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THE EFFECT OF *LUMBRICUS RUBELLUS* EXTRACTS ON IL-4, IL-10, IgE, AND EOSINOFIL LEVELS IN ATOPIC DERMATITIS PATIENT

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Abstract

Atopic dermatitis is a chronic, residive inflammatory skin condition characterized by severe pruritus. Pathophysiology of AD is a multifactorial, skin barrier disorder, and involves altered immune response, it can be seen in rural population which exposed from many worm infections with a low prevalence of allergic disease and has been shown in animal models by stimulating the formation of TGF β and interleukin-10 (IL-10) inhibiting IL-4, IL-5, IL13 by stimulating Treg. To determine the effect of Lumbricus Rubellus extract, it can increase interleukin 10 and reduce IL-4, immunoglobulin E and eosinophils and it can provide clinical improvement in Atopic Dermatitis patients. This research used "Pretest-Posttest Design" method of mild atopic dermatitis patients who were not infected with worms. Lumbricus Rubellus extract was given for 2 weeks and checked for eosinophils and ELISA to determine IL-10 and IgE levels on day 0, 8 and 15. Statistical test used non-parametric tests, such as Mann-Whitney test (U-Test) and Wilcoxon test to determine whether there was a difference between two different treatments or not. There was a difference ($p < 0.05$) between *Lumbricus Rubellus extract* group and the group without *Lumbricus Rubellus extract* on day 8, at day 15 there was no significant difference ($p > 0.05$) there was still an increase in IL levels. -10 and decreased levels of IgE and eosinophil. The side effects that appeared at research were intestinal disorders, such as nausea and bowel disorders of a research subject. *Lumbricus Rubellus* extract has an immune response effect towards people with atopic dermatitis.

Keywords: Earthworms, Lumbricus Rubellus, Atopic Dermatitis

INTRODUCTION

Skin consists of three layers, epidermi, dermis and hypodermis. The main component of epidermis is keratinocytes, which is formed from basal layer, spinous layer, granular layer and stratum corneum, which has a role of replacing the plasma membrane with an insoluble layer of

macromolecules called cornified enveloped (CE) that has a weak consentration inside stratum corneum or the one that dies outside the stratum corneum (1).

Epidermis is interspersed with Langerhans cells (LCs) derived from antigen presenting cells (APCs). Dermis is vascular layer consists of fibroblasts and dense connective tissue with collagen and elastic fibers, which inhabited by cells of hematopoietic derivatives which include dendritic cells, mast cells, macrophages, and several lymphocytes (1). Hypodermis is a layer of fat cells and long connective tissue. The main function of skin is to provide protection as physical barrier against the entry of outside substances including irritants, allergens, and pathogens, and control water loss. (1).

Atopic dermatitis (AD) is a chronic residive skin disease that most commonly occurs for children. The disease often associated with abnormalities of skin barrier function, allergen sensitization, and recurrent skin infections (2). Based on the data, The incident of atopic dermatitis occurs on 15-20% of children and 1-3% of adults (3). There are two hypotheses involving the pathogenesis of AD. The first hypothesis states that there is a disruption of epithelial cells of skin which causes a malfunctioning of skin barrier that produces an immune response. Another hypothesis indicated that there is an abnormality in the immune response that produces the domination of TH2 and IgE cells (4). Patients with AD experience the increase of spontaneous histamine release from basophils. This finding reflects systemic Th2 immune response in AD especially in patients who have elevated serum IgE levels. And the most important, peripheral blood skin overexpressing CD4 or CD8 spontaneously secretes IL-5 and IL-13, functionally prolonging eosinophil survival and inducing IgE synthesis. (2).

The pathophysiology of atopic dermatitis is multifactorial, it involves skin barrier disorders, change the immune response mediated by cellular and humoral immune systems and

hypersensitivity type I reactions that can cause the increase of IgE. (5). Human T cell categorized as T cell helper 1 (Th1) and T cell helper 2 (Th2) depends on the obtained cytokine profile. Repeated exposure to antigens will change the cytokine profile in skin lesions from Th1 to Th2 (6).

Pathophysiology of atopic dermatitis occurs due to several mechanisms, one of the dominant mechanisms comes from inflammation. This process happens due to Th2 cell-related cytokines such as IL-4 and IL-13, along with chemokines such as TARC (thymus and activation-regulated chemokine) and eotactions. Th2 cytokines, of IL-4 and IL-13, stimulate fibroblasts to produce periostine, a protein that causes keratinocytes to produce TSLP, which will induce the production of TARC / CCL17 by dendritic cells (Katayama *et al*., 2017). The high levels of IL-4 produced by T cells on their born day can increase the risk to develop atopic dermatitis. High IL-4 levels have also been found in children with atopic dermatitis. Several studies have confirmed that IL-4 is genes that have a role in atopic dermatitis outcome and targeted cytokine therapy in this case. (Yang *et al*., 2017).

Ever since thousands years ago, *Lumbricus rubellus* has been widely used by Chinese people as medicine for various diseases. (Mihara et al., 1991). For patients who is infected with worms, it can stimulate the formation of interleukin-10 (IL-10) and transforming growth factor-beta (TGF-β) through increased T regulatory cells (8). The increase of IL-10 and TGF- β can reduce TH2 which is increased in atopic dermatitis patients. The main function of IL-10 is to prevent extensive fiber damage after inflammation and infection (9).

IL-10 is an anti-inflammatory cytokine produced by T-reg cells. Although it is known that IL-10 regulates the immune system which minimizes fiber damage during inflammation, the data regarding its role in AD still conflicting. Several studies have shown the increased of IL-10

levels in peripheral blood mononuclear cells and skin lesions of AD patients. In contrast, other studies reported that IL-10 levels were inversely related to the severity of AD. The more severe Autopic Dermatitis condition, the lower IL-10 levels (10).

Laboratory testing is not required for evaluation routine and treatment of AD patients. IgE levels elevated in 70-80% of AD patients. It is related to sensitization toward concomitant inhalant and food allergens, allergic rhinitis and asthma that occurs at the same time. While for 20-30% AD patient with normal level of IgE, This AD subtype has less IgE sensitization toward food or inhalation allergens. However, several patients may have IgE sensitization to microbial antigens such as S. aureus toxin, and Candida albicans or Malassezia sympodialis that can be detected. Besides, some of those patients showed positive reactions of using atopy patch test even though the immediate skin test was negative (2).

