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Effect Of Giving Avocado (Persea americana M.) Juice on MDA and TNF-α Levels

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ABSTRACT

A Cigarette is one of the free oxygen radicals that interfere with a free radical's balance and antioxidant in the body. It involves oxidative stress. Oxidative stress involves a failure system that triggers an inflammation response in endothelial class blood vessels that have pinned exiting the inflammation reaction in the form of cytokines, especially TNF-α. It functioned to know the effect of awarding avocado juice to MDA and TNF-α. This research used the experimental laboratory method that approached *a post-test-only control group design*. The subject of this research used 20 Wister rats, which kind of inclusion. It is shared in 4 sections as random services. Such as K1, K2, K3 and K4. K1 has given a standard weft. It has been done without a smoking display. Meanwhile, K2 has given a standard weft that has used a smoking display for 14 days. K3 and K4 have been given avocado juice doses. It was as much as 2,7g/200g weight/day and 54g/200g weight/day. On the fifth day, the specimen has taken the blood. It could know the results of MDA and Tnf-a. The data analysis used the normality test by *Shapiro Wilk,* homogeneity, *Levene,* and *one-way ANOVA* test. The result of this research has the highest rate. The highest rate was K2. It could be proven for *the One-Way Anova* MDA and Tnf rate test. It showed a significant difference between categories, which is p=0,000 scores. The conclusion of this research has been giving avocado juice with 2,7g/200g weight/day and 5,4g/200g weight/day. Then, it could reduce the MDA and Tnf-a rate on the Wistar rat. It has been since the smoking display.

KEYWORDS

Avocado Juice; MDA; TNF; Smoking display

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Introduction

Cigarettes are a source of exogenous free radicals that disrupt the body's balance of free radicals and antioxidants. This causes oxidative stress (Fitria, Triandhini R, Mangimbulude JC, Karwur FF, 2013). In addition, cigarette smoke is also known to contain various toxic compounds such as nicotine compounds, tar, carbon monoxide, carbon dioxide, PAHs (Polynuclear Aromatic Hydrocarbons), and hydrogen peroxide. The harmful content of cigarettes is inhaled into the respiratory tract and then enters the lungs. ROS reactivity will damage DNA, proteins, and lipids that makeup cells.

Smoking can increase lipid peroxidation, induce antioxidant enzyme activity as a self-defence mechanism, and disturb the balance between oxidation and antioxidants (Susanti et al., 2017). Free radicals can cause increased lipid peroxidation, which then undergoes decomposition into Malondialdehyde (MDA) in the blood. MDA is a marker of cell damage caused by free radicals (Inoue M, 2001). Research conducted by Somwanshi et al. (2013) increased MDA levels due to exposure to cigarette smoke (Somwanshi et al., 2013). Reactive oxygen species (ROS) is an umbrella term for an array of derivatives of molecular oxygen that occur as a normal attribute of aerobic life. Increased ROS and lipid peroxidation increased MDA levels as a biomarker of oxidative stress found in smoker's blood serum (Kashinakunti et al., 2011).

Oxidative stress results in tissue damage, which triggers an inflammatory reaction in the endothelial cells of blood vessels, characterized by the release of inflammatory mediators in the form of cytokines, especially TNF-α (Rahman D, 2018). The inflammatory response through NF-KB regulation increases the number of leukocytes, especially macrophages, to synthesize TNF-α in maintaining the immune system (Fajri et al., 2015). Research conducted by Kusumastuti et al. (2015) found that the average TNF-α level, as measured by the ELISA method in rats exposed to cigarette smoke, was higher when compared to the group not exposed to cigarette smoke, namely 69.1 pg/ml in the group exposed to cigarette smoke and 36.4 pg/ml in mice not exposed to cigarette smoke. Research conducted by Oktaviana (2018) revealed that exposure to cigarette smoke caused an increase in TNF-α levels.

