

Effects of *Alpinia galanga* and prednisone on biomarkers and fetal development in asthmatic pregnancy: A rat model study

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Received 11 March 2025 ♦ Accepted 5 May 2025 ♦ Published 5 June 2025

Citation: Muflihah IS, Soetrisno, Reviono, Prayitno A, Wasita B, Pamungkasari EP, Dirgahayu P (2025) Effects of *Alpinia galanga* and prednisone on biomarkers and fetal development in asthmatic pregnancy: A rat model study. Pharmacia 72: 1–10. <https://doi.org/10.3897/pharmacia.72.e152691>

Abstract

Background: The combination of asthma and pregnancy worsens placental function and fetal growth, with severe asthma increasing the risk of maternal and fetal hypoxia. Maternal hypoxia disrupts placental blood flow, reducing oxygen and nutrient transport to the fetus and affecting birth weight. Current asthma treatments remain costly and may cause side effects, leading pregnant women to seek alternatives. This study evaluated the effects of *Alpinia galanga* extract (EAG) and prednisone in pregnant rats with ovalbumin-induced asthma.

Methods: Thirty-five pregnant asthmatic Wistar rats were divided into five groups and treated for 19 days.

Results: The combination of *Alpinia galanga* extract and prednisone significantly reduced IL-5 levels by 54% ($p < 0.001$) and MDA levels by 70% ($p < 0.001$), while PlGF levels increased by 36% ($p < 0.001$), and microvessel density improved by 123% ($p < 0.001$), with fetal weight 4.3 times higher ($p < 0.001$) in the combination group compared to the untreated asthmatic group, indicating enhanced placental function and fetal growth.

Conclusion: These results suggest that EAG shows potential as a complementary therapy based on preclinical outcomes. These findings may inform future preclinical investigations on adjunct therapies for asthma during pregnancy.

Keywords

Alpinia galanga, asthma, fetal growth, Interleukin-5 (IL-5), malondialdehyde (MDA), microvessel density, placental growth factor (PlGF), pregnancy

Introduction

Asthma is one of the complications that worsens pregnancy because pregnancy asthma affects placental function and fetal growth (Jones et al. 2020). Asthma in pregnancy increases the risk of premature birth, low birth weight (LBW), perinatal death, placenta previa, and preeclampsia (Kwah and Stevens 2019; Wang et al. 2020). Robijn et al. (2022) reported that pregnant individuals with asthma had a 54% risk of having LBW infants, a 24% increased risk of small-for-gestational-age (SGA) fetuses, and an increased risk of pre-eclampsia by 22% (Robijn et al. 2022). These outcomes are largely attributed to maternal and fetal hypoxia (Sly 2019; Gao et al. 2021), which disrupts placental blood flow and impairs oxygen and nutrient transport (Grieger et al. 2013; To et al. 2018), consequently affecting fetal growth and development, even fatally (Radzikowska and Golebski 2023). Studies of asthma progression during pregnancy (Rey et al. 2019) indicate that one in three women experience worsening asthma control during pregnancy, one in three experience improvement, and the remaining third show no change. (Dewyea and Nelson 2005; Rey et al. 2019; Popa et al. 2021)

Asthma is characterized by airway inflammation and oxidative stress, with ovalbumin being one of the primary allergens known to contribute to the generation of free radicals in the body (Chouni et al. 2018). When someone inhales an allergen, the airway epithelium is induced to issue an alarm, changing the allergen so that it triggers the release of inflammatory mediators into the blood, causing degranulation to release proinflammatory cytokines such as interleukin (IL)-5 (Pavón-Romero et al. 2021), which results in anatomical changes so that clinical manifestations of asthma arise that enter the body until they reach the placenta (Goldstein et al. 2020).

