

Effect of Vitamin E Against Levels Malondyaldehyde (MDA) and Estrogen Hormone Levels Rats (*Rattus Norvegicus*) Females Who Exposed to Cigarette Smoke.

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ABSTRACT

Indonesia is one of the countries with the greatest prevalence of smokers in the world. Exposure to cigarette smoke can lead to the physiological impact for women that can inhibit estrogen synthesis in the ovaries and increase levels of Malondyaldehyde (MDA). The purpose of this study to analyze the effect of vitamin E against MDA and estrogen rat (*Rattus norvegicus*) females were exposed to smoke. This research is an experimental design Post-Test Only Control Group. The total sample consisted of 30 rats females were divided into 5 groups: negative control group without treatment, the positive control group were given exposure to cigarette smoke 1 cigarettes / day and 3 treatment groups P1, P2 and P3 with vitamin E doses of multilevel: 100, 200 and 400 mg / kg / day and exposure to tobacco smoke 1 cigarettes / day. Research carried out for 21 days at Animal house and in the Biomedical Laboratory Faculty of Medicine, University of Andalas Padang. MDA and estrogen hormone levels were measured using ELISA method. Test of significance with One Way ANOVA followed by Bonferroni Multiple Comparisons types. The results showed significant differences ($p < 0.05$) MDA levels between the negative control group (0.53 ± 0.04) positive control group (0.67 ± 0.07). In the estrogen levels also differed significantly ($p < 0.05$) between the negative control group (0.27 ± 0.04) with P2 group (0.38 ± 0.07). In conclusion, there is no effect of vitamin E against MDA both in the negative control group and positive control group.

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1. Introduction

Cigarettes contain more than 4,000 chemicals of which 60 of them are carcinogenic. Cigarette smoke contains a mixture of toxins is very much a complex. Some of it is toxic free radicals (Pichandi, 2011). Free radical is an atom or molecule that has an unpaired electron arrangement that is highly unstable. To become stable, free radicals attack the cells to get their partner electrons and pass a chain reaction that causes extensive tissue damage (Haliwell, 2009).

At the molecular level, one cause of cell damage is oxidative stress due to product Reactive Oxygen Species (ROS) which is a derivative oxidizing compounds that are highly reactive oxygen made up of the free radicals and groups nonradikal. Oxidative stress can damage a variety of molecules including lipids, proteins and nucleic acids. Lipoproteins and membranes undergo membrane lipid peroxidation process, giving rise to a variety of products including short-chain aldehydes such as Malondyaldehyde (MDA). MDA high concentration indicates the oxidation process in the cell membrane. High antioxidant status is usually followed by decreased levels of MDA (Galano, 2015).

Malondyaldehyde (MDA) is an end product of lipid peroxidation, which is usually used as biomarkers of biological describes the degree of lipid peroxidation and oxidative stress metabolites result of lipid peroxidation by free radicals (Ayala, 2014).

Lipid peroxidation can lead to impaired synthesis and secretion of hypothalamic GnRH. This failure will cause failure of the pituitary to perform the synthesis and secretion of FSH and LH. If there is a disruption in hormones FSH and LH will not cause the egg cell is formed, the hormones estrogen and progesterone also would not be formed and decline. As one of the hormones that regulate the menstrual duties, estrogen levels in the body should be enough. Disturbances in metabolism will cause irregular menstruation (Beshay, 2013).

Vitamin E is one form of antioxidants that can neutralize free radicals before menyebabkan lipid peroxidation (Colombo, 2010). Vitamin E serves as a deterrent to oxidative stress and protect

cell membranes from radical attack bebas.7 the same study of vitamin E to prevent a chain reaction of lipid peroxidation in the cell membranes that prevent oxidative damage (Ryan, 2010).

2. Method

This research has been carried out for 21 days at Animal house and in the Biomedical Laboratory Faculty of Medicine, University of Andalas Padang. The total sample consisted of 30 rats females were divided into 5 groups: negative control group without treatment, the positive control group were given exposure to cigarette smoke 1 cigarettes / day and 3 treatment groups P1, P2 and P3 with vitamin E doses of multilevel: 100 mg / kg / day, 200 mg / kg / day and 400 mg / kg / day and exposure to tobacco smoke 1 cigarettes / day. The level of Malondyaldehyde (MDA) and the levels of the hormone estrogen which was measured using enzyme-linked immunosorbent assay (ELISA).

Data processing is performed by the method of editing, coding, entry and tabulating. Untuk look at the effect of vitamin E on levels Malondyaldehyde (MDA) and estrogen hormone levels tested using Shapiro Wilk normality then resumed One Way ANOVA to determine the differences in the test group used the Multiple Comparisons (post hoc test) Bonferroni types.