Earthworms also contain active compounds of alkaloid compound class. Alkaloid compounds in earthworms contain nitrogen atoms and alkaline (have the greater pH than 7) which also have antibacterial and antipyretic activity. The mechanism action of alkaloids in inhibiting bacterial growth by disturbing constituent components of peptidoglycan in bacterial cells, therefore the cell is not completely formed. (11)

Nowadays, there have been no research results that obtain a worm therapy that can affect immunological pathways and cause atopic dermatitis. And considering the number of worm extract preparations that have not been used, it is necessary to study alternative atopic dermatitis treatments with natural ingredients that widely developed in Indonesia.

This study aims to determine the effect of earthworm extract (Lumbricus Rubellus) on IL-10 and reduce IL-4, immunoglobulin E and eosinophils. Therefore it can provide clinical

improvement in patients with atopic dermatitis. Moreover, it can be used as an alternative therapy in management of atopic dermatitis.

METHODOLOGY

This research used "Pretest-Posttest Design" research type, which means research design that contains a pretest before being given treatment and a posttest after being treated by an experimental approach. Thus the obtained results are more accurate, because they can compare before being treated to obtain the effect of therapy on particular disease. This sresearch was conducted within 30 days, on atopic dermatitis patient that met the criteria. In this research, patients with atopic dermatitis were treated by Lumbricus Rubellus therapy.

This research was done on January 2020 to March 2020 untill the number of sample met the target. The research was conducted at Dermatology and Venereology Polyclinic on Hospital of Education affiliated with Department of Dermatology and Venereology of Hasanuddin University.

The population of this study were all patients who met the inclusion criteria that came to Dermatology and Venereology Polyclinic at the Hasanuddin University. The Sample was collectively taken from the time the patient came to Skin and Venereal Polyclinic and had a diagnosis of atopic dermatitis during January 2020 - March 2020. 3 cc of blood was taken and was checked by the levels of IL 10, IL 4, IgE and eosinophils, using Enzyme-linked immunosorbent assay (ELISA) method, as well as an examination of eosinophil type. Furthermore, samples were taken to laboratory of Hasanuddin University Educational Hospital in Makassar

On adult patients, specimens were taken directly intravenously from patient's blood, after patient signed the informed consent, Pediatric patient serum specimens were taken from pediatric

patient's blood and then parental consent of the informed consent. Total IgE examination was done using Enzyme-Linked Immunosorbent Assay (ELISA) method (ELISA kit for Human IgE total) to observe the total of IgE concentration on the examined blood samples.

Taking blood samples for each 1.5 cc tube, sample 1 and 2 were put into a tube contains EDTA to collect blood plasma, while the third sample put into microsentrifuge tube without EDTA to collect the serum. Furthermore, the blood samples were centrifuged to separate blood plasma and blood serum within the blood cells.

Data Analysis Technique

The data analysis technique was done by SPSS 19. To determine the basic characteristics of numerical variables, mean ± standard deviation is used if the data distribution is even, if the distribution not even, it used median. To observe the numerical baseline data, mean ± standard deviation is used when the data distribution is even. To observe the relationship between Earthworms (Lumbricus Rubellus) and IL-10, a paired T-Test analysis was used.

RESULT AND DISCUSSION

Result

Natural experimental research has been carried out to determine the effect of Lumbricus Rubellus extract on IL-4, IL-10, IgE, and eosinophils of atopic dermatitis patients who were divided into two groups. The first group (A) was the control group; consist of patients with atopic dermatitis and the second group (B) atopic dermatitis patients who were given Lumbricus Rubellus extract. Both groups monitored IL-4, IL-10, IgE and eosinophil levels on days 8 and 15.

Table 1: Sociodemographic characteristics of control group and the group given Lumbricus Rubellus extract

Sociodemographic Characteristics	Total	Precentage (%)
Gender		

Man	18	60 %
Woman	12	40 %
Age Range		
< 5	0	0 %
5- 14	9	30 %
15- 24	2	6,7 %
25- 44	15	50 %
45- 64	3	10 %
>64	1	3 %

Source: own study

Based on Table 1, it can be seen that the number of male research subjects is more than the number of female samples, with a ratio of 3: 2, and the largest age group is 25-44 years (50%) followed by the 5-14 year age group (30%).

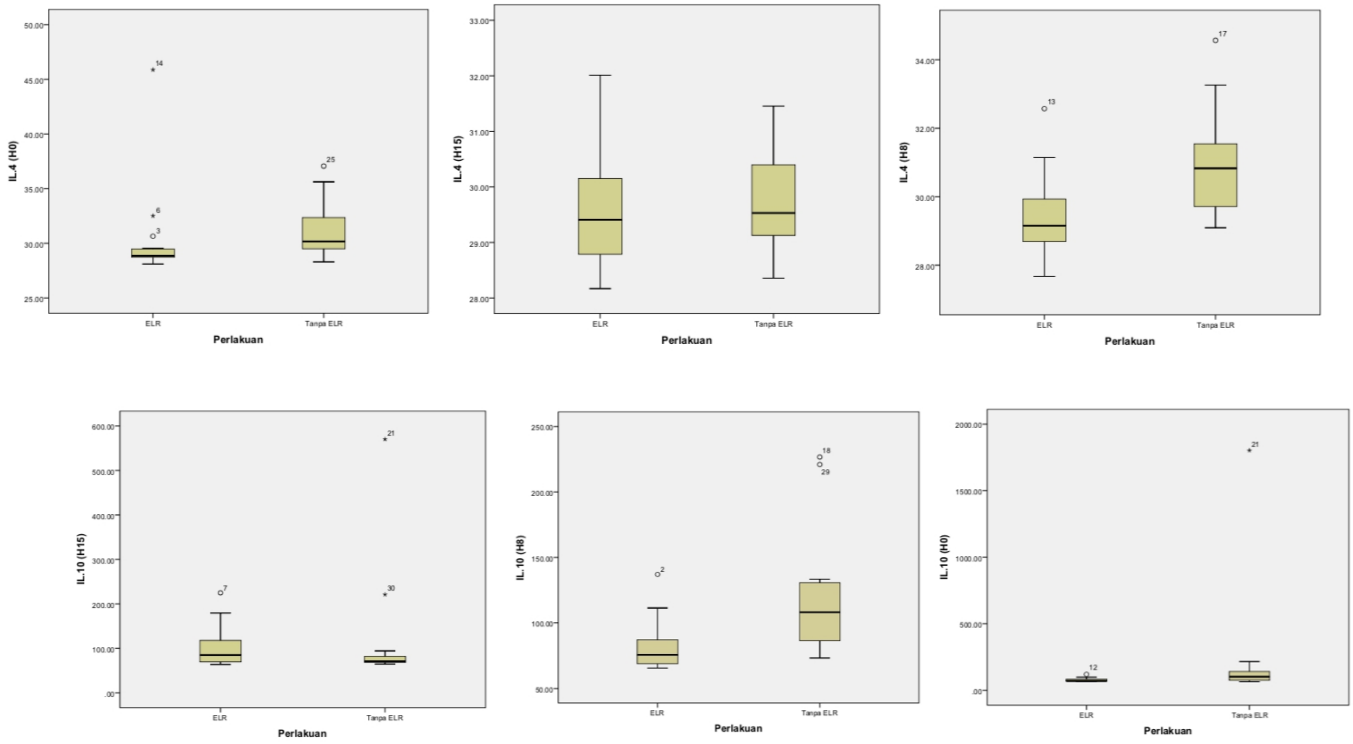
Before starting difference test, the analyst requirements are tested to test the normality and homogeneity.

