



Pulmonary Medicine

POLYPHYTOPHARMACA IMPROVING ACT VALUE, BIOMARKER (EOSINOPHILS AND MDA): QUASI EXPERIMENTAL TEST IN PATIENTS WITH ASTHMA

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ABSTRACT **Background:** Asthma is a heterogeneous disease marked with chronic inflammation of respiratory tract. Asthma control level is the success indicator of asthma treatment. Complex immunological mechanisms affects this condition including the role of MDA (malondialdehyde) as a marker of inflammation. This research aimed to determine the effect of polyphytopharmaca administration on the value of asthma control test (ACT), blood eosinophils level and markers of MDA serum inflammation in patients with asthma. **Method:** quasi experimental approach was conducted toward 15 stable asthma patients who were not fully controlled in Pulmonary Poly, Public Hospital of Dr. Saiful Anwar Malang. Assessments of ACT values, eosinophil levels and serum MDA levels were carried out before and after administration of polyphytopharmaca which contained a combination of 100 mg Nigella sativa extract, Kleinhovia hospita 100 mg, Curcuma xanthorrhiza 75 mg, and Ophiocephalus striatus 100 mg, three times daily with two capsules of two mg of Kleinhovia hospita for 12 weeks. The ACT value was determined by the researcher by asking the patient directly, blood eosinophil levels were calculated by analyzing blood type counts, and serum MDA levels were detected by the qPCR method. **Result:** there was a significant enhancement of ACT value (18.07 ± 2.57 to 22.06 ± 1.83 , $p = 0.001$), a significant decrease in blood eosinophils levels (653.15 ± 276.15 pg / mL to 460.66 ± 202.04 pg / mL, $p = 0.038$), and decreased serum MDA levels (109.64 ± 53.77 ng / ml to 78.68 ± 64.92 ng / ml, $p = 0.156$). **Conclusion:** administration of polyphytopharmaca can increase ACT value, decrease blood eosinophils levels and reduce MDA serum in stable asthma patients who are not fully controlled.

KEYWORDS : polyphytopharmaca, *Asthma control test*, eosinophils levels, MDA.

INTRODUCTION

Asthma is a heterogeneous disease marked with chronic inflammation of respiratory tract such as history of respiratory symptoms that vary in time and intensity, followed by limited expiratory air flow.¹ The prevalence of asthma increases in developing countries, which causes an increase in the burden of health costs, disability and mortality rates.² The prevalence of asthma in Indonesia was 4,5% of the entire population based on the assessment of symptoms in 2013.³

Despite the widely available medications and asthma treatment procedure, achieving controlled asthma is still a major problem for most patients. One cause of poor symptom control is the lack of adherence to the use of inhalation drugs.⁴ In addition, even though patients are adherence and use drugs according to medication procedure, nearly 50% of asthma patients had not yet achieved good control.¹

Many researches about the use of additional vitamin and nutrition for asthma. It shows many different result. Giving vitamin D has been shown to increase the value of ACT in asthma patients who have vitamin D deficiency.⁵ Various researches on the use of herbs have been reported such as Nigella sativa extract. The prophylactic effects on asthma and bronchitis have been described in animal and human models studies.⁶ Thymoquinone contained in Nigella sativa has an immunomodulatory effect. Significantly, thymoquinone can suppress IL-5 and IL-13 expression induced by lipopolysaccharides and proteins without causing changes in IL-10.7 production.

One way to assess asthma symptom control is using a numerical asthma control tool, the Asthma Control Test (ACT).⁷ ACT is very simple. This questionnaire is patient-oriented to assess asthma control.⁸ In the laboratory, there are various biomarkers which can be used. Blood eosinophil levels are easily accessible biomarkers.⁹ Eosinophils are fundamental and important inflammatory cells, marking or characterizing inflammation in asthma.¹⁰ In evaluating the effectiveness of using mepolizumab, the researchers chose to monitor blood eosinophils as a target for asthma treatment.¹¹

Mechanism of oxidative stress between reactive oxygen species (ROS) with polyunsaturated fatty acids will produce lipid peroxidation end products namely malondialdehyde (MDA) as a marker of inflammation. In cases of inflammatory diseases, including asthma, MDA levels usually increase.¹² High levels of MDA have been widely

studied in patients with various respiratory diseases including asthma, bronchiectasis and COPD.¹³

This study aims to prove the effect of polyphytopharmaca administration on levels of MDA inflammatory biomarkers, blood eosinophils and asthma control test (ACT) values in stable asthma patients who are not fully controlled.

