

Steroid Saponin in Ethanol
Extract Tuber of Purple Yam
(*Dioscorea alata* L.) as
Allergenic Agent on Sensitivity
Phase Balb/C Mice Model
Allergy on Transcription Factor
FoxP3 and Cytokine Profiles of
by Nurul Makiyah

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2nd ICHMS & 2nd LSC

PROCEEDING

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The 2nd International Conference of Medical and Health Sciences (ICMHS) and The 2nd Life Sciences Conference (LSC) 2016

*"Towards a Better Quality of Life
through Interdisciplinary Research"*

Yogyakarta, 9th-10th December 2016
The Alana Hotel and Convention Center

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**The 2nd International Conference of Medical & Health Sciences
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Committee of ICMHS & LSC 2016

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Chair	dr Iman Permana, M.Kes, Ph.D
Partner	Dr Zahid Iqbal, Ph.D
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Publication and Documentation section	dr April Imam Prabowo Arif Hadianto, ST
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**Chair person of The 2nd International Conference of Medical and
Health Sciences and The 2nd Life Sciences Conference 2016**



Welcome to Jogja, sugeng rawuh!

For the second time, the Faculty of Medicine and Health Sciences Universitas Muhammadiyah Yogyakarta is going to conduct the 2nd International Conference of Medical and Health Sciences (ICMHS) this December in vibrant Yogyakarta, Indonesia. This year we are going to collaborate with the Life Sciences Society of Pakistan for their 2nd Life Sciences Conference (LSC) with Dr. Zahid Iqbal as the general secretary.

This year's conference theme "Towards a better quality of life through interdisciplinary research" will be celebrating an era of seamless interdisciplinary integration and collaboration in scientific innovations with the involvement of more extensive topics and disciplines in the conference. We aim to exhibit the products of that kind of approach in solving challenges, improving the quality of life, and creating sustainable developments. We are happy to announce that our conference is filled with Invited speakers from Pakistan, United States of America, Uni Emirates Arab, Malaysia and Indonesia. Presentations will be conducted in oral as well as poster that covers topics from medicine, public health, dentistry, pharmacy, biomedical to agriculture. To put more credibility to the conference we are collaborating with Isra Medical Journal and the Asian Journal of Agriculture and Biology to publish selected papers from the event. Other paper will be published in the ISBN Proceeding book.

The last but not least, enjoy the conference, start networking and sharing ideas, and let immerse yourself to the heritage cultural ambient of Jogja, sumonggo!

Yogyakarta, 1st December 2016

dr. Iman Permana, M.Kes, Ph.D.

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**Dean of Faculty of Medicine and Health Sciences,
Universitas Muhammadiyah Yogyakarta**



Assalamu'alaikum Wr. Wb.

Science, especially in the areas of health and life growing more rapidly. We need to work together in the research of various disciplines to the advancement of science and to provide benefits to human life.

After successfully organized international scientific meeting last year, the Faculty of Medical and Health Sciences Universitas Muhammadiyah Yogyakarta, held the second scientific meeting ICMHS along with "2nd Life Sciences Conference". In this second scientific meeting, FKIK UMY collaborates with various researchers, among others from Pakistan, Malaysia, and the United States. Taking the theme "Towards a better quality of life through interdisciplinary research" we hope to establish cooperation with various parties to be able to contribute ideas to the civilization of human life.

Finally, we congratulate the scientific meeting in the city of Yogyakarta Indonesia. Enjoy the beautiful city of Yogyakarta with priceless historical relics. We hope that this meeting can run smoothly and provide benefits to the advancement of knowledge.

Wassalamu'alaikum Wr. Wb.

Yogyakarta, 1st December 2016

dr. Ardi Pramono, M.Kes, Sp.An.

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Rector of Universitas Muhammadiyah Yogyakarta



Assalaamu'alaikum Wr. Wb.

Ladies and Gentlemen,

Welcome to the 2nd International Conference on Medical and Health Science in conjunction with the 2nd Life Sciences Conference 2016

Welcome to Yogyakarta City of Tolerance

Our Faculty of Medicine and Health Sciences has been doing such international conference almost every year for the last ten years. This and other previous conferences are the things that supporting our vision as an excellence and Islamic university, a young and global university. We will always try to keep monitoring the development of science through sending more lecturers to do the sabbatical leave overseas, doing international research collaborations and also the international conference. Each department should do this strategy of internationalization so that each department has its own network. Faculty of medicine and health science is one of the most progressive units in implementing this strategy by inviting international experts on a regular basis. This program will certainly strengthen our vision.

