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**Original Article**

# *Combination of Eucheuma Cottonii and Corticosteroids has a Superior Immunomodulatory Effect on Asthma*

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***ABSTRACT***

*Asthma prevalence has been increasing globally which is commonly treated using corticosteroid as gold standard treatment. However, corticosteroids have adverse effects and cases of corticosteroid-resistant asthma are emerging. Eucheuma cottonii (EC) has been shown to potentially attenuate inflammatory response in type I hypersensitivity. This study aimed to investigate the effect of Eucheuma cottonii extract and methylprednisolone combination on IL-4, IL-10, and histamine levels in Ovalbumin-induced asthma BALB/c mice. Thirty female BALB/c mice were randomly assigned to 6 groups : (1) Sham; (2) Ovalbumin-induced asthma mice; (3) Ovalbumin-induced asthma mice treated with methylprednisolone (MP) 0.24 mg/day; (4) Ovalbumin-induced asthma mice treated with EC extract 300 mg/kgBW/day; (5) Ovalbumin-induced asthma mice treated with combination of both. Treatments were given respectively for 7 days. Plasma IL-4, IL-10, and histamine levels were measured using ELISA method. The combination group showed both the lowest IL-4 levels (89.30 ± 1.37 pg/ml) and the highest IL-10 levels (487.03 ± 20.57 pg/ml) compared to other asthma-like mouse groups ($p = 0.001$). No significant difference was observed in histamine levels among all treatments ($p > 0.05$). The administration of a combination of methylprednisolone and Eucheuma cottonii extract significantly affected plasma IL-4 and IL-10 levels compared to monotherapies. However, there was no significant difference in plasma histamine levels compared to monotherapies.*

***Keywords:*** *Eucheuma cottonii, corticosteroid, IL-4, IL-10, Histamin*

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## INTRODUCTION

The prevalence of asthma has been increasing globally, even in developed countries such as Indonesia [1,2]. Corticosteroid, such as methylprednisolone (MP) has been used widely as the gold standard treatment of asthma, however, this medication has some adverse effects, such as cushing syndrome, immunosuppression, and loss of bone density [3]. Moreover, there have been emerging cases of corticosteroid-resistant asthma in recent years[4].

Seaweed, particularly Eucheuma cottonii (EC), is a highly valued natural resource renowned for its potential applications as food and traditional medicine. It contains

carrageenan, a sulfated polysaccharides which has been demonstrated to potentially regulate gut microbiota which produce short chain fatty acid (SCFA) which had been previously demonstrated to upregulate regulatory T (Treg) cells which has anti-inflammatory properties through production of interleukin 10 (IL-10) [5,6]. Additionally, EC contains flavonoids that exhibit anti-inflammatory properties by inhibiting the JAK/STAT pathway which has crucial role in the secretion of interleukin 4 (IL-4) and interleukin 13 (IL-13) by T helper 2 (Th2) cells[7,8]. Previous studies have demonstrated the beneficial effects of Eucheuma cottonii extract in reducing inflammatory response in type I hypersensitivity [9]. However, to our knowledge, there are currently no study comparing EC extract to the gold standard treatment, corticosteroid. Moreover, the effects of EC extract as an adjuvant to corticosteroid in asthma treatment remain largely unexplored. Therefore, this research aims to investigate the synergistic effects of these substances on asthma-related markers such as IL-4, IL-10, and histamin in BALB/c mice.

## METHOD

This posttest-only control group study was conducted on 30 female BALB/c mice from February to March of 2023 in Center of Food and Nutrition Study of Gadjah Mada University. This study has passed the ethical review by the Ethics Committee of Medical Faculty of Sultan Agung University (No. 10/I/2023/Komisi Bioetik).

The Center of Food and Nutrition Study of Gadjah Mada University cultivated and identified EC. The seaweed was dried and processed into coarse powder. This powder was then soaked in 50% ethanol (1:10, w/v) in a dark room at a temperature of 28°C for 24 hours and stirred using a magnetic stirrer for 1 hour. Subsequently, the extract was filtered through filter paper to remove any solid particles. The resulting filtrate was then evaporated at 40°C until it transformed into a paste-like consistency. This paste was frozen using liquid nitrogen and stored in a dark glass bottle at a temperature of -20°C.

