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Ethyl p-methoxycinnamate isolated from *Kaempferia galanga L.* rhizome reduces airway remodeling in asthmatic rat models

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ABSTRACT

Background: Asthma causes changes in the airway structure called airway remodeling due to chronic inflammatory reactions. Transforming Growth Factor-β (TGF-β) is one of the inflammatory mediators which has a significant role in the remodeling and inflammatory process. One of the medicinal plants known to have anti-inflammatory potential is *Kaempferia galanga L*. This study aims to analyze the effect of ethyl p-methoxycinnamate isolated from *Kaempferia galanga L*. rhizome on the expression of TGF-β and fibrosis in the bronchial mucosa of asthmatic rat models.

Methods: We conducted an experimental laboratory study using the posttest only control group design. Thirty-six Wistar male white rats were allocated into six groups, NC (negative control), PC (positive control), T1 (treatment group), T2 (treatment group), T3 (treatment group), and T4 (treatment group) receiving 1% ovalbumin in alum (OVA); 1% OVA as well as 1 mg/kg steroid; 1% OVA, 1 mg/kg steroid, and 200 mg/kg ethyl p-methoxycinnamate isolated from *Kaempferia galanga L*. rhizome (KG-EPMC); 1% OVA, 1 mg/kg steroid, and 400 mg/kg KG-EPMC; 1% OVA and 200 mg/kg KG-EPMC; and 1% OVA as well as 400 mg/kg KG-EPMC, respectively. Data were analyzed with SPSS version 22.0 for Windows.

Results: The expressions of TGF-β and fibrosis were significantly lower in positive control as well as treatment groups than those of the negative control group (only receiving OVA) ($p<0.05$). There were no significant differences between positive control and treatment groups regarding TGF-β level and fibrosis ($p>0.005$).

Conclusion: Ethyl p-methoxycinnamate isolated from *Kaempferia galanga L*. rhizome exhibits antiasthma effect by reducing the expression of TGF-β and fibrosis in the bronchial mucosa of an asthmatic rat model. Hence, it can be a potential therapy for asthma.

Keywords: asthma, airway remodeling, TGF-β, Kaempferia galanga L.
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INTRODUCTION

Asthma is a chronic inflammation of the airways involving various inflammatory cells, such as eosinophils, T lymphocytes, macrophages, mast cells, neutrophils, and epithelial cells. These inflammatory cells produce inflammatory mediators, such as cytokines, chemokine, and growth factors which may cause persistent chronic inflammation and trigger bronchoconstriction and structural changes in the airways.[1] Airway remodeling is defined as a constellation of changes in the airway walls, such as its composition, content and cellular and molecular elements. In asthma, structural changes include epithelial damage, mucous cell hyperplasia, collagen and proteoglycans in subepithelial cell membranes, angiogenesis, and increased smooth muscle mass of the airways or airway smooth muscle (ASM). Airway remodeling is the most common cause of decreased lung function symptoms.[2]

Transforming Growth Factor-β is one of the inflammatory mediators known to play a role in the remodeling process.[3] It is also known for its chemotaxis, immune cell regulation, and extracellular matrix protein stimulation abilities.[1] In airway remodeling, TGF-β is responsible for the Epithelial-Mesenchymal Transition (EMT) process, which enhances airway remodeling, supports the differentiation and proliferation of fibroblasts as well as myofibroblasts which are the primary sources of extracellular matrix proteins producing subepithelial fibrosis, increases the proliferation of ASM, and plays a role in microvascular congestion.[4-7]

It is widely known that many medicinal plants have anti-asthma activity and various studies have been conducted on molecules that play a role in the mechanism.[7] One of them is *Kaempferia galanga L*. It has been suggested that this plant has anti-inflammatory potential.[8] However, studies on its anti-asthma activity are still limited. Therefore, this study aims to analyze the effect of ethyl p-methoxycinnamate isolated from

Kaempferia galanga L. rhizome (KG-EPMC) on airway remodeling in the asthmatic rat model.

METHODS

This is an experimental animal model study by using posttest only control group design. The subjects were 36 male Wistar white rats obtained from the Experimental Animal Center for the Inter-University Study Center, Universitas Gadjah Mada, Yogyakarta, Indonesia. Healthy rats aged 2 months weighing 150-250 grams were included in the study. They received standard BR1, ovalbumin free and drinks ad libitum. The rats were randomly allocated into 6 groups, i.e., negative control, positive control, and 4 treatment groups.