3. Results and Analysis

Table 1.

Normality Test The Effect of Vitamin E Against Malondyaldehyde levels (MDA) Rats (*Rattus norvegicus*) Females Exposed Smoke

MDA (nmol / ml) (Mean $\pm$ SD)	P
0.61 $\pm$ 0.08	0.53

From table 1 normality test results obtained Malondyaldehyde levels (MDA) $p > 0.05$, which means that the data Malondyaldehyde levels (MDA) normal distribution ($p = 0.53$).

Table 2.

Effect of Vitamin E Against Malondyaldehyde levels (MDA) (nmol / ml) Rats (*Rattus norvegicus*) Females In control group and treatment group (n = 25)

Group	N	MDA (nmol / ml) Mean $\pm$ SD	p
Negative control	5	0.53 $\pm$ 0.04	0.03
Positive control	5	0.67 $\pm$ 0.07	
treatment 1	5	0.63 $\pm$ 0.04	
treatment 2	5	0.58 $\pm$ 0.08	
treatment 3	5	0.62 $\pm$ 0.08	

In table 2 the mean levels obtained Malondyaldehyde (MDA) in the negative control and treatment groups showed no significant differences, whereas levels of Malondyaldehyde (MDA) in the negative control group is 0.53 ± 0.04 nmol / ml, positive control group is 0.67 ± 0.07 nmol / ml, group 1 was 0.63 ± 0.04 nmol / ml, group 2 is 0.58 ± 0.08 nmol / ml and 3 treatment groups is 0.62 ± 0.08 nmol / ml. In statistical tests found varying levels of Malondyaldehyde (MDA), which was significant between the control group and the treatment group $p < 0.05$.

Table 3.

Significance level Effect of Vitamin E Against Malondyaldehyde levels (MDA) Rats (*Rattus norvegicus*) Females Exposed Smoke in the control group and the treatment group

Group	KN	KP	P1	P2	P3
Negative control	-	0.03	0.23	1.00	0.34
Positive control	0.03	-	1.00	0.35	1.00
treatment 1	0.23	1.00	-	1.00	1.00
treatment 2	1.00	0.35	1.00	-	1.00
treatment 3	0.34	1.00	1.00	1.00	-

Table 3 shows that there is a significant difference between the negative control group against the positive control group where $p < 0.05$. These data indicate there is a difference with a given exposure to cigarette smoke only on the level of Malondyaldehyde (MDA) rats (*Rattus norvegicus*) females.

Table 4.Normality Test The Effect of Vitamin E Against Estrogen Levels Rats (*Rattus norvegicus*) Females Exposed Smoke

Estrogen Hormone levels (ng / L) (Mean ± SD)	P
0.34 ± 0.06	0.98

In Table 4 the results obtained estrogen normality test $p > 0.05$, which means that the data were normally distributed estrogen levels ($p = 0.98$).

Table 5.Effect of Vitamin E Against Estrogen Hormone levels (ng / L) Rats (*Rattus norvegicus*) Females in the control group and the treatment group (n = 25)

Group	N	Estrogen levels (ng / L) Mean ± SD	p
Negative control	5	0.27 ± 0.04	0.03
Positive control	5	0.31 ± 0.05	
treatment 1	5	0.34 ± 0.06	
treatment 2	5	0.38 ± 0.07	
treatment 3	5	0.37 ± 0.03	

In Table 5 obtained the mean levels of estrogen in the control and treatment groups showed no significant differences, where the levels of estrogen in the negative control group is 0.27 ± 0.04 ng / L, the positive control group was 0.31 ± 0.05 ng / L, the treatment group 1 was 0.34 ± 0.06 ng / L, the treatment group 2 is 0.38 ± 0.07 ng / L and 3 treatment groups, namely 0.37 ± 0.03 ng / L. In statistical tests found varying levels of estrogen were significantly between the control group and the treatment group $p < 0.05$.

Table 6.Significance level Effect of Vitamin E Against Malondyaldehyde levels (MDA) Rats (*Rattus norvegicus*) Females Exposed Smoke in the control group and the treatment group

Group	KN	KP	P1	P2	P3
Negative control	-	1.00	0.47	0.04	0.08
Positive control	1.00	-	1.00	0.67	1.00
treatment 1	0.47	1.00	-	1.00	1.00
treatment 2	0.05	0.67	1.00	-	1.00
treatment 3	0.08	1.00	1.00	1.00	-

In Table 6 shows that there are significant differences between the negative control group to the treatment group 2 where $p < 0.05$. These data indicate that administration of vitamin E against estrogen rat (*Rattus norvegicus*) females were exposed to smoke there is a difference when administered at doses of 2, but no significant difference if given the negative control group against the positive control group, the treatment group 1 and the 3 treatment groups.

