



The Effect of Kefir (Cow's Milk) Administration to Prevent Reduction of SOD (Superoxide Dismutase) Activity in Pregnant White Rats (*Rattus norvegicus*) Placenta with Cigarette Smoke Exposure

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ABSTRACT

*Free radicals are molecules that contain particles that have reactive unpaired electrons known as Reactive Oxygen Species (ROS). ROS can be formed through metabolic and enzymatic pathways, one source of free radicals is cigarette smoke. Toxic chemicals contained in cigarettes can trigger the formation of free radicals which cause biological oxidative damage. Oxidative damage occurs due to oxidants and antioxidants in the body, one of which is SOD (Superoxide Dismutase). SOD is an endogenous antioxidant that protects against cell damage by catalyzing superoxide radicals into hydrogen peroxide and oxygen. This study aimed to determine the effect of giving cow's milk kefir on preventing reduction in the SOD (Superoxide Dismutase) activity of the placenta of pregnant white rats (*Rattus norvegicus*) with exposure to cigarette smoke. This experimental study was using Post Test Only Control Group Design which was applied to 24 pregnant rats. The sample was divided into five groups; negative control (not exposed to cigarette smoke and no cow's milk kefir), positive control (exposed to cigarette smoke and no cow's milk kefir), and P1, P2, and P3 (exposed to cigarette smoke and administering 2.5 ml/kgBB of kefir; 5 ml/kgBB of kefir; 10 ml/kgBB of kefir). SOD activity was measured using spectrophotometry. One-way ANOVA test results monitoring several different significantly among the groups ($p=0.000$). The Post Hoc Test checks performed on P1, P2, and P3 can prevent the reduction SOD activity ($p=0.000$), and P3 can obtain SOD activity under normal conditions ($p=0.051$). There is a strong relationship ($r = 0.909$ and a value significantly <0.05). Kefir can affect SOD activity by 82.6%. The research conclusion shows that kefir can prevent a reduction in placental SOD activity against cigarette smoke.*

Keywords: Kefir, SOD (Superoxide Dismutase) Activity, Cigarette Smoke, Pregnant Rats (*Rattus norvegicus*)

ABSTRAK

Radikal bebas adalah molekul yang mengandung partikel dengan elektron reaktif tidak berpasangan, dikenal dengan Reactive Oxygen Species (ROS). ROS terbentuk melalui jalur metabolisme dan enzimatik, salah satu sumber radikal bebas terdapat pada asap rokok. Bahan kimia beracun yang terkandung dalam rokok dapat memicu terbentuknya radikal bebas yang menyebabkan kerusakan oksidatif biologis. Kerusakan oksidatif terjadi akibat oksidan dan antioksidan di dalam tubuh yang tidak seimbang, salah satunya SOD (Superoxide Dismutase). SOD adalah antioksidan endogen yang melindungi terhadap kerusakan sel dengan mengkatalisis radikal superoksida menjadi hidrogen peroksida dan oksigen. Penelitian ini bertujuan untuk mengetahui pengaruh pemberian kefir susu sapi terhadap pencegahan penurunan aktivitas SOD plasenta tikus putih (*Rattus norvegicus*) bunting dengan paparan asap rokok. Penelitian eksperimental ini menggunakan Post Test Only Control Group Design yang diterapkan pada 24 ekor tikus bunting. Sampel dibagi menjadi lima kelompok; kontrol negatif (tidak terpapar asap rokok dan tidak diberi kefir susu sapi), kontrol positif (dipapar asap rokok dan tidak diberi kefir susu sapi), serta P1, P2, dan P3 (dipapar asap rokok dan diberi kefir 2,5 ml/kgBB; 5 ml/kgBB kefir; 10 ml/kgBB kefir). Aktivitas SOD diukur menggunakan spektrofotometri. Hasil uji One Way ANOVA menunjukkan perbedaan secara signifikan antar kelompok ($p=0,000$). Pemeriksaan Post Hoc Test pada P1, P2, dan P3 dapat mencegah penurunan aktivitas SOD ($p=0,000$), dan P3 dapat memperoleh aktivitas SOD dalam kondisi normal ($p=0,051$). Terdapat hubungan kuat ($r = 0,909$ dan nilai signifikan $< 0,05$). Kefir dapat mempengaruhi aktivitas SOD sebesar 82,6%. Kesimpulan penelitian menunjukkan bahwa kefir dapat mencegah penurunan aktivitas SOD plasenta terhadap asap rokok.

