

Effect of infused lemon water (citrus limon) on Newborn Baby Weight (BBL) in pregnant rats (*rattus norvegicus*) exposed to electric cigarette vapor

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ABSTRACT

The electric cigarettes are said safe for health, in fact there are toxic substances, carcinogens, and unstable nicotine, it is suspected to cause low birth weight in pregnant women who are exposed to electric cigarette. Nicotine in electric cigarette is free radicals, so to ward off free radicals is required antioxidant. Infused lemon contains antioxidants that are higher than lime and are believed to resist free radicals from electric cigarettes. The purpose of research to determine the influence of infused lemon water in preventing low birth weight in pregnant rats (*Rattus norvegicus*) exposed by electric cigarettes. The study used 30 pregnant rats, divided into 5 groups; Negative control (KN), positive control (KP), Treatment (P1), Treatment (P2), Treatment (P3). Using electric cigarette liquid 9 mg, and the difference in the weight of lemon in the making of infused water (P (1): 25g, P (2): 50g, P (3): 100g lemon + 100ml water). The treatment is given on day 1 ovulation to day 18, sacrificed on the 19th day ovulation then calculated the average weight of the baby. The average weight baby KN: 5.502g, KP: 2.821g, P (1): 4.301g, P (2): 4.888g, P (3): 5.435g. The result of One-way ANOVA indicates there is a significant difference in the treatment group with the value $P=0,000$ ($P<0.05$). The KP group differs significantly from KN. Group P (1,2,3) is significantly different from KP. The KN group is significantly different from P (1), but does not differ significantly from P (2, 3). The group P (1) differs significantly with P (3). Infused lemon juice can prevent the occurrence of low birth weight in the child of the pregnant rats that's exposed by electric cigarettes especially in group P (3).

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INTRODUCTION

The biggest causes of neonatal death are babies born with Low Birth Weight (LBW) or preterm, intrapartum complications (asphyxia), and infections in newborns (WHO, 2017). The World Health Organization (WHO, 2017) estimates that 16% of all babies born worldwide are LBW babies.

Meanwhile, in Indonesia itself, based on Basic Health Research (Riskesdas), babies born with LBW in children aged 0-59 months have a prevalence of 6.2% (Baidho et al., 2021). One of the causes of LBW is exposure of pregnant women to cigarette smoke. This is in line with research conducted (Hanum & Wibowo, 2017), if pregnant women are exposed to cigarette smoke for >7 hours every day, the risk of giving birth to a LBW baby increases 2.4 times higher than that of pregnant women who are not exposed to cigarette smoke.

Based on the National Youth Tobacco Survey (NYTS), the use of e-cigarettes among high school students in 2011 was 1.5% and increased rapidly in 2014, reaching 13.4% (Ladesvita & Agustina, 2017). As an effort to prevent this from happening, the WHO Framework Convention on Tobacco Control (WHO-FCTC) was formed which is a solution to tobacco use (Damayanti, 2016). Namely by using the Nicotine Replacement Therapy (NRT) method (WHO, 2011). One type of NRT is electronic cigarettes. Electric cigarettes are a way of administering nicotine or chemical substances in the form of vapor using electricity from a battery, then the vapor will be inhaled by the user, WHO calls it an Electronic Nicotine Delivery System (ENDS) (Trtchounian et al., 2010)(Suhendra, 2016).

Initially, electronic cigarettes were considered safe for health because they did not contain tar, tobacco or the toxic substances found in tobacco cigarettes (Trtchounian et al., 2010). However, in reality, electronic cigarettes contain toxic substances, namely Tobacco Specific Nitrosamine (TSNA) and carcinogenic substances, namely Diethylene Glycol (DEG), according to the results of research by the Food and Drug Administration (FDA) in the United States in 2009. This was also found in electronic cigarettes. 4 times more chromium and nickel than tobacco cigarettes (Saffari et al., 2014). Finally, in 2010, e-cigarettes were no longer recommended as NRT by WHO, because several studies showed that e-cigarettes contained substances that could be toxic and carcinogenic, therefore WHO stated that e-cigarettes did not meet safety requirements (Suhendra, 2016).

