant women has been shown to trigger oxidative stress that impairs fetal growth and can result in low birth weight (LBW), a serious health problem because of its association with increased neonatal mortality. The antioxidants vitamin C and vitamin E may provide protective effects, but scientific evidence remains limited. This preliminary experiment in mice (*Mus musculus*) aimed to determine the effectiveness of vitamin C and vitamin E in preventing a decrease in the birth weight of offspring of pregnant mice exposed to cigarette smoke. An experimental study with a randomized post-test only control group design was conducted using 30 pregnant female mice divided into five groups (144 offspring weighed): negative control, positive control (exposure to cigarette smoke), and three treatment groups (exposure to cigarette smoke plus vitamin C, vitamin E, or a combination of vitamins C and E). Data were analyzed using one-way ANOVA followed by Bonferroni and Duncan post hoc tests ($p < 0.05$). The mean birth weight in the control group without exposure was 1.723 g, whereas in the group exposed to cigarette smoke without antioxidants it decreased to 1.552 g. Administration of vitamin C (1.665 g) or vitamin E (1.683 g) significantly increased birth weight compared with the positive control group, whereas the combination of vitamins C and E resulted in the lowest mean birth weight (1.335 g). ANOVA showed a significant difference between groups ($p < 0.001$). Exposure to cigarette smoke reduced the birth weight of mouse offspring. Vitamin C and vitamin E given separately protected against this reduction, while their combination did not show a synergistic effect.

Keywords: Antioxidants; Cigarette smoke; Low birth weight; Oxidative stress; Vitamin C; Vitamin E

INTRODUCTION

Low birth weight (LBW) remains one of the serious public health problems in the world. UNICEF (2013) reported that around 2.9 million babies died in the first month of life, and most of these deaths were related to LBW without optimal care. LBW is defined as a birth weight of less than 2500 g regardless of gestational age (WHO, 2014b). Babies with LBW have a 35 times higher risk of death than babies born with normal weight (WHO, 2019). More than 80% of neonatal deaths occur in babies with LBW, whether due to premature birth, small for gestational age (SGA), or a combination of both (WHO, 2015).

Globally, the prevalence of LBW reached 20.6 million cases (15.5%) in 2011 and 15.2% in 2012, with 95.6% of cases occurring in developing countries (WHO, 2014b). Babies born in developing countries are more vulnerable to intrauterine growth retardation because of poor maternal nutrition and high infection rates than babies in developed countries (Undela et al., 2019). In Indonesia, the Basic Health Research (Riskesdas) recorded a decrease in the prevalence of LBW from 11.5% in 2007 to 10.2% in 2013 (Kemenkes RI, 2018). In Palembang City, South Sumatra, 3,289 LBW cases were recorded in 42 primary health centres during 2011–2020, including 497 cases in 2012 (Irzanita et al., 2022).

Various studies show that the causes of LBW can be categorized into three main factors: maternal, fetal, and environmental factors. Maternal factors include high-risk pregnancy conditions such as pre-eclampsia, antepartum hemorrhage, premature rupture of membranes, and underlying diseases (Acharya et al., 2018; Ahankari et al., 2017). Fetal factors are related to congenital defects, intrauterine infections, and multiple pregnancies (Kasuga et al., 2021; Tellapragada et al., 2016; WHO, 2014a). Meanwhile, environmental factors such as exposure to air pollution, radiation, and cigarette smoke (environmental tobacco smoke, ETS) also play a significant role in increasing the risk of LBW (Huang et al., 2017; Pereira et al., 2017; Witt et al., 2018).

Exposure to cigarette smoke in the household environment is of particular concern because it has a more serious impact on passive smokers than on active smokers

(Huang et al., 2017). Pregnant women, as a vulnerable group, are at high risk of pregnancy complications due to exposure to cigarette smoke, such as premature birth, SGA, birth defects, and LBW (Abusalah et al., 2012; Hawsawi et al., 2015; Hoyt et al., 2018). Cigarette smoke contains around 4000 toxic chemicals, including nicotine, carbon monoxide, free radicals, and oxidants, which can trigger oxidative stress that damages cells and body tissues (Nagiah et al., 2015; Rabha et al., 2018). Oxidative stress due to cigarette smoke exposure may be prevented by consuming antioxidants, such as vitamin C and vitamin E, which counteract free radicals and reactive oxygen species (ROS) (Carr & Maggini, 2017; Galli et al., 2017; Lung & Destiani, 2017), but scientific evidence is still limited.

This preliminary experiment using mice (*Mus musculus*) was conducted to test this theory. Mice are often used in research because of the similarity of their organs to those of humans, thus providing an initial picture of the effectiveness of antioxidant protection against the impact of cigarette smoke exposure. If proven effective, vitamin C and E supplementation in pregnant women has the potential to become a protective strategy against the risk of LBW caused by cigarette smoke exposure during pregnancy.

METHODS

Study design and setting

This research was an experimental study with a randomized post-test only control group design using pregnant female mice (*Mus musculus*) as test subjects. The research was conducted at the Integrated Laboratory of the Faculty of Public Health, Universitas Sriwijaya, over one year (2023). The independent variables were the administration of vitamin C and vitamin E, and the dependent variable was the birth weight of the mouse offspring.