The primary resistance to oxidative stress can be achieved by providing antioxidants. *Antioxidants* are compounds that protect tissues from damage caused by oxidation. Antioxidants are needed to avoid increasing MDA levels due to exposure to cigarette smoke (Rahayu S, and Tjitraresmi A, 2016). Avocados contain antioxidant compounds that can protect the body from oxidative stress (Sutrisna et al., 2015). The content that is typical of this avocado is a source of vitamin B complex; besides that, it also contains vitamin A, vitamin C, and vitamin E, in addition to fat, protein, carbohydrates, minerals, tannins, and other compounds (Purnamayati L, 2008). Flavonoids, another ingredient in avocados, have potent antioxidant activity ((Sutrisna et al., 2015)). High levels of flavonoid compounds

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can be used as antioxidants, anti-inflammatories, anti-cancer, and anti-hypertension (Arukwe et al., 2012). Research conducted by Yuniastuti et al. (2015) showed that administering avocado juice (Persea americana M.) can reduce plasma MDA levels. Based on the above background, further research is needed regarding the effect of avocado juice on MDA and TNF-α levels in rats exposed to cigarette smoke.

Literature review

Malondialdehyde (MDA) is a metabolite resulting from lipid peroxidation by free radicals (Asni E et al., 2009). MDA is a dialdehyde compound that is the end product of lipid peroxidation in the body. This compound has three carbon chains with the molecular formula C3H4O2. MDA is also a decomposition product of amino acids, complex carbohydrates, pentoses, and hexoses. In addition, MDA is also a product produced by free radicals through ionization reactions in the body and a by-product of prostaglandin biosynthesis, which is the end product of membrane lipid oxidation. In addition, MDA is also a metabolite of cell components produced by free radicals. Therefore, a high MDA concentration indicates an oxidation process in the cell membrane. High antioxidant status is usually followed by decreased MDA levels (Winarsi H, 2007). Among the oxidative stress biomarkers most frequently used as laboratory parameters is malondialdehyde. MDA can be found in plasma, serum, and urine. Lipid peroxidation and cellular damage are indications of oxidative stress (Situmorang N, Zulham, 2020).

Malondialdehyde (MDA) can be formed when hydroxyl free radicals such as Reactive Oxygen Species (ROS) react with fatty acid components of cell membranes to cause a chain reaction known as lipid peroxidation. Lipid peroxidation will cause the fatty acid chains to break down into various toxic compounds and cause damage to cell membranes (Yunus M, 2001). A chain reaction occurs, which produces several lipid radicals and compounds that are highly cytotoxic to the endothelium. These lipid radicals react with free transition metals in the blood, such as Fe2+ and Cu2+, to produce toxic aldehydes, one of which is MDA. Eliminating MDA from the circulation with the help of the enzyme aldehyde dehydrogenase and thiolation in the liver occurs within 2 hours in rats. However, 10-30% is attached semi-permanently to protein and eliminated within 12 hours. The toxicity of MDA is increased due to its high reactivity, especially to proteins and DNA (Winarsi H, 2007).

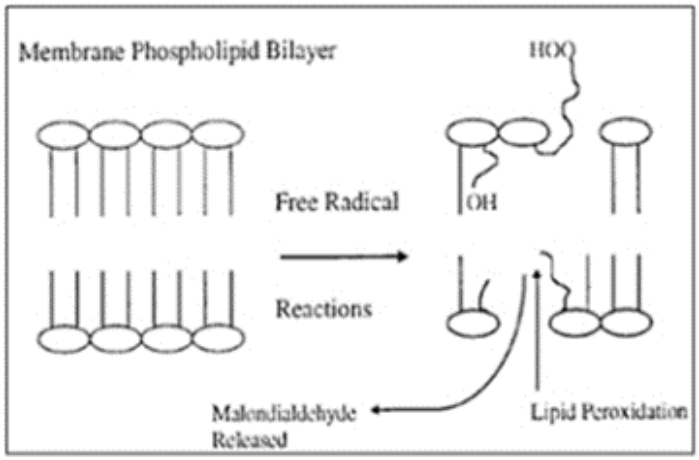

Figure 1. MDA Formation Mechanism (Marciniak A, et al, 2009)

MDA levels were measured using the TBARS (Thiobarbituric Acid Reactive Substance) method. The process of lipid peroxidation mediated by free radicals produces MDA compounds (Nayanatara AK et al., 2017). The working principle of MDA measurement is the reaction of a single molecule of MDA with two molecules of thiobarbituric acid (TBA) to form a pink colour measured at a spectrophotometer with a wavelength of 532 nm (Wahdaningsih S, and Untari EK, 2016).

