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The Effect of LumbricusRubellus Extracts on IL-4,IL-10, IgE, and Eosinofil Levels in Atopic Dermatitis Patient

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ABSTRACT
Atopic dermatitis (AD) is a chronic, residive inflammatory skin condition that is characterized by severe pruritus. The pathophysiology of this condition is a multifactorial, skin barrier disorder. Furthermore, it involves altered immune response, and it can be seen in rural population exposed to many worm infections with a low prevalence of allergic disease. This 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. Also, the effects of LumbricusRubellus extract can increase interleukin 10, and reduce IL-4, immunoglobulin E, and eosinophils. Furthermore, it can provide clinical improvement in Atopic Dermatitis patients. This study used "Pretest-Posttest Design" method of mild patients who were not infected with worms. The 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. The statistical test used non-parametric tests, such as Mann-Whitney (U-Test) and Wilcoxon test to determine whether there was a difference between the two treatments or not. There was a difference ($p < 0.05$) between LumbricusRubellus extract group and the group without theextract on day 8. Meanwhile, at day 15, there was no significant difference ($p > 0.05$), and there was still an increase in IL levels and decreasedIgEand eosinophil. The side effects that appeared were intestinal disorders, such as nausea and bowel disorders of the subjects. Inaddition, LumbricusRubellus extract has an immune response effect towards people with atopic dermatitis.

Keywords: Earthworms, LumbricusRubellus, Atopic Dermatitis

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INTRODUCTION
Skin ismade up of three layers, namely epidermis, dermis, and hypodermis. The main component of epidermis is keratinocytes, which is formed from thebasal, spinous, and granular layer. The stratum corneum has a role in replacing the plasma membrane with an insoluble layer of macromolecules called cornified enveloped (CE), which has a weak concentration inside stratum corneum (Oyoshi, He, Kumar, Yoon, & Geha, 2009).
The epidermis is interspersed with Langerhans cells (LCs) derived from antigen presenting cells (APCs). Furthermore, the dermis is a vascular layer, which consists of fibroblasts and dense connective tissue with collagen and elastic fibers. It is inhabited by cells of hematopoieticderivativeswhich include dendritic cells, mast cells, macrophages, and several lymphocytes (Oyoshi et al., 2009).The hypodermis is a layer of fat cells and long connective tissue. Therefore, the main function of skin is to provide protection as physical barrier against externalagents such as irritants, allergens, as well as pathogens, and also control water loss (Oyoshi et al., 2009).
Atopic dermatitis (AD) is a chronic residive skin disease that commonly occurs in children.This condition is often associated with abnormalities of skin barrier function, allergen sensitization, and recurrent skin infections(Leung DYM, Eichenfield LF, 2008). Accordingto data, atopic dermatitis occurs in 15-20% of children and 1-3% of adults (Nutten, 2015). There are two hypotheses about the pathogenesis of AD. The first stated that there is anepithelial cells disruption of skin which causes malfunctioning of the 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 (Watson &Kapur, 2011). Meanwhile, patients with AD experience an increase of spontaneous histamine release from basophils. This finding reflects systemic Th2 immune response in AD, especially in patientswith elevated serum IgE levels. Also, the peripheral blood skin overexpressing CD4 or CD8 spontaneously secretes IL-5 and IL-13, thereby functionally prolonging eosinophil survival and inducing IgE synthesis. (Leung DYM, Eichenfield LF, 2008).
The pathophysiology of atopic dermatitis is multifactorial, and it involves skin barrier disorders, change in immune response mediated bycellular and humoral immune systems, and hypersensitivity type I reactions that can cause IgE increase(Madhu, 2015).Also, human T cell categorized as T cell helper 1 (Th1) and T cell helper 2 (Th2) depends on the obtained cytokine profile. Therefore, repeated exposure to antigens will change the cytokine profile in skin lesions from Th1 to Th2 (Mizutani, 2006).
Atopic dermatitis pathophysiology occurs due to several mechanisms, one of whichcomes from inflammation. This process is 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 cytokinesof IL-4 and IL-13, stimulate fibroblasts to produce periostine, a protein that causes keratinocytes to produce TSLP, which induces TARC / CCL17 production by dendritic cells (Katayama *et al.*, 2017). Also, the high levels of IL-4 produced by T cells can increase the risk of atopic dermatitis. Furthermore, high IL-4 levels have been found in children with this condition. 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).
Over thousands years ago, *Lumbricus rubellus* has been widely used by the Chinese people as medicine for various diseases. (Mihara et al., 1991). For worm infected patients, it can stimulate the formation of interleukin-10 (IL-10) and transforming growth factor-beta (TGF-β) through increased

T regulatory cells (Taylor, van der Werf, & Maizels, 2012). Also, 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 (Boyman, Werfel, & Akdis, 2012).