Animals and sample size

The population consisted of pregnant female mice aged ≥ 8 weeks with a gestational age of no more than 6 days before treatment. The minimum sample size was estimated using the WHO method for determining the number of test animals, $(t - 1)(n - 1) \geq 15$ (Federer, 1966). Thirty mice were divided into five groups: negative control (Group K); positive control (Group P1), exposed to cigarette smoke; treatment 1 (Group P2), exposed to cigarette smoke and given vitamin C routinely; treatment 2 (Group P3), exposed to cigarette smoke and given vitamin E routinely; and treatment 3 (Group P4), exposed to cigarette smoke and given a combination of vitamins C and E routinely. Based on the calculation, each group required at least five pregnant mice, or at least 25 mice in total. To anticipate the loss of experimental units, a dropout correction of 10% was applied, so that the minimum sample was six mice per group.

At the preparation stage, 30 mice that met the inclusion criteria were adapted to the laboratory conditions for one week. Adaptation aimed to avoid stress during treatment and to standardize eating patterns in the new environment. The mice were kept in cages with rice husk bedding and were provided with pellet feed and water *ad libitum*. Their health was monitored daily until delivery, and mice that became sick were excluded from the study. After one week, the mice were randomly divided into five groups (K, P1, P2, P3, and P4).

Antioxidant doses

Vitamin C and vitamin E were administered orally. The conversion of human doses to animal doses was calculated using the conversion table of Laurence & Bacharach (1964). The daily vitamin C dose for pregnant women is 85 mg/day; using the conversion table, the dose of vitamin C for mice with an average body weight of 20 g was 0.221 mg/20 g body weight. Pure vitamin E powder was assumed to be 1 IU = 0.666 mg, and the daily human dose of 120 IU/day (80 mg/day) was converted to 0.208 mg/20 g body weight for mice.

Cigarette smoke exposure

Group K was placed in a separate room without exposure to cigarette smoke. During exposure, groups P1, P2, P3, and P4 were placed in a smoking chamber and inhaled sidestream smoke from burning cigarettes placed under each chamber, following the procedure of Özkol et al. (2012). Exposure was conducted twice a day for approximately 1 hour, with each group exposed to two cigarettes at 09:00 and two cigarettes at 15:00, until the mice gave birth. Vitamin C and/or E was administered in the morning after the exposure procedure (09:00 WIB) to ensure proper metabolism. The newborn mice were weighed to determine their birth weight.

Statistical analysis

The birth weight of each pup was recorded individually. In total, 144 pups were born and weighed: 37 in group K, 37 in P1, 27 in P2, 33 in P3, and 10 in P4. The individual pup was used as the unit of analysis. Data normality was tested using the Kolmogorov–Smirnov test and homogeneity of variance using Levene's test. Differences in mean birth weight among the five groups were analyzed using one-way ANOVA, followed by the Bonferroni post hoc test and Duncan's multiple range test to identify homogeneous subsets. Statistical significance was set at $p < 0.05$. Because pups from the same dam are not fully independent, analysis at the pup level may overestimate statistical significance; this is acknowledged as a limitation of the study.

RESULTS AND DISCUSSION

Birth weight of mouse offspring

The mean birth weight of pups in the control group (K), which was not exposed to cigarette smoke, was 1.723 g. In contrast, the group exposed to cigarette smoke without antioxidants (P1) experienced a reduction in mean birth weight to 1.552 g (Table 1). Cigarette smoke, which contains more than 4000 toxic chemicals, including carbon monoxide, benzo(a)pyrene, and other carcinogenic compounds, can reduce oxygen transport and induce oxidative stress that disrupts fetal growth (Hawsawi et al., 2015; Kalayasiri et al., 2018). This finding is consistent with

epidemiological evidence in humans that exposure to cigarette smoke during pregnancy increases the risk of LBW and other pregnancy complications (Hanum & Wibowo, 2016; Sari & Yunamawan, 2019).

Table 1. Descriptive analysis of mouse birth weight

Group	n (pups)	Mean (g)	Median (g)	Min (g)	Max (g)
Control (K)	37	1.723	1.757	1.310	1.937
P1	37	1.552	1.565	1.207	1.784
P2	27	1.665	1.687	1.242	1.878
P3	33	1.683	1.696	1.338	1.866
P4	10	1.335	1.332	1.216	1.472

Note: K = control (no exposure); P1 = cigarette smoke exposure without antioxidants; P2 = exposure + vitamin C; P3 = exposure + vitamin E; P4 = exposure + vitamins C and E.

Administration of vitamin C to mice exposed to cigarette smoke (P2) increased the mean birth weight to 1.665 g (Table 1). Vitamin C acts as a non-enzymatic, water-soluble antioxidant capable of neutralizing free radicals and protecting cells from oxidative damage (Carr & Maggini, 2017). These results suggest that vitamin C consumption can protect against the negative effects of cigarette smoke exposure during pregnancy. In line with this, the consumption of antioxidant-rich fruits and vegetables is recommended for pregnant women as a preventive measure (Dewantari, 2013; Lung & Destiani, 2017).