The animal model used were 30 female BALB/c mice weighted ±20g. Female mice were used due to their higher susceptibiliy to allergic reaction [10]. Combination of 20 µg/mL ovalbumin (OVA) and 1 mg aluminium hydroxide (Biosm, Indonesia) were administered intraperitoneally at day 0 and 14. Furthermore, OVA inhalation was performed at a concentration of $3mg/m^3$/day from day 22 to day 24. Confirmation of asthma was performed using histopathology examination by 2 trained pathologists. The model were then randomized and divided into 6 groups: (1) Sham: healthy mice, (2) OVA: OVA-induced asthma mice, (3) MP: OVA-induced asthma mice treated with oral MP 0.24 mg/day (4) EC: OVA-induced asthma mice treated with oral Eucheuma cottonii extract 300 mg/kgBW/day, (5) MP+EC: OVA-induced asthma mice treated with combination of both. The treatments were administered orally by dissolving EC extract and/or MP into 2 cc of aqua at day 25 to 31. Termination was performed on the 32th day by inhalation of carbon dioxide and blood samples were taken from the orbital sinus and placed in EDTA tubes, then centrifuged at a speed of 3000 rpm for 20 minutes. The plasma was taken and stored at $-70^0$C.

Histamine were measured spectrophotometrically using an ELISA kit (Bioenzy, Indonesia) according to the manufacturer's instructions.

The plasma levels of IL-4, IL-10, and Histamine were measured spectrophotometrically using an ELISA kit (Bioenzy, Indonesia) according to the manufacturer's instructions. The lungs and tracheobronchial tree were harvested for histopathological assessment and fixed using formaldehyde solution for 24 hours before preparation according to standard protocols. The samples were cut to a thickness of 5 µm, and then stained with haematoxylin and eosin (H&E). The samples were observed under light microscope (magnification 100x) and blinded assessment was done by a trained pathologist.

The data for IL-4, IL-10, and histamine are presented descriptively and tested for normality and homogeneity using shapiro-wilk and levene's test respectively. Data were tested using one way ANOVA and tukey post-hoc test to determine differences between each groups with p-value < 0,05 considered significant. The data obtained were processed using computerized methods, and the analysis was performed using SPSS 25.0 for Windows.

## RESULTS

Figure 1 demonstrates the histopathological findings of the OVA-induced asthma groups are consistent with histological changes in asthma. The findings include leukocyte infiltration (white arrowhead), respiratory epithelial hyperplasia (black arrowhead), and bronchi smooth muscle hypertrophy (red arrowhead).

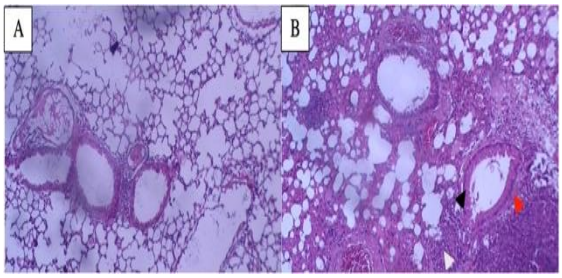


**Figure 1 Lung and bronchi histopathology (H&E. 100x magnification). (A) normal lung and bronchi; (B) lung and bronchi in ovalbumin-induced asthma**

The plasma IL-4 level analyses were presented in Figure 2. The lowest IL-4 level were observed in MP+EC group compared to other OVA-induced asthma groups (p=0.001). MP and EC groups had significant lower level of IL-4 compared to OVA group (p=0.001) However there were no statistically significant difference in IL-4 level between the two groups (p=0.227).

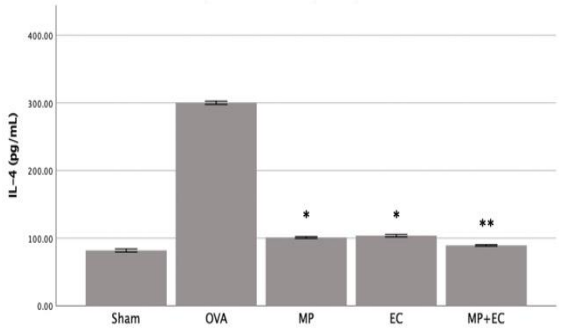


**Figure 2 Plasma IL-4 analyses. *Mean values were significantly different compared to OVA group. ** Mean values were significantly different compared to OVA, MP and EC groups.**

The plasma IL-10 level analyses were presented in Figure 3. The highest IL-10 level were observed in MP+EC group compared to other OVA-induced asthma groups (p=0,001). Furthermore, EC group had a significantly lower level of IL-10 compared to MP group.