The development of the fetus in the womb can be affected by free radicals due to exposure to e-cigarette smoke. This can occur due to the presence of nicotine which has been proven to have a bad effect on the reproductive process, fetal body weight and child brain development. Nicotine can affect the fetus's weight because nicotine can inhibit nutrition from the mother to the fetus, and in turn this will have an impact on the condition and birth weight of the baby during delivery (Suhendra, 2016)(Julianti et al., 2018). Exposure to cigarette smoke will have a negative effect on the mother and fetus because exposure to cigarette smoke can damage DNA (Deoxyribo Nucleic Acid) which is triggered by oxidative stress, smoking can also cause a decrease in antioxidant levels in the blood, and increase the release of superoxide radicals (Fitria et al., 2013).

One of the herbal plants that is often used in everyday life is lemon (*Citrus limon*). Lemon (*Citrus limon*) contains many bioactive compounds such as flavonoids, carotenoids, limonoids, tannins and terpenoids, these bioactive compounds each have antibacterial and antioxidant properties (Russo et al., 2014). When using lemon (*Citrus limon*), many people use this fruit as a mixture in infused water drinks.

Herbal ingredients or fruit slices that are given water without adding sugar or other artificial sweeteners are called infused water. Pregnant women also need water to balance the fluids in their bodies, therefore drinking infused water with sour fruits or lemon (*Citrus limon*) is expected to balance the acid base in the body of pregnant women, a better source of dehydration and more comfort when drinking it. compared to ordinary water (Surati & Qomariah, 2017). This research was conducted to prove the effect of infused lemon water (*Citrus limon*) on the weight of newborn babies (BBL) in pregnant white rats (*Rattus novergicus*) exposed to e-cigarette vapor).

RESEARCH METHOD

This research uses an experimental research design (true experiment design) using pregnant white rats (*Rattus novergicus*) and the research design is Randomized Post Test Only Control Group Design. There were 5 groups in this study, each group consisting of 6 mice. In the negative control

group (which will hereinafter be referred to as KN) the experimental animals will not be given any treatment. Meanwhile, the positive control group (which will hereinafter be referred to as KP) will be given e-cigarette vapor using 9 mg nicotine e-cigarette liquid with 1x exposure (3x evaporation) given 6 ml of liquid and a vape device at 55 W and 0.20 Ω . The treatment group is divided into 3 groups, namely P1, P2 and P3, they will be treated with 3 ml/day of lemon infused water with differences in the weight of lemons in making infused water, namely P(1): 25 g lemon/100 ml water, P(2): 50 g lemon/100 ml water, P(3): 100 g lemon/100 ml water, and exposure to e-cigarette vapor. Lemon water infusion is made using a plastic glass container and left in the refrigerator for 12 hours.

The infused water and e-cigarette vapor treatment was given on the first to 18th day of pregnancy. The mouse surgery will be carried out on the 19th day of pregnancy, and at that time the baby mice will be taken and then the birth weight of the baby mice will be measured. Then a comparison will be carried out between the effects of giving infused water on the birth weight of mice that have been exposed to e-cigarette vapor in all treatment groups.

Data Analysis Technique

Data analysis uses the SPSS 23.0 program, data normality test uses the Shapiro-Wilk test, if the data is normally distributed then continues with the data homogeneity test using the Levene statistical test. If the data is normally distributed and homogeneous then proceed with the One-way ANOVA test, this is carried out for each treatment group to compare the average values and find out which groups are significantly different, at least two treatment groups. Next, a post hoc test was carried out which aimed to find out which groups were significantly different from the ANOVA test that had been carried out previously. The Tukey HSD test is a post hoc test used and the significance level is 95% ($p < 0.05$).