Table 2: The results of Shapiro-Wilk IL-4, IL10, IgE and Eosinophil normality tests of various groups on days 0, 8, and 15

Treatment		Shapiro-Wilk						Explanation
		Min	Max	Mean	Deviation-Standard	Median	Sig.	
IL.4 (H0)	ELR	28.11	45.88	30.3148	4.44300	28.8488	0.000	Not Normal
	Without ELR	28.29	37.06	31.2330	2.56034	30.1494	0.050	Not Normal
IL.4 (H8)	ELR	27.67	32.57	29.4275	1.22954	29.1583	0.132	Normal
	Without ELR	29.10	34.56	30.9243	1.65818	30.8316	0.101	Normal
IL.4 (H15)	ELR	28.17	32.01	29.6214	1.07464	29.4059	0.420	Normal
	Without ELR	28.35	31.45	29.7657	0.93316	29.5298	0.518	Normal
IL.10 (H0)	ELR	64.39	119.48	77.3157	15.45106	70.5219	0.001	Not Normal
	Without ELR	64.74	1802.47	219.7086	439.96753	102.1230	0.000	Not Normal
IL.10 (H8)	ELR	65.61	137.05	83.1810	20.33293	75.5281	0.005	Not Normal

	Without ELR	73.19	226.66	118.0581	47.20522	108.2801	0.002	Not Normal
IL.10 (H15)	ELR	63.70	224.84	103.2225	49.54104	85.2536	0.002	Not Normal
	Without ELR	64.91	570.09	116.0181	131.49605	70.8765	0.000	Not Normal
Ig.E (H0)	ELR	175.46	1057.21	576.1896	255.78367	513.9254	0.506	Normal
	Without ELR	58.99	521.13	230.1328	162.60585	199.9720	0.043	Not Normal
Ig.E (H8)	ELR	82.72	996.37	440.8090	315.38880	379.3188	0.111	Normal
	Without ELR	60.90	457.38	232.4674	142.84166	199.2657	0.051	Normal
Ig.E (H15)	ELR	37.33	816.47	370.3055	262.32755	294.3760	0.173	Normal
	Without ELR	89.77	781.19	257.5831	192.89845	173.4884	0.003	Not Normal
Eosinophil (H0)	ELR	2.10	16.60	6.3600	3.75610	5.9000	0.009	Not Normal
	Without ELR	1.20	10.60	4.0800	2.56493	3.5000	0.032	Not Normal
Eosinophil (H8)	ELR	1.70	16.50	6.2267	3.74480	5.5000	0.024	Not Normal
	Without ELR	1.00	11.30	4.1400	2.74169	3.8000	0.039	Not Normal
Eosinophil (H15)	ELR	1.40	13.20	5.4933	2.98100	5.2000	0.062	Normal
	Without ELR	1.50	12.00	5.2933	3.09619	4.9000	0.080	Normal

Source: SPSS data analysis



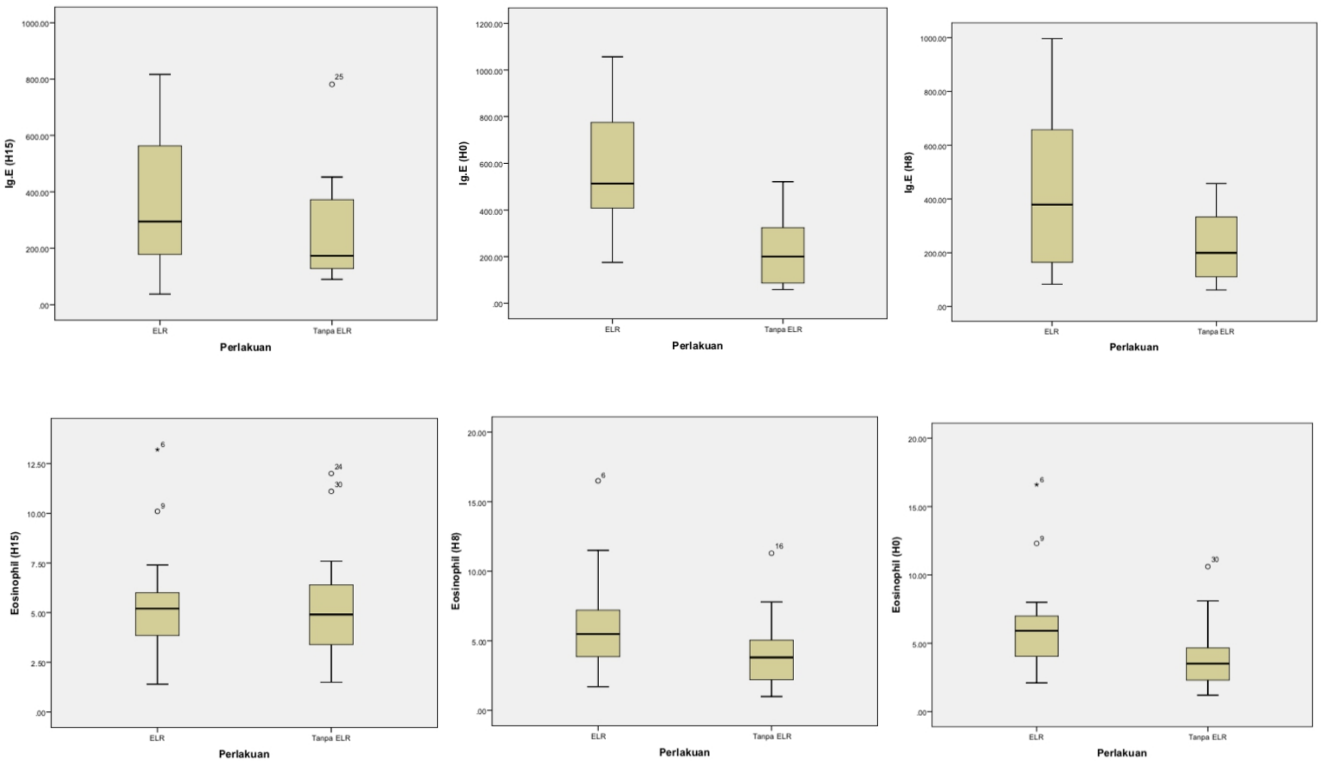


Figure 1: Hasil analisis distribusi kadar IL-4, IL-10, IgE, dan Eosinofil hari ke 0,8, dan 15

Based on Table 2 and figure 1, it can be seen that the normality test was carried out using Shapiro-Wilk formula; it was found that the data distribution of two groups, both the intervention group and control group, was abnormal within the Asymp value. Sig Asymp. Sig. (2-tailed) ≤ 0.05.