Kata kunci: Kefir Susu Sapi, Aktivitas SOD (Superoxide Dismutase), Asap Rokok, Tikus (*Rattus norvegicus*) Bunting.

INTRODUCTION

Maternal Mortality Rate (MMR) is one indicator that is sensitive to the quality and accessibility of health service facilities. Based on the Indonesian Demographic and Health Survey (SDKI) in 2012, MMR (which relates to pregnancy, childbirth and postpartum) was 359 per 100,000 live births. In 2012 the IDHS again recorded a significant increase in MMR, namely from 228 to 359 maternal deaths per 100,000 live births (Department of Health of the Republic of Indonesia, 2012). In East Java, MMR achievements have tended to increase in the last five years. In 2012 it reached 97.43 per 100,000 live births (Department of Health of the Republic of Indonesia, 2012).

One thing that influences body health is environmental factors related to life behavior, namely smoking habits. Currently, smoking is not only done by men but also by women. Data from the Indonesian Ministry of Health's Health Research and Development Agency (2013) states that the smoking population aged 15 years and over has not decreased from 2007 to 2013. It increased from 34.2% in 2007 to 36.3% in 2013, 64, 9% of men and 2.1% of women still smoked cigarettes in 2013. 1.4% smokers aged 10-14 years, 9.9% of smokers in the non-working group, and 32.3% in the lowest ownership index quintile group.

Sucking or inhaling cigarette smoke is certainly dangerous for the body. Women who smoke or inhale cigarette smoke continuously during pregnancy will have a negative impact on the health of the mother and fetus, such as low birth weight, risk of spontaneous abortion, increased rate of premature birth, risk of disease in the placenta, increased risk of death of the newborn, increased risk of ectopic pregnancy, placenta previa, and congenital anomalies such as cleft lip (Liszewski et al., 2007).

Exposure to cigarette smoke is exposure to smoke inhaled by someone who is not a primary smoker (Passive Smoker). Cigarette smoke is more dangerous for passive smokers because the chemical content is higher than in active smokers (Zisovska, 2010), five times more carbon monoxide, four times more tar and nicotine (Simpson, 2009). Carbon monoxide can have a negative impact on the breathing of pregnant women so that the fetus will lack oxygen (Ross, 2006). Every time you inhale cigarette smoke, intentionally or not, you also inhale more than 4000 chemicals, 200 of which are toxic, such as CO (carbon monoxide) and nicotine (Sirajuddin et al, 2011).

Cigarette smoke can induce the formation of free radicals so that free radicals will increase. Physiologically, this will be anticipated by an endogenous enzyme, namely Superoxide Dismutase (SOD). SOD is the primary antioxidant in the body (intracellular). SOD indicates antioxidant status and is used as a bioindicator of oxidative stress conditions (Herdiani, 2016). SOD plays an important role as the first defense against free radicals by dismuting superoxide anions into hydrogen peroxide and oxygen (Marks *et al*, 2000). This activity of SOD has been found in the placenta (Zhang *et al.*, 2002).

If exposure to free radicals continues, the endogenous enzyme/SOD in the body is insufficient. This imbalance in the production of free radicals/ROS (Reactive Oxygen Species) and endogenous antioxidant status is called oxidative stress (Halliwell, 2006). In connection with the potential toxicity of free radical compounds, the body needs external antioxidants which function to neutralize and accelerate the degradation of free radical compounds to prevent damage to cell macromolecular components and help increase enzymatic antioxidants in the body.