Tumor necrosis factor alpha (TNF-α) is a cytokine that supports the occurrence of the inflammatory process. Cytokines in the body are proteins that function as regulators of giving information between cells by driving immune reactivity, specific and nonspecific. TNF-α is a cytokine produced by the activation of macrophages and monocytes besides that TNF-α is also produced by the activation of several other cells, such as T cells, mast cells, B cells, and fibroblasts (Abbas A et al., 2016).

Tumor necrosis factor alpha (TNF-α) has a role as a growth factor in B cells and maturation processes in dendritic cells, whereas, in T cells, TNF-α acts as a stimulus for apoptosis. TNF-α regulates negative TCR (T cell receptor) against autoreactivity by T cells and induces apoptosis in T cells in peripheral blood. TNF-α can also stimulate anti-apoptotic molecules and activate NF-Kb against apoptosis mediation. TNF-α activates secondary inflammation, which is characterized by necrosis and tissue damage so that it can affect immunity (Wu Y, and Zhou BP, 2010).

Many studies have found that the inflammatory response of the lung to exposure to a gas or cigarette smoke is characterized by an increase in the number of neutrophils, macrophages, and T lymphocytes, which CD8+ dominates, increased concentrations of proinflammatory cytokines such as leukotrienes B4, IL-8, TNF-α and evidence that oxidative stress is caused by inhaled cigarette smoke or activated by inflammatory cells. An increased number of T lymphocytes dominated by CD8+ is found in lung tissue and paratracheal lymph nodes (Agustı A, 2007).

Avocado (Persea americana M.) is an important tropical fruit source of lipophilic phytochemicals such as monounsaturated fatty acids, carotenoids, vitamin E, and sterols. Avocados contain bioactive molecules that protect human body cells against free radicals. Phytochemical analysis showed the presence of alkaloids, terpenoids, saponins, tannins, phenolics, and flavonoids in avocados (Marciniak et al., 2009).

Cigarette smoke has a high exogenous oxidant content for the body. The mechanism of increasing oxidant compounds in the body caused by cigarette smoke includes: 1) through direct exposure to oxidants found in cigarette smoke in the tar and gas phases, 2) Indirectly this cigarette smoke activates macrophages and neutrophils which will release endogenous oxidant compounds, and 3) Endogenous oxygen radical compounds that are formed physiologically through respiratory chain reactions in mitochondria (Ambrose et al. RS, 2004). Free radical levels can lead to the formation of oxidative stress conditions and stimulate lipid peroxidation in cell membranes, which will produce Malondialdehyde (MDA). MDA is used as a biomarker of free radical levels in the body. The negative impact caused by free radicals can be neutralized by antioxidants in the body (Kefer JC et al., 2009).

Method

The type of research used is experimental research. The research design was the Post Test Only Control Group on experimental male Wistar Rats. Samples were taken from 20 male rats weighing 150-200 grams and eight weeks old. The variables used are the independent variable: avocado juice, the dependent variable: levels of SOD and interleukin-6; and the precondition variable: cigarette smoke.

This type of research was laboratory experimental with a post-test-only control group design approach using experimental male Wistar rats exposed to cigarette smoke. The aim was to determine MDA and TNF-α levels in the control and treatment groups after being given avocado juice. The study population was male Wistar rats aged eight weeks, weighing 150-200 grams, obtained from the Laboratory of the Center for Food and Nutrition Studies, UGM Yogyakarta. Mice were maintained with standard Comfeed AD II feed and drinking water in the form of plain water. The maintenance room temperature ranges from 28–30 ^{0}C with adequate ventilation and room. Mice were then adapted for seven days before being given treatment.