International conference on medicine and health sciences is a smart choice to offer our lecturers access to the most recent development of the subjects. The participants will also gain the same knowledge and latest information on medicine and health sciences. As everyone knows that the development of science and technology are faster today compared to the previous period. Information technology, computer, and other development have fastened the transformation of medicine and health science into the different and more complex stage.

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Cellular technology, for instance, can be used for several functions including those that directly impacts our daily life. There is no long distance call anymore today because cellular phone can do everything we need to contact other people far from where we stand anytime anywhere. People will finally innovate cellular phone for the sake of personal health services. We will in the future using our simple cellular phone to detect our body temperature, blood pressure, even how much fat we have in our body and how much it is supposed to be. We may also be able to check the health of our body without leaving our house and order medicine without going into the drug store. Everything is almost possible as long as we think hard for the better of people in the future. Enjoy the conference and don't forget to visit our rich tourist destinations, mountains, beaches or caves (underground waterways).

Thank you

Wassalaamu'alaikum Wr. Wb.

Prof. Dr. Bambang Cipto, MA

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Keynote Speech

**by Head of Provincial Health Office Special Region of Yogyakarta
in International Conference
of Medical and Health Sciences and Life Sciences Conference**

The Alana Hotel and Convention Center, Yogyakarta, December 9-10, 2016

The honorable:

- Rector of Muhammadiyah University of Yogyakarta,
- The Dean of Medical and Health Sciences Muhammadiyah University of Yogyakarta,
- The chairman of organizing committee of the international conference of medical and health,
- Distinguished guests and colleagues.

Assalamu'alaikum Warahmatullahi Wabarakatuh,

First of all, we thank God for His blessings that today we may attend the International Conference of Medical Health Towards a Better Quality of Life Through Interdisciplinary Research in Yogyakarta.

My distinguished colleagues,

In Indonesia National Long Term Development Plan (2005-2024), the Indonesian Ministry of Health have determined a paradigm shift that have governed health services in health development plan. There has been a shift from Curative Health Services to Preventive and Promotive Health Services.

Recently, Indonesia suffers from a triple burden of diseases as health development challenges. The triple burden of diseases are: 1) the backlog of common infections, undernutrition, and maternal mortality; 2) the emerging challenges of non-communicable diseases (NCDs), such as cancer, diabetes, heart disease; and 3) mental illness, and the problems directly related to globalization, like pandemics and the health consequences of climate change.

Dear colleagues,

Here are some data that show several health problems in Indonesia:

1. Maternal mortality rate in 2015 is 4,809 cases, infant mortality rate in 2015 is 22,267 cases;
2. Regarding to children under the age of five, the national stunting rate is 37.2% which consists of 18% for very short dan 19.2% for short (Riskesdas 2013);

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3. HIV testing coverage is 14% dan antiretroviral (ARV) therapy coverage is 65.58% (Directorate General of Disease Control and Prevention Ministry of Health, 2015);
4. Tuberculosis (TB) notification rate in 2015 is 73.5% and tuberculosis treatment success rate is 72% (Directorate General of Disease Control and Prevention Ministry of Health, 2015).

Distinguished guests,

Indonesia Health Development Program in 2015-2019 strengths in improving human quality life through Health Indonesia Program with family approach. The Indonesian Ministry of Health issued The Minister of Health Regulation (Permenkes) No. 39 Year 2016 as a Guideline of Implementation of Health Indonesia Program with Family Approach. This program has 12 main indicators as markers of a family health status. Currently, many health programs have been implemented by Indonesian Ministry of Health, Provincial Health Offices, and District Health Offices. However, many health problems, some as mentioned above, still become health burdens. We may ask a question whether the programs that we conducted have answered the health problems we have in Indonesia.

It would be better if all health programs that we implement based on scientific health research, especially interdisciplinary research. The research should be related to detection, prevention, and treatment of diseases or problem solving for better health.

My dear colleagues,

Being a province with speciality, Special Region of Yogyakarta placed Traditional Medicine as one of the priority programs in Provincial Medium Term Development Plan (2017-2022). We still encounter many challenges in developing Traditional Medicine, especially in providing services which are based on scientific evidence.

Distinguished colleagues,

We look forward to results of interdisciplinary research which would support health problem solving, especially by developing traditional medicine in Yogyakarta. We believe that collaboration in interdisciplinary research would improve quality of human life.