Antioxidants from outside the body can be obtained by consuming foods or drinks that contain antioxidants. One of them is "Kefir". Research entitled "Antioxidative Activities of Kefir" states that kefir is a potential antioxidant that interacts directly with its various antioxidant activities which are responsible for oxidative damage and become a natural antioxidant supplement in the human diet (Anim, 2005).

Kefir also provides a more protective antioxidant effect compared to vitamin E in research conducted on experimental animals given carbon tetrachloride (CCl₄) (Güven & Gülmez, 2003). The use of kefir is recommended as an antioxidant, antimicrobial, antitumor and immunomodulator. Research continues to be carried out to produce developments in kefir products to preserve healthy consumers (Maria R. Prado, 2015).

Kefir, which has been tested as an additional antioxidant, has not been studied in pregnant women. Based on this description, researchers are interested in researching kefir which has been proven to be an antioxidant, are they able to increase SOD (Superoxide Dismutase) in white rat placenta (*The rat is a newborn*) as pregnant experimental animals with exposure to cigarette smoke. It is hoped that this research can prevent the negative impacts of free radicals, especially exposure to cigarette smoke during pregnancy.

METHOD

This research is research that uses a True Experimental design with a Post Test Only Control Group Design research design. In this study, the experimental animals will be divided into negative control groups (exposed to cigarette smoke and without cow's milk kefir), positive control (exposed to cigarette smoke without cow's milk kefir and treatment groups 1, 2, 3 (exposed to cigarette smoke and given cow's milk kefir with dose 2.5 ml/kgBW; 5 ml/kgBW; 10ml/kgBW).

The exposure to cigarette smoke and giving cow's milk kefir was carried out from the 5th to the 18th day of pregnancy. Measurement of placental SOD activity will be carried out on the 19th day of pregnancy. The assessment was carried out on a post-test by comparing SOD activity (Superoxide Dismutase) placenta in pregnant rats from control and treatment groups.

Inclusion Criteria

- a. Kind of rat *Rattus norvegicus strain Wistar* pregnant females.
- b. The rats are in good health, characterized by clean, non-shedding fur, good appetite, and normal activity.
- c. Rats must be at least 8 – 9 weeks old
- d. Body weight is around 130-160 grams

Exclusion Criteria

- a. Rats whose condition deteriorated and/or died during the study.
- b. Rats that give birth quickly (miscarriage or premature).

Trial Animal Maintenance

Experimental animals were maintained and adapted to the laboratory environment for seven days at constant room temperature. Experimental animals are kept in a plastic box measuring 31.5 x 23 x 9 cm which is covered with a wire lid and husks which will be replaced every Monday and Thursday. Each cage was occupied by 1 pregnant rat and fed 40g/day/head.

Rat mating time is carried out during the estrus phase which is characterized by the desire for copulation between female and male rats. The estrus phase can be stimulated using pheromone stimulation (Meziana, 2007). Then mix female rats and male rats in a 1:1 ratio. In the morning a vaginal plug examination will be carried out to see the first day of pregnancy (Kaufman, 1992). Rats that are pregnant are given a label (permanent board marker) on the tail, while those that are not pregnant are mixed again and seen the next day (Samsuria, 2009).

Giving Cow's Milk Kefir

Cow's milk kefir is given orally on the 5th to 18th day of pregnancy using a sonde. In the journal, it is said that cow's milk kefir can also be used for anti-apoptotic and radio-protective *activities* the result ($p < 0.05$) with a cow's milk kefir dose of 5ml/kgBW given to rats, so the researchers used doses of 2.5 ml/kgBW, 5 ml/kgBW, and 10ml/kgBW in the three treatment groups.

Exposure To Cigarette Smoke In Experimental Animals

Exposure to cigarette smoke was carried out on the 5th to 18th day of pregnancy after giving cow's milk kefir. Exposure to 1 cigarette/day for 7.5 minutes.