In gestational asthma, placental abnormalities have been observed, including a reduction in both the volume and length of placental capillaries. There is strong evidence that oxidative stress, resulting from an imbalance between the reducing and oxidizing systems, plays a critical role in the pathogenesis of gestational asthma. These vascular impairments stem from a diminished capacity of blood vessels to undergo dilation and constriction, which consequently affects fetal cardiovascular development. This imbalance is characterized by excessive Reactive Oxygen Species (ROS) production and an antioxidant defense system marked by elevated malondialdehyde (MDA) levels (Raviraja Shetty and Monisha 2015; Eram et al. 2019). Concurrently, cytokine dysregulation, marked by decreased placental growth factor (PlGF) and increased levels of anti-angiogenic factors sFlt-1 (soluble fms-like tyrosine kinase 1), disrupts vascular remodeling. Specifically, inadequate trophoblast invasion of the decidual spiral arteries leads to a reduction in the intraluminal diameter, along with thickened and inelastic arterial walls. These changes compromise placental function, impairing its role in oxygen and nutrient transport to the fetus (Bobic et al. 2012; Chaiyana et al. 2022).

Previous studies have demonstrated the anti-inflammatory and antioxidant properties of *Alpinia galanga* in non-pregnant models. However, its potential effects on placental function and fetal development, particularly in the context of asthma, remain largely unexplored. *A. galanga* contains various bioactive compounds, including essential oils, flavonoids, phenolics, saponins, and terpenoids, with major active components such as 1'-Acetoxychavicol Acetate (ACA), kaempferol, and cineole. These phytochemicals have been shown to exhibit strong antioxidant activity, primarily attributed to their redox properties, which enable them to scavenge free radicals, donate hydrogen atoms, and neutralize reactive oxygen species (ROS). Flavonoids, in particular, contribute to the suppression of inflammatory mediators by inhibiting Th2 cell activation and reducing eosinophilic infiltration, which are key factors in asthma pathogenesis. In ovalbumin-sensitized murine models of asthma, phenolic compounds from *A. galanga* have demonstrated the ability to decrease IgE levels, suppress airway remodeling, prevent goblet cell hyperplasia, and downregulate Th2-mediated inflammatory cytokines (Khairullah et al. 2020; Kojima-Yuasa and Matsui-Yuasa 2020).

Previous studies have demonstrated the anti-inflammatory and antioxidant properties of *Alpinia galanga* in non-pregnant models, but its effects on placental function and fetal development in the context of asthma remain unexplored. Prednisone, a commonly prescribed corticosteroid for asthma control, functions by suppressing the immune response and reducing inflammation. However, long-term corticosteroid exposure can have adverse effects (Ryu et al. 2018). Previous studies have suggested that while prednisone remains an essential component of severe asthma management, its adverse effects underscore the need for alternative or complementary therapeutic approaches. Given the limitations of corticosteroid therapy, the antioxidant and anti-inflammatory properties of *A. galanga* present a promising natural adjunct to prednisone, potentially mitigating oxidative stress and inflammation in gestational asthma while minimizing steroid-associated risks (Khairullah et al. 2020).

Asthma treatment is often expensive and associated with significant side effects, leading many pregnant women to avoid chemical drugs due to concerns about potential risks to both mother and fetus. Therefore, there is an urgent need to identify affordable, accessible, and safe therapies that can complement standard asthma treatments. Natural compounds, such as galangal (*Alpinia galanga* Linn.), offer a promising alternative due to their abundance, ease of cultivation, and potential therapeutic benefits for improving placental function and fetal weight (Seo et al. 2013; Ahlina et al. 2020). To date, no studies have examined the synergistic effects of *A. galanga* and prednisone on placental angiogenesis and fetal weight in asthma-complicated pregnancy. This study aims to investigate the effects of *Alpinia galanga* extract (EAG) and prednisone on biomarker levels (IL-5, MDA, PlGF), microvessel density, and fetal weight in pregnant rats with asthma models.

