

The Effect of Ethanolic Extract of Katuk Leaf (*Sauropus Androgynus*) on Malondialdehyde (MDA) Levels in Ovalbumin-Induced Atopic Dermatitis Model Mice

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Abstract

Consensus guidelines have been developed to provide concise, evidence-based recommendations for general practitioners and dermatologists in the Asia-Pacific region regarding the management of pediatric and adult AD. Previous studies have reported differences in MDA levels between AD and non-AD patients, suggesting the potential role of oral antioxidants in reducing MDA levels. Antioxidants are present in many traditional medicinal plants used in Indonesia, including *katuk* leaves. This study aimed to analyze the effect of the ethanolic extract of *katuk* leaves (*Sauropus androgynus*) on MDA levels in a mouse model of atopic dermatitis induced by ovalbumin. An experimental method was applied using 36 mice divided into treatment groups given *katuk* leaf extract at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg. Plasma and skin tissue MDA levels were assessed using the Wills method. Data were analyzed with One-Way ANOVA and the Kruskal-Wallis test. The results showed that plasma MDA levels in AD model mice (570.5 nmol/L) and tissue MDA levels (1727 nmol/g) were higher than in non-AD controls. However, statistical analysis revealed p-values >0.05 for both plasma and tissue MDA, indicating no significant differences between groups.

Keywords: Atopic Dermatitis (AD); Malondialdehyde (MDA); Antioxidants; Ethanol Extract

INTRODUCTION

Atopic dermatitis (*DA*) is an inflammatory skin disease that manifests as chronic dermatitis, characterized by reactivity, itching, and its occurrence on specific parts of the body (Djuanda et al., 2018). The prevalence of *DA* in Southeast Asia varies from 1.1% among children aged 13–14 years in Indonesia to 17.9% at age 12 years in Singapore (Herwanto and Hutomo, 2016). Current consensus guidelines have been developed to provide recommendations based on clear evidence and clinical experience, directed at general practitioners and dermatologists in the Asia-Pacific region regarding the management of both pediatric and adult *DA* (Rubel et al., 2013). The countries involved in the formulation of these guidelines include Australia, Hong Kong, India, Indonesia, Malaysia, the Philippines, Singapore, and Taiwan (Rubel et al., 2013).

The clinical symptoms and disease course of *DA* vary widely, with persistent itching being one of the most bothersome manifestations (Ayuningati et al., 2018). Until now, the etiology of *DA* has been considered multifactorial, but its exact pathogenesis is still under investigation (Kouli, Torsney, & Kuan, 2018). Researchers have focused on fields such as genetics as well as external and internal influences, including disruption of the skin barrier (Djuanda et al., 2018). Causative factors of *DA* involve both genetic predisposition and

environmental influences, such as impaired skin function, infections, and stress (Ayuningati et al., 2018).

Malondialdehyde (MDA) is a dialdehyde compound and the final product of lipid peroxidation in the body, produced through both enzymatic and non-enzymatic processes (Całyniuk et al., 2016; Mohideen et al., 2021; Simsek, Durmus, Yildiz, & Ozcelik, 2018). The role of Reactive Oxygen Species (ROS) has been explored in eczema and other skin diseases, but their role in DA has been less clearly investigated (Ayuningati et al., 2018). Oxidative stress occurs when the production of free radicals exceeds the antioxidant capacity of cellular defenses (Sinaga, 2016). Common biomarkers of oxidative stress used in laboratory parameters include lipid peroxidation and protein carbonylation (Ayuningati et al., 2018).

Research comparing MDA levels in patients with DA and non-DA groups conducted by Ayuningati et al. (2018) showed significantly higher MDA levels in DA patients. Trimbake (2013) reported that administering antioxidants such as vitamin C, vitamin E, and carotenoids derived from fruits and vegetables reduced MDA levels in leprosy patients. Similarly, in cases of leprosy and acne vulgaris, MDA levels decreased after oral antioxidant administration (Ayuningati et al., 2018). These findings suggest the potential value of administering oral antioxidants in reducing MDA levels in DA patients. Other antioxidants under consideration include melatonin, vitamins A, C, D, and E, as well as oxytocin (Trimbake et al., 2013).