Table 3: The results of IL-4, IL10, IgE and Eosinophil homogeneity tests of various groups on day 0, 8, and 15.

Homogeneity Variation Test					
	Levene Statistic	df1	df2	Sig.	Exp
IL.4 (H0)	0.147	1	28	0.704	Homogen
IL.4 (H8)	1.606	1	28	0.215	Homogen
IL.4 (H15)	0.304	1	28	0.586	Homogen
IL.10 (H0)	4.083	1	28	0.053	Homogen
IL.10 (H8)	4.373	1	28	0.046	Homogen

IL.10 (H15)	1.576	1	28	0.220	Homogen
Ig.E (H0)	5.778	1	28	0.023	Not Homogen
Ig.E (H8)	8.992	1	28	0.006	Not Homogen
Ig.E (H15)	2.215	1	28	0.148	Homogen
Eosinophil (H0)	0.665	1	28	0.422	Homogen
Eosinophil (H8)	0.654	1	28	0.426	Homogen
Eosinophil (H15)	0.090	1	28	0.766	Homogen

Based on Table 3 above, homogeneity test above was carried out to determine whether the data obtained from two groups had homogeneous variant or not, and the results obtained from the data of two groups, both intervention group and control group, were not homogeneous within the Asymp value. Sig Asymp. Sig. (2-tailed) ≤ 0.05.

Based on two previous tests above, the obtained data from both control group and intervention group were not normally distributed and not homogeneous, therefore the hypothesis testing used non-parametric testing, such as Mann-Whitney test (U-Test) and Wilcoxon test.

Differences in IL-4 levels in atopic dermatitis patients of two groups on days 0, 8 and 15

To determine the effect of lumbricus rubellus extract toward IL-4 in atopic dermatitis patients, Mann-Whitney test (U-Test) was performed to determine whether there was a difference between two different treatments (given lumbricus rubellus extract treatment and not given lumbricus rubellus extract treatment) on days 0, 8, and 15. The results of the test within the Mann-Whitney test can be seen in Table 4 and graph 2 below:

Table 4: Differences in IL-4 levels in atopic dermatitis patients of two groups

Treatment		Mann-Whitney Test						Explanation
		Min	Max	Mean	Deviation Standard	Median	P	
IL.4 (H0)	ELR	28.11	45.88	30.3148	4.44300	28.8488	0.011	Differences
	Without ELR	28.29	37.06	31.2330	2.56034	30.1494		
IL.4 (H8)	ELR	27.67	32.57	29.4275	1.22954	29.1583	0.006	Differences
	Without ELR	29.10	34.56	30.9243	1.65818	30.8316		
IL.4 (H15)	ELR	28.17	32.01	29.6214	1.07464	29.4059	0.547	No Differences
	Without ELR	28.35	31.45	29.7657	0.93316	29.5298		

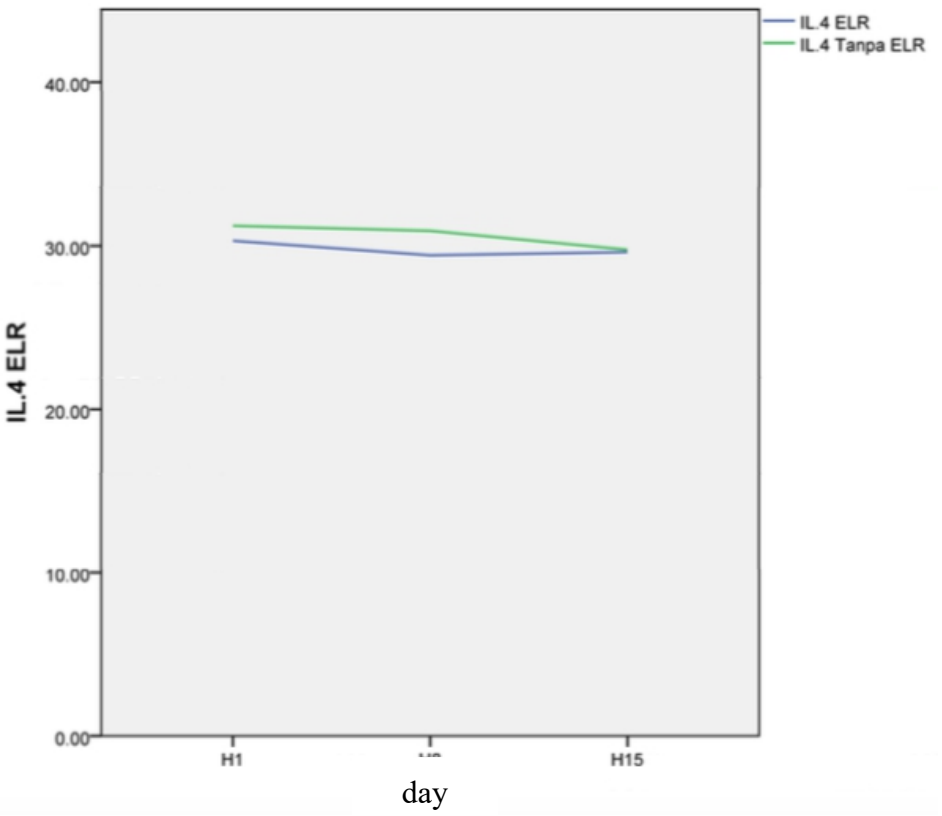


Figure 2. IL-4 levels of control groups and intervention day 0, 8, and 15

Based on Table 4 and figure 2, it can be seen that, in Mann-Whitney test that there is a difference ($p < 0.05$) of IL-4 levels between ERL group and without ERL group on day 0 and day 8, however it is different on day 15. There was a difference ($p > 0.05$) in IL-4 levels in ERL group and the one without ERL.

