



## ETHANOL EXTRACTS OF TUBERS *DIOSCOREA ALATA L.* AS ANTIALLERGIC AGENT ON MICE BALB/C INDUCED WITH OVALBUMIN

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### ABSTRACT

This study aimed to analyse the effects of ethanol extract tuber *Dioscorea alata L.* (EEDA) on mice induced by Ovalbumin using the measurement of IgE, IL-4 and TNF- $\alpha$  level. The experimental research on 25 Balb/C mice divided into 5 groups including: the control, and treatment groups which given EEDA dose 0.31, 0.62, 1.24 g/kg and antihistamine drug. Treatment groups were induced the allergic model using Ovalbumin. On the 29<sup>th</sup> day, the mice were sacrificed and sera were processed with Elisa methods. The data were analyzed with Anova continued with Tukey test. The IgE level in EEDA 0.62 and 1.24 g/kg decreases with insignificantly difference with antihistamin drug. IL-4 level in EEDA is insignificant different. TNF- $\alpha$  levels in EEDA 1,24 g/kg increases with insignificantly difference with antihistamin drug. The conclusion is EEDA dose 0.62 and 1.24 g/kg is able to decrease the IgE level and EEDA dose 1,24 g/kg is able to increase TNF- $\alpha$  levels.

**KEYWORDS:** Balb/C mice, *Dioscorea alata L.*, Ovalbumin, IgE, IL-4, TNF- $\alpha$



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## INTRODUCTION

Purple yam (*Dioscorea alata* L.) is the biological source of the tubers that has not been used optimally. *D. alata* L. has potency as a source of carbohydrates. It contains phenolic compounds, namely anthocyanin that has high antioxidants.<sup>1</sup> Anthocyanin is part of the flavonoid. Flavonoid has many benefits for health such as antibacterial agents, antiviral, antiinflammatory, antiangiogenic, analgesic, hepatoprotective, cytostatic, apoptotic, estrogenic/antiestrogenic and anti-allergenic<sup>2,3</sup>. Allergens that come into the body will be processed by antigen presenting cells (APC). Peptide of allergen which is presented by APC induces activation of Th0 lymphocyte. Th0 lymphocyte further activates Th2 lymphocytes to produce its cytokines<sup>4</sup>. IL-4 plays an important role in the differentiation of naive T cells into Th-2 cells; IL-4 also plays an important role in the regulation of allergic conditions. IL-4 is a major stimulus for the formation of Th-2 and suppresses the formation of Th-1<sup>5</sup>. The production of Th2 cytokines, especially IL-4 suppresses Th1 development primarily through IFN- $\gamma$  that will maintain the allergic phenotype. The production of Th1 cytokines especially TNF- $\alpha$  will suppress the development of Th2<sup>6,7</sup>. TNF- $\alpha$  is a cytokine produced by CD4+ Th1 cells, as well as IFN- $\gamma$  and lymphotoxin that play a role in the cell-mediated immunity. Allergic reactions involve specific IgE antibodies<sup>8</sup>. IgE is a key molecule that acts as a mediator of allergic responses (asthma, rhinitis, food allergy, atopic dermatitis, etc.)<sup>9</sup>. The purpose of this study was to analyse the effects of ethanol extract of tuber *Dioscorea alata* L. on mice induced by Ovalbumin using the measurement of IgE, IL-4 and TNF- $\alpha$  level.

## MATERIALS AND METHODS

This study was approved by the Research Ethics Committee of the Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta. This was an experimental research on Balb/C mice with posttest only

