THE EFFECTIVENESS OF DEWANDARU (EUGENIA UNIFLORA L.) LEAF CRUDE EXTRACT ON IMMUNOGLOBULIN E LEVEL IN WISTAR RATS WITH ALLERGIC RHINITIS

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ABSTRACT Background: Dewandaru plant (Eugenia uniflora L.) is a plant that contains flavonoids with anti-inflammatory, anti-allergy and antioxidant effects that have various clinically proven benefits. Aims: To determine the effectiveness of Dewandaru (Eugenia uniflora L.) leaf crude extract on total IgE levels in Wistar rats with ovoalbumin-induced allergic rhinitis. Methods: This is a true experimental study with a randomized post-test only control group design. This study used 28 male Wistar rats randomly divided into 4 groups (n=7 in each group), i.e. negative control group (without therapy), 100 mg/kg BW in extract group, 200 mg/kg BW in extract group, and positive control group (mometasone furoate). Rats were sensitized with ovoalbumin intraperitoneally for 14 days, followed by intranasal ovoalbumin exposure on the 15th day for 14 days with intervention. Nasal symptom scores and IgE serum levels were assessed in all groups. Results: There were significant differences in nasal symptoms scores between groups. Nasal symptoms scores were significantly lower in the positive control group and treatment group than in the negative control group (p<0.001), and there was no significant difference between the positive control group and treatment group (p=0.362 and p=0.457). However, there was no significant difference in mean IgE levels (p=0.705). Conclusion: Dewandaru leaf crude extract is not effective in reducing total IgE levels, but it effectively reduces symptoms of allergic rhinitis and has similar effects to topical corticosteroids.

KEYWORDS Allergic rhinitis, rat, flavonoid

Introduction

The Dewandaru plant (Eugenia uniflora L.) treats fever, rheumatism, gastric disease, digestive system disorder, hypertension, and gout. It is also postulated to reduce body weight and blood pressure and act as a diuretic.1 Flavonoids from dewandaru leaf extract in the form of quercitrin, quercetin, myristicin and myricetin found in the re-
allergy in reducing immunoglobulin E (IgE) in Wistar rats with allergic rhinitis.

Methods
This is a true experimental study with Randomized Post-test Only Control Group Design by evaluating dewandaru (Eugenia uniflora L.) crude extract at doses 100 mg/kg BW and 200 mg/kg BW.

Dewandaru leaf was extracted at the Laboratory Service Unit of the Faculty of Agricultural Technology, Udayana University. Then, the animals were treated at Animal Laboratory Unit, Department of Pharmacology, Udayana University, followed by measuring total serum IgE levels in the Immunology Laboratory, Faculty of Veterinary Medicine, Udayana University. This study was conducted from June 2018 to August 2018.

Results
This true experimental study tested dewandaru (Eugenia uniflora L.) leaves crude extract at doses of 100 mg/kg BW and 200 mg/kg BW. The study was conducted at the Animal Laboratory Unit of Pharmacology Department, Faculty of Medicine, Udayana University, from July 2018 to August 2018. This study involved 28 male Wistar rats with a Randomized Post-test Only Control Group Design.

Characteristics of the Subjects’ Nose Symptoms Score
According to the study groups, the results showed that nasal symptoms scores in Wistar rats revealed varying characteristics. A comparison of nasal symptoms scores between each study group can be seen in Table 1 below.

Based on Table 1 above, there were differences in nasal symptoms scores between the study groups. The nasal symptoms score in the negative control group was compared with the positive control group, the extract group at doses of 100 mg / kg BW and 200 mg / kg BW. There were significant differences with p-value <0.001 (p <0.05). Whereas in the positive control group compared to the extract group at dose of 100 mg / kg BW and 200mg / kg BW, there was no significantly difference with p-value = 0.362 and p = 0.457 (p> 0.05), respectively.