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 an increased IL-10 levels in peripheral blood mononuclear cells and skin lesions of AD patients. However, other studies reported that IL-10 levels were inversely related to ADseverity. Therefore, the more severe AD condition, the lower the IL-10 levels(Girard-Madoux, Kel, Reizis, & Clausen, 2012).

Laboratory testing is not required for evaluation routine and treatment of AD patients. Moreover, IgE levels are elevated in 70-80% of the patients. This is related to sensitization towards concomitant inhalant and food allergens, allergic rhinitis, and asthma that occurs at the same time. Meanwhile, 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 suchas S. aureus toxin, and Candida albicans orMalassezia sympodialis which can be detected. Besides, some of thepatients showed positive reactions of using atopy patch test even though the immediate skin test was negative (Leung DYM, Eichenfield LF, 2008).

Earthworms also contain active alkaloid compounds, which contain nitrogen atoms and alkaline (have the greater pH than 7) which have antibacterial and antipyretic activity. The alkaloids mechanism of action in inhibiting bacterial growth is by disturbing constituent components of peptidoglycan in bacterial cells, therefore the cell is not completely formed. (Yusriana, 2018)

Nowadays, there have been no research that obtained a worm therapy which can affect immunological pathways and cause atopic dermatitis. Meanwhile, 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 are widely developed in Indonesia.

Therefore, this study aims to determine the effect of earthworm extract (Lumbricus Rubellus) on IL-10 and reduce IL-4, immunoglobulin E, and eosinophils. This can provide clinical improvement in patients with atopic dermatitis. In addition, it can be used as an alternative therapy in management of atopic dermatitis.

METHODOLOGY

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

This studywas conducted from January to March 2020 untill the number of sample met the target. The location was Dermatology and Venereology Polyclinic in Hospital of Education affiliated withDermatology and Venereology department, Hasanuddin University.

The populationwere patients who met the inclusion criteria atthe Dermatology and Venereology Polyclinic, Hasanuddin University. Furthermore, the sample was collectively obtained from the time the patient came to the Skin and Venereal Polyclinic, and had a diagnosis of atopic dermatitis during January 2020 - March 2020. Also, 3 cc of blood was taken and examined for 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. In addition, the samples were taken to laboratory of Hasanuddin University Educational Hospital, Makassar

In adult patients, specimens were intravenouslyobtained from the patients blood, after they had signed the informed consent. Meanwhile, pediatric patients serum specimens were taken from their blood, followed by informed parental consent. Total IgE examination was conducted using Enzyme-Linked Immunosorbent Assay (ELISA) method (ELISA kit for Human IgE total) to observe the total IgE concentration on the examined blood samples.

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

Data Analysis Technique

The technique used "Pretest-Posttest Design" method of mild patients who were not infected with worms. The 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. The statistical test used non-parametric tests, such as Mann-Whitney (U-Test) and Wilcoxon test to determine whether there was a difference between the two treatments or not.

RESULT AND DISCUSSION

Result

Natural experimental research has been conducted 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, which consisted of atopic dermatitis patients. Meanwhile, the second group (B) were patients who received 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 subjects was more than female, with a ratio of 3:2, and the largest age group was 25-44 years (50%) followed by the 5-14 year (30%).

