ORIGINAL ARTICLE

Malondialdehyde (MDA) Levels on Mice Atopic Dermatitis Treated with Pearl Grass (*Hedyotis corymbosa* (L.) Lamk) Extract Cream

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ABSTRACT

Introduction: Atopic dermatitis is a skin ailment caused by a reaction of immunoglobulin E. One of the factors that could cause complications with this condition is oxidative stress. Pearl Grass is an herb that has been used as a traditional medicine believed to be able to cure many ailments, such as allergies. This study aims to discover the malondialdehyde (MDA) levels in blood serum on mice atopic dermatitis that is treated with pearl grass extract cream. **Methods:** This study was done during September-November 2018. This study uses an experimental method with completely randomized design. 35 mice blood samples were used and divided to 7 treatment groups. To calculate MDA levels in blood a Thiobarbituric Reactive Substance (TBARS) method is used. The MDA levels data gained was then analyzed with an ANOVA test using SPSS 21.0. **Results:** The result shows that there is a decrease in MDA blood levels on every treatment groups but no significant difference between the malondialdehyde level of atopic dermatitis mice in each group. The malondialdehyde level were obtained ranged from 0.79 nmol/ml to 1.22 nmol/ml. **Conclusion:** The MDA level in blood serum of atopic dermatitis mice is not affected by treatments with pearl grass extract cream (Sig>0,05).

Keywords: Malondialdehyde, Atopic dermatitis, Pearl grass (*Hedyotis corymbosa*)

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INTRODUCTION

Atopic dermatitis is an immune disease that is induced by an imbalance of T Helper2 cells (Th2). So far, medical treatment of atopic dermatitis uses steroids, antihistamines, immunosuppressive agents (1). However, several studies have stated that this treatment was a long-term treatment and can cause various side effects, such as damage to the liver, kidneys, and arterial hypertension (2). Oxidative stress plays a role in the inflammatory process of atopic dermatitis (3). Oxidative stress is a condition of an imbalance between oxidants and antioxidants, that is, if the production of ROS exceeds the oxidant capacity and has the potential to cause damage (4). Activation of inflammatory cells will release free radical, so that this process plays a role in the pathogenesis of atopic dermatitis (5). Study on Canine atopic dermatitis and human atopic dermatitis have shown that there was an increase in MDA production in response to oxidative stress due to excess production of ROS during the inflammatory process (3,6). Malondialdehyde (MDA) can be formed through lipid peroxidation (Lox) and the cyclooxygenase (Cox) pathway (7,8).

Pearl grass (Hedyotis corymbosa (L.) Lamk) is a plant that is used as herbal medicine, which has anti-pyretic, anti-inflammatory, anti-bacterial, anti-cancer, smoothen blood circulation, increases white blood cell phagocytes and hormonal immunity (9,10). Pearl grass (Hedyotis corymbosa (L.) Lamk) contains hentriacontane, stigmasterol, β-sitosterol, sitisterol-D-glucoside, p-coumaric acid, flavonoid glycoside, baihuasheshecaosu, iridoid, alizarin, chorogenin, antragalol bonding, olenolic acid and ursolate acid (10,11). Pearl grass has been used as an anti-inflammatory, but there are no specific studies regarding the ability of pearl grass extract in treating allergies, especially on the skin. Therefore, this study

was conducted to find out the effect of administration of pearl grass (Hedyotis corymbosa (L.) Lamk) extract cream (PGEC) on atopic dermatitis mice.

MATERIALS AND METHODS

Setting and time of research

This research was conducted at the Animal House and Animal Physiology Laboratory of the Biology Study Program, Faculty of Mathematics and Sciences, Universitas Negeri Jakarta, the making of pearl grass extract cream was carried out at the Biochemistry Laboratory of the Faculty of Animal Husbandry, Institut Pertanian Bogor, and malondialdehyde (MDA) test was carried out in the Biochemistry and Molecular Biology Laboratory, Faculty of Medicine, Universitas Indonesia. Activities carried out from September to November 2018.