Instruments

Research material: 1) Giving Material: Avocado Juice (Persea americana M.). 2) Ingredients for Making Clove Cigarette Smoke. 3) MDA Examination Materials: Rat serum, 0.37% TBA solution in 0.25 N HCl, and 15% TCA solution. Research equipment: a) Mouse cages with feed containers with sizes L: 40 cm, W: 30 cm, H: 30cm. b) Rat scales "Nigushi Scale." c) Small scissors. d) Razor blade. e) Oral probe. f) Gloves. g) Counter cotton. h) Drop pipette. i) Eppendorf tube. j) cuvette. k) Waterbath with a temperature of 95oC. l) Spectrophotometer. m) Centrifuge. n) Micropipette. o) ELISA readers. p) Digital camera. and q) Glass box for exposure to cigarette smoke.

Data analysis

Data on average levels of MDA and TNF-α levels are presented descriptively in the form of tables and graphs. Then, the data were tested for normality with the Shapiro-Wilk test and the data homogeneity test with the Levene test. The distribution of data on MDA levels and TNF-α levels were normal and homogeneous, so it was continued with the parametric test One Way Anova test, which was obtained with a p-value <0.05 so that it was continued with the posthoc test with the Tukey test.

Results

This study used a sample of 20 male Wistar rats, divided into four groups, each consisting of 5 male Wistar rats, namely one control group and three treatment groups. Group 1 is the control group with standard feed without exposure to cigarette smoke; Group 2 is the treatment group with standard feed given exposure to cigarette smoke for 14 days; Group 3 is the treatment group given standard feed and avocado juice with a dose of 2.7 gram/200g BW/day exposed to cigarette smoke for 14 days, and group 4, namely the treatment group was given standard feed and given avocado juice at a dose of 5.4 g/200g BW/day which was given exposure to cigarette smoke for 14 days. Research on giving avocado juice (Persea Americana M) on MDA levels and TNF-α levels in male Wistar rats exposed to cigarette smoke for 14 days.

Results of Measurement Analysis of MDA and TNF-α

MDA and TNF-α levels were measured in male Wistar rats exposed to cigarette smoke for 14 days. The research obtained the measurement results presented in Table 1.

Table 1. Results of MDA and TNF-α measurements

Groups		MDA	Mean ± SD	TNF	Mean ± SD
K1	1	1.65	1.88 ± 0.20	6.21	5.58 ± 0.31
	2	1.89		5.52	
	3	2.02		6.01	
	4	2.14		6.31	
	5	1.71		5.82	
K2	1	9.35	9.90± 0.42	18.58	18.89 ± 0.28
	2	9.6		19.07	
	3	10.34		18.87	
	4	9.97		19.27	
	5	10.28		18.67	
K3	1	5.19	4.85 ± 0.26	10.86	10.92 + 0.30
	2	4.57		11.36	
	3	5.06		10.56	
	4	4.81		11.06	
	5	4.63		10.76	
K4	1	3.14	3.00 ± 0.34	8.29	7.34 ± 0.48
	2	2.89		7.89	
	3	2.45		7.6	
	4	3.32		8.59	
	5	3.2		7.4	
One Way Anova		0.000			0.000