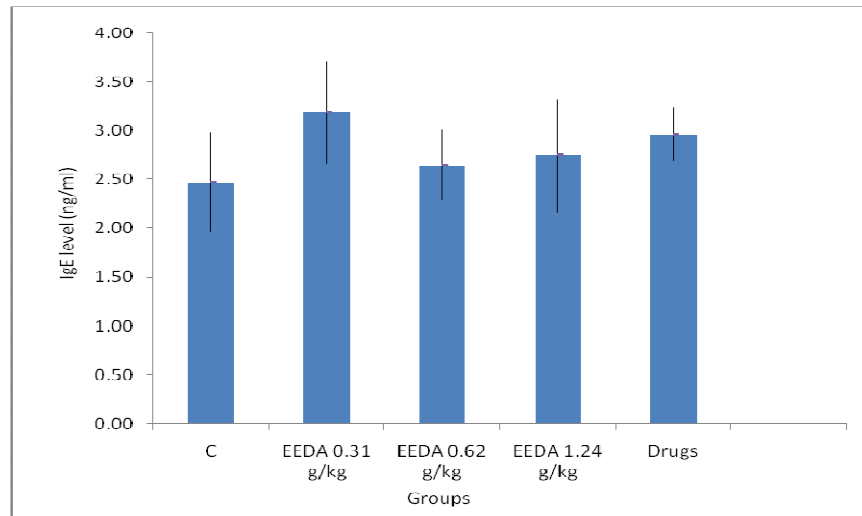
control group design. The independent variable is the ethanol extract of tubers *Dioscorea alata* L. dose 0.31 g/kg, 0.62 g/kg and 1.24 g/kg. The dependent variable is the IgE, IL-4 and TNF- $\alpha$  levels measured with Elisa methods. Twenty five Balb/C mice were divided into five groups namely control (C) group, treatment groups were treated with ethanol extract tubers of *D. alata* L. dose 0.31 g/kg, 0.62 g/kg, 1.24 g/kg (T<sub>1</sub>– T<sub>3</sub>), and antihistamine drug 0,4 mg/mice/day (T<sub>4</sub>) for 28 days. Treatment groups of mice were induced and challenged using allergic models with Ovalbumin 0.048 mg/mouse on the 15<sup>th</sup> day and 0,037 mg/mouse on the 22<sup>nd</sup> day i.p. and then induced with 0,15 mg/mouse Ovalbumin orally on 23<sup>rd</sup> to 28<sup>th</sup> day<sup>10,11</sup>. Mice were sacrificed on the 29<sup>th</sup> day, and then intracardiac blood was taken and processed to measure the levels of IgE, IL-4 and TNF- $\alpha$  using ELISA method. Ethanol extract tuber of *D. alata* L. was obtained from purple yam which was cleaned, thinly sliced, dried, pulverized and extracted with 70% ethanol liquid using a maceration method. The result of measurement IgE, IL-4 and TNF- $\alpha$  levels was previously tested for normality by using the Kolmogorov Smirnov nonparametric test. Furthermore, the data levels of IgE, IL-4 and TNF- $\alpha$  were tested using one way ANOVA and continued with Tukey's test if the result of one way Anova tests significant<sup>10</sup>.

## RESULTS

The result of IgE, IL-4 and TNF- $\alpha$  levels measurement in blood sera of mice after the administration of ethanol extract tuber of *D. alata* L. with various doses and antihistamines drug for 28 days and induced with Ovalbumin can be seen in Figure 1 until Figure 3.

**Figure 1**

***IgE, levels in blood sera of mice after administration of ethanol extract tuber of *D. alata L.* (EEDA) with various doses and antihistamines drug as Control positive for 28 days and induced with Ovalbumin***