Before starting difference test, the analyst requirements were analyzed 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

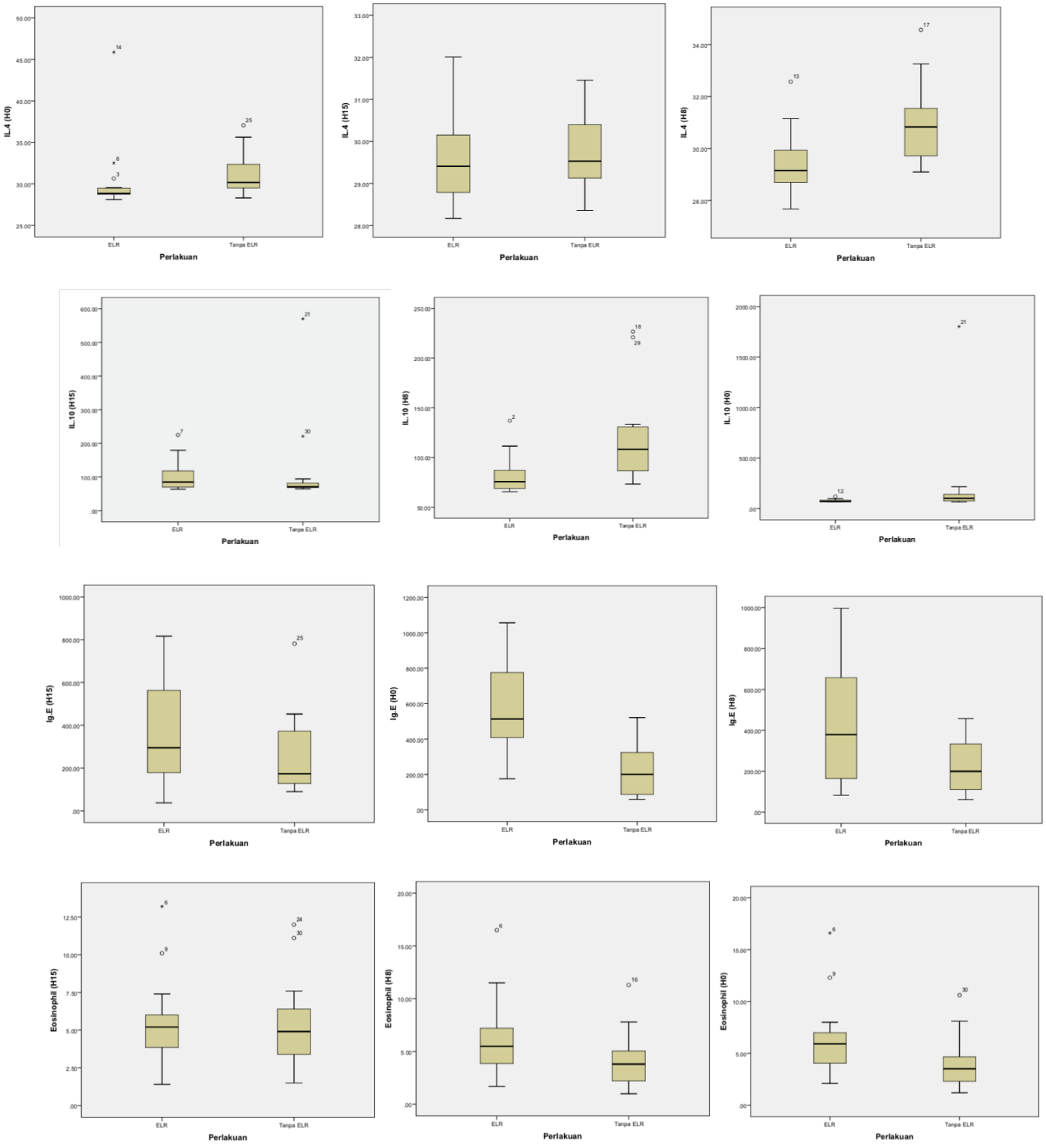

Figure 1:The analysis results on the distribution of levels IL-4, IL-10, IgE, and Eosinophils on days 0, 8, and 15

Based on Table 2 and figure 1, it can be seen that the normality test was conducted using Shapiro-Wilk formula. Furthermore, it was found that data distribution of boththe intervention and control groupwas 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 was conducted to determine whether the data obtained from the two groups had homogeneous variant or not. The results obtained from both intervention 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 groups were not normally distributed and not homogeneous, therefore the hypothesis testing used non-parametric testing, such as Mann-Whitney (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 on IL-4 in atopic dermatitis patients, the 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 the treatment) on days 0, 8, and 15. The results 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	
	Without ELR	28.29	37.06	31.2330	2.56034	30.1494		Differences
IL.4 (H8)	ELR	27.67	32.57	29.4275	1.22954	29.1583	0.006	
	Without ELR	29.10	34.56	30.9243	1.65818	30.8316		Differences
IL.4 (H15)	ELR	28.17	32.01	29.6214	1.07464	29.4059	0.547	
	Without ELR	28.35	31.45	29.7657	0.93316	29.5298		No Differences