To determine the difference in IL-4 levels before and after the implementation of lumbricus rubellus extract at day 0 (before implementation), day 8 (eight days after the implementation), and day 15 (15 days after implementation) within the Wilcoxon test. The result of wilcoxom test can be seen as table 5 below:

Table 5: The differences in Il-4 levels before and after the implementation of lumbricus rubellus extract on days 0, 8, and 15

	Mean	N	Std. Deviation	Std. Error Mean	Sig. Wilcoxon	explanation

Pair 1	IL.4 ELR (day 8)	29.4275	15	1.22954	0.31747	0.955	no differences
	IL.4 ELR (day 1)	30.3148	15	4.44300	1.14718		
Pair 2	IL.4 ELR (day 15)	29.6214	15	1.07464	0.27747	0.470	no differences
	IL.4 ELR (day 1)	30.3148	15	4.44300	1.14718		
Pair 3	IL.4 ELR (day 15)	29.6214	15	1.07464	0.27747	0.733	no differences
	IL.4 ELR (day 8)	29.4275	15	1.22954	0.31747		

Table 6: Differences in IL-4 levels without the application of lumbricus rubellus extract on days 0, 8, and 15

		Mean	N	Std. Deviation	Std. Error Mean	Sig. Wilcoxon	Ket
Pair 1	IL.4 Without ELR (day 8)	30.9243	15	1.65818	0.42814	0.910	No Differences
	IL.4 Without ELR (day 1)	31.2330	15	2.56034	0.66108		
Pair 2	IL.4 Without ELR (day 15)	29.7657	15	0.93316	0.24094	0.053	No Differences
	IL.4 Without ELR (day 1)	31.2330	15	2.56034	0.66108		
Pair 3	IL.4 Without ELR (day 15)	29.7657	15	0.93316	0.24094	0.031	No Differences
	IL.4 Without ELR (day 8)	30.9243	15	1.65818	0.42814		

Source: SPSS Analysis result

Based on Tables 5 and 6, it can be seen that Wilcoxon test of ERL group there was no difference ($p > 0.05$) in IL-4 levels before and after the implementation of lumbricus rubellus extract on day 0 (before implementation), day 8 (after implementation), and day 15 (after implementation). On the group without ERL, there was no difference ($p > 0.05$) of IL-4 levels on days 0, 8 and 15.

Differences in IL-10 levels of atopic dermatitis patients in two groups on day 0, 8 and 15

To determine the effect of lumbricus rubellus extract on IL-10 in patients with atopic dermatitis, Mann-Whitney test (U-Test) was performed to determine whether there was a difference between two different treatments (given lumbricus rubellus extract treatment and not given lumbricus rubellus extract treatment) on day 0, 8, and 15. The results of test within the Mann-Whitney test can be seen in Table 7 below:

Table 7: Differences in IL-10 levels of atopic dermatitis patients in two groups

Perlakuan		Mann-Whitney Test						Explanation
		Min	Max	Mean	Std. Deviation	Median	P	
IL.10 (H0)	ELR	64.39	119.48	77.3157	15.45106	70.5219	0.010	
	Without ELR	64.74	1802.47	219.7086	439.96753	102.1230		Differences
IL.10 (H8)	ELR	65.61	137.05	83.1810	20.33293	75.5281	0.006	
	Without ELR	73.19	226.66	118.0581	47.20522	108.2801		Differences
IL.10 (H15)	ELR	63.70	224.84	103.2225	49.54104	85.2536	0.395	
	Without ELR	64.91	570.09	116.0181	131.49605	70.8765		
	Without ELR	1.50	12.00	5.2933	3.09619	4.9000		No Differences

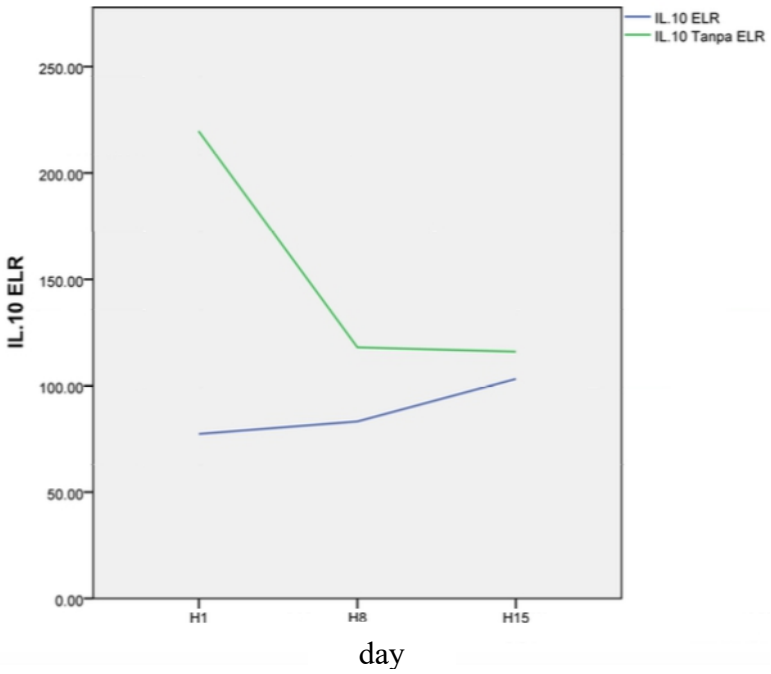


Figure 3: IL-10 levels in control groups and intervention days 0.8, and 15

Based on Table 7 and figure 3, it can be seen in Mann-Whitney test that there is a difference ($p < 0.05$) in IL-10 levels between the treatment with ERL group and without ERL group on day 0 and 8, but different from day 15. There was a difference ($p > 0.05$) in IL-10 levels within the group with ERL and without ERL.

To determine the differences in IL-10 before and after application of lumbricus rubellus extract at H0 (before implementation), H8 (after implementation), and H15 (after implementation) by Wilcoxon test. The results of Wilcoxon test can be seen in Table 8 below:

Table 8: The differences in IL-10 levels before and after the implementation of lumbricus rubellus extract on days 0, 8, and 15

		Mean	N	Std. Deviation	Std. Error Mean	Sig. Wilcoxon	Explanation
Pair 4	IL.10 ELR (H8)	83.1810	15	20.33293	5.24994	0.156	No Differences
	IL.10 ELR (H1)	77.3157	15	15.45106	3.98945		

Pair 5	IL.10 ELR (H15)	103.2225	15	49.54104	12.79144	0.140	No Differences
	IL.10 ELR (H1)	77.3157	15	15.45106	3.98945		
Pair 6	IL.10 ELR (H15)	103.2225	15	49.54104	12.79144	0.281	No Differences
	IL.10 ELR (H8)	83.1810	15	20.33293	5.24994		

Table 9: Differences in IL-10 levels without the implementation of lumbricus rubellus extract on day 0, 8, and 15