Placenta Recovery

On the 19th day of pregnancy, surgery was performed, the placenta was removed, and SOD activity (U/100 μ L) was measured in all rats in the control and treatment groups. Before the rats were dissected, they were sacrificed by injecting 0.1-0.2 cc of ketamine regularly intramuscular

SOD Measurement

After the placenta is removed, wash the placental tissue using PBS (*phosphatase*) buffered saline, PH 7.4 to remove blood and blood clots. Then weigh 200 mg of placenta for each group, and homogenize by chopping and finely grinding using a mortar.

Dilute with PBS to clean the placental organ from fat, erythrocytes, and other impurities. Then add PBS to the sample in a ratio of 1:1, collect in ependorf then vortex, cold centrifuge 4000rpm 4°C for 15 minutes to reduce the sediment. Take the supernatant, put it in a test tube then add the reagent EDTA 100 mm 200 μ L, NBT 25 units 100 μ L, Xanthine 25 mm 100 μ L and XO 1 unit 100 μ L, and add PBS to make 1 ml. Then incubate at 37°C for approximately 30 minutes, centrifuge 3000 rpm for 10 minutes at room temperature take a solution without colloids add distilled water to make 3 cc, and read the absorbance with a spectrophotometer with λ 580 nm.

Determination of SOD enzyme activity is carried out using a protocol that has the following theoretical basis: if xanthine is reacted with the xanthine oxidase enzyme, superoxide free radicals ($O_2^{\bullet-}$). Superoxide will reduce NBT (*NitroBlue Tetrazolium*).

RESULTS AND DISCUSSION

RESULTS

In this study, the total sample was divided into 5 groups, namely the negative control group (K-) was a group of pregnant rats that did not receive cow's milk kefir and exposure to cigarette smoke, the positive control group (K+) was a group of pregnant rats that only received exposure to cigarette smoke without cow's milk kefir, treatment group 1 (P1) is a group of pregnant rats that received cow's milk kefir at a dose of 2.5 ml/kgBW and exposure to cigarette smoke, treatment group 2 (P2) was a group of pregnant rats that received cow's milk kefir at a dose of 5 ml/kgBW and exposure to cigarette smoke, treatment group 3 (P3) was a group of pregnant rats that received cow's milk kefir at a dose of 10ml/kgBW and exposure to cigarette smoke.

Cow's milk kefir was given according to the dose per kg of body weight of the rat and cigarette smoke was exposed 1 day/cigarette for 7.5 minutes from day 5 to day 18 of pregnancy. On the 19th day of pregnancy, surgery was performed to take samples of the placenta and calculate placental SOD activity. The number of samples dissected and tested for placental SOD activity was 24 samples, in the negative control group, positive control, treatment 1, and treatment 2 each there were 5 samples, while in treatment group 3 there were 4 samples.

SOD activity measurement results (Superoxide Dismutase) can be seen in the following table and figure:

Group	(n)	SOD activity (U/100 μ L)
		Mean SOD Activity \pm Std. Deviation
K-	5	53,13 \pm 1,355
K+	5	23,63 \pm 2,141
P1	5	35,79 \pm 3,523
P2	5	43,12 \pm 1,932
P3	4	44,36 \pm 2,618

Table 1. Results of SOD Activities(Superoxide Dismutase) from Several Control and Treatment Groups