METHOD

The research used quasi experimental design, pre and post treatment for stable asthma patients who were not fully controlled. Subjects were bronchial asthma patients who were not fully controlled with age range of 18-59 years. The research was conducted at the Lung Poly and Clinical Pathology Laboratory of Dr. Hospital Saiful Anwar Malang in November 2018 to April 2019. Samples were obtained consecutively in asthma patients who met the inclusion and exclusion criteria. Every procedure undertaken had been approved by the ethics committee of the Public Hospital of Dr. Saiful Anwar Malang. Patients voluntarily participated in this research and signed an informed consent.

ACT Assessment

ACT assessment was conducted in the beginning and after the administration of treatment for 12 weeks to find out the level of asthma control. The research subjects filled out the ACT questionnaire consisting of 5 questions with 5 answer choices for each question. The lowest value was 5, and the highest value was 25. It was referred to as fully controlled asthma.

Measurement of Blood Eosinophils and MDA Serum Level

Venous blood sample was taken twice, in the beginning of research and after giving treatment for 12 weeks to measure the absolute value of blood eosinophils and serum MDA levels. Three milliliters of blood was used for eosinophil test through a count of white blood cell types with flow cytometry which worked automatically. Two milliliters of blood was used for test of serum MDA levels. MDA levels were measured by the ELISA (Human Malondialdehyde ELISA kit) method in the Clinical Pathology laboratory of Dr. Hospital. Saiful Anwar Malang.

Statistic Analysis

Data obtained were noted in research medical record to then processed and conducted analysis and interpretation. Whether it was normal or not, Data distribution were determined by the Saphiro-Wilk test. The

effect of polyphytopharmaca administration on ACT values, blood eosinophil levels, and serum MDA levels were tested by paired T-tests on normal data distribution. In the abnormal distribution of data, the Wilcoxon test was used. The measurement results of the variables measured in this research were carried out using Microsoft Office Excel 2010, and statistical analysis using SPSS 25.0 series.

RESULT

16 stable asthma patients were considered meeting research requirement. One patient did not complete the research until the final because he did not take polyphytopharmaca supplements regularly as agreed at the start of the research. Thus, there were 15 subjects who meet the requirements for statistical analysis at the end of the research. Socio-demographic characteristics can be seen in Table 1. All research subjects routinely used asthma control therapy which was a combination of inhaled corticosteroids and long-acting β 2 agonists, but not all subjects used additional lozenges (SABA).

Asthma Control Test (ACT)

The administration of polyphytopharmaca for 12 weeks was proven to be able to improve ACT value from 18.07 ± 2.57 to 22.06 ± 1.83 , and statistically significant with a value of $p = 0.001$ as shown in Figure 1 below. The number of subjects who had an ACT value of less than 20 decreased, initially 60%, to only 6.7% after receiving polyphytopharmaca supplementation.

Table 1. Characteristic Of Sociodemography And Clinical Of Research Subjects

Characteristic	n	Percent (%)
Age (year)		
18-40	5	33
41-59	10	67
Gender		
Male	1	7
Female	14	93
Education		
Low education	9	60
High education	6	40
Employment		
Worker	7	47
Non-worker	8	53
History of atopic		
Yes	14	93
No	1	7
History of atopic in family		
Yes	10	67
No	5	33
History of SABA Treatment		
Yes	9	60
No	6	40
Smoke exposure		
Yes	9	60
No	6	40
Eusinofil		
Yes	2	13
No	13	87
Eosinofil darah		
< 300 pg/mL	6	40
\geq 300 pg/mL	9	60

We also conducted the statistic analysis toward each question from five questions of ACT. There was an increase in the value of each question after the combination of extracts of *Nigella sativa*, *Kleinhovia hospita*, *Curcuma xanthorrhiza*, and *Opiocephalus striatus* for 12 weeks. It was statistically significant with $p < 0.05$ as presented in table 2 below.

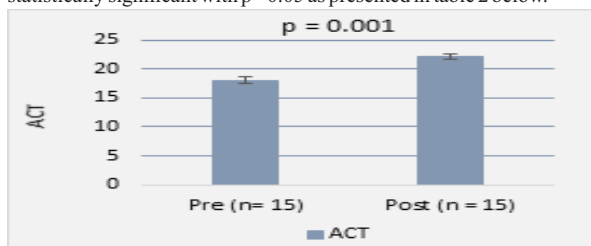


Figure 1. The change of ACT value before and after administration of polyphytopharmaca