Finally,

Thank you for your attention. We wish you a successful conference.

Wassalamu'alaikum Warahmatullahi Wabarakatuh,

On behalf of
the Head of Provincial Health Office
Special Region of Yogyakarta

Drg. Pembajun Setyaningastutie, M.Kes

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**SPEAKER OF
INTERNATIONAL CONFERENCE**

Zahid Iqbal

Al-Nafees Medical College Isra University Islamabad Campus Islamabad, Pakistan
"One Health Program for Public Health Benefit"

Prof. Dr. Abdul Khaliq

Professor, Department of Agronomy, University of Agriculture, Faisalabad
"Role of Agriculture in Poverty Alleviation of Rural Areas"

Fitri Arofati

Universitas Muhammadiyah Yogyakarta, Indonesia
"Continuing Professional Development of Practicing Nurses in Indonesia"

Tri Wahyuliati

Universitas Muhammadiyah Yogyakarta, Indonesia
"Diabetic Neuropathy - A Chance Towards A Better Treatment"

Mohammad Khalid Ashfaq

University of Mississippi, USA
"Natural Products –Use or Misuse"

Muhammad Mukhtar

American University of Ras Al Khaimah, United Arab Emirates
"Emerging Biotechnologies and Genomic Medicines in Human Health and Well-Being"

Muhammad Sasmito Djati

Brawijaya University Malang, Indonesia
"Herbal Medicine a Holistic Approach: in case of food supplement formulation of Sauropusandrogynus and Elephantopuscaberto modulate immune and hormonal system in pregnant Salmonella typhi infected mice"

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REVIEWER

1. Dr. Zahid Iqbal, Ph.D (Isra University, Islamabad, Pakistan)
2. Prof. Dr. Abdul Khaliq (University of Agriculture, Faisalabad)
3. Dr. Mohammad Khalid Ashfaq, DVM, DTVM, MS, Ph.D (University of Mississippi, USA)
4. Dr. Muhammad Mukhtar, Ph.D (American University of Ras Al Khaimah, United Arab Emirates)
5. Dr. Ir. Muhammad Sasmito Djati, MS. (Brawijaya University Malang, Indonesia)
6. Fitri Arofiati, S.Kep., Ns., MAN., Ph.D (Universitas Muhammadiyah Yogyakarta, Indonesia)
7. Dr. SN Nurul Makiyah, S.Si., M.Kes (Universitas Muhammadiyah Yogyakarta, Indonesia)
8. dr. Iman Permana, M.Kes, Ph.D (Universitas Muhammadiyah Yogyakarta, Indonesia)
9. Dr. dr. Ikhlas M. Jenie, M.Med, Sc (Universitas Muhammadiyah Yogyakarta, Indonesia)
10. Dr. dr. Arlina Dewi, M.Kes, AAK (Universitas Muhammadiyah Yogyakarta, Indonesia)
11. dr. Oryzati Hilman, M.Sc, CMFM (Universitas Muhammadiyah Yogyakarta, Indonesia)
12. Dr. Dra. Yoni Astuti, M.Kes, Ph.D (Universitas Muhammadiyah Yogyakarta, Indonesia)
13. Dr. drg. Tita Ratya Utari, Sp. Ort (Universitas Muhammadiyah Yogyakarta, Indonesia)
14. Dr. dr. Tri Wahyuliati, Sp.S, M.Kes (Universitas Muhammadiyah Yogyakarta, Indonesia)
15. Dr. Elsy Maria Rosa, M.Kep (Universitas Muhammadiyah Yogyakarta, Indonesia)
16. Dr. dr. Titiek Hidayati, M.Kes (Universitas Muhammadiyah Yogyakarta, Indonesia)
17. Dr. Shanti Wardaningsih, M.Kep., Ns., Sp.Kep.J., Ph.D. (Universitas Muhammadiyah Yogyakarta, Indonesia)
18. Dr. dr. Sri Sundari, M.Ke (Universitas Muhammadiyah Yogyakarta, Indonesia)
19. Dra. Lilis Suryani, M.Kes (Universitas Muhammadiyah Yogyakarta, Indonesia)
20. Drh. Tri Wulandari K, M.Kes (Universitas Muhammadiyah Yogyakarta, Indonesia)
21. Dr. dr. Wiwik Kusumawati, M.Kes (Universitas Muhammadiyah Yogyakarta, Indonesia)
22. Sabtanti Harimurti, S.Si., M.Sc., Ph.D., Apt. (Universitas Muhammadiyah Yogyakarta, Indonesia)