All groups were sensitized with 10 mcg of ovalbumin in 1 mg of alum (OVA) (2 ml, intraperitoneal (i.p)) on days 0 and 12. Chronic inflammation was induced by aerosolized 1% OVA administration daily from day 18 to day 25 and 3 times a week started from day 26 to day 34. This experiment was modified from the method of McMillan SJ et al.[9]

The negative control group (NC) received no treatment, and the positive control group (PC) received 1 mg/kg of budesonide. T1, T2, T3, and T4 (treatment groups) received 1 mg/kg budesonide and KG-EPMC 200 mg/kg, budesonide 1 mg/kg and KG-EPMC 400 mg/kg, KG-EPMC 200 mg/kg, and KG-EPMC 400 mg/kg respectively. The treatments were administered 3 times a week before 1% OVA exposure, starting from day 26 to day 34. The KG-EPMC was processed at the Integrated Research and Testing Laboratory, Universitas Gadjah Mada, Yogyakarta, Indonesia.

The rats were terminated on day 35. Their bronchial mucosa was examined with the immunohistochemical method using TGF-β reagent kits (Santa Cruz Biotechnology, Inc) and histopathological method using Trichrome Masson (Zigma) in Anatomical Pathology, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia. The sample was examined with a light microscope (Olympus CX21). Immunohistochemical score readings of TGF-β were conducted based on the following criteria 0: negative, 1: weak, 2: moderate, 3: strong with a positive result was demonstrated by a brownish-colored image.[10] Meanwhile, histopathological score readings of fibrosis were conducted based on the following criteria 0: no fibrosis; 1: 1-25% fibrosis; 2: 26-50% fibrosis; 3: 51-75% fibrosis; and 4: 76-100% fibrosis. The histopathological score was modified from McMillan SJ et al. study.[9]

All data were analyzed with the Kruskal-Wallis and Mann-Whitney tests. We used SPSS 22.0 to analyze the data statistically, with a p-value of 0.05 was considered significant.

RESULTS

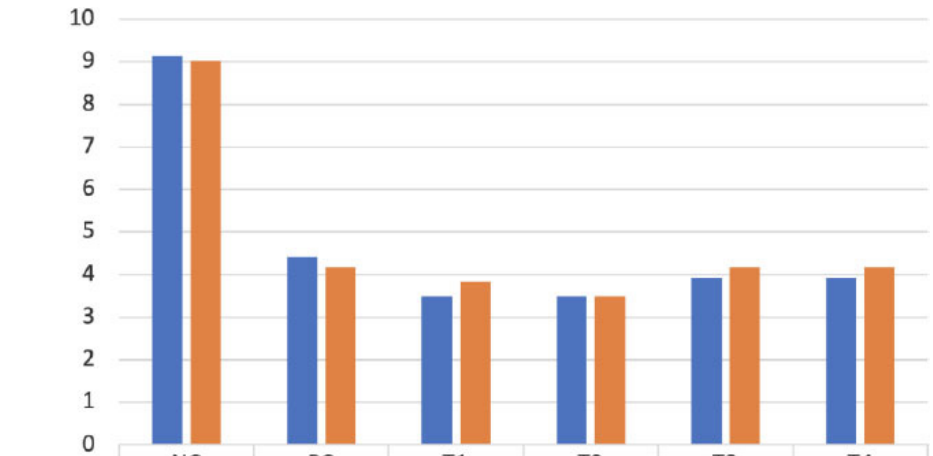
We observed higher expression of TGF-β and fibrosis in group NC, indicating the airway remodeling process after OVA administration. This study showed that the expressions of TGF-β in the groups PC (p=0.013), T1 (p<0.001), T2 (p<0.001), T3 (p=0.003), and T4 (p=0.001) were lower than those of in negative control group (NC). Meanwhile, TGF-β expressions in the positive control (PC) and treatment groups (T1, T2, T3, T4) were comparable. The mean rank of NC, PC, T1, T2, T3, and T4 are 9.15, 4.42, 3.5, 3.5, 3.92, and 3.92, respectively (Figure 1 and 3).

There was a significant decrease in the level of fibrosis in groups PC (p=0.007), T1 (p=0.001), T2 (p<0.001), T3 (p=0.007), and T4 (p=0.003) as compared to that of the negative control group (NC). However, no significant differences were found between positive control (PC) and treatment groups (T1, T2, T3, T4). The mean rank of NC, PC, T1, T2, T3, and T4 are 9.03, 4.17, 3.83, 3.5, 4.17, and 4.17, respectively (Figure 1 and 2).