Table 2. The Effect Of Treatment For Each ACT Question

Question (P)	Before treatment	After treatment	p
P1 (Daily functional)	3.80 ± 0.68	4.73 ± 0.46	0.004
P2 (Frequent of asthma)	3.60 ± 0.91	4.47 ± 0.52	0.006
P3 (Night symptom)	4.07 ± 1.22	4.80 ± 0.41	0.026
P4 (Treatment of reliver)	3.00 ± 1.19	4.00 ± 1.00	0.011
P5 (all controls)	3.53 ± 0.64	4.07 ± 0.46	0.023

Blood Eosinophils Level of research subjects before treatment was 450.34 ± 334.46 pg/ mL, while blood eosinophil levels after receiving treatment was 400.09 ± 182.18 pg/mL. Through the paired T-test the value of $p = 0.820$ obtained. Thus, it can be concluded that the combination of extracts of *Nigella sativa*, *Kleinhovia hospita*, *Curcuma xanthorrhiza*, and *Opiocephalus striatus* can reduce blood eosinophil levels in the research subjects but not statistically significant as shown in Figure 2 below.

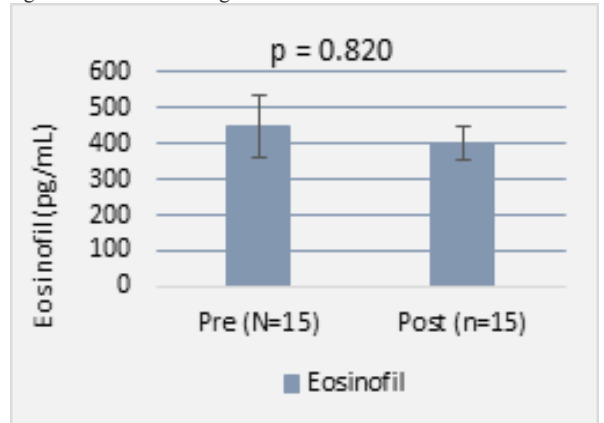


Figure 2. The Change Of Blood Eosinophil Level Before And After The Administration Of Polyphytopharmaca For All Subject

We also analyzed group of subjects with blood eosinophil level at the beginning of the examination ≥ 300 pg / mL (n=9). It was obtained that blood eosinophil levels in the research subjects before treatment was 653.15 ± 276.15 pg/mL, decreased to 460.66 ± 202.04 pg/mL after administration of polyphytopharmaca with a p-value = 0.038. Thus, it can be concluded that administration of polyphytopharmaca can significantly reduce blood eosinophil levels as shown in Figure 3 below.

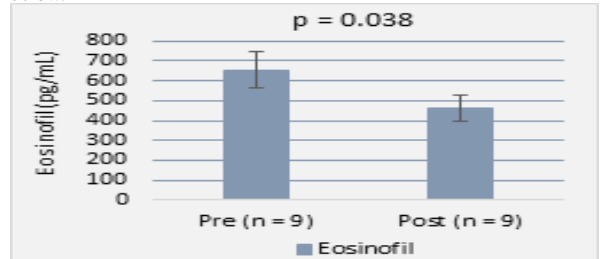


Figure 3. The change of blood eosinophils level before and after administration of polyphytopharmaca insubjects with initial eosinophil of ≥ 300 pg/mL

MDA Serum Level

The average of MDA before administration of oral polyphytopharmaca was 109.64 ± 53.77 . After oral administration of fitofarmaka was 78.68 ± 64.92 . After the Wilcoxon test, it was known $p = 0.156$. It can be concluded that oral phytopharmaca administration does not significantly affect MDA as shown in Figure 4 below.

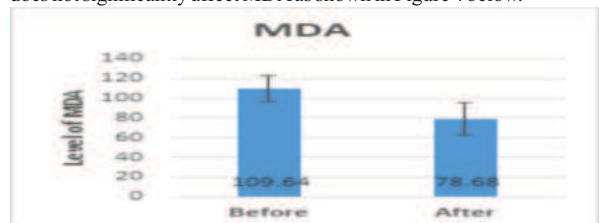


Figure 4. Te change of MDA serum level before and after the

administration of polyphytopharmaca for all subject

DISCUSSION

Polyphytopharmaca Increasing Asthma Control Test Value

In this research, administration of polyphytopharmaca could increase ACT value. The number of subjects who initially had an ACT value of less than 20% was 60%, decreasing to only 6.7%. Nigella sativa contained in polyphytopharmaca inhibits cyclooxygenase and 5-lipoxygenase pathways from arachidonic acid metabolism caused decreased thromboxan synthesis and leukotrienes.¹⁴ Leukotriene receptor antagonists had been used for the treatment of bronchial asthma, because besides producing minimal bronchodilator effect and decreasing bronchoconstriction due to allergens, sulfur dioxide and exercise, it also had anti-inflammatory effects.¹⁰ Nigella sativa could also prevent the release of histamine from mast cells and basophils and had an anti-inflammatory effect, which further provides a therapeutic effect on asthma.¹⁵