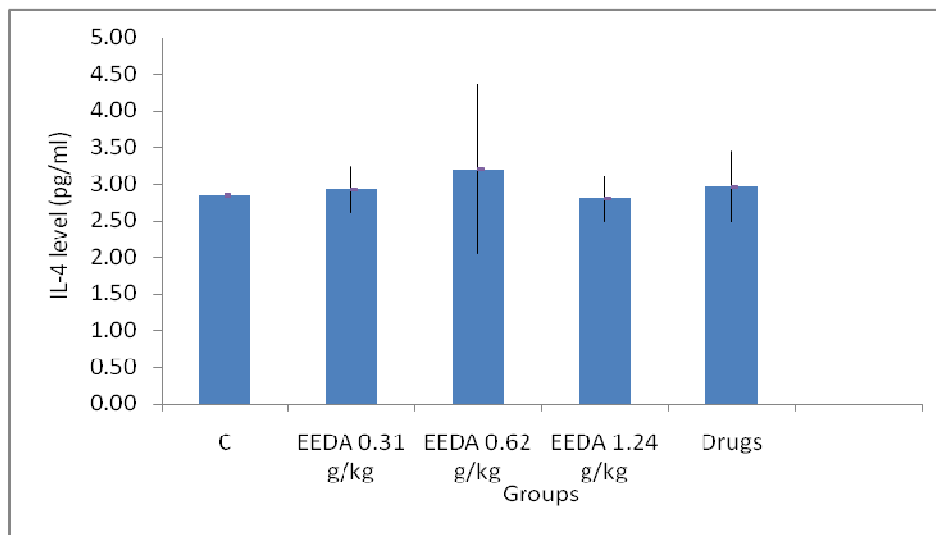


This study shows that the treatment group that was given the ethanol extract tuber of *D. alata L.* and induced the allergy with Ovalbumin showed a decline of IgE levels that were in accordance with the increasing dose. The lowest IgE levels were in the group that was

given the ethanol extract tuber of *D. alata L.* dose of 0.62g/kg followed by the second lowest group in the group that was given ethanol extract tuber of *D. alata L.* dose 1,24 g/kg, and slightly lower than the treatment group that was given antihistamine drug.

**Figure 2**

***IL-4 levels in blood sera of mice after administration of ethanol extract tuber of *D. alata L.* (EEDA) with various doses and antihistamines drug as Control positive for 28 days and induced with Ovalbumin***

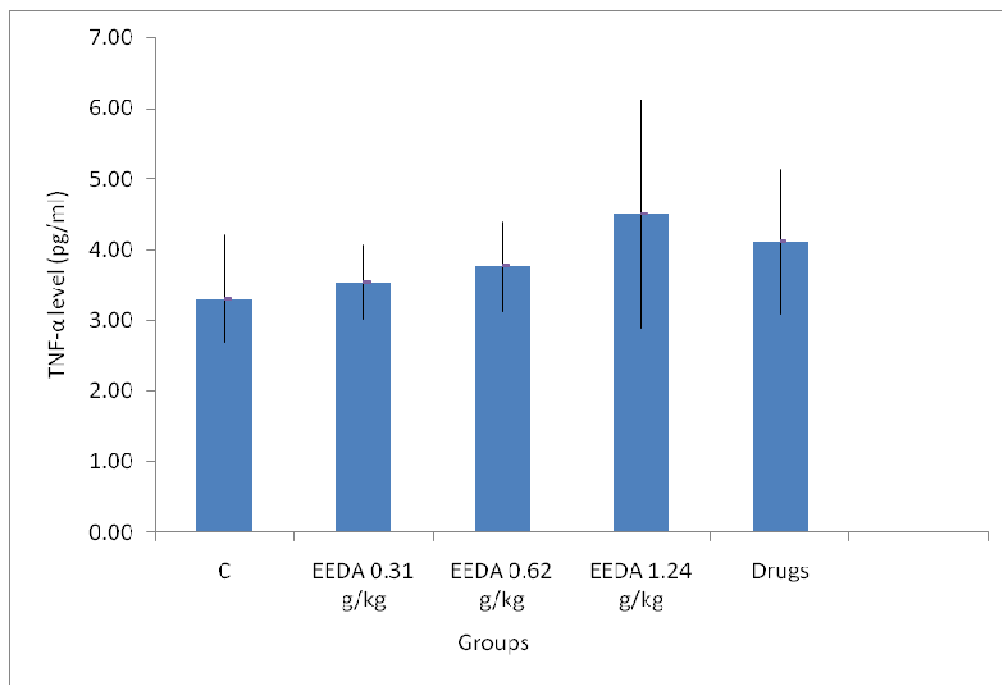


In this study, CD4+ Th2 cytokines was measured in the IL-4 cytokine. The control group has the lowest levels of IL-4. The IL-4 levels in the treatment group decreases in concordance with the increasing dose of ethanol extract tuber of *D. alata L.*. The lowest

levels of IL-4 were those of the ethanol extract of tubers of *D. alata L.* dose of 0,62g/kg bw and it was 1,24g/kg lower than the treatment group that were given antihistamines and it was also slightly lower than the levels of IL-4 of the control group.