3.1. Analysis

The results of this study indicate that there is a significant difference in the levels of Malondyaldehyde (MDA) in the positive control group (cigarettes) in rats (*Rattus norvegicus*) female with a value of $p < 0.03$. Increased levels of Malondyaldehyde (MDA) in the blood caused by elevated levels of lipid peroxidation caused by free radicals.

Exposure to cigarette smoke is one source of free radicals to the body. Therefore, if the body is often exposed to smoke then the free radicals in the body to increase much later lead to increased levels Malondyaldehyde (MDA) in the body. This is because very much cigarette smoke contains a mixture of complex toxins. Some of it is toxic free radicals (Pichandi, 2011).

High antioxidant status is usually followed by decreased levels of MDA (Galano 2015). Increased levels of Malondyaldehyde (MDA) by free radicals can be prevented by giving antioxidants. Antioxidants help neutralize free radicals and prevent cell damage that does not happen then the process of lipid peroxidation prevent Malondyaldehyde levels (MDA) increased.

This study is in line with research Yadav and Jaggi (2015), found that there are significant differences in the levels of Malondyaldehyde (MDA) smokers than non-smokers (Jaggi, 2015). Malondyaldehyde levels (MDA) in the blood could serve as biomarkers for assessing oxidative

stress, with rigorous assessment of the marker can be known pathological condition that occurs in a person's body (Zorawar, 2014).

Based on research Hartamto & Idris (2006) on the effects of smoke cigarettes on the cellular immunity female mouse strains using a randomized block design with four different types of treatments: the long exposure of cigarette smoke each 7, 14, 21 of the estrous cycle and the group without the exposure of cigarette smoke using blasttransformasi parameter T lymphocytes, find cellular immunity will be disrupted after smoke exposure 21 estrous cycle. Possible nicotine received by the rats exposed to 21 cycles is sufficient concentrations to effect a decrease in the proliferation of lymphocytes T. This means that substances contained in cigarette smoke can lower blasttransformasi T lymphocytes (Idris, 2006).

In the estrogen levels p value is 0.03. It was concluded there are significant difference in estrogen levels, especially in the treatment group 2 (200 mg) were given vitamin E in rats (*Rattus norvegicus*) females.

Vitamin E is one form of antioxidants that can neutralize free radicals before menyebabkan lipid peroxidation (Colombo, 2010). Vitamin E as a source of fat-soluble antioxidant and easily provide hydrogen from hydroxyl group (OH) on the ring structure to free radicals. The workings of vitamin E by finding, reacting, and damaging chain reaction of free radicals and prevent lipid peroxidation of unsaturated fatty acids in cell membranes and helps the oxidation of vitamin A and fertility (Rizvi, 2013).

Antioxidants are substances or molecules that can slow or prevent the damage caused by the oxidation of other molecules such as free radicals. In general, antioxidants as reducing agents (compound electron donors) prevent cell damage by preventing the formation of ROS, by stopping (deactivation, detoxification) of free radicals that have been formed before they damage more important component of body cells (Galano, 2015),

Rizvi et al, (2013) suggested that vitamin E serves as a deterrent to oxidative stress and protect cell membranes from free radical attack (Rizvi, 2013). In the same study of vitamin E to prevent a chain reaction of lipid peroxidation in the cell membranes that prevent oxidative damage (Ryan, 2010).

The research line is also carried out by Rahmawati et al, 2014 found that there were significant differences in the administration of α -tocopherol various doses (100 mg, 200 mg and 400 mg) in preventing oxidative damage and loss of ovarian function due to exposure to tobacco smoke is characterized by decreased levels of MDA, increased primary and secondary follicles, as well as the hormone estradiol, hereinafter also increase the thickness of the endometrium (Rahmawati, 2014).

Based on research Duran et al, (2015) found that the content in cigarette smoke can inhibit aromatase activity in granulosa cell formation, it is considered as one of the mechanisms underlying infertility among women exposed to secondhand smoke. Vitamin E, the bonding of free radicals in the early stages protecting cell membranes. Vitamin E plays an important role in the function and structure of the membrane, has the property to protect the unsaturated fatty acids in cell membranes. Therefore, the antioxidant vitamin E supplements had a curative effect (Duran, 2015).

4. Conclusion

Conclusion exposure to cigarette smoke significantly increases the levels of Malondyaldehyde (MDA) and vitamin E significantly increase the levels of estrogen in rats (*Rattus norvegicus*) females.

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