Plants represent a rich source of antioxidants, especially those used in traditional medicine (Xu et al., 2017; Zafar et al., 2023). In Indonesia, the use of traditional medicine is an important cultural practice that dates back centuries; however, its efficacy and safety are not always fully supported by scientific studies (Sen & Chakraborty, 2017; Sokouti, Shafiee-Kandjani, Sokouti, & Sokouti, 2023). Various Indonesian plants are used medicinally, one of which is the katuk leaf (*Sauropus androgynus*). In Indonesia, katuk leaves are traditionally consumed by postpartum mothers to help restore uterine and abdominal size (Khoo et al., 2015). A study by Darsono et al. (2014) demonstrated that katuk leaf extract significantly increased the expression of prolactin and oxytocin-encoding genes in rat brains. Furthermore, katuk leaves have long been used in India for managing skin problems (Khoo et al., 2015).

Research by the National Working Group on Indonesian Medicinal Plants identified several active compounds in katuk plants, including papaverine alkaloids, proteins, fats, vitamins, minerals, saponins, flavonoids, and tannins (FACHRUDDIN, SUPRAYOGI, & HANIF, 2025; Khairani et al., 2021). Flavonoids, tannins, and other phenolic components found in plant-derived foods are well-recognized antioxidants (Nahak and Sahu, 2010). Several of these compounds present in katuk leaves are also known for their medicinal properties. To date, however, according to Ayuningati et al. (2018), no study has described a reduction of MDA levels in DA patients following oral antioxidant administration.

The katuk plant is widely known in the community, particularly among mothers, and is recognized as having strong antioxidant activity (Arista, 2013). *Sauropus androgynus* possesses higher antioxidant activity compared to *Erythrina variegata* L. (dadap plant) (Laveena and Chandra, 2018). Among eleven Indonesian plants examined by Andarwulan et al. (2010)—including kenikir, Chinese kedondong, antanan, basil, beluntas, mangkokan, gingseng leaves, pohpohan, kecombrang, and purslane—katuk showed the highest flavonoid content. Due to their antioxidant properties, katuk leaves can be identified as a rich natural

antioxidant source. Ethanol solvents are used in this extraction for their non-toxic qualities (Arista, 2013).

Based on this background, students aim to carry out further studies on the effect of administering ethanolic extracts of *katuk* leaves (*Sauropus androgynus*) as antioxidants on *MDA* levels in ovalbumin-induced *DA* model mice.

The general objective of this study is to analyze the effect of *katuk* leaf ethanolic extract on *MDA* levels in ovalbumin-induced atopic dermatitis model mice. Specifically, the study seeks to identify plasma *MDA* levels in the control group, measure plasma *MDA* levels in the treatment group, and determine tissue *MDA* levels in the treatment group. The expected benefits of this research include providing a theoretical basis for further studies, contributing knowledge for researchers and readers, and supporting the development of traditional medicine in Indonesia. In the medical field, the outcomes are expected to facilitate treatment approaches for *DA*. For the community, this research aims to provide alternative strategies for managing atopic dermatitis.

RESEARCH METHODS

The design of this study is experimental with a type of posttest only control group, which involves six groups consisting of one negative control group, one positive control group, and four experimental groups with low, medium, and high doses and comparison drugs. The research was conducted at the Animal Laboratory of Nahdlatul Ulama University Surabaya (UNUSA) and the Biochemistry Laboratory of Hang Tuah University (UHT) Surabaya, with a implementation time from September 2019 to March 2020. The study population was a white male mouse (*Mus musculus* L.) and the sample was determined using Federer's formula, resulting in a minimum of four mice per group, so a total of 36 mice were needed. The inclusion criteria included male mice aged two to three months with a healthy body weight of 20-40 grams and diagnosed with atopic dermatitis, while the exclusion criteria included maladaptive mice and diagnosed with fungus. The study framework involved six groups, with different treatments, including *katuk* leaf ethanol extract and vitamin C, and ovalbumin-induced mice on day 7 after adaptation. *MDA* levels are measured after treatment, with appropriate instruments and materials, as well as data processing that includes editing, coding, processing, and cleaning. Data analysis used normality and homogeneity tests, followed by the Kruskal Wallis test or ANOVA. The research ethics were submitted to the UNUSA Health Research Ethics Commission, with appropriate animal rearing and ethical euthanasia procedures. The limitations of the study include time constraints and researchers' experience in certain procedures, but assisted by teams from the UNUSA Animal Laboratory and the UHT Laboratory.