Materials and methods

Materials

Galangal rhizome (*A. galanga*) was obtained from UPT Yankestrad Tawangmangu Dr. Sardjito General Hospital/Center for Research and Development of Medicinal Plants and Traditional Medicines Kalisoro, Tawangmangu, Karanganyar Regency, Central Java, and extracted using the maceration method at the Integrated Research and Testing Laboratory Unit 2, Gadjah Mada University, Yogyakarta. Maceration was carried out at a temperature of 50 °C for 12 hours. Ovalbumin, a protein derived from egg whites, was used as an asthma allergen in experimental animals. The ovalbumin used in this study was obtained from chicken egg albumin (Grade V) with a purity of > 98%. Ovalbumin and Al(OH)₃ were obtained from SIGMA (St. Louis, USA). Intraperitoneal induction of ovalbumin (75 mcg) combined with aluminum hydroxide (7.5mg) was administered on days 0 and 14, followed by a second exposure to 1% ovalbumin (OVA) via inhalation for 30 minutes per session over a period of four weeks. Ketamine-Xylazine (Troy Laboratories Australia and Interchemie Holland). Phosphate buffered saline (PBS). IL-5 ELISA Kit (Zhejiang, China, Catalog No. EO134Ra Rats Interleukin-5 ELISA Kit). PIGF ELISA Kits were obtained from Zhejiang, China, Catalog No. E0579Ra, Rats Placenta Growth Factor. MDA Elisa Kit from Zhejiang, China, Catalog No. E0156Ra Rats, malondialdehyde, and CD34 Polyclonal Antibody No. catalog E-AB-60105.

Animal experimental study design

All experimental procedures were approved and conducted under the supervision of the Faculty of Medicine of Universitas Sebelas Maret Ethics Committee Team (30/UN27.06.11/KEP/EC/2024) and followed their guidelines. Female Wistar rats (*Rattus norvegicus*), aged 6–8 weeks with a body weight of 170–200 grams, were obtained from the animal center of the Integrated Research and Testing Laboratory Unit 4, Gadjah Mada University, Yogyakarta. All rats were housed in a controlled environment with regulated light and humidity with unlimited access to

food and drink. They were acclimatized for one week before the experiment. The sample size was determined using the Steel and Torrie Formula (1997), resulting in seven female Wistar rats per group (Steel 1997). Animals were randomly grouped, and researchers assessing outcomes were blinded to treatments to reduce bias. There were five groups with a total of 35 female Wistar rats. The rats were divided into 5 groups, each group consisting of 7 female Wistar rats (n = 7), including:

- Group 1: Normal – Healthy pregnant control rats without asthma (terminated on day 20),
- Group 2: Negative Control (KN) – Pregnant asthma model rats that did not receive any treatment (terminated on day 20),
- Group 3: Positive Control (KP) – Pregnant asthma model rats treated with prednisone (0.4 mg/kg BW) for 19 days, from the first day of pregnancy until day 19 (terminated on day 20),
- Group 4: Treatment Group 1 (KP1) – Pregnant asthma model rats treated with *Alpinia galanga* rhizome extract (800 mg/kg BW) for 19 days, from the first day of pregnancy until day 19 (terminated on day 20),
- Group 5: Treatment Group 2 (KP2) – Pregnant asthma model rats treated with a combination of prednisone (0.4 mg/kg BW) and *Alpinia galanga* rhizome extract (800 mg/kg BW) for 19 days, from the first day of pregnancy until day 19 (terminated on day 20).

Rats were euthanized using ketamine (100 mg/kgBW) and xylazine (15 mg/kgBW) anesthesia, after which blood and tissue specimens were collected.

Blood sampling and serum isolation

Blood samples were collected from the orbital vein and left at room temperature for 30 minutes before centrifugation at 1000 g for 15 minutes. The serum was then isolated and stored for further analysis. Serum was separated and stored at -80 °C for other cytokine examinations. IL-5 and MDA were measured in serum using an enzyme-linked immunosorbent assay (ELISA) trophometric-based kit. All plates were analyzed on an automatic plate reader (Zhejiang, China).