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**SPEAKER OF
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ICMHS-O-1-43

**Steroidal Saponin in Ethanol Extract Tuber of Purple Yam
(*Dioscorea alata* L.) as Allergenic Agent on Sensitivity Phase
Balb/C Mice Model Allergy on Transcription Factor FoxP3 and
Cytokine Profiles of T reg**

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Muhammad Sasmito Djati³

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²Pharmacy Study Programme, Faculty of Medicine & Health Science,
Universitas Muhammadiyah Yogyakarta

³Biology Department, Faculty of Mathematic and Sciences, Brawijaya University

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¹
Abstract

Purple yam (*Dioscorea alata* L.) is a source of biological tubers not been used optimally. Diosgenin is steroids saponin compounds that most important because it has multiple biological functions, such as a¹ allergenic activity. The objective of this research was to analyze the potential allergenic ethanol extract tuber of *D. alata* L. (EEDA) on BALB/c mice on sensitivity phase with measure the profile of transcription factor FoxP3 Treg cells and cytokine profile of Treg and IgE and IgG1 B cells. An experimental study using BALB/c mice divided into 7 groups: control group (I), the treatment group (II-V) ethanol extract of tuber *Dioscorea alata* L. dose of 0.00; 0.17; 2.01; 10.04 g/kg bw, the treatment group antihistamines drug and Diosgenin (VI-VII). For 17 consecutive days the group II-VII were treated in accordance with the group and with Ovalbumin induced allergy models. Mice were sacrificed on day 18. Spleen is removed, lymphocyte isolated and analyze the transcription factor FoxP3 Treg cells, cytokine profile of T reg cells and IgE and IgG1 B cells on spleen using Flowcytometry FACS Calibur. The results showed EEDA able to inhibit the production of B220IgE and B220IgG1, trigger Treg cells (CD4CD25) and the transcription factor FoxP3 (CD4CD25FoxP3) and profile of the cytokines produced by T reg cells. The conclusion is ethanol extract tubers of *Dioscorea alata* L (EEDA) does not trigger Treg (CD4CD25) and the transcription factor FoxP3 (CD4CD25FoxP3) and cytokine profiles produced by Treg cells namely CD4IL-10, CD8IL-10, CD4TGF- β and CD8TGF- β in the sensitivity phase.

⁶
Keywords: Saponin steroid of ethanol extract of the tubers of *Dioscorea alata* L.; allergenic agent; Treg cells; the transcription factor FoxP3; lymphocyte.

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INTRODUCTION

Purple yam (*Dioscorea alata* L.) is a source of biological tubers that has not been used optimally. Some nutrients contained in *Dioscorea* species are carbohydrates, essential amino acids, minerals, polyphenols, mucilage (glycoprotein), a purine derivative (e.g. allantoin) and steroidal saponin.¹⁻¹⁰ *Dioscorea alata* L. also contains diosgenin¹¹ which is the main aglycon steroidal saponin which acts as steroid intermediates in the pharmaceutical industry. Steroidal saponin is the most important bioactive compounds because it has multiple biological functions, including the activity of anticarcinogenic, antithrombotic, antiviral, haemolytic, hypocholesterolemic, hypoglycemic, immunostimulatory, antitumorogenic, antimutagenic, immunomodulatory and anti-inflammatory depending on its structure.⁵⁻⁸ Some researcher also suggest that steroidal saponins have hypoallergenic activity.¹²

The prevalence of food allergies is estimated between 5-10% and its prevalence has increased over the last ten years worldwide.^{13,14} Immunological responses have a tendency to induce both Th1 or Th2 cytokine profile. Th2 lymphocytes predominantly produce IL-4 and IL-5 while predominantly Th1 lymphocytes produce IL-2, TNF- α and IFN- γ .¹⁵ Someone with allergies have a dominant response to Th2 lymphocytes and specific IgE antibodies against the protein due to failure of the normal tolerance response as occurs in normal individuals.¹⁶ Th2 lymphocytes play an important role in the development of allergic diseases. Th2 lymphocyte phenotype characterized by the production of the cytokine IL-4, IL-5 and IL-13, directly activate and accumulation of eosinophils, basophils and transform B lymphocytes to produce IgE.^{17,18} IL-4 is the most important Th2 cytokine required by the B lymphocytes to produce IgE, mast cell activation and differentiation of Th2 lymphocytes.¹⁹