In the positive control group, both doses of extract groups showed a decrease in nasal symptoms score compared to the negative control. Furthermore, both groups found that both doses were equally effective in reducing nasal symptoms scores. These characteristics can be illustrated graphically in Figure 1.

As shown in Figure 1, the nasal symptom score in Wistar rats with allergic rhinitis in four weeks of study, at first was not much different from the average score of 1-2. After 14 days, the negative control showed that nasal symptoms score began to increase, especially on the 15th, 16th, 20th and 25th day. Whereas in the other three groups, i.e positive control group, extract group at dose of 100mg / kgBW and 200mg / kgBW showed a different trend from the negative control, which showed an increase on day 15th but thereafter it was tended to decrease.

Comparison of Average Total IgE Levels in Wistar Rats Suffering from Allergic Rhinitis After Administering Dewandaru Leaves Coarse Extract (Eugenia uniflora L.)
In this study, the normality of total IgE level was tested using the Shapiro-Wilk, and showed normal distribution with p> 0.05. Then, data was analyzed using One-Way ANOVA test. Mean IgE levels could be seen in Table 2.

Based on the results of One-Way ANOVA test, it was found that the total IgE levels in negative control group was 255.0 ± 59.1 and in positive control group was 241.1 ± 53.2, while in dose of 100mg / kgBW group was 251.1 ± 70.0 and in dose of 200 mg / kgBW dose group was 276.1 ± 30.7. There was no statistically significant difference between total IgE levels in the four study groups; p value = 0.705 (p> 0.05). The comparison of mean total IgE levels between each study group could be seen in Table 3.

Based on Table 3, the results of Post Hoc for IgE levels in each study group found no significant difference with p> 0.05.

Discussion
Characteristics of Subjects
Allergic rhinitis in an experimental animal model can be assessed by nasal symptoms score after the subjects were induced with ovalbumin. In this study, Wistar rats with allergic rhinitis were initially induced by intraperitoneal ovalbumin for 14 days during the sensitization phase, then continued re-exposure of ovalbumin intranasally for 14 days. Repeated administration of intranasal ovalbumin can cause symptoms of allergic rhinitis such as sneezing and scratching the nose, and rats are good experimental animal models for allergic rhinitis study.6–8

Table 1 Comparison of nasal symptoms score of each study group

| Comparison between groups | p-value |
|---------------------------|---------|
| Negative control group    | < 0.001 |
| 100 ml/kgBW of extract group |         |
| 200 ml/kgBW of extract group |         |
| Positive control group    | < 0.001 |
| 100 ml/kgBW of extract group |         |
| 200 ml/kgBW of extract group |         |
| Negative control group    | <0.001  |
| Positive control group    | 0.362   |
| 100 ml/kgBW of extract group |         |
| 200 ml/kgBW of extract group |         |
| Negative control group    |         |
The results of this study demonstrated that nasal symptoms score in Wistar rats suffering from allergic rhinitis within four weeks of study in the negative control group was higher than the positive control group, the extract group at the dose of 100 mg/kg BW and dose of 200mg / kg BW, where the three groups had significantly the same value. It could be said that the Dewandaru leaves extract group had an effect similar to the drug used as a positive control. The Dewandaru plant (Eugenia uniflora L.) is one of Indonesia’s local wisdom known to have many clinically tested benefits. The leaves contain flavonoids including myricetin, myristicin, gallocatechin, quercetin and quercitrin.2,4

The positive control group used intranasal corticosteroid, mometasone furoate, in this study. Mometasone effectively suppresses antibody production by directly affecting nasal immune cells such as antigen-presenting cells and specific T cell antigens. Mometasone has an immunosuppressive effect and has a direct effect on inhibiting allergic reactions, leading to an inhibitory effect on nasal symptoms and IgE production even after treatment has been completed. It is known that serum IgE levels decrease when allergen exposure is eliminated. It is also postulated that a decrease in IgE levels can be associated with the additional effect of corticosteroids. As a result, therapeutic interventions can reduce inflammation in the airways and reduce serum IgE levels by decreasing airway permeability.6