Treatment of experimental animals

Mice were randomly separated into 7 treatment groups, namely group 1 (positive control), group 2 (negative control), group 3 (atopic dermatitis mice with 0.025% prednisolone cream), group 4 (atopic dermatitis mice with 2% PGEC), group 5 (atopic dermatitis mice with 5% PGEC), group 6 (atopic dermatitis mice with 7% PGEC) and group 7 (atopic dermatitis mice with 10% PGEC), each group consisted of 5 mice. Mice were wounded (curettage) using a scalpel on the tail with a length of 2 cm. The administration of cream to mice was done topically for 14 days and once a day. On the 14th day, blood samples were taken from the heart as much as 1ml.

Extract cream making

The pearl grass simplicia was extracted using the maceration method with 70% ethanol solution, the comparison of pearl grass simplicia with 70% ethanol was 1:20. The maceration process was carried out 3 times. The filtrate was evaporated with a rotary vacuum evaporator to form a paste. Pearl grass extract cream (Hedyotis corymbosa (L.) Lamk) was made in 4 doses, namely 2%,5%,7%,10% in 5 grams of cream base.

Calculation of MDA level

The calculation of MDA level was adapted from the thiobarbituric acid reactive substance (TBARS) method. A total of 100 µl of blood serum was added to the micro tube, 0.5 ml of 20% trichloroacetic acid (TCA) solution and 1 ml of 0.67% barbituric acid (TBA) solution were added and homogenized. The homogeneous solution was heated in a water bath at 95ºC for 15 minutes and cooled to room temperature. The solution was centrifuged at 3000rpm for 10 minutes. The absorbance of the pink supernatant was measured using a spectrophotometer at a wavelength of 532 nm. The absorbance value and the concentration of the standard solution were plotted into a linear regression graph, then the regression equation obtained was used in determining the value of the serum MDA concentration. The MDA levels data analyzed with an ANOVA test using SPSS 21.0.

RESULTS

Malondialdehyde level was measured from blood serum using the Thiobarbituric Acid Reactive Substance (TBARS) method (17). Making of the standard solution was used to find out the linear equation, by making standard solution in various concentrations and seeing the adsorption, so that the following equation was obtained: $y = 0.0656x - 0.0004$ ($R^2=1$). This equation means that there was an increase in absorbance of 0.0656 for every increase of 1M standard solution. The level of malondialdehyde (MDA) in blood serum was calculated by entering the absorbance results into a predetermined linear regression equation. The results of observing the malondialdehyde level in mice blood serum can be seen in Table I.

Table I : The test results of the blood serum MDA level of mice were administered pearl grass (Hedyotis corymbosa) extract cream topically for 14 days

Treatment	Mean of MDA level (nmol / mL) ± SE
Positive Control	1.04 ± 0.27
Negative Control	0.99 ± 0.15
Prednisolone Cream (K1)	1.22 ± 0.06
Pearl Grass 2% (K2)	0.98 ± 0.06
Pearl Grass Cream 5% (K3)	1.16 ± 0.12
Pearl Grass Cream 7% (K4)	0.87 ± 0.04
Pearl Grass 10% (K5)	0.79 ± 0.05

The results of statistical test used ANOVA that is, there was no significant difference between the malondialdehyde level of atopic dermatitis mice in each group (sig> 0.05). The malondialdehyde level were obtained ranged from 0.79 nmol/ml to 1.22 nmol/ml. It was known that the malondialdehyde level of normal mice ranged from 0.8 nmol/ml to 1.1 nmol/ml (12).