**Figure 3**

***TNF- $\alpha$  levels in blood sera of mice after administration of ethanol extract tuber of *D. alata L.* (EEDA) with various doses and antihistamines drug as Control positive for 28 days and induced with Ovalbumin***



This study also measured the TNF- $\alpha$  level. The results showed that the lowest levels of TNF- $\alpha$  was found in the control group. The TNF- $\alpha$  in the treatment groups increase and it is in line with the increase in the dose. The Kolmogorov Smirnov nonparametric test shows that IgE, IL4 and TNF- $\alpha$  levels had a normal distribution. Anova test shows there is a significant difference in the level of IgE and TNF- $\alpha$  level, while the IL-4 level result was not significantly different. The result of Tukey test shows, the IgE level was significantly different between the control group and the treatment groups. However, the level of IgE between control group and treatment group which was given antihistamin drug is not significantly different.. The IgE level between the treatment groups was not significantly different. Tukey test

showed that TNF- $\alpha$  level was significantly different between the control group and the treatment groups. There was no significantly difference between the control group and the treatment group of ethanol extract tuber of *D. alata L.* dose of 0.31 g/kg ethanol and treatment group of ethanol extract tuber of *D. alata L.* dose of 0.62 g/kg. There was no significantly difference between the treatment groups of ethanol extract tuber of *D. alata L.* dose 1.24 g/kg and the treatment groups with antihistamine drugs.

## DISCUSSION

In this study IgE level was increased on mice treated with Ovalbumin compared to IgE level in the control group. This is consistent with the

previous studies which found that the administration of Ovalbumin is able to increase the production of IgE.<sup>12, 13</sup> It is known that IgE is identified as a key molecule that acts as a mediator of the type-1 hypersensitivity reactions (allergic asthma, allergic rhinitis, food allergy, atopic dermatitis, various forms of allergy medications and allergy to insects) in which IgE are strongly related to the allergen. IgE has a very short half-life (<1 day) and IgE concentration in the circulations very low; it is lower than most of Immunoglobulins<sup>12</sup> due to the destruction of IgE in the endosome, where as the IgG in endosomal compartment is protected by FcγRn. Despite the low concentration of IgE in circulation, it has an extreme biological activity. This is caused by the IgE antibodies that bound to the receptors, which have high affinity to the surface of the mast cell or basophil<sup>14</sup>. The results of this study support previous study, which found that the repeated exposure of Ovalbumin can increase CD4+ lymphocyte cells to further stimulate B cells to increase the production of IgE. IgE is a cytokine that plays an important role in the development of allergic reactions<sup>15, 16</sup>. When there is the development of an allergic reaction, there will be an increase in the release of inflammatory mediators, including histamine. High levels of histamine will increase the secretion of CD4+Th2 cytokines, such as IL-4, IL-5, IL-6, IL-10 and IL-13. CD4+ Th2 cells play an important role in the allergic-inflammatory reactions. It is known that CD4+ T cells can become two different effector cells, namely CD4+Th1 and Th2 CD4+ cells. CD4+ Th1 cells produce interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α) and lymphotoxin that play a role in cell-mediated immunity. Meanwhile CD4+ Th2 secrete the interleukin-4 (IL-4), IL-5, IL-6, IL-10 and IL-13, which plays a role in the humoral immune response<sup>15, 16, 17</sup>. The imbalance between CD4+ Th1 cells and CD4+ Th2 is a factor that greatly affects the occurrence of immunological diseases, including infectious diseases, autoimmune and allergic<sup>18, 19, 20</sup>.

This study measured the levels of TNF-α. TNF-α is a cytokine produced by CD4+ Th1 cells, IFN-γ and lymphotoxin that plays a role in