In this study, based on the nasal symptoms score, it can be said that Dewandaru leaves extract has a similar effect in reducing allergic rhinitis symptoms. In this case, it is probably due to the flavonoids in the extract, one of which is quercetin, which works as a mast cell stabilizer and has an effect on the rapid phase of allergic reactions that can inhibit the inflammatory process.10

In this study, Dewandaru leaf crude extract was used as a whole since no one had examined the effect of this extract as an anti-allergy. The crude extract of Dewandaru leaves contains quercetin and other flavonoids such as quercitrin, myricetin, and myristicin, which have also been studied to have anti-allergic effects. Treatment with the medicinal plant approach, often called the “herbal shotgun” approach, is very different from the modern therapeutic approach, often referred to as the “magic bullet” approach, which uses one of the pure components to target specific cells or certain physiological pathways.11

Based on the approach in most chronic diseases involving multiple pathways, the “herbal shotgun” approach is appropriate for treatment. It is known that flavonoids have immunomodulatory and anti-inflammatory activities and antioxidant effects. Several studies have found that bee pollen and some flavonoids can inhibit rapid types of allergic reactions by inhibiting mast cell activation. Flavonoid components such as myricetin, kaempferol, quercetin, and luteolin can inhibit mast cell degranulation and IgE-related allergy. Flavonoids can suppress intracellular calcium and inhibit calcium phosphorylation in human mast cells. This component inhibits cyclic AMP phosphodiesterase and calcium-dependent ATPase, which is responsible for the release of histamine from mast cells and basophils.12

Flavonoids are also said to interfere the initial chain of free radicals on the surface of the cell membrane which will prevent the progressive formation of free radicals. This process will inhibit the occurrence of further inflammatory processes.13 Similar results to this study were obtained in the study by Sagit, where it was found that the symptoms of allergic rhinitis could be suppressed in the steroid and quercetin groups. It is said that quercetin may reduce these symptoms by suppressing the inflammatory pathway and has almost the same results as steroids.6

It is stated that there is much evidence that giving oral quercetin and its isoquercetin derivatives to allergic patients can modify the clinical condition of the disease. This quercetin therapy mode may be partly due to its suppressive effect on inflammatory cell activation. This study found that the oral administration of quercetin of more than 25 mg/kg for 5 days in mice sensitized with TDI significantly inhibited the development of nasal allergy symptoms such as sneezing and nasal scratching movements induced by exposure to allergens.14

Mast cells are the main key in developing and managing allergic diseases and become the main target in the choice of therapy for allergic diseases such as asthma, allergic rhinitis, and allergic conjunctivitis. Mast cells have a very clear role in
the immunopathology of rapid-type hypersensitivity reactions that occur in response to contact with certain allergens. An allergic reaction can be prevented or attenuated by interfering with specific molecule signals in mast cell formation. Flavonoids are mast cell stabilizer agent that comes from natural sources.13

Quercetin flavonoids are found in many plants. Quercetin is more effective as a mast-cell stabilizer compared to chloromol in more than one in vitro study. Quercetin has a relevant allergy effect on patients with eosinophilia or chronic allergics, including inhibitory effects on the over-expression of histamine-1, 5-lipoxygenase receptors, cyclo-oxygenase, abnormal or excessive dendritic cells activity, excessive mucus production (without inhibiting cilia movement) and many others.16

Ebihara et al. revealed that quercetin exerted an inhibitory effect on IL-4, which induced nitric oxide production in human nasal epithelial cells. Quercetin is reported to inhibit the production of inflammatory cytokines, chemokines and chemical mediators from mast cells and eosinophils in allergic diseases. In the model of allergic rhinitis rats, quercetin can reduce clinical symptoms such as sneezing and scratching the nose through suppression of substance P, calcitonin gene-related peptide and nerve growth factor in the nasal cavity after exposure to nasal allergens with tolune diisocyanate.17 Zhang et al. found that quercetin was significantly able to increase transepithelial chloride ion transport and ciliary shocks frequency in nasal septal epithelial murine and human sinonasal epithelial culture.18