In the group exposed to cigarette smoke and given vitamin E (P3), the mean birth weight reached 1.683 g (Table 1). Fat-soluble vitamin E inhibits the formation of ROS during lipid oxidation and thus protects cell membranes from oxidative damage (Galli et al., 2017; Lung & Destiani, 2017). These results reinforce the role of vitamin E as an effective antioxidant in maintaining fetal growth despite exposure to cigarette smoke.

However, in the group exposed to cigarette smoke and given a combination of vitamins C and E (P4), the mean birth weight was lower (1.335 g) than in the groups that received only one of the vitamins. This finding indicates an antagonistic effect when vitamins C and E are given simultaneously, reducing their protective benefit against cigarette smoke exposure. This result differs from the initial hypothesis of a synergistic effect; further research is therefore needed to explore the pharmacological interactions between these antioxidants.

Normality, homogeneity, and group comparisons

The normality test showed Kolmogorov–Smirnov p-values > 0.05 in all groups (Table 2); therefore, the data were considered normally distributed and met the assumption of normality.

Table 2. Normality test of mouse birth weight data

Group	Kolmogorov–Smirnov			Shapiro–Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Control (K)	0.119	37	0.200	0.942	37	0.052
P1	0.092	37	0.200	0.953	37	0.116
P2	0.146	27	0.145	0.909	27	0.022
P3	0.139	33	0.109	0.914	33	0.012
P4	0.143	10	0.200	0.956	10	0.734

Note: Variable: mean birth weight (g).

The homogeneity of variance test showed a p-value of 0.149 (> 0.05) (Table 3). Thus, the variances between groups were homogeneous, and the data met the assumption of homogeneity required for ANOVA.

Table 3. Homogeneity of variance test (Levene's test)

Variable	Levene statistic	df1	df2	Sig.
Mean birth weight	1.720	4	139	0.149

One-way ANOVA showed a p-value < 0.001 (Table 4), indicating a significant difference in mean birth weight among the treatment groups. A post hoc test was then conducted to identify which groups differed significantly.

Table 4. Distribution of mean birth weight by treatment group (one-way ANOVA)

Group	n	Mean (g)	SD	F	p-value
Control (K)	37	1.723	0.135	19.402	< 0.001
P1	37	1.552	0.161		
P2	27	1.665	0.146		
P3	33	1.683	0.128		
P4	10	1.335	0.083		

The Bonferroni post hoc test was used to determine which groups had significantly different mean birth weights. Table 5 shows that significant differences were found between the control group and groups P1 and P4, and between group P1 and groups P2 and P3, as well as between group P4 and groups P1, P2, and P3.

Next, Duncan's post hoc test was conducted (Table 6) because the sample sizes of the groups were unequal. This test also allowed the mean birth weights of the groups to be compared in homogeneous subsets, showing which exposure group had the greatest effect on mean birth weight.

Table 5. Significance of differences in mean birth weight between treatment groups (Bonferroni post hoc test)

Comparison	p-value	Conclusion
Control vs P1	< 0.001	Significant
Control vs P4	< 0.001	Significant
P1 vs P2	0.019	Significant
P1 vs P3	0.002	Significant
P1 vs P4	< 0.001	Significant
P2 vs P4	< 0.001	Significant
P3 vs P4	< 0.001	Significant

Note: Only significant pairwise comparisons (p < 0.05) are shown.

Table 6. Homogeneous subsets of mean birth weight (Duncan post hoc test)

Group	Subset for α = 0.05		
	1	2	3
P4 (exposure + vitamins C and E)	1.335		
P1 (exposure without antioxidants)		1.552	
P2 (exposure + vitamin C)			1.665
P3 (exposure + vitamin E)			1.683
Control (K)			1.723

Note: Values are mean birth weight (g).

Table 6 shows that there were significant differences in mean birth weight among the treatment groups. Groups P1 and P4 had lower birth weights than the other groups, indicating that cigarette smoke exposure reduced birth weight. Of these two groups, the greatest difference was found in group P4, which was exposed to cigarette smoke and routinely given the combination of antioxidants (vitamins C and E), whereas groups P2 and P3 were in the same subset as the control group.

CONCLUSION

Exposure to cigarette smoke during pregnancy reduced the birth weight of mouse offspring: the mean birth weight in the exposed group without antioxidants (P1; 1.552 g) was significantly lower than in the unexposed control group (1.723 g). Vitamin C (P2; 1.665 g) or vitamin E (P3; 1.683 g) given separately significantly increased birth weight compared with P1, to values in the same Duncan subset as the control group. In contrast, the combination of vitamins C and E (P4; 1.335 g) produced the lowest birth weight, indicating no synergistic protective effect at the doses and schedule used. Vitamin C or vitamin E given separately therefore shows potential as a protective measure against LBW caused by cigarette smoke exposure. Further studies are needed to explain the interaction between the two antioxidants, to confirm these findings with the litter as the unit of analysis, and to measure oxidative stress markers before the results are extrapolated to pregnant women, who should also be protected from cigarette smoke exposure at home.

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