DISCUSSION

Atopic dermatitis canine has increased in level of malondialdehyde due to oxidative stress during the inflammatory process in atopic dermatitis (3). The skin that was wounded will experience oxidative stress which leads to an increase in malondialdehyde level so that the malondialdehyde level can be used as a bio-indicator for wound

healing from atopic dermatitis mice. High levels of MDA indicate inflammation that occurs in the body. In acute cases of atopic dermatitis, inflammation occurs at the beginning of the acute phase, namely on day 3 to day 7. In this study, the application of pearl grass extract cream was carried out for 14 days because prednisolone cream as an atopic dermatitis drug should only be used for a maximum of 2 weeks (18).The skin is the outermost organ as a barrier to the body and as the main target for oxidative stress due to the continuous reaction to antigens from outside the body (4). The occurrence of inflammation will stimulate the release of free radical to destroy pathogen or other foreign objects that enter the body (13). An excessive increase in free radical can damage skin cells and slow down the wound healing process. During the inflammatory process in atopic dermatitis, lymphocyte, monocyte and eosinophil infiltration occured, where these three agents secrete pro-inflammatory cytokines and reactive oxygen species (ROS).

In this research used pearl grass as a treatment for atopic dermatitis. The treatment group with pearl grass extract cream had malondialdehyde level ranging from 0.79 to 1.16 nmol/ml. Previous research has used starflower oil (borage seeds oil) and virgin coconut oil in the herbal treatment of atopic dermatitis (14). Antioxidant is compound that can prevent the lipid oxidation process and the Cox-2 reaction so that the formation of MDA can be prevented.

The anti-inflammatory properties of pearl grass are thought to be derived from antioxidants such as the flavonoid and gallic acid groups. Antioxidant (flavonoid) works by inhibiting the Cox-2 process, the release of histamine and the radical scavenging activity of a molecule. So the inflammatory process and the formation of MDA can be inhibited. This was reinforced by previous research that flavonoid can decrease the Cox-2 reaction (13,14).

Flavonoid is phenolic compound that belong to the same group as polyphenol. Flavonoid can prevent free radical by inhibiting oxidation reaction. Flavonoid can bind superoxide, hydroxyl and peroxyl radical that affect the cyclooxygenase or lipooxygenase pathways (14,15). Gallic acid is a phenolic compound that has antioxidant properties. Gallic acid can react with free radical and can inhibit the oxidation process further. This was in accordance with Junaidi (2018) if a material contained flavonoid and gallic acid, then the antioxidant activity is very high and works well.

The negative control group had level of malondialdehyde (MDA) of 0.99±0.153 nmol/mL. This was due to the innate immune reaction where the mice did self-recovery. Self-recovery begins with a signal in the form of an antigen that enters the body, then the Antigen Presenting Cells (APC) will capture the antigen that can directly carry out phagocyte activity or release cytokines and chemokines. This stage is characterized by the occurrence of an inflammatory process that begins in the injured cells that release inflammatory mediators such as leukotriene, histamine and prostaglandin. The bond between the mediator and the endothelial cell receptor causes vasodilation and diapedesis. In particular, prostaglandin will bind blood and platelets so that clots can close the wound. This was in accordance with Ranneh (2017) that the body experienced tissue repair mechanism and defense against antigen that enter the body.

All groups did not have significantly different results (Sig ≥ 0.05). Each treatment group experienced a decrease in malondialdehyde (MDA) level which almost resembled the positive control group. The results were not significantly different in this study because the samples were taken on day 14 where the inflammation that occurred had subsided and started to enter the tissue healing process. For that, it is necessary to do further research with sampling on days 3 to 7 as additional data for the effectiveness of pearl grass extract cream. In this research the use of 2% pearl grass extract cream was sufficient to cure atopic dermatitis allergy, compared to the use of prednisolone which can cause ongoing allergic reaction and various side effects.

CONCLUSION

All atopic dermatitis mice were declared cured on the 14th day. The level of malondialdehyde in the blood serum of atopic dermatitis mice was not affected by the administration of pearl grass extract cream. It takes measurement of other parameters, namely Immunoglobulin-E (IgE) to find out the results of metabolism through cyclooxygenase in atopic dermatitis.

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