Allergens that enter in the body will be processed by APC (antigen presenting cells). Peptides of allergen are presented by APC induce lymphocyte activation T naive cells, T naive cells further activates lymphocytes to produce Th2, its cytokines.²⁰ Each T lymphocytes express the TCR (T cell receptor) which is unique to the cell surface as a result of clonal selection in the process of maturation in the thymus. Mature T lymphocytes known as naïve T cells, circulating through the blood and lymphatic systems and are naïve T lymphocytes in secondary lymphoid organs.²¹

The objective of this research was to analyze the allergenic potency of ethanol extract tuber of *Dioscorea alata* L. (EEDA) on BALB/c mice model digestive tract allergy with measure the IL-4 density of blood sera.

1 **MATERIALS AND METHODS**

An experimental study using BALB/c mice divided into 7 groups: control group (I), the treatment group (II-V) ethanol extract of tuber *Dioscorea alata* L. dose of 0.00;

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0.17; 2.01; 10.04 g/kg bw, the treatment group with antihistamines drug and Diosgenin (VI-VII). For 30 consecutive days the group II-VII were treated in accordance with the group and with Ovalbumin induced digestive tract allergy models.

The research was conducted at the Laboratory of Animal Physiology, Laboratory of Molecular Biology Department of Biology, Faculty of Mathematics and Natural Sciences and Biomedical Laboratory, Faculty of Medicine, University of Brawijaya, Malang, East Java, Indonesia.

Experimental animals are BALB/c mice (*Mus musculus*), 6- 8 weeks, with a healthy condition. This research has taken the certificate of eligibility of ethics (Ethical Clearance) of the Research Ethics Committee (Animal Care and Use Committee) University of Brawijaya number KEP-144-UB.

Ethanol extract tubers of *Dioscorea alata* L. was made according to a previous study by Lee et al.²² The tuber of *Dioscorea alata* L. peeled, washed, cut thin, then dried in the sun and covered with a black cloth to dry further pulverized in a blender into tiny particles known as simplicia. Simplicia is made an extract with a solution of 70 % ethanol for seven days with maceration method.

Dose of ethanol extract of the tubers of *Dioscorea alata* L. converted from human dose with 70 kg body weight into 20 g of mice, multiplied by the conversion value 0.0026, so we get the first dose of 0.17 g/kg, second doses of 2.01 g/kg and third doses of 10.04 g/kg.

Mice Model Allergic Digestive Tract. BALB/c mice intraperitoneally sensitized and challenged with OVA (ovalbumin). Mice immunized with intraperitoneal injections on day 15 with 0.15 ml OVA in Al(OH)₃ which is made of 2.5 mg OVA dissolved in 7.75 ml of aluminium hydroxide. On day 22, there was intraperitoneal injection with OVA in 0.15 ml of distilled water per 2.5 mg OVA mice was dissolved in 10 ml of distilled water. On day 23 until day 30, mice received oral booster with OVA in 0.15 ml of distilled water which is made by mixing 2.5 mg OVA in 10 ml of distilled water.^{23,24} On day 18, day 25 and day 31, three mice from each group were taken of its blood from retroorbital vein.

Isolation of Blood Sera. The blood is isolated from retroorbital vein of mice collected in Eppendorf 1 ml. Blood centrifuged at 2,500 rpm, 4°C for ten minutes to obtain serum which will be used in determining IL-4 protein density with Western blotting methods.