		Mean	N	Std. Deviation	Std. Error Mean	Sig. Wilcoxon	Explanation
Pair 4	IL.10 Without ELR (H8)	118.0581	15	47.20522	12.18834	0.820	No Differences
	IL.10 Without ELR (H1)	219.7086	15	439.96753	113.59913		
Pair 5	IL.10 Without ELR (H15)	116.0181	15	131.49605	33.95213	0.036	Differences
	IL.10 Without ELR (H1)	219.7086	15	439.96753	113.59913		
Pair 6	IL.10 Without ELR (H15)	116.0181	15	131.49605	33.95213	0.078	No Differences
	IL.10 Without ELR (H8)	118.0581	15	47.20522	12.18834		

Based on Tables 8 and 9, it can be seen that the Wilcoxon test of ERL group there was no difference ($p > 0.05$) in IL-10 levels before and after the implementation of lumbricus rubellus extract on day 0 (before implementation), day 8(after implementation), and day 15 (after implementation). In the group without ERL there was no difference ($p > 0.05$) in IL-10 levels on days 0, 8 and 15.

Differences in IgE levels of atopic dermatitis patients in two groups on days 0, 8 and 15

To determine the effect of lumbricus rubellus extract on IgE in atopic dermatitis patients, Mann-Whitney test (U-Test) was performed. Whether there was a difference between two different treatments (given lumbricus rubellus extract treatment and not given lumbricus rubellus extract treatment) or not on day 0, 8, and 15. The results of Mann-Whitney test can be seen in Table 10 below:

Table 10: Differences in IgE levels of atopic dermatitis patients

Treatment		Mann-Whitney Test						Explanation
		Min	Max	Mean	Std. Deviation	Median	P	
Ig.E (H0)	ELR	175.46	1057.21	576.1896	255.78367	513.9254	0.001	Differences happen
	Without ELR	58.99	521.13	230.1328	162.60585	199.9720		
Ig.E (H8)	ELR	82.72	996.37	440.8090	315.38880	379.3188	0.059	Differences Happen
	Without ELR	60.90	457.38	232.4674	142.84166	199.2657		
Ig.E (H15)	ELR	37.33	816.47	370.3055	262.32755	294.3760	0.272	No Differences
	Without ELR	89.77	781.19	257.5831	192.89845	173.4884		

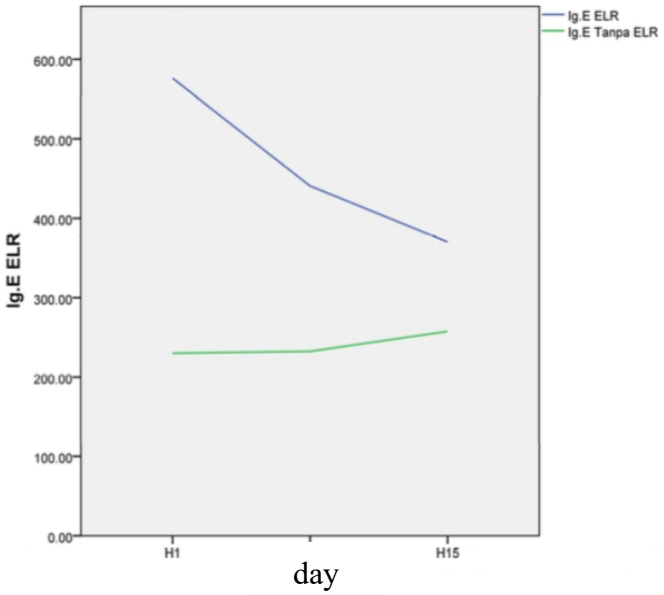


Figure 4: IgE levels in two control groups and intervention days 0, 8, and 15

Based on Table 10 and figure 4, it can be seen that Mann-Whitney test there is a difference ($p < 0.05$) in IgE levels between ERL group and without ERL group on day 0 and day 8, however on day 15 there is no difference ($p > 0.05$) IgE levels in the group with ERL and without ERL.

To determine the difference in IgE levels before and after implementation of lumbricus rubellus extract at H0 (before implementation), H8 (after implementation), and H15 (after implementation) by Wilcoxon test. The results of Wilcoxon test can be seen in table 11 below:

Table 11: The differences in IgE levels before and after the implementation of lumbricus rubellus extract on days 0, 8, and 15

		Mean	N	Std. Deviation	Std. Error Mean	Sig. Wilcoxon	Explanation
Pair 7	Ig.E ELR (H8)	440.8090	15	315.38880	81.43304	0.191	No Differences
	Ig.E ELR (H1)	576.1896	15	255.78367	66.04306		
Pair 8	Ig.E ELR (H15)	370.3055	15	262.32755	67.73268	0.100	No Differences
	Ig.E ELR (H1)	576.1896	15	255.78367	66.04306		
Pair 9	Ig.E ELR (H15)	370.3055	15	262.32755	67.73268	0.460	No Differences
	Ig.E ELR (H8)	440.8090	15	315.38880	81.43304		

Table 12: Differences in IgE levels without the implementation of lumbricus rubellus extract on day 0, 8, and 15

		Mean	N	Std. Deviation	Std. Error Mean	Sig. Wilcoxon	Explanation
Pair 7	Ig.E Without ELR (H8)	232.4674	15	142.84166	36.88156	0.776	No Differences
	Ig.E Without ELR (H1)	230.1328	15	162.60585	41.98465		
Pair 8	Ig.E Without ELR (H15)	257.5831	15	192.89845	49.80617	0.865	No Differences

	Ig.E Without ELR (H1)	230.1328	15	162.60585	41.98465		
Pair 9	Ig.E Without ELR (H15)	257.5831	15	192.89845	49.80617	0.733	No Differences
	Ig.E Without ELR (H8)	232.4674	15	142.84166	36.88156		

Based on Tables 11 and 12, it can be seen in Wilcoxon test within the ERL group that there is no difference (p> 0.05) in IgE levels before and after the implementation of lumbricus rubellus extract on day 0 (before implementation), day 8 (after implementation), and day 15 (after implementation). In the group without ERL group there was no difference (p> 0.05) in IgE levels on days 0, 8 and 15.