RESULTS AND DISCUSSIONS

Average Body Weight (BW) of Newborn Rat Babies

In the KN group, the average body weight of newborn mice was 5.502 grams. In the KP group, there was a reduction in the average body weight of newborn mice of up to 2.821 grams. So it can be concluded that the KN group is the lowest weight group for baby mice compared to other groups. In treatment group 1 (which will hereinafter be referred to as P1), the average body weight of newborn mice was found to be 4.301 grams, which is the lowest average baby weight among the treatment groups, then there was an increase in treatment group 2 (which will hereinafter be referred to as P2) mean weight. the body weight of newborn mice was 4.888 grams, and there was another increase in treatment group 3 (which will hereinafter be referred to as P3) with the result that the average body weight of newborn mice was 5.435 grams.

Data Analysis

The normality test result is $p = 0.747$ ($p > 0.05$), so each group is declared normally distributed. Then, in the homogeneity test, the result was $p = 0.306$ ($p > 0.05$) and the data distribution could be declared homogeneous. In the one-way ANOVA test, the value of $p = 0.000$ ($p < 0.05$) was obtained and these results can state that there were significant differences in all treatments (P1, P2 and P3) after giving infused lemon water with a ratio of lemon and water. varies over 18 days.

After carrying out the one-way ANOVA test, a Post Hoc test was carried out using the Tukey HSD test to determine which groups were significantly different from the one-way ANOVA test that had been carried out previously. In table 1 it can be concluded that the KP group with a value of $p = 0.000$ is significantly different from the KN group. The P1 group is significantly different from the KN group with a p value = 0.001. The treatment groups (P1, P2, and P3) with a p value = 0.000 were significantly different from the KP group. Group P3 with a result of p value = 0.002 is significantly different from group P1. However, there was no significant difference

between the KN group and the P2 and P3 groups, this also happened in the P2 group which was not significantly different from the P1 group, and there was no significant difference between the P3 group and the P2 group.

Table 1. Tukey HSD test results on baby weight data

<i>p-value</i>	KN	KP	P1	P2	P3
KN		0,000*	0.001*	0.155	0.999
KP			0,000*	0,000*	0,000*
P1				0.185	0.002*
P2					0.241
P3					

Note: *p-value* < 0.05 is significant (*)

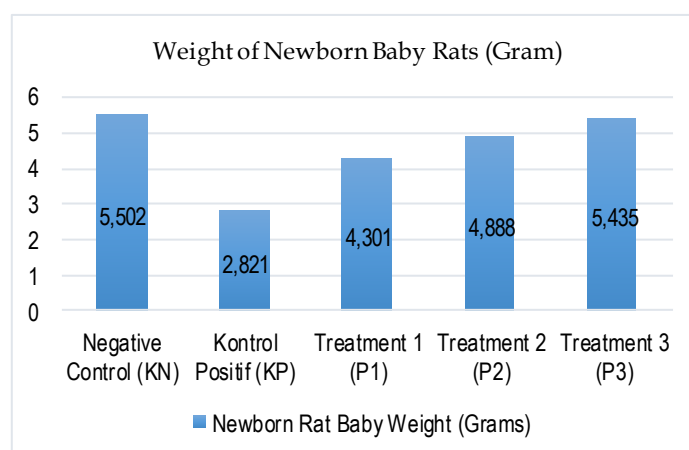


Figure 1. Graph of average body weight of newborn rats

Discussion

The Effect of Exposure to E-Cigarette Vapor on the Body Weight of Baby Rats

The KN group had an average weight of newborn mice of 5.502 grams. This result was very different from the KP group whose average weight of newborn mice was 2.281 grams. Quantitatively, the two groups had quite a big difference, namely 3.221 grams. The results of statistical tests using the Tukey HSD test also support this difference. It can be concluded that between the KP group with a significance value of $p = 0.000$ ($p < 0.05$) and the KN group, there is a significant difference. This happened due to the effect of free radicals in the e-cigarette vapor given to the KP group.