RESULTS AND DISCUSSION

The research was carried out from January to March 2020 at the Animal Laboratory of Nahdlatul Ulama University Surabaya. A total of 24 mice were adapted to meet the research criteria for 7 days from January 23 – 29, 2020. The K(+), P1, P2, P3, and P4 groups were given sensitization treatment by injection and ovalbumin patches according to the previous dosage and study protocol. Followed by the administration of therapy using *katuk* leaf ethanol extract

and vitamin C orally from February 22 to March 4, 2020 routinely once a day except for the K(+) group. The predetermined dosage was adjusted to each group based on the group's average body weight. Mouse sampling was carried out after all research protocols were implemented, sampling was carried out on March 5, 2020, exactly on the 43rd day of the study. The samples taken were blood samples and skin tissue of the back of mice. Then the sample was sent to the Biochemistry Laboratory of Hang Tuah University Surabaya to measure the MDA level of each group.

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Before the data is processed, a descriptive test is carried out first. From the descriptive test, the average value of plasma MDA levels in each group was as follows:

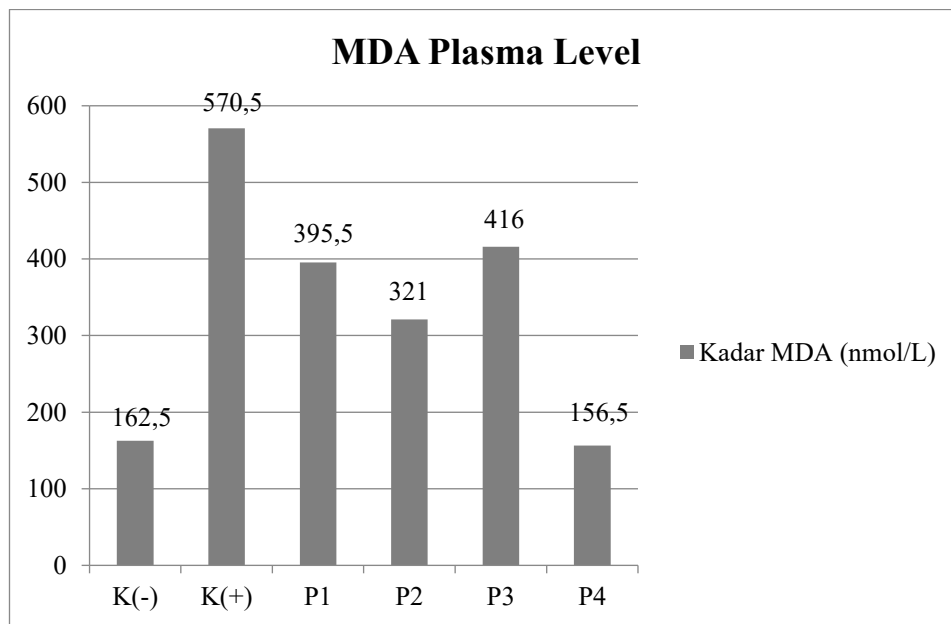


Figure 1. The average plasma MDA levels of each group

Descriptive data can be used to see the average and standard deviation. Both are important to see the distribution of research data. It can be concluded that from the above average, it can be seen that the K(+) group has the highest average Plasma MDA levels, while P4 has the lowest average Plasma MDA levels as explained in table 1.

Table 1. Analysis of Plasma MDA Descriptive Test

Group	Quantity (n)	Average	Standard Deviation	Min.	Max.	Middle Value
K(-)	4	162,50	117,162	54	324	136
K(+)	4	570,50	360,282	214	1063	502,5
P1	4	395,50	207,619	114	559	454,5
P2	4	321,00	177,016	138	558	294
P3	4	416,00	27,725	375	436	426,5
P4	4	156,50	74,263	56	217	176,5

From these data, it shows that the average plasma MDA level in the K(+) group is much higher than in the group that was given therapy. Even the P4 group given vitamin C therapy

had lower average plasma MDA levels compared to the K(-) group. However, the plasma MDA levels of individual mice in the K(-) group had lower compared to the P4 group.