Differences in Eosinophil levels of atopic dermatitis patients in two groups on days 0, 8 and 15

To determine the effect of lumbricus rubellus extract on eosinophils in atopic dermatitis patients, Mann-Whitney test (U-Test) was performed to determine whether there was a difference between two different treatments (given lumbricus rubellus extract treatment and not given lumbricus rubellus extract treatment) on day 0, 8, and 15. The results of test with Mann-Whitney test can be seen in Table 16 below:

Table 16: The Differences in eosinophil levels of atopic dermatitis patients

Treatment		Mann-Whitney Test						Explanation
		Min	Max	Mean	Std. Deviation	Median	P	
Eosinophil (H0)	ELR	2.10	16.60	6.3600	3.75610	5.9000	0.036	Differences Happen
	Without ELR	1.20	10.60	4.0800	2.56493	3.5000		
Eosinophil (H8)	ELR	1.70	16.50	6.2267	3.74480	5.5000	0.056	No Differences
	Without ELR	1.00	11.30	4.1400	2.74169	3.8000		
Eosinophil (H1	ELR	1.40	13.20	5.4933	2.98100	5.2000	0.740	No Differences
	Without ELR	1.50	12.00	5.2933	3.09619	4.9000		

5)								

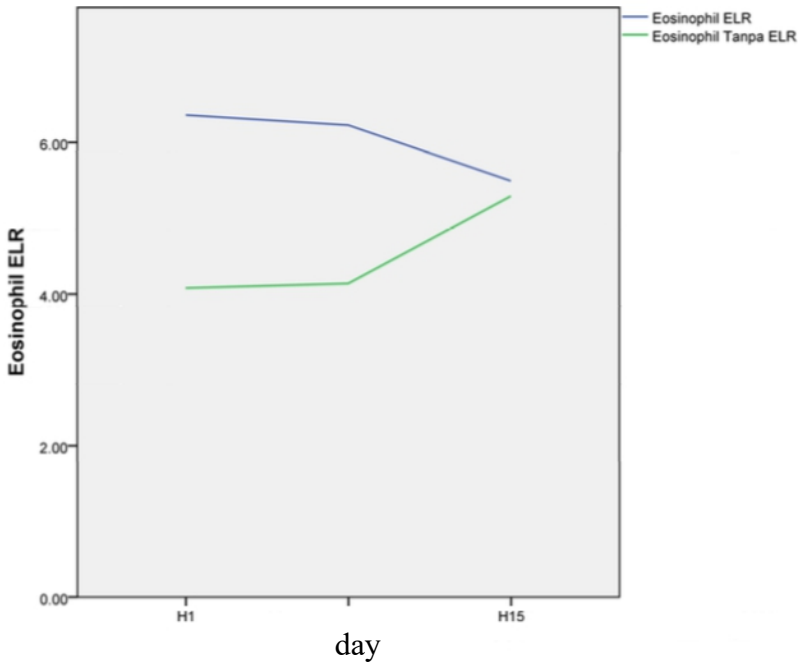


Figure 5: Eosinophil levels in the control and intervention groups day 0.8 and 15

Based on Table 16 and figure 5, it can be seen in Mann-Whitney test that there is a difference ($p < 0.05$) in eosinophil levels between the group with ERL and without ERL on day 0 and day 8, however on day 15 there is no difference. ($p > 0.05$) IgE levels in the group with ERL and without ERL.

To determine the difference in eosinophil levels before and after the implementation of lumbricus rubellus extract at day 0 (before the implementation), day 8 (after the implementation), and day 15 (after the implementation) by Wilcoxon test. The results of Wilcoxon test can be seen in Table 17 below:

Table 17: The difference in Eosinophil levels before and after the implementation of lumbricus rubellus extract on days 0, 8, and 15

		Mean	N	Std. Deviation	Std. Error Mean	Sig. Wilcoxon	Explanation
Pair 10	Eosinophil ELR (H8)	6.2267	15	3.74480	0.96690	0.550	No Differences
	Eosinophil ELR (H1)	6.3600	15	3.75610	0.96982		

Pair 11	Eosinophil ELR (H15)	5.4933	15	2.98100	0.76969	0.001	Differences
	Eosinophil ELR (H1)	6.3600	15	3.75610	0.96982		
Pair 12	Eosinophil ELR (H15)	5.4933	15	2.98100	0.76969	0.016	Differences
	Eosinophil ELR (H8)	6.2267	15	3.74480	0.96690		

Table 18: P Differences in Eosinophil levels without the implementation of lumbricus rubellus extract on day 0, 8, and 15

		Mean	N	Std. Deviation	Std. Error Mean	Sig. Wilcoxon	Explanation
Pair 10	Eosinophil Without ELR (H8)	4.1400	15	2.74169	0.70790	0.955	No Differences
	Eosinophil Without ELR (H1)	4.0800	15	2.56493	0.66226		
Pair 11	Eosinophil Without ELR (H15)	5.2933	15	3.09619	0.79943	0.002	Differences
	Eosinophil Without ELR (H1)	4.0800	15	2.56493	0.66226		
Pair 12	Eosinophil Without ELR (H15)	5.2933	15	3.09619	0.79943	0.349	No Differences
	Eosinophil Without ELR (H8)	4.1400	15	2.74169	0.70790		

Based on Tables 17 and 18, it can be seen that the Wilcoxon test in ERL group there was no difference (p> 0.05) in Eosinophil levels before and after the implementation of lumbricus rubellus extract on H0 (before implementation), H8 (after implementation), and H15 (after implementation). In the group without ERL there was no difference (p> 0.05) in eosinophil levels on days 0, 8 and 15.

Discussion

Atopic dermatitis (AD) is residual chronic disease characterized by clinical symptoms of itching, which generally affects children. Pathogenesis of AD includes skin barrier disorders, which include disruption of filaggrin gene expression, genetics, environment and abnormalities of immune system. Skin's protection structure distruption can reduce the ability and function of skin, causes an immune response and an inflammatory reaction (12). The function of skin barrier minimizes water loss of epidermis and protects from the outside factors such as heat or cold, penetration of potentially harmful substances, and colonization of pathological bacteria. (13). Protective structure of epidermis consists of corneocytes (cells of stratum corneum), lipids, and natural moisturizing factors that were produced during corneocyte formation process. (14). skin's natural moisturizer functions is to absorb and bind water to protect the skin layer of epidermis (15). In Atopic Dermatitis patients, they lost a lot of water and cause an increase in Trans Epidermal Water Loss (TEWL) which causes the skin to become dry (xerosis) (1).

A study indicated higher risk of Atopic Dermatitis related to the maternal atopy of mother rather than father (16). Genetic abnormalities in cytokines that play important role in the immune response on pathogenesis of AD, where Interleukin (IL) -4 *tumor necrosis factor* (TNF), *stem cell factor* (SCF), IL-4 receptor (IL-4R), IL-13 promoter, and IL-12 receptor has been previously reported (16).