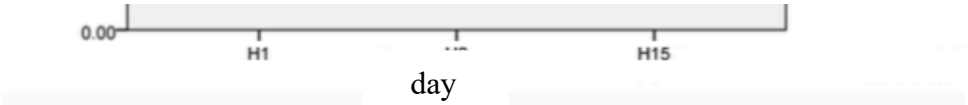

Figure 2. IL-4 levels of control groups and intervention day 0, 8, and 15

Based on Table 4 and figure 2, it can be seen that in Mann-Whitney test, there was a difference (p <0.05) of IL-4 levels between ERL group and without ERL group on day 0 and 8, however it was different on day 15. Also, there was a difference (p> 0.05) in IL-4 levels in ERL group and the group without ERL.
Therefore, the difference in IL-4 levels before and after the implementation of lumbricus rubellus extract was

determined at day 0 (before implementation), 8 (eight days after the implementation), and 15 (15 days after implementation) with the Wilcoxon test. The wilcoxom test results can be seen in 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 in Wilcoxon test of ERL group, there was no difference ($p > 0.05$) in IL-4 levels before and after the implementation of the extract on day 0 (before implementation), 8 (after implementation), and 15 (after implementation). Meanwhile, in 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 effect of the 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 the treatment) on day 0, 8, and 15. The Mann-Whitney test results 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	Differences
	Without ELR	64.74	1802.47	219.7086	439.96753	102.1230		
IL.10 (H8)	ELR	65.61	137.05	83.1810	20.33293	75.5281	0.006	Differences
	Without ELR	73.19	226.66	118.0581	47.20522	108.2801		
IL.10 (H15)	ELR	63.70	224.84	103.2225	49.54104	85.2536	0.395	No Differences
	Without ELR	64.91	570.09	116.0181	131.49605	70.8765		
	Without ELR	1.50	12.00	5.2933	3.09619	4.9000		

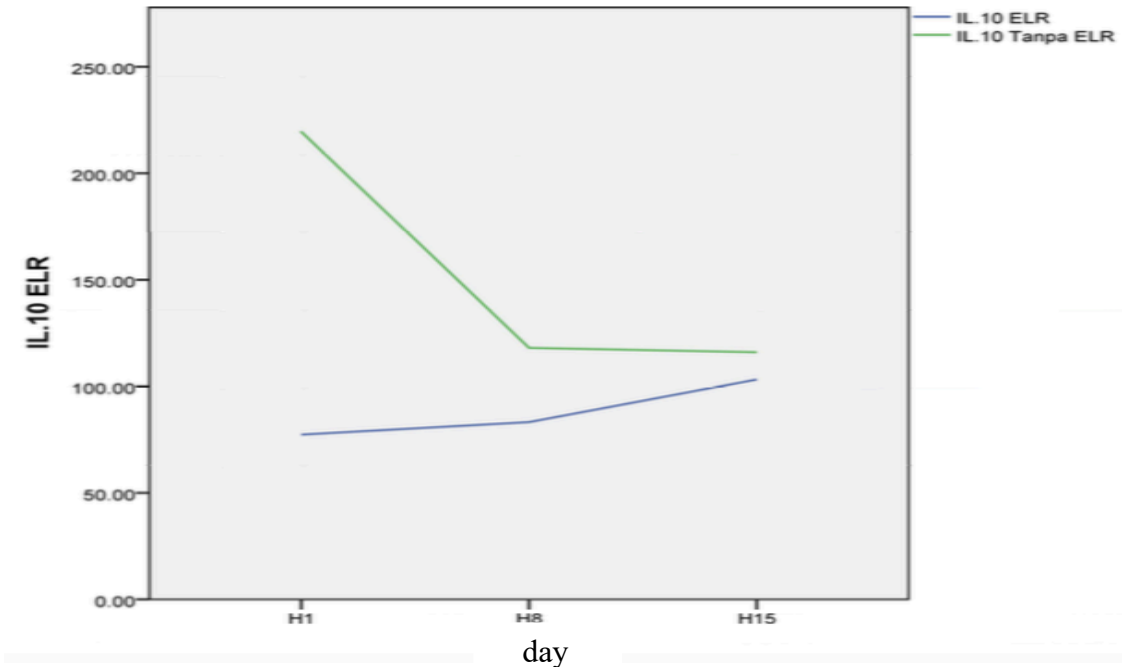

Figure 3:IL-10 levels in control groups and intervention days 0.8, and 15

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. Also, there was a difference (p> 0.05) in IL-10 levels within the group with ERL and without ERL.