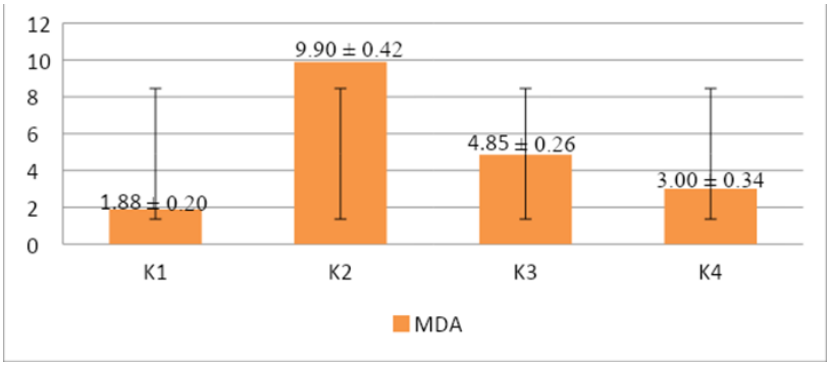

Figure 1. Graph of the average value of MDA levels in each group
MDA levels

Figure 1. shows the average and standard deviation of MDA levels in Wistar rats from the four groups. the control group K1 had an average MDA of 1.88 ± 0.20, the K2 treatment group had an average MDA of 9.90 ± 0.42, the K3 treatment group had an average -average MDA 4.85 ± 0.26. The K4 treatment group had an average MDA of 3.00 ± 0.34.

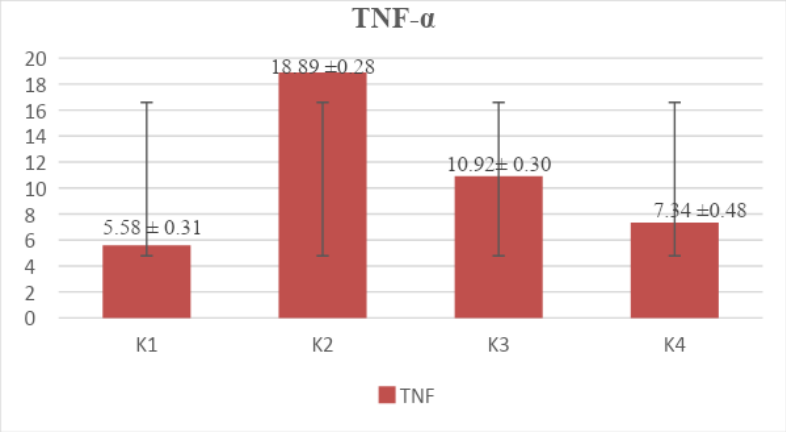

Figure 2. Graph of the average value of TNF-α levels in each group

TNF-α levels

Figure 2. informs the average and standard deviation of TNF-α levels in Wistar rats from the four groups the control group K1 has an average TNF-α 5.58 ± 0.31, the K2 treatment group has an average TNF-α 18.89 ± 0.28, the K3 treatment group had an average TNF-α of 10.92 + 0.30, and the K4 treatment group had an average TNF-α of 7.34 ± 0.48. Based on the descriptive analysis of the four groups in this study, it can be seen that the K2 treatment group, i.e. giving cigarette smoke without giving avocado juice (Persea americana M), has the highest levels of MDA and TNF-α. In contrast, the control group K1, without giving cigarette smoke, has an average of The lowest MDA and TNF-α, which means that free radicals are formed in the body very slowly and slowly under normal conditions.

Normality test

The normality test for MDA and TNF-α levels in rats exposed to cigarette smoke by giving avocado juice (Persea americana M.) can be seen in the following table:

Table 2. Normality Test (Shapiro-Wilk)

MDA Level	P	TNF-α Level	P
Group 1	0.727	Group 1	0.802
Group 2	0.526	Group 2	0.794
Group 3	0.554	Group 3	0.940
Group 4	0.382	Group 4	0.816

Based on Table 2. it can be seen that the normality test of Shapiro Wilk found that the levels of Malondialdehyde (MDA) and Tumor Necrosis Factor Alpha (TNF-α) with a significance value greater than 0.05 p> 0.05, so this can be interpreted that the data distribution is declared normal.

Homogeneity test

Homogeneity test for MDA and TNF-α levels in Wistar rats exposed to cigarettes based on avocado juice obtained the following results:

Table 3. Homogeneity Test

Level	P
MDA	0.283
TNF- α	0.306

Based on Table 3. it can be seen that the homogeneity test for MDA and TNF-α levels on the effect of giving avocado juice (Persea americana M.) exposed to cigarette smoke shows that the sig value on MDA and TNF-α levels has a value greater than p-value 0, 05, it can be concluded that the data is stated to have a homogeneous variety.