Natural immune system and innate immune system both contribute to the pathogenesis of AD. TH2 cells have a major role on increasing eosinophils and IgE Atopic Dermatitis patients. In AD acute lesions, releasing TH2 is characterized by dermal infiltration of $CD4^+$ T cells and eosinophils by increasing the derivative products of eosinophils in form of increased expression on cytokines IL-4, IL-5, IL-13, and few expression of IFN-γ. Whereas in chronic AD there is a

transition of TH2 to TH1, an increase of IFN-γ, IL-12, GM-CSF expression and tissue remodeling with increased collagen deposition and skin thickening. (17).

Specific antigens that can penetrate the skin due to skin barrier disorders was captured by antigen-specific IgE on inflammatory dendritic epidermal cells and Langerhans cells (LC). Specific IgE mostly reacts within the environmental and bacterial antigens. LC of AD patients secreted primarily at Th2 cytokine IL-10 rather than Th1 cytokine IL-12 (18).

The decreased exposure toward the infection after birth can move the Th2-cell balance response toward Th2. The result of imbalance response will cause an excessive eosinophil and IgE response; both are related to the allergic reactions and atopy. Microbial exposure can affect the balance of Th1 and Th2 by increase Th1 response and decrease Th2 response. Th1 cells are related to the response of infection and production of interferon-∂. Th2 cells induce IgE production and maturation of mast cells, basophils and eosinophils therefore Th2 cells generally associated to the atopic immune responses (19).

The Role of Cytokines in Atopic Dermatitis begins from an adaptive immune response that was mediated by T cells and B cells and associated with antigen-presenting cells (APC). The adaptive immune system consists of cellular immune system and humoral immune system. (18). T cells were produced in the bone marrow and grow up in thymus gland. The T cell receptor (TCR) will recognize specific peptides that bind to Major Histocompatibility Complec (MHC) / Human Leukocyte Antigen (HLA), which is cell surface molecule of infected APCs. This bond will activate T cells to proliferate. In Atopic Dermatitis, MHC class II in lymphoid tissue take a role by removing proteins that exist inside lysosomes, endosomes or extra-cellular. T lymphocytes activate T Helper (CD4) by secreting cytokines to assist T cells, B cells and macrophages. Th1 cells took a major role in the activation of macrophages. Th1 cells produce

cytokine profiles IL-2 (T cell proliferation) and IFN-γ (stimulate and activate NK cells) while dominant Th2 cells are associated with activation of B cells and produce antibody. Th2 cells produce cytokine profiles IL-4, IL-5 (synthesizing IgE and activation of eosinophils) and IL-10 (inhibiting proliferation of Th1). Th17 cells have an important role of fungal infections by secreting the cytokine IL-17 profile (activating neutrophils to kill fungus) (17). B cells are produced and grow up in marrow bone; plasma cells will produce various kinds of antibodies for IgA, IgD, IgG, IgM, and IgE.

Allergens are captured by dendritic cells and presented to T cells. Therefore, it will be an imbalance between TH1 and TH2. TH2 cells induce B cells to provide the production of immunoglobulin E (IgE). The allergen-specific IgE binds to the receptor for IgE (FcεRI) on mast cells (17).

IgE production in atopic disease by B cells is depend on the support of T helper 2 (TH2) cells, which produces interleukin-4 (IL-4), IL-5, IL-9 and IL-13. In general, TH1 cells promote a cellular immune response rather than humoral immune response, and have a greater role in chronic infections, such as Crohn's disease and psoriasis (20). Reexposure to similar allergens toward sensitive mucosa will cause bonding between IgE molecules on mast cells and allergens to stimulate mucosal mast cell degranulation by releasing histamine, leukotriene, heparin and other toxic products. (17).

Eosinophils derived from hematopoietic stem cells. Under the influence of interleukin-5 (IL-5) and some of the effects on IL-3 and GM-CSF, the hematopoietic cell progenitors differentiate into mature cells in bone marrow. Adult eosinophils are cells that remain in the fibers only in a small portion circulate in the blood circulation. (19)

Earthworms also contain active compounds of alkaloid compound class. Alkaloid compound in earthworms contains nitrogen atoms and has an alkaline characteristic (pH greater than 7) which also have antibacterial and antipyretic activity. The mechanism action of alkaloids in inhibiting bacterial growth by disturbing the constituent components of peptidoglycan in bacterial cells, thus the cell walls are not completely formed.(11)

In this research, the results of Shapiro-Wilk distribution test stated that the levels of IL-4, IL-10, IgE and Eosinophils had uneven distribution data both in ERL group and the group without ERL, hypothesis testing by non-parametric testing, such as Mann-Whitney test (U- Test) and Wilcoxon test. In research results, there was a difference ($p < 0.05$) between the ERL group and the group without ERL on the eigth day of ERL administration, although at day fifteenth ERL administration there was no significant difference ($p > 0.05$) there was an increase of IL levels 10 and decreased levels of IgE and eosinophils. However, in contrast to IL-4 levels, which decreased at day 8 ERL administration, it was increased on day 15. The side effects that appeared at the time of research were intestinal disorders, such as nausea and bowel disorders in a research subject.

Deworming therapy is possible as an adjuvant treatment for alergic patient. Secara Epidemiologically, it is indicated that the areas with rural populations are heavily exposed to worm infections with a low prevalence of allergic diseases and have been proven by studies of animal models by stimulating the formation of TGF β and interleukin-10 (IL-10) inhibits IL-4, IL-5, IL13 by stimulating Treg.

Conclusion

The ability of Lumbricus rubellus extract to hold any bacterial growth happen due to the bioactive compounds contained inside, it is known as Lumbricin-I which is a peptide compound composed of complete amino acids, especially proline, which is able to inhibit the negative gram bacteria, positive gram bacteria, and several function. Lumbricin-I inhibits bacterial growth by providing pores in bacterial cell. Therefore, it can cause cytoplasm of bacterial cells to be exposed on the outside environment and cause a bacterial death.

Deworming therapy is possible as an adjuvant treatment for atopic dermatitis patient. Epidemiologically, it is indicated that the areas with rural populations are heavily exposed to worm infections with a low prevalence of allergic diseases and have been proven by studies of animal models. Worms Therapy can stimulate the formation of interleukin-10 (IL-10) and it can suppress TH2 cells to reduce cytokines IL-4, IgE and Eosinophils that take a role in atopic dermatitis patients.

Based on the results of previous research and discussion, it can be concluded that the extract of earthworms (Lumbricus rubellus) can increase IL-10 levels and reduce IgE and Eosinophils on days 0.8, and 15 in atopic dermatitis patients.

Suggestion

Based on the conclusion above, there are several suggestions implied as below:

1. Further research is required to determine the side effects of long-term administration of lumbricus rubellus extract in atopic dermatitis patients.
2. Long-term research is required to determine the effect of other cytokine levels from giving lumbricus rubellus extract to the atopic dermatitis patient.

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