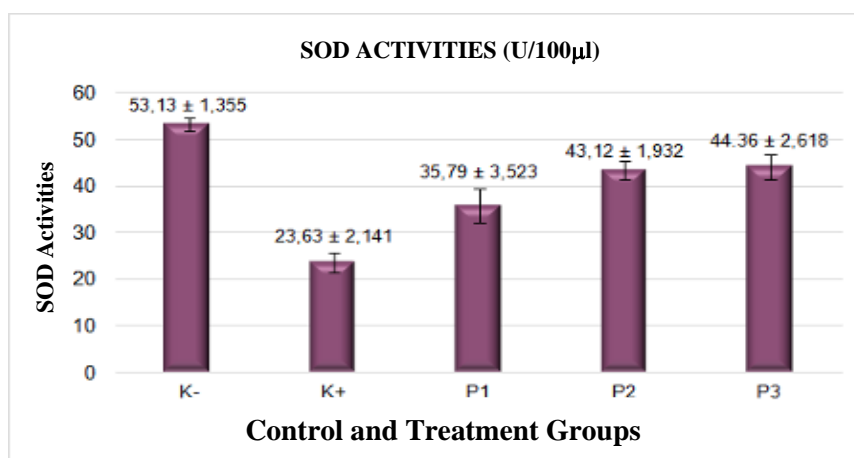


Figure 1. Results of SOD Activities(Superoxide Dismutase) from Several Control and Treatment Groups

Information:

- a. Negative Control Group (K-): Without exposure to cigarette smoke and cow's milk kefir
- b. Positive Control Group (K+): Exposed to cigarette smoke without cow's milk kefir
- c. Treatment Group 1 (P1): Exposed to cigarette smoke and given cow's milk kefir at a dose of 2.5 ml/kgBB
- d. Treatment Group 2 (P2): Exposed to cigarette smoke and given cow's milk kefir at a dose of 5 ml/kgBB
- e. Treatment Group 3 (P3): Exposed to cigarette smoke and given cow's milk kefir at a dose of 10ml/kgBB

Data analysis was carried out using the SPSS computer program (*Statistical Product and Service Solution*) 16.0 for Windows. The data analysis used is the method *One way Anova*. Conditions for using the method *One Way Anova* is that the data distribution must be normal in the normality test and the data variance must be the same in the homogeneity test. Based on the normality test using *Shapiro-Wilk* The significant value obtained in the five groups was above 0.05 ($p > 0.05$), meaning that the data distribution in this study was normal, while the homogeneity test used *Levene statistic* obtained a sig value of 0.359 ($p > 0.05$), meaning that the data variance between groups is the same. With that in mind, test it *One Way Anova* can be done.

From the results of statistical calculations using analytical methods *One Way Anova*, the result was a significant value of 0.000 ($p < 0.05$), with these results meaning that there was a minimal significant difference between two different groups after 14 days of administration of cigarette smoke and cow's milk kefir at different doses. Meanwhile, to find out which groups are different, statistical calculations can be carried out using analytical methods *After this* with test *Tukey HSD* with a significance level of 95% ($p < 0.05$). With the conclusion of the test results *Tukey HSD* is as follows:

1. The positive control group (K+) had a significantly lower difference in SOD activity values than the negative control group (K-) ($p=0.000$)
2. Groups P1, P2, and P3 all had significantly higher differences in SOD activity values compared to the positive control group (K+) ($p=0.000$)
3. Groups P1 and P2 all had significantly lower SOD activity values compared with a negative control group (K-) ($p=0.000$; $p=0.002$)
4. The P3 group did not have a significant difference in SOD activity values from the negative control group (K-) ($p=0.051$)
5. Group P1 did not have a significant difference in SOD activity values with group P2 ($p=0.13$)
6. The P3 group had a significant difference in SOD activity compared to the P1 group ($p=0.000$).
7. The P3 group did not have a significant difference in SOD activity with the P2 group ($p=0.493$)

From the results of statistical calculations using the correlation test Pearson The following results were obtained:

1. The correlation value is (r)=0.909, which means the correlation between giving cow's milk kefir and SOD activity is very strong.
2. The direction of the correlation is positive, meaning that the higher the cow's milk kefir, the higher the placental SOD activity of pregnant white rats.
3. P value = 0.000 ($p < 0.05$), which indicates a significant correlation between giving cow's milk kefir and SOD activity.