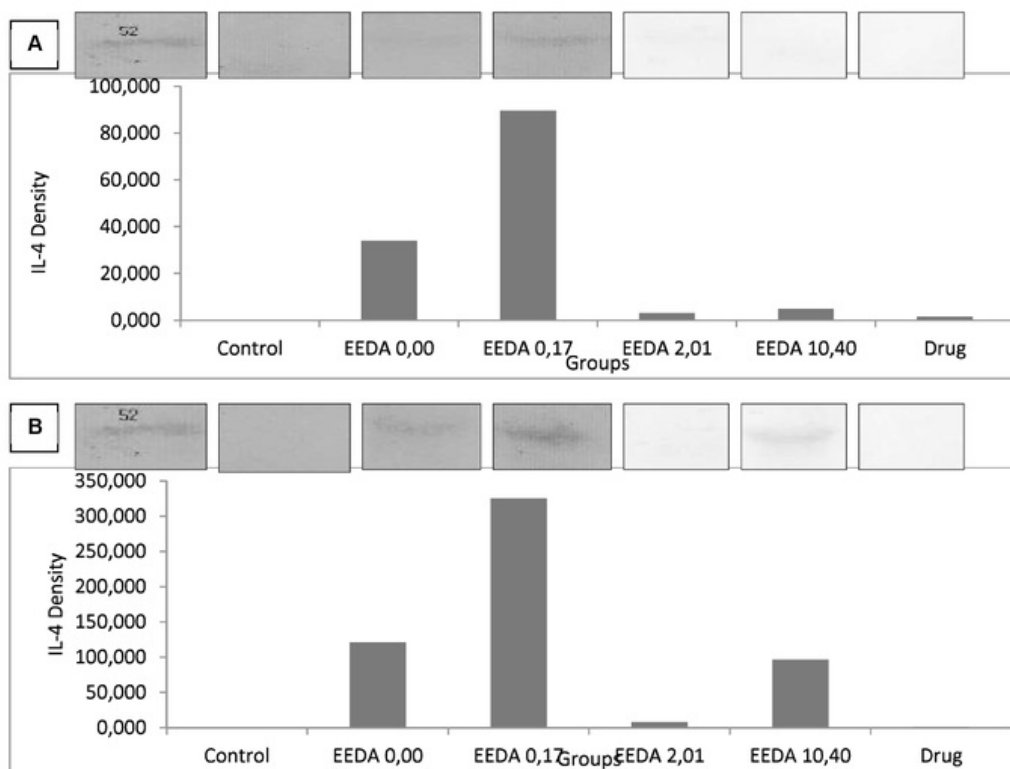
Detection of IL-4 protein by Western blotting. Blood sera was suspended with buffer containing 20 mM TrisHCl, 1 mM EDTA, and 0.1 mM PMSF. Blood sera were centrifuged 12,000 rpm on 4° C for 5 minutes, centrifuged again 15,000 rpm on 4° C for 1 hour. Samples separated with 15% SDS-PAGE gel. The results of gel electrophoresis are washed with distilled water and soaked in blotting buffer. NC membrane (nitrocellulose) are cut, then moistened with PBS for 10 minutes at room temperature.

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Membrane immersed in blotting buffer before blotting process. Furthermore, the transfer was performed for 12 hours at 25 volts at 4° C. After completion of the transfer, the membrane are blocking in PBS-T Skim milk 5% for 1 hour while shaken. Washed 3x5 minutes in PBS-T. Incubation with primary antibodies diluted in PBS-T Skim milk 5% (1: 200) overnight at 4° C. Washed 3x 5 minutes with TBS. Incubated with AP conjugated secondary antibody (1: 2500 in TBS) for 1 hour at room temperature. Washed with PBS-T for 4x5 minutes. Furthermore, protein or antigen detected band with the addition of the substrate Western Blue (in the dark) membrane for overnight or until visible color bands. The reaction was stopped by washing the membrane in distilled water.²⁵ Western Blot qualitative data were analyzed by using ImageJ Software and measured the density of the protein IL-4.

RESULTS

Mice were sacrificed on day 18 as sensitivity phase, day 25 as challenge phase and day 31 as digestive tract allergy phase, blood sera is isolated and analyzed the IL-4 density using Western Blotting.