Free radicals that cannot be balanced by antioxidants in the body will result in oxidative stress (Widayati, 2012). Oxidative stress in pregnant women will cause maternal vascular endothelial dysfunction due to decreased placental perfusion (Setiawan & Insani, 2004). The main effect of nicotine in pregnancy is vasoconstriction of the uteroplacental blood vessels, which causes reduced blood flow to the placenta, while the blood carries oxygen and nutrients so that the delivery of oxygen and nutrients from the mother to the fetus will be disrupted or even reduced, this is what causes Low Birth Weight (LBW) (Wickstrom, 2007).

This is in accordance with research conducted by (Gupta & Sreevidya, 2004) stating that newborn babies whose mothers were exposed to cigarette smoke, their birth weight will be 105 grams lighter than mothers who were not exposed to cigarette smoke during pregnancy. However, in contrast to research conducted by (Bowker et al., 2018), it was stated that e-cigarettes are seen as less dangerous than conventional cigarettes, because e-cigarettes are useful as a tool to reduce and stop smoking habits. Research conducted by (Bruin et al., 2010) stated the same thing as research

by Bowker et al¹⁹, in the research it was stated that Nicotine Replacement Therapy (NRT), one of which is electronic cigarettes, has been developed as pharmacotherapy to stop smoking habits and is considered a safer alternative for women to smoking during pregnancy.

Electronic cigarettes have a relatively smaller dose of nicotine than conventional cigarettes and are considered safer for pregnant women, however there is no safe dose of nicotine during pregnancy (Saunders, 2012). Pregnant women who use e-cigarettes during pregnancy also still have concerns about the safety, dependence and long-term effects of nicotine, even though nicotine is the most abundant component in e-cigarettes (Bowker et al., 2018)(Bruin et al., 2010).

The Effect of Giving Lemon Water Infusion on the Weight of Baby Rats

The results obtained in this study were differences between all treatment groups and the KP group. The P1 group produced an average body weight of newborn mice of 4.301 grams, while in the KP group the average body weight of newborn mice was 2.821 grams. The results of the post hoc test using the Tukey HSD test also provide supporting results, namely $p = 0.000$ ($p < 0.05$), which means there is a significant difference between KP and P1. So it can be concluded that the P1 group was able to increase the body weight of newborn mice significantly compared to the KP group.

If the P1 group and the KN group are compared, the statistical test results will also be significantly different, namely $p = 0.001$ ($p < 0.05$). This shows that by administering lemon water infusion with a ratio of 25 grams of lemon/100 ml of water, it can increase the weight of newborn mice compared to the KN group.

Group P2 had a mean newborn of 4.888 grams. If the KP group is compared with P2, the statistical test results are significantly different, $p = 0.000$ ($p < 0.05$). So, giving infused lemon water in a ratio of 50 grams of lemon/100 ml of water can significantly increase the body weight of newborn mice when compared to mice that were exposed to e-cigarette vapor without giving infused lemon water.

The KN group had an average body weight of newborn mice of 5.502 grams when compared with the P2 group, the statistical test results were $p = 0.155$ ($p < 0.05$), so it can be concluded that there was no significant difference between the P2 and KN groups. These results stated that there was no difference between the KN group and the P2 group. The KN group is a group that was not given any treatment, and is considered to have a normal birth weight of newborn mice. If the statistical results state that there is no significant difference between P2 and KN, then give a lemon water infusion of 50 grams of lemon/100 ml of water. exposed to e-cigarette vapor, the average body weight of newborn mice was sufficient to be comparable to normal conditions. This is because vitamin C is one of the micronutrients needed by pregnant women to fulfill the nutrition of pregnant women during their pregnancy. In research conducted by (Prihati & Kostania, 2017) it was stated that based on the average value (mean) administration of Multi Micro Nutrients (MMN) can increase weight. newborn baby's body is 0.18 grams.