Furthermore, a normality test and a homogeneity test were carried out. The average results of Plasma MDA levels tested for normality using the Saphiro-Wilk test showed a normal distributed group with a reference value of $p > 0.05$. Then followed by a homogeneity test using the Levene test showed that the data of the homogeneous group with a reference value of $p > 0.05$.

Table 2. Results of Plasma MDA Level Normality Test (Saphiro-Wilk test).

Group	Quantity (n)	The <i>Saphiro-Wilk Test</i>	Information
K(-)	4	0,604	P > 0,05 Normal Distributed
K(+)	4	0,704	
P1	4	0,308	
P2	4	0,815	
P3	4	0,071	
P4	4	0,369	

Table 3. Results of Plasma MDA Level Homogeneity Test (Levene test)

Result	Uji Homogenitas <i>Levene Test</i>	Information
Based on average	0,110	P > 0.05 Homogeneous
Based on the middle value	0,155	
Based on the middle value and <i>adjusted df</i>	0,221	
Based on <i>trimmed mean</i>	0,110	

Based on the results of normality and homogeneity testing, the data obtained includes parametric data. The data has qualified for a one-way ANOVA test which aims to find out the average difference between groups.

Table 4. Results of one-way ANOVA differential (comparative) tests

Result	Uji <i>one way ANOVA</i>	Information
Intergroup	0,053	P > 0.05 H0 accepted H1 rejected

The interpretation of the ANOVA one-way test is that if $P < 0.05$, H0 is rejected and H1 is accepted. If $P > 0.05$ then H0 is accepted and H1 is rejected. In this study, the one-way ANOVA test showed that $P > 0.05$ with a value of $P = 0.053$ so H0 was accepted and H1 was rejected. In addition, the results show that H0 is accepted and H1 is rejected, so the hypothesis of this study is rejected. These results did not show the effect of administering katuk leaf ethanol extract on the reduction of plasma MDA levels in mice in a significant atopic dermatitis model.

From the results of the data testing, it can be concluded that there is no difference in the effect of katuk leaf ethanol extract on the significant level of Plasma MDA. So there is no need to continue for the T-test.

Malondialdehyde Levels of Skin Tissue

Before the data is processed, a descriptive test is carried out first. From the descriptive test, the average value of tissue MDA levels in each group was as follows:

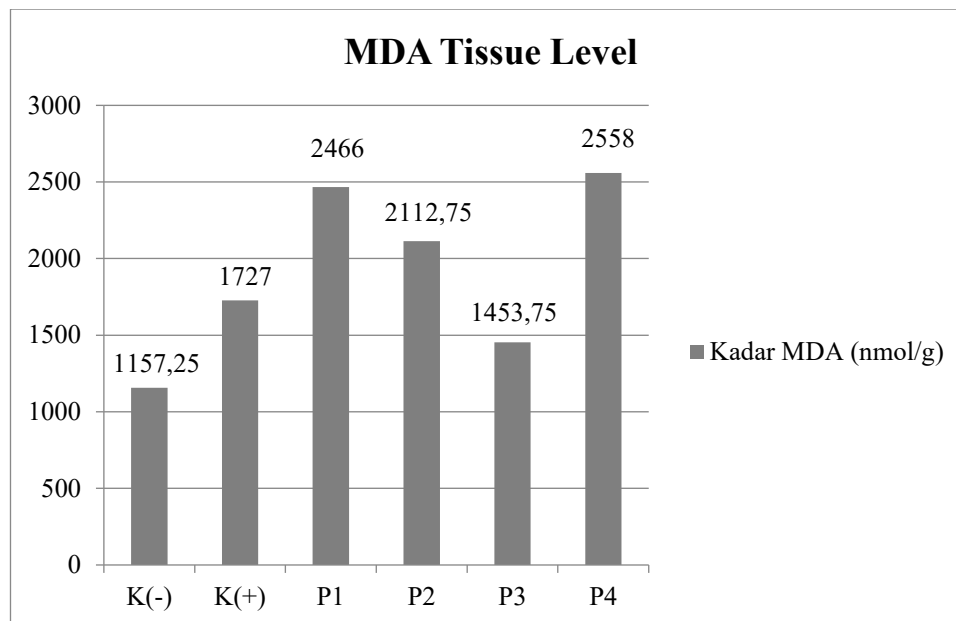


Figure 2. The average level of network MDA of each group

From these descriptive data, it can be concluded that the lowest average tissue MDA levels were found in the K(-) group, while the highest average MDA levels were in the P4 group. The average tissue MDA levels in the P4 group showed results that were inversely proportional to the previous average plasma MDA levels.

Table 5. Descriptive Test Analysis of Network MDA

Group	Quantity (n)	Average	Standard Deviation	Min.	Max.	Middle Value
K(-)	4	1157,25	457,844	626	1734	1134,5
K(+)	4	1727,00	550,841	1362	2529	1508,5
P1	4	2466,00	427,564	2119	3064	2340,5
P2	4	2112,75	994,065	631	2759	2530,5
P3	4	1453,75	1153,293	393	2756	1333
P4	4	2558,00	319,977	2223	2977	2516

In the treatment group that was given katuk leaf ethanol extract therapy with varying doses, there were sequential tissue MDA levels from the smallest dose to the largest dose. The P1 group with a dose of 100 mg/KgBB had the highest levels of tissue MDA compared to the P2 and P3 groups. This shows that the larger the dose given, the more likely it is to suppress the level of MDA in the tissue.

Next, normality and homogeneity tests were carried out. The normality test used the Saphiro-Wilk test and showed a group that was not normally distributed, considering that the normal distributed criterion was $P > 0.05$. In the test results, the P value in the P2 group was 0.026. Then followed by a homogeneity test using the Levene test showed that the data of the homogeneous group with a reference value of $p > 0.05$. In the homogeneity test, a P value of < 0.05 was obtained, so the data obtained was not homogeneous.

Table 6. Results of Tissue MDA Level Normality Test (Saphiro-Wilk test).

Group	Quantity (n)	The <i>Saphiro-Wilk Test</i>	Information
K(-)	4	0,947	P < 0.05 Not Normally Distributed
K(+)	4	0,086	
P1	4	0,351	
P2	4	0,026	
P3	4	0,344	
P4	4	0,884	

Table 7. Results of Tissue MDA Level Homogeneity Test (Levene test)

Result	Uji Homogenitas <i>Levene Test</i>	Information
Based on average	0,022	P < 0.05
Based on the middle value	0,281	Not
Based on the middle value and <i>adjusted df</i>	0,340	Homogeneous
Based on <i>trimmed mean</i>	0,034	

Considering that the requirements for the one way ANOVA test are normal and homogeneous distributed data (parametric data), then in the next test the Kruskal Wallis test is used to find out the average difference between groups.

Table 8. Results of Kruskal Wallis Difference Test (Comparison)

Group	Quantity (n)	Uji <i>Kruskal Wallis</i>	Average Network MDA Levels
K(-)	4	P = 0,106 (Reference value P < 0.05)	1157,25
K(+)	4		1727
P1	4		2466
P2	4		2112,75
P3	4		1453,75
P4	4		2558

The results of the Kruskal Wallis test (Sig. 0.106 > 0.05) showed insignificant results. Where it can be concluded that the results of the Kruskal Wallis test do not have a significant difference in the average percentage of the total level of tissue MDA levels between the sample groups.

In addition, the results show that H0 is accepted and H1 is rejected, so the hypothesis of this study is rejected. These results did not show any effect of administering katuk leaf ethanol extract on reducing tissue MDA levels in atopic dermatitis model mice.