One Way Anova test

Testing the effect of giving avocado juice (Persea americana M.) on MDA and TNF-α levels in rats exposed to cigarette smoke can be seen in the following table:

Table 4. One Way Anova Different Test Results

Kadar	P
MDA	0.000
TNF- α	0.000

Table 4 informs that the different tests of the effect of giving avocado juice (Persea americana M.) on MDA

and TNF-α levels in rats exposed to cigarette smoke produced F test statistics of 604,016 for MDA and 1261,114 for TNF-α during the sig. 0.000. So based on the ANOVA test, it was found that for MDA and TNF-α levels with a sig value of 0.000, which means that there is an effect of giving avocado juice (Persea americana M) on MDA levels and TNF-α levels.

One Way Anova test

Table 5. Results of Analysis of MDA Levels with Post Hoc Tukey Test

	K2	K3	K4
K1	0,000	0,000	0,000
K2		0,000	0,000
K3			0,000

Based on Table 5, it was found that there were significant differences between each group in MDA levels (P <0.05). these differences are found in K1 and K2 with a value of 0.000, K1 and K3 with a value of 0.000, K1 and K4 with a value of 0.000. In addition, K2 with K3 with a value of 0.000, K2 with K4 with a value of 0.000, and K3 with K4 with a value of 0.000. From these values, it can be seen that each group has a significant difference in MDA levels.

Table 6. Results of Analysis of TNF Levels with Post Hoc Tukey Test

	K2	K3	K4
K1	0,000	0,000	0,000
K2		0,000	0,000
K3			0,000

Based on Table 6 above, it was found that there was a significant difference between each group in TNF-α levels (P <0.05). these differences are found in K1 and K2 with a value of 0.000, K1 and K3 with a value of 0.000, K1 and K4 with a value of 0.000. In addition, K2 with K3 with a value of 0.000, K2 with K4 with a value of 0.000, and K3 with K4 with a value of 0.000. From these values, it can be seen that each group has a significant difference in TNF-α levels.

Discussion

This study aims to determine the effect of avocado juice (Persea et al.) on MDA and TNF-α levels in male Wistar rats exposed to cigarette smoke within 14 days. Twenty male Wistar rats that met the inclusion criteria were divided into four groups: the control group (K1), without cigarette smoke. At the same time, there were three treatment groups, namely the treated group with standard feed given exposure to cigarette smoke for 14 days (K2), the group treated was given standard feed and given avocado juice at a dose of 2.7 gram/200g BW/day and given exposure to cigarette smoke for 14 days (K3), the treatment group was given standard feed and given avocado juice at a dose of 5.4 gram/200g BW/day and given exposure to cigarette smoke for 14 days (K4).

In this study, MDA levels were measured in the treatment group to determine MDA levels in the treated conditions and compared with the control group. Based on descriptive analysis, the average MDA level in the control group K1 ranged from 1.88 ± 0.20, while the treatment group without avocado juice K2 ranged from 9.90 ± 0.42. In contrast, the K3 group had 4.85 ± 0.26, and K4 had 3.00 ± 0.34, namely the treatment groups given cigarette smoke and administration of avocado juice (Persea americana M.) with different doses. The results of the four groups showed that the highest MDA level was the treatment group giving cigarette smoke (K2) without giving avocado juice; the data showed that giving cigarette smoke as a form of treatment had an impact on increasing free radicals in the body of Wistar rats which indicated MDA levels as a marker of stress. Oxidative. Cigarette smoke contains chemical compounds that are toxic or carcinogenic (Wulandari, Erni, 2016).

We usually think of these compounds as free radicals (reactive oxygen species/ROS), which results in smoking or exposure to cigarette smoke, which can be one of the factors that cause an increase in free radical levels in the body, apart from the production of free radicals themselves naturally by the body. Under normal conditions, these free radicals can be removed, captured, cleaned, and retained for their formation by converting them into a radical compound with a more stable form so that it does not reach the formation of a chain reaction by attacking other compounds (Adwas A et al., 2019). However, exposure to free radicals is often too high due to human activities such as exposure to chemicals, for example, smoking, both intentionally (active smokers) and not (passive smokers), and high physical activity (Harun Iriyanti et al., 2017). Conditions where there is an imbalance between the concentration of free radicals and antioxidants in the body can produce oxidative stress (Burlakova et al., 2010).