the cell-mediated immunity. in a state of being induced, the allergic cytokines produced by CD4+ Th2 increased, on the contrary, cytokines produced by CD4+ Th1 (such as TNF-α) will decrease. Cellular immune response is activated to eliminate the allergens Ovalbumin as the allergic agents. Allergen provocation affects lymphocytes as cells that have the ability to regulate immune responses through modulation of T cells and local tissue inflammation. The exposure to antigen presenting cells (APC) by Ovalbumin will initiate the activation of CD4+ Th2 cells and IgE synthesis, better known as the stage of allergic sensitization. Oral albumin exposure will lead to the recruitment and activation of inflammatory cells and release of mediators. This will cause an allergic response to both fast and slow phase. In the early stages of an allergic response, a few minutes after contact with an allergen, allergy occurs accompanied by the release of mast cell degranulation of inflammatory mediators. These mediators include histamine, leukotrienes and cytokines that will increase the permeability of blood vessels, smooth muscle contraction, and mucous production. Chemokines produced by mast cells and other cells will directly lead to the recruitment of inflammatory cells. These chemokine will contribute to the slow phase of the allergic response characterized by the number of cells CD4+ Th2 and eosinophil<sup>21</sup>. IL-4 expression often provides clues to the presence of IgE production and allergic inflammation caused by the presence of cytokines produced by Th2 cells. Th2 cytokines will increase the occurrence of allergic diseases through various mechanisms. IL-4 and IL-13 induces B cells to differentiate themselves into mast cells and IgE production. IgE is instrumental in the development of allergic reactions<sup>15, 16</sup>. Ethanol extract tuber of *D. alata L.* is able to suppress proinflammatory cytokine production because it contains phenolic compounds (flavonoids) such as anthocyanin. Flavonoids are natural antioxidants that can capture free radicals that produce proinflammatory cytokines. Flavonoids release hydrogen atoms from the hydroxyl groups which can stop the production of

cytokines and decrease the IgE levels. In addition, flavonoids have the ability as cyclooxygenase inhibitor that reduces the production of proinflammatory mediators, and then provide feedback to the hypothalamus axis li-nes that capable to suppress IgE production<sup>23, 24</sup>.

Another mechanism is the presence of phenolic compounds in the tuber of *D. alata L.* which binds to receptors on the cell surface of lymphocytes, which are composed of proteins. According to Albert et al. (1994)<sup>25</sup> and Tejasari (2007)<sup>26</sup> phenolic compounds can bind the receptors in lymphocytes because phenolic compounds can bind to the protein. The existence of this bond can activate G proteins which then activate the phospholipase C enzyme. Phospholipase C breaks phosphatidylinositol bisphosphate (PIP2) into diacylglycerol (DAG) and inositol triphosphate (IP3) in the membrane. IP3 diffuses from the membrane to the cytosol and binds to a receptor protein on the cytoplasmic surface of the calcium sequestering compartment. This binding causes an increase in the concentration of Ca<sup>+2</sup> ion cytosolic. Diacyl glycerol and increased concentrations of Ca<sup>+2</sup> activates the enzyme protein kinase C. Activated protein kinase C is phosphorylate or transfer of a phosphate group to a specific serine or threonine residues of membrane proteins that activate exchange of Na<sup>+</sup>, H<sup>+</sup>, resulting in an increase in pH. The increasing of pH gives a signal the cell to proliferation activities. The activation of protein kinase C stimulates the production of interleukin-2 (IL-2) that activates B cells to proliferate. The ability mechanism of ethanol extract tuber of *D. alata L.* in allergic reactions

still require further research so it is expected that the ethanol extract tuber of *D. alata L.* can be used as a standardized herbal medicine. Ethanol extract tuber of *Dioscorea alata L.* dose of 0.64 g/kg is the best dose in this study because the dose of 0.64 g/kg had the lowest IgE levels and did not differ significantly with IgE levels of the control group and did not differ significantly in the treatment group with antihistamine drugs. IgE levels become an important parameter in this study because of allergic reactions involving specific IgE antibodies<sup>7</sup> and IgE is a key molecule that acts as a mediator of allergic responses (asthma, rhinitis, food allergy, atopic dermatitis, etc.<sup>8</sup>). The limitations of this study are that the ethanol extract of tubers of *Dioscorea alata L.* consists of several compounds and the entire compounds act as immunomodulatory compounds, so we need a further research to isolate the compounds which act as allergenic agents. Ethanol extract tuber of *Dioscorea alata L.* dose 0.62 g/kg and 1.24 g/kg can reduce levels of IgE and ethanol extracts tuber of *D. alata L.* dose of 1.24 g/kg is able to raise the TNF- $\alpha$  levels that is not significantly different with antihistamine drug dose 0.4 mg/mouse.

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