Therefore, the differences in IL-10 before and after the extract application at H0 (before implementation), H8 (after implementation), and H15 (after implementation) was determined by Wilcoxon test. The Wilcoxon test results 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 in the Wilcoxon test of ERL group, there was no difference (p> 0.05) in IL-10 levels before and after the extract implementation on day 0 (before implementation), 8(after implementation), and 15 (after implementation). Meanwhile, 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 extract effect on IgE in atopic dermatitis patients, Mann-Whitney test (U-Test) was performed. This was done to determine whether there was a difference between two different treatments (given lumbricus rubellus extract treatment and not given) or not on day 0, 8, and 15. The Mann-Whitney test results 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		

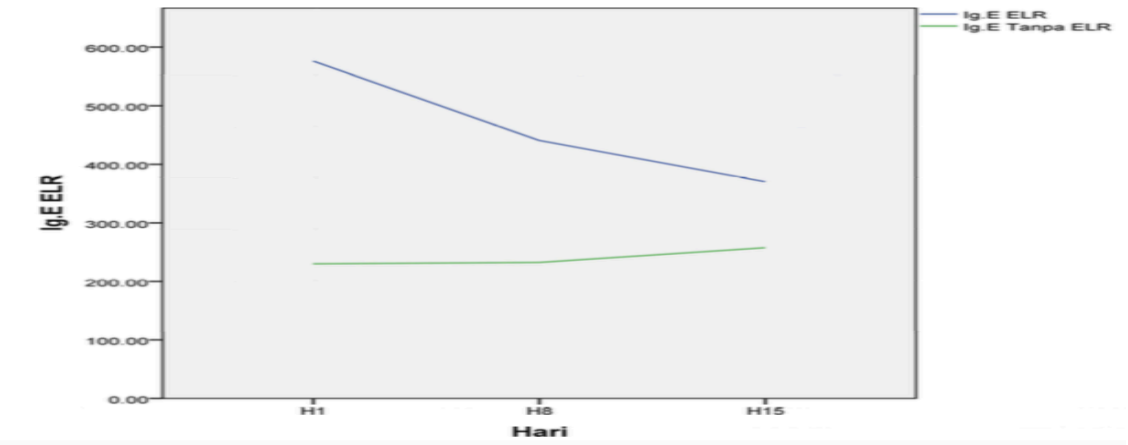

Figure 4:IgE levels in two control groups and intervention days 0, 8, and 15 day

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

Therefore, the difference in IgE levels before and after the extract implementation at H0 (before implementation), H8 (after implementation), and H15 (after implementation) was determined by Wilcoxon test. The Wilcoxon test results 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 that in Wilcoxon test within the ERL group, there was no difference (p> 0.05) in IgE levels before and after the implementation of lumbricus rubellus extract on day 0 (before implementation), 8 (after implementation), and 15 (after implementation). In the group without ERL, 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. This was done to determine whether there was a difference between two different treatments (given lumbricus rubellus extract treatment and not given) on day 0, 8, and 15. The Mann-Whitney test results can be seen in Table 13 below:

Table 13: 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 (H15)	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		