Based on the study results, group K2 had the highest average MDA of 9.90 ± 0.42; this was due to high exposure to free radicals (within 14 days) not accompanied by the assistance of exogenous antioxidant intake. This shows that the body's defence mechanisms cannot compensate for exposure to free radicals because of their high concentration, and the body falls into a state of oxidative stress. In addition to MDA levels, this study also measured TNF-α levels. The results were obtained in the control group K1 5.58 ± 0.31, the treatment group without avocado juice K2 18.89 ± 0.28, the treatment group with avocado juice, different doses, namely K3 10.92 + 0.30, and K4 7.34 ± 0.48. The research resulted in the highest TNF-α levels in the treatment group without giving avocado juice (K2); this was due to continuous exposure to free radicals for 14 days, not accompanied by giving antioxidant therapy so that no one caught free radicals from cigarette smoke.

Giving cigarette smoke works by causing the movement of macrophages, neutrophils, and T lymphocytes into the respiratory tract and triggering the activation of various inflammatory mediators and chemotactic factors, namely TNF-α, IL-6, IL-8, MCP-1, leukotriene LTB4, ROS, and secretion of proteolytic enzymes such as MMP-9 and MMP-12 (Chen L et al., 2018). TNF-α is secreted by macrophages activated by cigarette smoke through the classic MAPK pathway, which meditates the formation of proteases and inflammatory cytokines so that exposure to cigarette smoke can cause TNF-α activation and initiate an inflammatory response10. Mitogen-activated protein kinase (MAPK) is a highly conserved serine and threonine protein kinase in eukaryotes and is involved in signal transduction that regulate cellular physiological and pathophysiological responses. When free radicals (cigarette smoke) produce ROS (Reactive Oxygenated Species), an imbalance occurs between the number of free radicals and endogenous antioxidants, resulting in oxidative instability (stress). Oxidative stress causes excessive lipid peroxidation and results in increased MDA levels.

The K3 and K4 treatment groups, namely the groups that were given treatment but were given avocado juice (Persea americana M.) with different doses, had MDA and TNF-α levels between the control group (K1) and the treatment group without avocado juice (K2), this was due to Avocado juice contains vitamin A, vitamin B, vitamin C and vitamin E, fat, carbohydrates, folic acid and protein, where vitamin C is a water-soluble antioxidant, where these antioxidants can react with free radicals.

Another content of flavonoids has potent activity against antioxidants so that they can protect against toxic substances in an organ and prevent cell damage. It can be concluded that there is an effect of giving avocado juice on MDA and TNF-α levels. The higher the dose of avocado juice given to rats, the lower the MDA and TNF-α levels, with a dose of 200 mg/kg BW avocado juice can reduce MDA and TNF-α levels. Cigarette smoke produces large amounts of ROS (Reactive Oxygenated Species), which activate intracellular signals in endothelial cells that trigger gene activation and the formation of inflammatory mediators resulting in inflammatory cytokines. These inflammatory cytokines are also caused by activating macrophages and other molecules NF-kB and phosphorylation of IkB, which increases TNF-α levels. There are several limitations in this study, including the need to examine levels of Malondialdehyde (MDA) in the histology of lung tissue as a marker of oxidative stress due to exposure to cigarette smoke, levels of avocado juice (Persea americana M.) in the body of rats were not previously measured, and research is needed—more about the different types of cigarettes in male Wistar rats exposed to cigarette smoke.

Conclusion

Based on the results of the above study, it can be concluded that the administration of avocado juice (Persea americana M.) affected MDA levels in male Wistar rats exposed to cigarette smoke for 14 days. In addition, the administration of avocado juice (Persea americana M.) also affected TNF-α levels in male Wistar rats exposed to cigarette smoke for 14 days.

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