To find out the percentage of SOD activity that is influenced by cow's milk kefir, the R² regression test is used, the R² value is 0.826, if calculated R square x 100% = 82.6%, which means cow's milk kefir can influence placental SOD activity by 82.6%.

DISCUSSION

This research is an experimental study which aims to prove the effect of giving cow's milk kefir on preventing a decrease in the placental SOD (Superoxide Dismutase) activity of pregnant white rats with exposure to cigarette smoke. This study has 5 sample groups divided into control and treatment groups which have been measured in each group. Based on the measurement results of the negative control group (K-) or normal group, it was found that the average placental SOD activity was $53.13 \pm 1,355$ U/100 μ L. This negative control group had the highest average SOD activity compared to the other groups. This condition describes the normal condition of the body which does not receive any exposure from the outside, including free radicals due to cigarette smoke. Free radicals that enter the body have a natural antidote in the form of antioxidants, one of which is in the placental organ. The placental organ has natural protection against the dangers of free radicals, one of which is antioxidants, namely Cu/Zn-SOD and MnSOD which are part of Superoxide Dismutase in cells (Wresdiyati, 2006).

This SOD works by quickly giving hydrogen atoms to radical compounds so that they are more stable and inhibit the formation of radicals by breaking the chain reaction (polymerization). SOD works by catalyzing the dismutation reaction of the O₂ superoxide anion radical⁻ into H₂O₂, then assisted by the enzymes catalase and glutathione peroxidase to convert H₂O₂ into H₂O and O₂ (Winarsih, 2007). The results of measuring SOD activity in the positive control group (K+) were $23.63 \pm 2,141$ U/100 μ L. The average results of measuring SOD activity in the positive control group were the lowest compared to other groups, where this group was a group of pregnant rats that were exposed to free radicals in the form of cigarette smoke for 14 days. Based on the Tukey HSD test, a significant difference was found between the positive control group and negative control (p=0.000). These results illustrate that exposure to free radicals (cigarette smoke) can reduce placental SOD activity in pregnant rats. This decrease in SOD activity is due to the presence of toxic chemicals in cigarette smoke which can trigger the formation of free radicals, causing oxidative biological damage (Valavanidis, 2009). The content in cigarettes is very reactive and relatively stable which has the potential to stimulate cells to produce ROS (Nopya, 2013). ROS in cigarettes triggers the destruction of endogenous antioxidants (vitamins and enzymes) and reduces the body's defense effects by antioxidants (Valavanidis, 2009).

This can increase the risk of oxidative damage, which is not only caused by physiological increases in free oxygen levels during pregnancy but also by radicals due to cigarette smoke (Chelchowska et al., 2011). With the low molecular weight of ROS and the permeable nature of the placenta, it can easily pass through the placenta, which not only damages placental cells but will also have an impact on the fetus.

Based on the results of the Tukey HSD test in the treatment group, cow's milk kefir as an exogenous antioxidant was able to provide different results in preventing a decrease in placental SOD activity. This is proven by the significant comparison between the positive control group (K+) and treatment groups 1, 2, and 3 of p=0.000.

In groups P1 and P2 when compared with the positive control group (K+) there is a significant difference (p=0.000; p= 0.000) and when compared with the negative control group (K-) there is also a significant difference of (p=0.000; 0.002), this shows that in groups P1 and P2 with a dose of 2.5 ml/kgBW; 5 ml/kgBW can prevent a decrease in placental SOD activity but is not able to achieve SOD activity under normal conditions. The increased SOD activity in treatment groups 1 and 2

compared to the positive control group (K+) was because giving kefir to the treatment group was proven to be an additional antioxidant from outside the body which could prevent damage caused by free radicals in cigarette smoke. The lactic acid contained in kefir can form organic acids that are synergistic by providing H⁺ ions to free radicals so that it can increase the amount of primary antioxidant activity and antioxidant stability (Pratimasari, 2009).