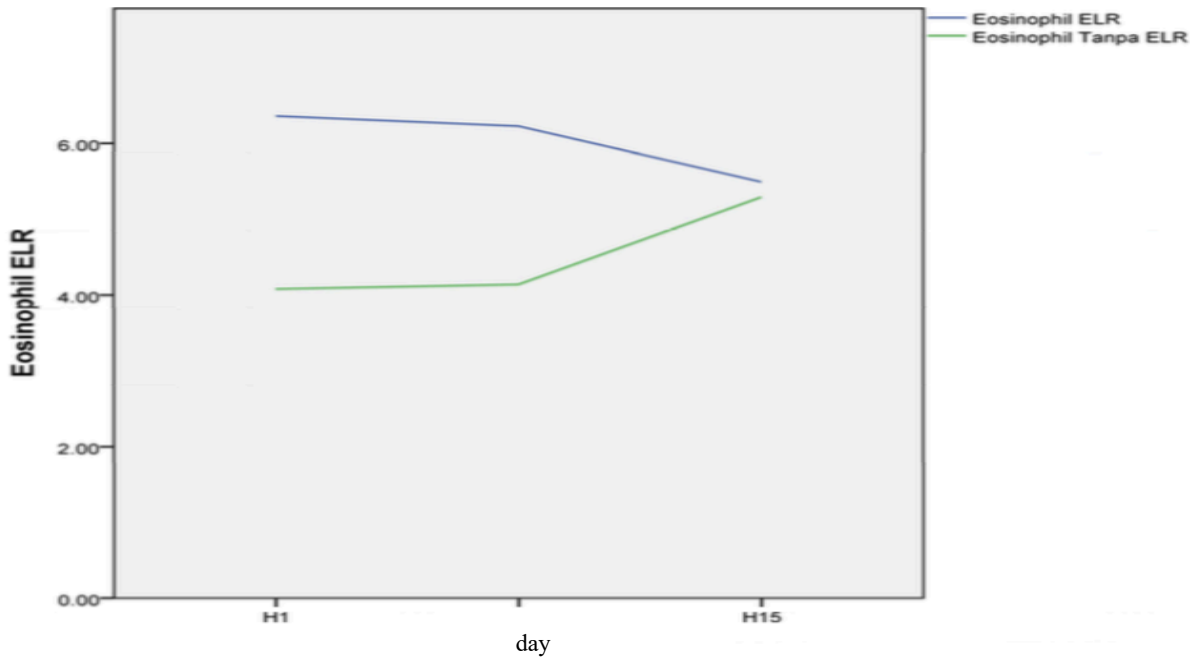

Figure 5:Eosinophil levels in the control and intervention groups day 0.8 and 15

Based on Table 13 and figure 5, it can be seen in Mann-Whitney test that there was a difference (p <0.05) in eosinophil levels between the group with ERL and without ERL on day 0 and 8. However, on day 15 there was no difference (p>0.05) of IgE levels in the group with ERL and without ERL.

The difference in eosinophil levels before and after the extract implementation at day 0 (before the implementation), 8 (after the implementation), and 15 (after the implementation) was determined by Wilcoxon test. The Wilcoxon test results can be seen in Table 14 below:

Table 14: 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 15: PDifferences 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 14 and 15, it can be seen that in the Wilcoxon test in ERL group, there was no difference (p> 0.05) in Eosinophil levels before and after the extract implementation on H0 (before implementation), H8 (after implementation), and H15 (after implementation). Meanwhile, 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 a residual chronic disease characterized by clinical symptoms of itching, which generally affects children. The pathogenesis includes skin barrier disorders, which include disruption of filaggrin gene expression, genetics, environmental and immune system abnormalities. The disruption of skin protection structure can reduce the ability and skin function. This leads to an immune response and inflammatory reaction (Czarnowicki, Krueger, & Guttman-Yassky, 2014). Also, the skin barrier minimizes water loss from the epidermis and protects from the external factors such as heat or cold, penetration of potentially harmful substances, and pathological bacteria colonization. (Nowicka & Grywalska, 2018). Meanwhile, the protective structure of epidermis consists of corneocytes (cells of stratum corneum), lipids, and natural moisturizing factors that were produced during corneocyte formation process. (Le Lamer et al., 2015). The function of skin's natural moisturizer is to absorb and bind water to protect the epidermis (Jungersted et al., 2010). 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) (Oyoshi et al., 2009).

A study showed higher risk of Atopic Dermatitis is related to the maternal atopy of mother rather than father (Bin & Leung, 2016). Also, genetic abnormalities in cytokines that play important role in the immune response of AD pathogenesis, where 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 (Bin & Leung, 2016).

Natural and innate immune system both contribute to AD pathogenesis. The TH2 cells have a major role in increasing eosinophils and IgE in 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. Meanwhile, in chronic AD, there is a transition of TH2 to TH1, an increase of IFN-γ, IL-12, GM-CSF expression, as well as tissue remodeling with increased collagen deposition and skin thickening. (Kay, 2001).