MDA Levels of Negative and Positive Control Groups

Up to MDA Plasma

MDA is one of the results of lipid peroxidation. The reaction between ROS and polyunsaturated fatty acids (on the cell wall) will result in the formation of aldehydes, such as MDA, through the process of lipid peroxidation. Several previous studies have shown that MDA is a stable and accurate component of lipid peroxidation measurements, and has helped explain the role of oxidative stress in a number of diseases, including DA, which plays a role in the inflammatory process. In previous studies, it has been said that dermatitis patients have higher plasma MDA levels compared to non-DA (Ayuningati et al., 2018). This is in accordance with the results obtained in this study, showing that there was an average plasma MDA level of negative control group that had a lower value of 162.5 nmol/L compared to the

positive control group with a value of 570.5 nmol/L. So there was a difference between DA and non-DA mice.

The stratum layer, as the skin layer is most exposed to a variety of environmental factors, is particularly susceptible to oxidative stress. Given the high lipid content of the stratum corneum, lipophilic antioxidants such as α -tocopherol play a major role in the ROS generated during oxidative stress (Okayama, 2005). Atopic dermatitis is more played by a type I hypersensitivity immune reaction, where a direct stimulus to mast cells causes mast cells to degranulate and release inflammatory mediators. Okayama (2005) said that ROS can act as a second messenger carrier in inducing several biological responses in mast cells. Exact ROS levels are difficult to measure in vivo. However, in an in vitro study, the production of superoxide anions (including ROS) was higher in cell samples obtained from patients with severe atopic dermatitis than in samples from normal control subjects or patients with allergic rhinitis (Okayama, 2005).

In the very early phase of the development of psoriasis lesions, macrophages appear in the epidermis followed by lymphocytes. Infiltrated and activated leukocytes can cause the release of ROS through processes such as respiration. Polymorphocyte Leukocytes (PMN) have the potential to damage surrounding tissues by releasing superoxide anion radicals generated through NADPH oxidase/myeloperoxidase which further give rise to other active oxygen species, all of which are known to trigger lipid peroxidation. ROS in turn also stimulates PMN recruitment by increasing PMN adhesion to the endothelium (Pujari, 2014). Pujari (2014) said the increase in ROS generation, by increased infiltration and activation of PMN may target cellular polyunsaturated fatty acids for lipid peroxidation, which may be indicated by an increase in the concentration of MDA in the serum of psoriasis patients.

Network MDA Rate

The results showed that the average level of tissue MDA in the negative and positive control groups was different. Similar to plasma MDA levels, the mean tissue MDA levels in the negative group showed lower results (1157.25 nmol/g) compared to the positive control group (1727 nmol/g). There was a numerical difference in the average level of tissue MDA obtained in the control group itself. During the wound healing process inflammatory cells such as neutrophils, macrophages, endothelial cells and fibroblasts produce superoxide. The activation of neutrophils and macrophages produces large amounts of superoxides and their derivatives via the phagocytic isoform NADPH oxidase. Thrombin and TNF- α will stimulate the release of superoxide from endothelial cells. Meanwhile, interleukin 1 (IL-1) and TNF- α stimulate the release of superoxide from fibroblasts (Arief and Widodo, 2018). Arief and Widodo (2018) also mentioned that ROS has a role in wound healing, there is an increase in ROS levels in chronic wounds.

MDA Levels of Treatment Groups

Up to MDA Plasma

Plasma MDA in the treatment group showed a different average plasma MDA result for each group. The highest plasma MDA levels in the treatment group were found in the P3 group (416 nmol/L) with the highest dose of katuk leaf ethanol extract, followed by the P1 group (395.5 nmol/L) with the lowest dose and the P2 group (321 nmol/L) with the medium dose.

Meanwhile, the P4 treatment group with vitamin C therapy had the lowest average plasma MDA levels compared to other treatment groups with an average level value of 156.5 nmol/L. This shows that plasma MDA levels of the group given vitamin C had much lower levels compared to the group given katuk leaf ethanol extract, although statistically tested the results did not say that there was a significant difference between groups. So it can be said that there is no significant change in levels before the mice get sick with the administration of ethanol extract of katuk leaves.