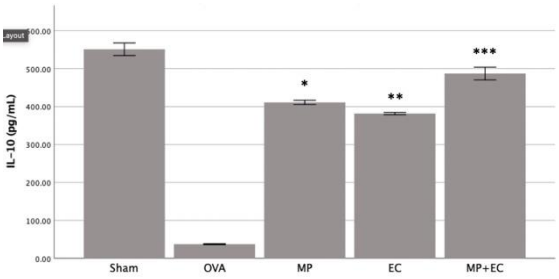


**Figure 3 Plasma IL-10 analyses. ** Mean values were significantly different compared to OVA and MP groups. *** Mean values were significantly different compared to OVA, MP, and EC groups.**

The plasma histamine level analyses were presented in Figure 4. All treatment groups had significantly lower histamine level compared to the OVA group (p=0,001). However, there were no statistically significant differences between the treatment groups (p>0,05).

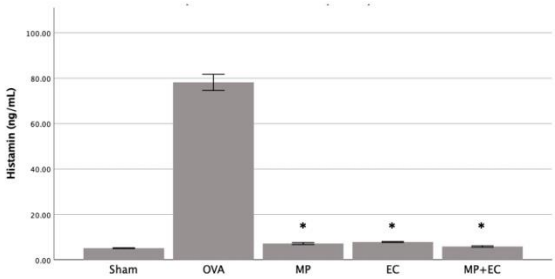


**Figure 4 Plasma Histamin analyses. *Mean values were significantly different compared to OVA group.**

## DISCUSSION

This study is the first to investigate the effect of EC and methylprednisolone combination in asthma. The results demonstrates superior effects of combined therapy in attenuating inflammatory response in asthma compared to monotherapies. The administration of combined therapy resulted in a significantly lower plasma IL-4 level while simultaneously having higher plasma IL-10 level compared to monotherapies. This study also demonstrates the anti-inflammatory properties of EC which were comparable to methylprednisolone. However, there were no statistically significant difference in histamine

level of all treatment groups which may be attributed by other cells histamine-producing cells which are not influenced by IL-4 and IL-10 level such as enterocromaffin-like (ECL) cells and histaminergic neuron. Histamine secretion by ECL cells were influenced by somatostatin and gastrin, while histaminergic neuron were influenced by neurotransmitter such as dopamine and serotonin [10,11]. These findings are consistent with previous studies investigating the effect of EC in several condition mediated by type I hypersensitivity, such as asthma and food allergy [9,12].

EC contains flavonoids such as quercetin and kaempferol which had been previously proven to downregulate the JAK/STAT pathway. Previous study has shown quercetin ability in downregulating GATA3 transcription factor [7]. Other study also showed that kaempferol had the ability to inhibit the activity of the JAK3 protein, thereby downregulate the expression of STAT6 and subsequent inhibition of GATA3 activity [8]. The downregulation of this pathway reduces IL-4 production by Th 2 cells.

Furthermore, the carrageenan in EC acts as a prebiotic that can alter the composition of the gut microbiota, leading to increased production of short-chain fatty acids (SCFAs)[13]. These SCFAs have the ability to decrease the expression of the HDAC9 gene, resulting in increased expression of the transcription factor FOXP3 [14]. This transcription factor plays a role in upregulating Treg cells expression, leading to an increase in the anti-inflammatory cytokine IL-10. This cytokine suppresses the differentiation of CD4+ cells into Th2 cells, thereby reducing the production of IL-4. The IL-4 levels is further reduced with the administration of methylprednisolone, a corticosteroid preparation that reduces IL-4 levels in asthma by suppressing the activity of the transcription factor STAT6 [15].

## CONCLUSION

This study demonstrates the promising outcome of EC adjuvant to corticosteroid as the gold standard treatment. These findings also provide the data supporting comparable anti-inflammatory effect of EC which may reduce corticosteroid use in the treatment of asthma. However, it is important to note the potential adverse effect of EC administration which remains largely unexplored. Further study is recommended to evaluate the long term adverse effects of EC administration. This animal study can provide valuable insights in treatment for asthma, however the findings in this study may not be replicated in human subject, thus clinical trial on human subject is necessary to determine the potential clinical implications of this combination.

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## CONFLICT OF INTEREST

This study is independently funded by the author. The author declares no conflict of interest.

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