The P3 group had a mean of 5.435 grams when compared, the KP and P3 groups produced a statistically significant difference of $p = 0.000$ ($p < 0.05$). This means that giving infused lemon water in a ratio of 100 grams of lemon/100 ml of water exposed to e-cigarette vapor can significantly increase the body weight of newborn mice when compared to rats exposed to e-cigarette vapor without giving infused lemon water. When the P3 group is compared with the KN group, the statistical test result is $p = 0.999$ ($p < 0.05$) that there is no significant difference between P3 and KN. Statistical tests stated that there was no significant difference between the P3 group and the KN group. This is because the P3 group was given a lemon water infusion of 100 grams of lemon/100 ml of water and exposed to free radicals. The weight of their newborn mice was comparable to the condition of mice that were not given any treatment or normal mice. This is because antioxidants, one of which is vitamin C, which is found in lemon water infusion, functions to ward off free radicals in e-cigarette vapor. According to the results of research conducted by

(Sidauruk, 2018), the levels of vitamin C, antioxidant activity, and pH in the resulting infusion water will increase. increases with the length of soaking time.

Comparison between treatment groups, namely, treatment groups 1, 2 and 3 with 3 different weights of lemon for each lemon water infusion making, namely 25 gr lemon, 50 gr lemon and 100 gr lemon, gave different results. In group P1, the average weight of newborn mice was 4.301 grams, in group P2 it was 4.888 grams, in group P3 it was 5.435 grams. Based on statistical tests, when group P1 is compared with P2, $p = 0.185$ ($p < 0.05$), so there is no significant difference between group P1 and group P2, this is due to the lack of lemon compounds infused in the water. Meanwhile, between the P1 group and the P3 group with $p = 0.002$ ($p < 0.05$), there is a significant difference between the groups, because the P3 group has more lemons which will diffuse with water, and more antioxidant content in the fruit lemon infused in water (Sidauruk, 2018). If there are more antioxidants in the lemon water infusion, then the weight of the offspring of pregnant mice exposed to e-cigarette vapor will be higher and significantly different.

The antioxidant content of lemons is high enough to ward off free radicals in the environment. In fact, according to Muaris²⁴, consuming 1.5-3 lemons can provide daily vitamin C for adults. Apart from the high vitamin C content, lemons also contain other antioxidants such as citric acid, flavonoids, quercetin and potassium which help to ward off free radicals (Muaris, 2014).

Research conducted by (Nimse & Pal, 2015) shows that natural antioxidants, namely vitamin C and flavonoids, can overcome free radicals in the body due to exposure to cigarette smoke. Infused lemon water is also able to ward off free radicals caused by exposure to e-cigarette vapor and maintain the body's immune system because it contains vitamin C and antioxidants (Harifah et al., 2016). The results of research by (Harifah et al., 2016) show that infused water with a combination of lemon and black grape treatments has the highest antioxidant activity and vitamin C. This is also directly proportional to research conducted by (Permata et al., 2018) which states that lemon (*Citrus limon*) has higher antioxidant activity than lime (*Citrus aurantiifolia*).

CONCLUSION

Infused lemon water (*Citrus limon*) can prevent the occurrence of Low Birth Weight (LBW) in rat offspring (*Rattus novergicus*) from pregnant mothers who are exposed to e-cigarette vapor. The weight gain of newborn mice given infused lemon water and exposed to e-cigarette vapor was P1: 4.301 grams, P2: 4.888 grams, P3: 5.435 grams. There was a significant increase in the treatment groups, namely P1, P2 and P3 when compared to the positive control group (KP) and the most optimal group was treatment group 2 (P2). Further research is needed by administering different doses of lemon water infusion with a smaller dose range so that it can increase the weight of newborn mice. Future research is expected to increase the sample size. Further research regarding side effects and toxicity effects at higher doses of lemon water infusion and/or e-cigarette vapor. Pregnant women should avoid exposure to e-cigarette vapor, both active and passive.

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