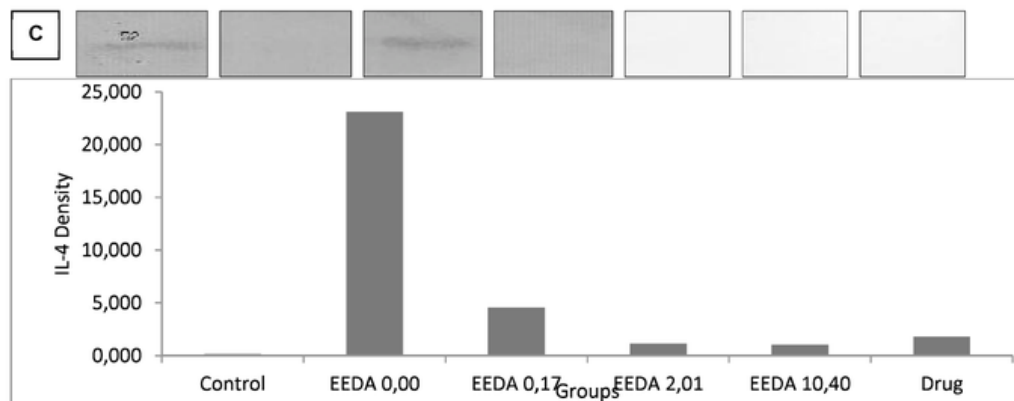


Figure 1. The density of IL-4 blood sera on EEDA 2.01; 10.04 g/kg and Drug groups decreased on the sensitization phase (A), the challenge phase (B) and the phase of the digestive tract allergies (C). The density of blood serum IL-4 in the sensitization phase and challenge phase EEDA group 0.17 g/kg increased, while in the group phase of the digestive tract allergy EEDA 0.17 g /kg decreased.

In the sensitization phase, the highest IL-4 density of blood sera in the treatment group of EEDA0,17 g/kg followed with treatment group of EEDA 0.00 /kg and the lowest of IL-4 density of blood sera in the untreated control group followed with the Drug treatment group, the treatment group with EEDA 2.01 g/kg and EEDA 10.40 g/kg . There has been a decreased in the IL-4 density of blood sera in the treatment group with EEDA 2.01; 10.04 g/kg and Drug treatment group compared with treatment group with EEDA 0.00 g/kg, but the density of blood serum IL-4 in the treatment group with EEDA 0.17 g/kg was increase.

Similarly in the challenge phase, the highest IL-4 density of blood sera in the treatment group of EEDA0,17 g/kg followed with treatment group of EEDA 0.00 /kg and the lowest of IL-4 density of blood sera in the untreated control group followed with the Drug treatment group, the treatment group with EEDA 2.01 g/kg and EEDA 10.40 g/kg . There has been a decreased in the IL-4 density of blood sera in the treatment group with EEDA 2.01; 10.04 g/kg and Drug treatment group compared with treatment group with EEDA 0.00 g/kg, but the density of blood serum IL-4 in the treatment group with EEDA 0.17 g/kg was increase.

The digestive tract allergy phase, the highest IL-4 density of blood sera in the treatment groups EEDA 0.00 g/kg followed with treatment group EEDA 0.17 g/kg and Drugs treatment group , while the lowest IL-4 density of blood sera in the untreated

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control group followed with treatment group EEDA 2.01 g/kg and treatment group EEDA 10.40 g/kg. It has been a decline in the IL-4 density of blood sera in the treatment group EEDA 0.17; 2.01; 10.04 g/kg and Drug treatment group compared with treatment group EEDA 0.00 g/kg.

Anti Histamine Drug (AHD) effect on density of IL-4 blood sera of mice in the sensitization phase, the challenge phase and digestive tract allergic phase showed a decrease the density of IL-4.

DISCUSSION

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Interleukin (IL) -4 is a key cytokine in the pathogenesis of asthma and other allergic reactions. Expression of IL-4 plays a role in stimulating the differentiation of CD4 + T cells naïve become Th2 cells, proliferation or clonal expansion Th2 cells, stimulates switching lymphocytes-B in producing IgE, increased expression of IgE receptor (FcεRI) on B-lymphocytes and cells mononuclear phagocytes, and FcεRII on mast cells and basophils after antigen exposure.^{19,26,27} In asthma, IL-4 stimulates the fibroblasts to eotaxin and cytokine-producing pro-inflammatory cytokines that cause airway remodeling and increased the expression of VCAM-1 on vascular endothelium. Through the interaction of VCAM-1, IL-4 has led directly to the migration of T lymphocytes, monocytes, eosinophils, and basophils to sites of inflammation.²⁸ Interleukin (IL) -4 are also able to inhibit apoptosis of T lymphocytes. Persistence and activation of T lymphocytes (Th2 cells) have positive feedback (positive feedback) with increasing the production of IL-4 followed by an increase in differentiation and clonal expansion of Th2 cells.²⁹

Diosgenin has suppressive effect against intestinal Th2 response associated with an increased of Treg cell immunity. Diosgenin reduce the expression of IL-4 and GATA-3 intestinal in BALB/c mice which were sensitized with Ovalbumin. This indicates that the suppressive effect of Diosgenin against intestinal Th2 response allergen-induced immunity is closely related to the upregulation of Treg cells at sites of inflammation (Huang et al., 2010).³⁰