DISCUSSION

Asthma is a chronic inflammation of the airways involving various inflammatory cells, cytokines, chemokine, and growth factors. This can gradually lead to structural changes in the airway called airway remodeling. Such changes can be characterized by epithelial and cilia damage to the airways, goblet cell hyperplasia, thickening of the lamina reticularis and reticular basal membranes, and increased myofibroblasts and fibrocytes in the airway subepithelial, thickening of the smooth muscles of the airway, and increased vascularization.[10,11]

Conventional therapies used for asthma today include inhaled corticosteroids, β2-agonists, leukotriene receptor antagonists, to monoclonal antibody anti-IL-5.[12] Nevertheless, complementary and alternative therapies as antiasthma were still widely used among asthma patients around the world.[13] A previous study on the content of medicinal plants and their properties for asthma has been conducted.[7] *Kaempferia galanga L.* rhizome is one of the medicinal plants widely studied for its content and benefits for various diseases.[14] The most significant content



	NC	PC	T1	T2	T3	T4
TGF-β	9,15	4,42	3,5	3,5	3,92	3,92
Fibrosis	9,03	4,17	3,83	3,5	4,17	4,17

Figure 1. Expression of TGF-β and fibrosis in all groups of asthma rat models.

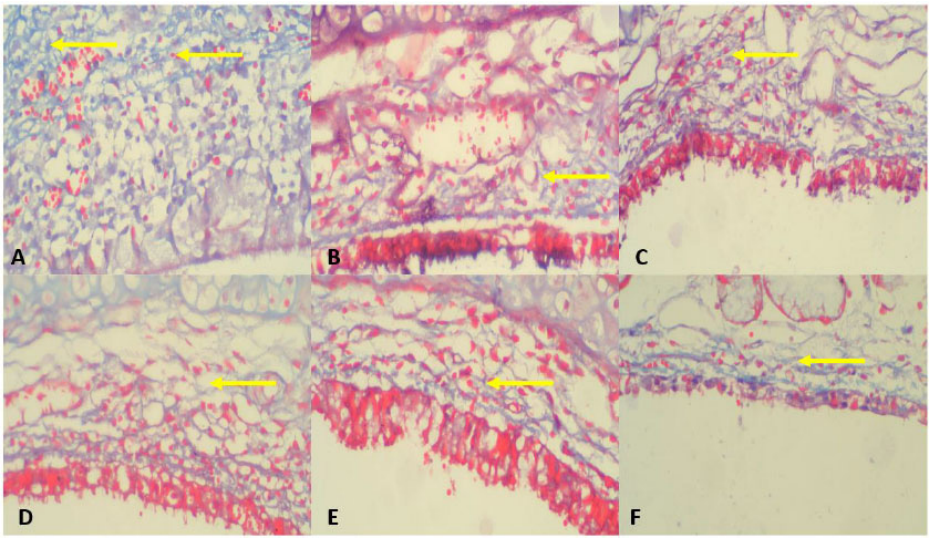


Figure 2. Expression of fibrosis in bronchial mucosa of asthma rat model. A: NC scored 2, B: PC scored 1, C: T1 scored 1, D: T2 scored 1, E: T3 scored 1, F: T4 scored 1. Yellow arrows show fibrotic area (Trichome-Masson preparation, magnification 400x).