Specific antigens that can penetrate the skin due to skin barrier disorders are captured by antigen-specific IgE on inflammatory dendritic epidermal cells and Langerhans cells (LC). Meanwhile, specific IgE mostly reacts within the environmental and bacterial antigens. LC of AD patients are primarily secreted at Th2 cytokine IL-10 rather than Th1 cytokine IL-12 (Aiba, Manome, Yoshino, & Tagami, 2003).

The decreased exposure toward the infection after birth can move the Th2-cell balance response toward Th2. Also, the result of imbalance response will cause an excessive eosinophil and IgE response, both of which are related to the allergic reactions and atopy. Microbial exposure can affect the Th1 and Th2 balance by increasing Th1 response and decreasing Th2 response. Furthermore, the Th1 cells are related to infection response and interferon-∂ production. Th2 cells induce IgE production and maturation of mast cells, basophils, and eosinophils, therefore Th2 cells are generally associated with atopic immune responses (Lambrecht & Hammad, 2017)

The Role of Cytokines in Atopic Dermatitis begins from an adaptive immune response that is mediated by T and B cells, and associated with antigen-presenting cells (APC). Meanwhile, the adaptive immune system consists of cellular and humoral (Aiba et al., 2003). Furthermore, T cells are produced in the bone marrow and mature in the thymus gland. The T cell receptor (TCR) will recognize specific peptides that bind to Major Histocompatibility Complex (MHC) / Human Leukocyte Antigen (HLA), which is a 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. Also, T lymphocytes activate Helper T (CD4) by secreting cytokines to assist T cells, B cells and macrophages. In fact, Th1 cells have 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 B cells activation and produce antibody. The Th2 cells produce cytokine profiles IL-4, IL-5 (synthesizing IgE and activation of eosinophils) and IL-10 (inhibiting proliferation of Th1). Furthermore, Th17 cells have an important role of fungal infections by secreting the cytokine IL-17 profile (activating neutrophils to kill fungus) (Kay, 2001). In addition, B cells are produced and mature in

the bone marrow, while plasma cells produce various kinds of antibodies for IgA, IgD, IgG, IgM, and IgE.

Allergens are captured by dendritic cells and presented to the T cells. Therefore, it will be an imbalance between TH1 and TH2. Meanwhile, the TH2 cells induce B cells to provide immunoglobulin E (IgE) production. The allergen-specific IgE binds to the receptor for IgE (FcεRI) on mast cells (Kay, 2001).

IgE production in atopic disease by B cells is dependent on the support from 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, and have a greater role in chronic infections, such as Crohn's disease and psoriasis (Cookson, 2004). Meanwhile, re-exposure 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. (Kay, 2001).

Eosinophils are 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 the bone marrow. Meanwhile, adult eosinophils are cells that remain in the fibers in a small portion circulate in the blood. (Lambrecht & Hammad, 2017)

Earthworms contain a class of active alkaloid compounds. These compounds contains nitrogen atoms and has an alkaline characteristics (pH greater than 7) which also have antibacterial and antipyretic activity. The mechanism of alkaloids action in inhibiting bacterial growth is by disturbing the constituent components of peptidoglycan in bacterial cells, hence the cell walls are not completely formed (Yusriana, 2018).

In this study, the results of Shapiro-Wilk distribution test showed 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. The results showed that there was a difference ($p < 0.05$) between the ERL group and the group without ERL on the 8 day of administration. Although at day 15, there was no significant difference ($p > 0.05$), and there was an increase of IL-10 levels and decreased levels of IgE and eosinophils. However, in contrast to IL-4 levels, which decreased at day 8, it was increased on day 15. In addition, the side effects that appeared at the time of the study were intestinal disorders, such as nausea and bowel disorders in a the subjects.

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

CONCLUSION

The ability of Lumbricus rubellus extract to prevent any bacterial growth is due to the content bioactive compounds. This is known as Lumbricin-I which is a peptide compound composed of complete amino acids, especially proline, which can inhibit both negative and positive gram bacteria, and several function. Furthermore, Lumbricin-I inhibits bacterial growth by providing pores in bacterial cell. Therefore, it can expose the bacterial cell cytoplasm and cause 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. This have been proven by studies of animal models. Also, 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 studies and discussion, it can be concluded that earthworm extract (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 patients.

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