Diosgenin has allergenic activity on RBL-3H3 cells, the aglican group has higher activity than the molecular diglucosilation, substitution with rhamnoglucosida seems reduced its allergenic activity. Furthermore, the effects dioscorealida A, dioscorealide B and dioscoreanone induced with the antigen which influence release of IL-4 and TNF-α on the late phase of an allergic reaction has also been performed by Tewtrakul & Itharat 2006.³¹ One of the parameters used to analyze the development of allergic process is a Th1-Th2 balance. In allergic conditions, it increased Th2 cytokine profile. The results of this study demonstrate that Ovalbumin as allergens success to create mice model of allergic indicated with Ovalbumin capable to increased the profile of Th2 cytokines

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in the sensitization phase, challenge phase and digestive tract allergic phase BALB/c mice groups DA 0,00 g/kg which is only injected Ovalbumin compared to DA group 0.17; 2.01; 10.04 g/kg; and Drug.

There has been a decreased in the IL-4 density of blood sera in the treatment group with EEDA 2.01; 10.04 g/kg and Drug treatment group compared with treatment group with EEDA 0.00 g/kg, but the density of blood serum IL-4 in the treatment group with EEDA 0.17 g/kg was increase on the sensitization phase and challenge phase. The digestive tract allergy phase, it has been a decline in the IL-4 density of blood sera in the treatment group EEDA 0.17; 2.01; 10.04 g/kg and Drug treatment group compared with treatment group EEDA 0.00 g/kg.

The results of this study indicate that the *Dioscorea alata* L. has Saponin Steroids as active compounds that it useful as allergenic agent so it can decrease the Th2 cytokine profile that is density of IL-4 blood sera of mice in the sensitization phase, the challenge phase and digestive tract allergic phase was not significantly different to the Control groups and Drug groups. Diosgenin is a Saponin Steroids active compound which is content on *Dioscorea* species. Anti Histamine Drug (AHD) effect on density of IL-4 blood sera of mice in the sensitization phase, the challenge phase and digestive tract allergic phase showed a decrease the density of IL-4.

The results of this study according to Huang et al. (2010) which showed that giving Diosgenin reduce intestinal IL-4 expression in BALB/c mice were sensitized Ovalbumin.³⁰

The results of this study demonstrate that the Th2 cytokine profile that IL-4 increased after stimulation with Ovalbumin intraperitoneal injection on day 15 and day 22 and orally on day 23 until day 30. High expression of IL-4 corroborates the hypothesis⁶ that increased Th2 cytokine production in BALB/c mice model of food allergy (Leung et al., 2008; Cardoso et al., 2008; Nakajima-Adachi et al., 2006).^{32,33,34}

Steroid Saponins can reduce the differentiation and proliferation of inflammatory cells that can act as an immunosuppressant. Steroidal Saponins can inhibit inflammation by activating the glucocorticoid receptor that inhibits the bond between the nuclei of proinflammatory DNA-binding transcription factors such as activator protein (AP-1) and the Nuclear Factor-kappa Beta (NF-κB) (Karin, 1998; Ito et al., 2000).^{35,36} Glucocorticoids are known to inhibit the formation of cytokines through the JAK-STAT pathway (Bianchi et al., 2000).³⁷

Steroidal saponins suppress allergic inflammatory reaction through genomic mechanisms that occur through activation of annexin-1 (lipocortin-1) and mitogen-activated protein kinase (MAPK) phosphatase 1. The addition of steroid saponins also increase anti-inflammatory gene transcription secretory leucoprotease inhibitory (SLPI), interleukin-10 (IL-10) and inhibitory nuclear factor-κB (INκB-α). Annexin-1 inhibits

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the release of arachidonic acid thus decreasing production of inflammatory mediators (prostaglandins, thromboxane, prostacyclin and leukotrienes). The enzyme MAPK phosphatase 1 cause MAPK 1 is inactive, so the activation of T cells, dendritic cells and macrophages inhibited. Other genomic mechanisms such as inhibition of transcription factors that play a role in the production of inflammatory mediators, namely the nuclear factor- κ B (NF- κ B) and activator protein-1 (AP-1). NF- κ B and AP-1 regulates gene expression of cytokines, inflammatory enzymes, proteins and receptors that play a role in inflammation (IFN- γ , TNF- α and IL-1). Inhibition of NF- κ B and AP-1 will decrease the production of inflammatory mediators (Sitompul, 2011).³⁸

CONCLUSION

EEDA has a potency as allergenic agents of mice BALB/c model digestive tract allergy model on sensitivity phase, challenge phase and digestive tract allergy phase through decreased of the IL-4 density of blood sera.

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