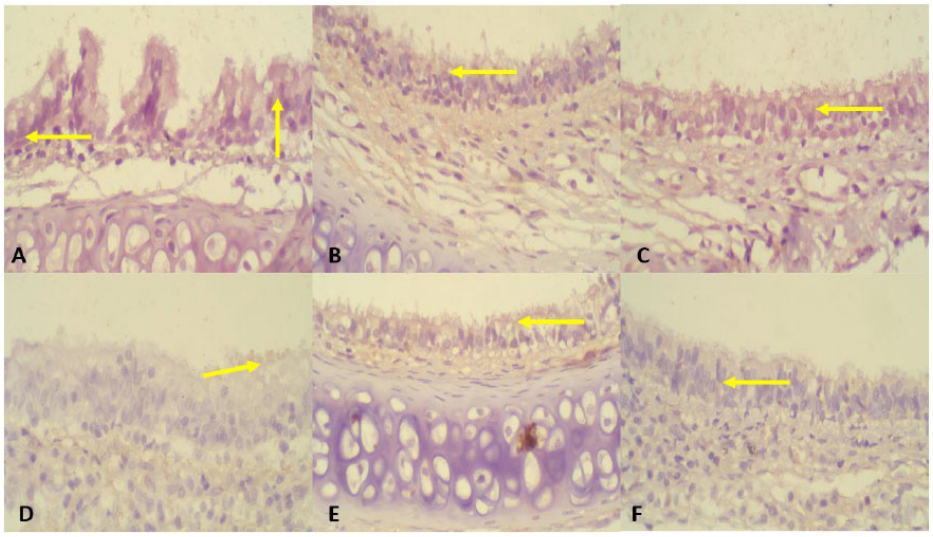


Figure 3. Expression of TGF-β in the bronchial mucosa of rat asthma mouse model. A: NC scored 2, B: PC scored 1, C: T1 scored 1, D: T2 scored 1, E: T3 scored 1, F: T4 scored 1. Yellow arrows show the expression of TGF-β that is stained in immunohistochemical preparation (Immunohistochemical preparation of TGF-β, magnification 400x).

of *Kaempferia galanga L.* rhizome, ethyl p-methoxycinnamate, has been known to have anti-inflammatory potential.[8,15] However, the anti-inflammatory effects and mechanisms of ethyl p-methoxycinnamate from *Kaempferia galanga L.* rhizome in asthma are still unknown.

TGF-β level was significantly higher in the negative control group than those of the positive control group as well as treatment groups. This finding is in line with a study by Ge Y et al. in 2019 that reported an increase in TGF-β expression in the bronchial mucosa of OVA-induced asthma rat models.[16] Previous studies also showed that administration of ovalbumin to induce asthma in mice could lead to autophagy processes.[17,18] Such a process may lead to activation of NF-ƙB, which is one of the transcription factors of TGF-β.[17,18] Administration of budesonide was shown to decrease TGF-β expression in this study significantly. It is supported by a study conducted by Tang X et al., which showed lower TGF-β expression in the budesonide group of OVA-induced asthmatic rats.[19] Corticosteroids as the primary therapy in chronic asthma, inhibit various cytokines, including TGF-β, by inducing gene transcription process, which leads to protein synthesis that plays a role in suppressing the occurrence of inflammation, one of which is an NF-ƙB inhibitor.[12,20]

Fibrosis expression in the negative control group was higher than those of the positive control group as well as treatment groups. It is likely associated with increased TGF-β expression. This finding is supported by Wnuk D et al., which demonstrated an increase in TGF-β in asthmatic patients that leads to fibrosis, one of which is through the activation of pro-fibrotic pathway TGF-β Smad 2/3.[5] This activation causes the transition of fibroblasts into myofibroblasts, the primary source of extracellular matrices which has a significant role in the formation of subepithelial fibrosis.[5] Another study by Jaffer et al. found that increased ROS in the murine asthma model activated TGF-β, stimulating collagen production by fibroblasts, resulting in fibrosis in the airways.[21] Budesonide given in this study reduced fibrosis significantly. This is in line with a study conducted by Qian J et al., which demonstrated budesonide inhibits the TGF-β/Smad signaling pathway in OVA-induced asthmatic mice, resulting in decreased fibrosis.[22]

Animal models receiving KG-EPMC in this study had lower expression of TGF-β and fibrosis. This indicates the anti-asthmatic effect of KG-EPMC. The expression of TGF-β and fibrosis in the positive control and treatment groups were comparable in this study. It is possible that steroids and KG-EPMC may share a similar mechanism of action.

CONCLUSION

Hexane fraction of Kaempferia galanga L. rhizome (ethyl p-methoxycinnamate) can significantly decrease the expression of TGF-β and fibrosis in airway remodeling of an asthmatic rat model. Hence,

Kaempferia galanga L. rhizome could be a potential therapy for asthma. This study was still limited to an animal model. A further study in humans should be conducted to evaluate the effect of the fraction of *Kaempferia galanga L.* rhizome in airway remodeling.

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ETHICS CONSIDERATION

This study has been approved by the Ethics Committee of Dr. Moewardi Hospital (letter of ethical clearance no. 930/VII/HREC/2019).

CONFLICT OF INTEREST

There is no conflict of interest in conducting and reporting the study.

FUNDING DISCLOSURE

The authors self-funded the study.

AUTHORS CONTRIBUTION

The authors have equal contribution in conducting and reporting the study from the conceptual framework, data acquisition, data analysis until reporting the study results through publication.

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