Quercetin can inhibit eosinophil activation, particularly chemokine production and, as a result, will inhibit the development of the eosinophil inflammatory response. Quercetin is reported to be an anti-allergy agent through inhibition of histamine and chemicals that cause allergic reactions that are released from allergic inflammatory cells such as mast cells. In several studies, the concept has been established that eosinophils are important in developing allergic immune responses and are associated with the severity of the disease. Upper airway remodelling may occur in the nasal mucosa of patients with allergic rhinitis but appears to be less extensive than the lung appearance in asthma. Several studies have indicated that airway remodelling and adaptation occur after exposure to air pollutants, allowing protection against hyper-responsive airways.19 Prolonging the time and amount of allergen exposure will induce upper airway remodelling. Thus, long-term exposure to allergens will reduce nasal hyperresponsiveness and the development of nasal remodelling. This remodelling of the nasal mucosa may be associated with adaptation to nasal hyperresponsiveness due to defensive mechanisms such as in bronchial mucosa.20

Comparison of Mean Total IgE Levels in Wistar Rats
There was no statistically significant difference between total IgE levels in the four study groups. Therefore, the results of this study demonstrated that there was an increase in total IgE levels in the four study groups. However, changes in total IgE levels before and after treatment could not be known because this study only examined one measurement. This was probably because, at the beginning of the study, it was possible that the total IgE levels in the mice were already high, which could be due to other factors.

Similar results were obtained in the study by Sagit et al. There was no significant increase in total IgE levels at the end of the study in the steroid and quercetin groups. However, there was a significant increase in OVA-specific IgE levels in all groups compared to normal. Total serum IgE levels and OVA-specific IgE levels were increased in experimental animals, which is the ovalbumin-induced allergic rhinitis model.6 IgE levels can be increased due to allergic diseases, worm infections, and inflammatory conditions, malignancy and immunodeficiency. Extreme elevations in serum total IgE levels are typically associated with atopic eczema, allergic broncho-pulmonary aspergillosis, helminth infections and rare immunodeficiency diseases. Worm infections are known to induce Th2 immunity. Several viruses such as cytomegalovirus, Epstein-Barr virus and human immunodeficiency virus can also increase IgE levels.21 Worm infections associated with increased IgE have been observed since the 1960s, and increases in helminth infections IgE - associated also increase the number of mast cells and eosinophils. No stimulus was more effective than worm infections which could produce high IgE-specific antibodies and total IgE levels.22

Parasitic infections unless protozoa are known to induce hyperproduction of IgE and peripheral blood eosinophils in mammalian hosts. This is because the immune response that occurs is almost the same as the response in allergic diseases. Mesoces-toides corti is a parasite that is common in dogs and humans in North and Central America. The larvae of these parasitic worms are called tetrahyridium larvae and have been studied in the peritoneal cavity of wild rodents. This larval infection in rodents has been accepted as a producer of eosinophilia in peripheral blood and hyperproduction of IgE.14

Conclusion
Based on the results obtained, it can be concluded that the total serum IgE levels in this study were not different between groups. Therefore, this showed that in this study, the crude extract of dewandaru leaves had not been proven effective in reducing total IgE levels in Wistar rats suffering from allergic rhinitis.

There were differences in the nasal symptoms score between study groups in this study. However, the positive control and treatment groups had significantly lower nasal symptom scores than the negative control groups. This showed that the crude ex-tract of dewandaru leaves at a dose of 100 mg/kg or 200 mg/kg was effective in reducing symptoms of allergic rhinitis and had an effect that was not different from topical corticosteroids.

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Conflict of interest
There are no conflicts of interest to declare by any of the authors of this study.

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