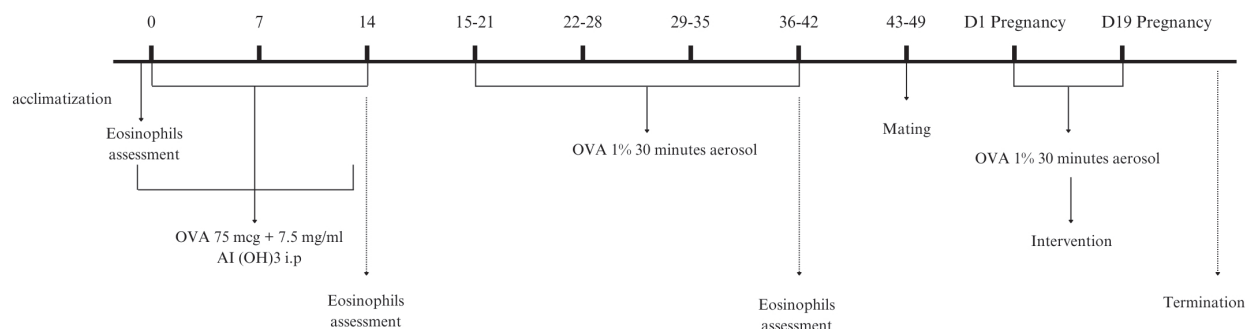


Figure 1. Study protocol with subjects underwent OVA sensitization, aerosol challenges, and eosinophil assessments before mating. Pregnant subjects received OVA aerosol and intervention.

Placental tissue ELISA

Placental tissue was rinsed with PBS with pH 7.4 to remove red blood cells/sediment/clots, tissue homogenization in cold buffer with a concentration of 20 mM, pH 7.2 containing 1 mM EGTA, 210 nM mannitol, and 70 mM sucrose, centrifuged for 20 minutes with $1,000 \times g$ at a temperature of 2–8 °C, and supernatant was taken for testing. PLGF levels were measured from placental homogenate using ELISA.

Histopathological analysis

Placental tissue was dissected, washed with NaCl, and immersed in 10% formaldehyde solution. Placental specimens were sectioned and stained with CD34 polyclonal antibody, and selected images were taken at 200 $\times$.

Statistical analysis

Data were analyzed using SPSS 29.0 (Windows vers.). Analysis of variance (ANOVA) was used for IL-5, MDA, PLGF, MVD, and fetal weight, with Tukey post-hoc where applicable. A p-value of <0.05 was considered statistically significant. If a significant difference was obtained, continue with the Tukey. Histopathology of placental tissue with CD34 polyclonal antibodies between the normal group and the treatment group to obtain a picture of angiogenesis placenta (microvessel density) is included. The analysis was performed using GraphPad Prism 10 Version Update 10.4.0 Windows 10 (GraphPad Software, San Diego, CA, USA).

Results

A. galanga rhizome extract test

The *A. galanga* rhizome used in this study was obtained from the UPT Yankestrad Sardjito General Hospital, Tawangmangu. The plant was identified as *Alpinia galanga* Linn. (Khairullah et al. 2020) and tested at the Integrated Research and Testing Laboratory Unit 2, Gadjah Mada University, Yogyakarta. Ethanol extraction was carried out using 96% ethanol solvent through a maceration process.

The 1,1-Diphenyl-2-picrylhydrazyl (DPPH) test indicated a free radical scavenging activity with an IC₅₀

of 2.124 mg/mL. The past study from Cahyono (2020) demonstrated that antioxidant activity testing using UV-Vis revealed that *Alpinia galanga* extract IC₅₀ of 2.980 mg/mL demonstrated stronger antioxidant activity than quercetin IC₅₀ value of 1,622.14 mg/mL (Cahyono 2020), while *Alpinia galanga* extract in the present study exhibited an IC₅₀ of 2.124 mg/mL. The lower IC₅₀ value indicates superior free radical scavenging capacity, suggesting *Alpinia galanga* may be a more potent antioxidant source compared to the quercetin compound.

In 100 grams of *A. galanga* rhizome extract were found to contain the following nutrients: vitamin A (5000 mcg), thiamine (0.08 gr), riboflavin (0.06 mg), niacin (0.3 mg), vitamin C (50 mg), calcium (50 mg), phosphorus (50 mg), iron (2 mg), sodium (24 mg), potassium (137 mg), and zinc (0.3 mg). Gas chromatography-mass spectrometry (GCMS) analysis identified the presence of (S)-4-(1-acetoxyallyl)phenyl acetate, phenol, 2-ethoxy-4-(2-propenyl)-, and (E)-3-(4-acetoxyphenyl)allyl acetate in the extract. Furthermore, liquid chromatography-mass spectrometry (LCMS) analysis confirmed the presence of 1'S-1'-acetoxychavicol as a compound in the rhizome extract of *A. galanga* (EAG).