In the treatment group 3 (P3) with a dose of 10ml/kgBW, a significant difference was found when compared with the positive control group (K+), namely $p=0.000$, but a non-significant difference was found when compared with the negative control group (K-) at $p=0.051$. Thus, a dose of 10ml/kgBB of cow's milk kefir as an exogenous antioxidant can prevent a decrease in SOD activity and can return SOD activity to normal conditions. The increase in antioxidant activity is due to the activity of yeast and lactic acid bacteria in kefir. Yeast can produce the enzyme vinyl phenol reductase. *Lactococcus* spp and *Streptococcus thermophilus* bacteria can express the activity of the antioxidant enzyme superoxide dismutase (SOD). The bacterial flora in lactic acid in kefir is homofermentative so it has a positive influence on superoxide anions (Kesenkaşa et al., 2011). The increase in antioxidant activity in this study means that the prevention of a decrease in SOD activity due to kefir by exposure to cigarette smoke as free radicals is also caused by the presence of protein and peptide derivatives that act on kefir.

And finally, the comparison of treatment group 3 (P3) with treatment group 1 (P1) had a significant difference of ($p=0.000$), and it was also proven that when compared with the negative control group (K-) there was no significant difference ($p= 0.051$), which means that this shows that P3 can reach normal conditions. Thus it can be seen that treatment group 3 (P3) 10ml/kgBW can be the optimal dose compared to treatment 1 (P1) 2.5 ml/kgBW and treatment 2 (P2) 5 ml/kgBW.

From these results, it can be concluded that exposure to free radicals due to smoking can reduce placental antioxidants as measured by placental SOD activity. Cow's milk kefir which contains antioxidants was proven in this study to be able to neutralize free radicals thereby supporting the prevention of decreased placental SOD activity and treatment group 3 (P3) can be called the treatment group with an optimal dose of 10ml/kgBB.

Other research states that kefir supplementation given to experimental animals using lead for six weeks of treatment can increase glutathione peroxidase and reduce malondyaldehyde (Ozcan and K, 2009). This supports the hypothesis that kefir can be a potential tool in controlling oxidative stress. Based on the results of the Pearson correlation test, it was found that the correlation value was (r) = 0.909 with a positive correlation direction and a significant p -value of $p= 0.000$ ($p<0.05$), meaning that there was a very strong relationship between giving cow's milk kefir and SOD activity. where the higher the dose of cow's milk kefir given, the higher the results of placental SOD activity and the lower the effects due to cigarette smoke.

Based on the results of the regression test using linear regression, the results were 82.6%, which means that placental SOD activity was influenced by cow's milk kefir by 82.6%.

This correlation and regression test confirms the research results that cow's milk kefir as an exogenous antioxidant in preventing a decrease in SOD (endogenous antioxidant) activity in the placenta of white rats exposed to cigarette smoke as free radicals has a very strong relationship and influences each other.

Thus, based on the facts and results of the data analysis study above, the hypothesis stating that giving cow's milk kefir can prevent a decrease in SOD (Superoxide Dismutase) activity in the placenta of pregnant white rats (*Rattus norvegicus*) exposed to cigarette smoke is proven.

LIMITATION OF THE STUDY

The limitation of this study is that it is not known whether the dose between treatment 2 (5 ml/kgBB) and treatment 3 (10 ml/kgBB) can achieve SOD activity under normal conditions. Apart from that, in this study, no control group was only given cow's milk kefir without cigarette smoke, so it still cannot be determined to what extent cow's milk kefir can influence SOD activity without the presence of free radicals and is safe for pregnancy.

CONCLUSIONS AND SUGGESTIONS

Based on the research conducted, it was concluded that giving cow's milk kefir could prevent a decrease in the placental SOD activity of white rats exposed to cigarette smoke and a dose of 10 ml/kgBW could be the optimal dose.

ETHICAL CONSIDERATIONS

Funding Statement

This article's author did not receive any financial support for its research, writing, or publication.

Conflict of Interest Declaration

The author declares that there are no prospective conflicts of interest in the writing or publication of this article.

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