The effect of Nigella sativa extract in respiratory tract was the stimulating effect of β -adrenergic receptors considering the effects of bronchodilation through stimulation on nonadrenergic nervous system blockages, barriers to noncholinergic stimulation, and inhibitions on phosphodiesterase, all contribute to the anti-asthma effects of the Nigella sativa extract.¹⁵

Koshak et al. had previously examined the effect of Nigella sativa extract in asthma patients for 4 weeks, at the end of the research, there was a significant increase in average ACT values in the treatment group compared to the placebo group.

Polyphytopharmaca Reducing Blood Eosinophil Levels

The administration of Polyphytopharmaca can reduce blood eosinophils level. Similar to the results of the research by Koshak et al, Administration of Nigella sativa extract could significantly reduce the amount of blood eosinophils compared to placebo in stable asthma patients who are not fully controlled. However, in that research, the dose of Nigella sativa given was greater without combination with other extracts.⁶ These results are different from the results of other previous researches with allergic rhinitis subjects, in which there was no significant decrease in the percentage of blood eosinophils after administration of Nigella sativa extract. In that research, it did not use absolute eosinophil levels, and the percentage of eosinophils of the subjects before treatment was within the normal range of values.¹⁶

The decrease in eosinophils could be caused by the anti-inflammatory effects contained in the polyphytopharmaca given. Nigella sativa, Kleinhovia hospita and Curcuma xanthorrhiza had anti-inflammatory effects through different pathways. Antiviral, antibacterial and increased immunity effects (immunoglobulins in Ophiocephalus striatus) could reduce the likelihood of new inflammation and exacerbations by triggering infection.¹⁷ Thymoquinone was stated to suppress IL-5 and IL-13 induced by lipopolysaccharides and proteins.⁷ Emphasis on infection, cytokines IL-13 will limit the withdrawal of eosinophils and some inflammatory mechanisms in the respiratory tract of asthmatic patients.

Polyphytopharmaca Reducing MDA serum levels

Our research presented the reduce of MDA Serum level with clinical improvement and decreased blood eosinophil levels after supplementation with Nigella sativa extract, Kleinhovia hospita, Curcuma xanthorrhiza, and Ophiocephalus striatus for 12 weeks, especially in subjects with initial blood eosinophil levels ≥ 300 pg/mL. It is very likely that the decrease in serum MDA is correlated with a decrease in inflammation characterized by a decrease in blood eosinophil levels and improved asthma control symptoms characterized by an increase in ACT values.

Various literature showed that the administration of curcumin, the active ingredient contained in Curcuma xanthorrhiza had many benefits which be used as an antioxidant and anti-inflammatory. Curcumin plays role as an anti-inflammatory agent by inhibiting the activation of NF-KB, cyclooxygenase, lipoxygenase and iNOS expression. Previous researches revealed that administration of curcumin in two different doses to mice tried to reduce MDA levels.¹⁸ While other studies also explained that administration of polyphytopharmaca (Boswellia serrata, Glycyrrhiza glabra and Curcumin) three times a day for 4 weeks in asthma patients was successful decreased serum MDA levels although it was not statistically significant compared with the placebo group.¹⁹

Whereas other researches explained that Nigella sativa administration of 3 mg/kg in mice with ovalbumin-induced asthma succeeded in reducing MDA levels compared to the control group.²⁰

This research has several limitations, including the number of subjects in this research. it is relatively less than the minimum number of subjects planned due to limited research time. Researchers did not specifically analyze the research subjects for the onset of asthma symptoms, duration of use of controlling drugs and lozenges. Only one mediator for MDA was used which was blood eosinophil levels. Thus, it is relatively difficult to explain the relationship with inflammation in more depth.

CONCLUSION

This research shows that administration of polyphytopharmaca containing a combination of Nigella sativa extract, Kleinhovia hospita, Curcuma xanthorrhiza, and Ophiocephalus striatus can improve the degree of asthma control by increasing the value of the Asthma Control Test (ACT), decreasing blood eosinophils, and decreasing serum MDA levels in stable asthma patients who are not fully controlled.

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Conflict Of Interest

Researchers declare that there is no conflict of interest. Drugs for research are produced by PT. Royal Medika, and the company does not provide input either on the research design or interpretation of research results.

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