According to Okayama (2005) Although ROS plays a role in human allergic skin diseases, their biological targets and pathological actions are not fully understood. In addition, useful strategies for the therapeutic management of ROS activity in human skin are not available. However, the recognition that ROS can act as a secondary messenger in inducing some biological responses has led many researchers to focus on the possible effects of antioxidants in many pathological processes. Antioxidants, which act on upstream or downstream specific targets, can interfere with redox transcription factors. Enzymatic and non-enzymatic antioxidants have been shown to activate or inhibit protein-1 activator (AP-1) at the transcriptional or post-transcriptional level (Briganti and Picardo, 2003).

The P2 (medium dose) treatment group was a group that had lower average levels compared to the P1 and P3 groups. This can happen because of the possibility of a ceiling effect on katuk leaf ethanol extract. In a literature review, it has been mentioned that the anti-inflammatory properties of katuk leaf ethanol extract have almost the same effect as sodium diclofenac. In addition, in the previous study of Patonah et al. (2017) it was also said that the best dose for antiobesity on the epididimal and perirenal fat indexes was a dose of 200 mg.

Network MDA Rate

The tissue MDA levels of the treatment group varied widely, the P4 group had the highest average level of tissue MDA compared to the other groups. The P4 group had the highest average level (2558 nmol/g) followed by the P1 group (2466 nmol/g), the P2 group (2112.75 nmol/g), and the P3 group (1453.75 nmol/g). Based on the dosage of ethanol extract, katuk leaves given by groups P1, P2, and P3 have sequential average levels ranging from highest to lowest.

Changes in antioxidant patterns, found in the skin of DA patients, increased α -tocopherol levels and decreased lipid peroxide have been found in non-leneous skin as a consequence of an adaptive response to chronic inflammatory states (Briganti and Picardo, 2003). Increased oxidative stress occurs in chronic wounds because ROS has a role in the wound healing process. In the process of healing fibroblast wounds produce and at the same time overhaul the collagen-rich matrix, some of these fibroblasts will turn into myofibroblasts which will help the wound contraction, and ROS plays a role in changing fibroblasts into myofibroblasts. Wounds that have contracted will help in the reepithelialization process so that it is faster to bring the edge of one wound to the edge of another (Arief and Widodo, 2018).

Excessive ROS has a damaging effect on chronic wounds because it causes epidermal inactivation of enzymatic antioxidants even though there is an increase in enzymatic antioxidant expression in the wound, so that this condition will deplete the levels of nonenzymatic antioxidants that are still present in the wound tissue (Arief and Widodo, 2018). The results obtained have shown that the treatment group of katuk leaf ethanol extract with low

to high doses showed sequential results. So there is an idea that ROS levels are lower if the dose given is higher.

In the study, Zuhra et al. (2008) stated that a compound is said to be a very strong antioxidant if the IC₅₀ value is less than 50 ppm, strong for IC₅₀ is 50-100 ppm, moderate if it is 100-150 ppm, and weak if the IC₅₀ value is 151-200 ppm. The IC₅₀ value obtained was 80.81 ppm, this means that flavonoids from katuk leaves (*Sauropus androgynus* (L) Merr) have the ability to be powerful antioxidants (Zuhra et al., 2008). In a study conducted by Arista (2013), it was stated that in previous studies, katuk leaves were known to have high antioxidant activity which was shown by a small EC₅₀ value. But in his research, a large EC₅₀ value was obtained. The chemical content of plants can vary, depending on various factors, for example: genetic factors, climate, soil fertility (Arista, 2013).

CONCLUSION

The study on the effect of *katuk* leaf (*Sauropus androgynus*) ethanol extract on MDA levels in ovalbumin-induced atopic dermatitis (AD) model mice concluded that while differences in plasma and tissue MDA levels were observed between atopic and non-atopic groups, administration of the extract did not significantly reduce either plasma or tissue MDA levels in the treatment groups. Since this study did not examine the correlation between plasma and tissue MDA levels in AD, future research is recommended to investigate this relationship, as it may provide deeper insights into the role of oxidative stress and antioxidant therapy in managing atopic dermatitis.

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The Effect of Ethanolic Extract of Katuk Leaf (*Sauropus Androgynus*) on Malondialdehyde (MDA) Levels in Ovalbumin-Induced Atopic Dermatitis Model Mice

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