Characteristics of research animals

Characteristics of experimental animals

This study conducted an examination on the gestational day of pregnancy by measuring body weight, age, eosinophil D-0, eosinophil D-14, and eosinophil D-40 in pregnant rats, as shown in Table 1. The age and body weight of the pregnant rats did not differ significantly, as indicated by a p-value > 0.05, suggesting that these parameters were comparable across all groups. In contrast, eosinophil counts showed a statistically significant difference with a p-value < 0.05. This finding indicates that on day 0, prior to ovalbumin (OVA) induction, all rats were in a normal and healthy condition. However, by day 14 following OVA exposure, eosinophil levels had increased, and by day 40, they had risen further. This progressive increase in eosinophil count confirms that all groups transitioned from a healthy state to an asthmatic condition following OVA induction. In other words, it was ensured that all groups had developed asthma before receiving prednisone and *Alpinia galanga* extract treatment.

Table 1. Characteristics of experimental animals. Units are expressed in grams (g) or days; p-values are from one-way ANOVA.

Variable	Normal (n = 7)	KN (n = 7)	KP (n = 7)	KP1 (n = 7)	KP2 (n = 7)	p-value
			mean $\pm$ SD			
Weight (g)	190 $\pm$ 4.42	189 $\pm$ 6.93	188 $\pm$ 6.93	191 $\pm$ 4.80	193 $\pm$ 3.79	0.535
Age (days)	55.71 $\pm$ 1.25	55.43 $\pm$ 0.97	55.29 $\pm$ 1.25	55.43 $\pm$ 0.97	55.29 $\pm$ 1.25	0.740
Eosinophil D-0 (10 ³ /L)	0.57 $\pm$ 0.53	0.42 $\pm$ 0.78	0.42 $\pm$ 1.13	0.71 $\pm$ 1.25	0.57 $\pm$ 1.13	0.001*
Eosinophil D-14 (10 ³ /L)	0.71 $\pm$ 0.48	37.71 $\pm$ 0.11	32.28 $\pm$ 8.36	29.28 $\pm$ 4.92	30.00 $\pm$ 4.61	0.001*
Eosinophil D-40 (10 ³ /L)	0.71 $\pm$ 0.48	139.42 $\pm$ 56.10	145.28 $\pm$ 59.29	143.28 $\pm$ 58.58	138.14 $\pm$ 56.87	0.001*

Data are presented as mean $\pm$ SD (n = 7); *p < 0.05.

Abbreviations: Normal = pregnant control rats, KN = pregnant with asthma, KP = prednisone treatment, KP1 = EAG treatment, KP2 = prednisone + EAG treatment

Effects of prednisone and *A. galanga* extract on inflammation

The effects of prednisone and EAG on inflammation, oxidative stress, and placental function in asthma during pregnancy were evaluated by measuring IL-5, MDA, and PlGF levels (Fig. 3). IL-5 was reduced by 54% in the KP2 group compared to KN ($p < 0.0001$), indicating anti-inflammatory efficacy. Similarly, oxidative stress levels, assessed through MDA, showed that the combination therapy group (Prednisone + EAG) had a significant reduction of 70% ($p < 0.000$), approaching the baseline level of the healthy control group (0.80 ± 0.68), suggesting enhanced antioxidant effects. Additionally, placental function, as indicated by PlGF levels, was improved by 36% in the KP2 group (62.00 ± 6.34) compared to the KN group ($p < 0.000$), demonstrating its role in restoring angiogenic balance. These results suggest that the combination of prednisone and *A. galanga* extract exerts a synergistic effect in mitigating inflammation and oxidative stress while improving placental function in asthma during pregnancy.

Angiogenesis placenta (microvessel density/MVD)

MVD is a potential indicator of angiogenesis activity that plays an important role in the supply of oxygen and nutrients because it determines the formation of new blood

vessels. The growth of new blood vessel tissue is very important in proliferation. Several variations in MVD were identified as shown in Fig. 2. The results showed that in the healthy control group, the average MVD was 56.39 ± 18.56 . In contrast, the KN group showed a much lower MVD, with an average of 31.53 ± 10.15 . The group that received combination therapy of prednisone and EAG (KP2) showed a 123% increase in MVD compared to the KN group ($p < 0.0001$), with an average MVD of 70.39 ± 9.77 , indicating enhanced placental angiogenesis.

Fetal weight test

Fetal birth weight is a critical indicator of placental function and fetal development. Significant variations were observed among the experiment groups. The positive control group (KN) showed much lower fetal growth with a mean of 1.61 ± 0.25 . The group of pregnant asthmatics receiving prednisone therapy (KP) had an average BW of 5.67 ± 0.26 , and the group treated with *A. galanga* extract (KP1) had an average of 5.61 ± 0.35 . Notably, the group receiving combination therapy of prednisone and *A. galanga* extract (KP2) had a significantly ($p < 0.000$) increased fetal weight that was approximately 4.3 times higher in the KP2 group than in the KN group (6.89 ± 0.43). The results showed the potential for combination intervention in improving placental dysfunction and angiogenic balance in asthmatic pregnancy was effective and meaningful compared to other groups.

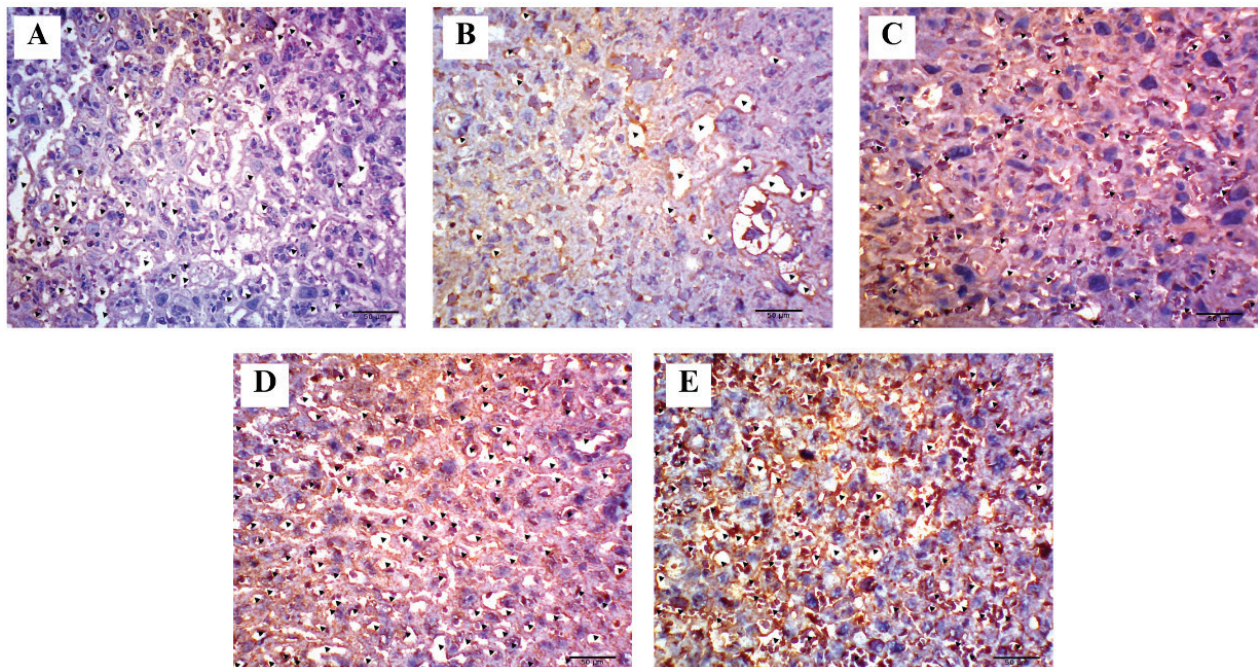


Figure 2. Immunohistochemistry (IHC) staining using antibody CD34 (vascular marker) revealed brown-stained areas at the edges, indicating newly formed microvessels, while non-brown areas represented old endothelial tissue. Arrows highlight the appearance of new microvessel growth in the placental tissue. The highest microvessel density was observed in the KP2 group (E) (prednisone + EAG), while the lowest density was found in the positive control group KN (B). A. Pregnant control rats; B. pregnant with asthma; C. Prednisone treatment; D. EAG treatment; E. prednisone + EAG treatment. Abbreviations: Ovalbumin (OVA). Scale bars: 50 μ m (A–E).

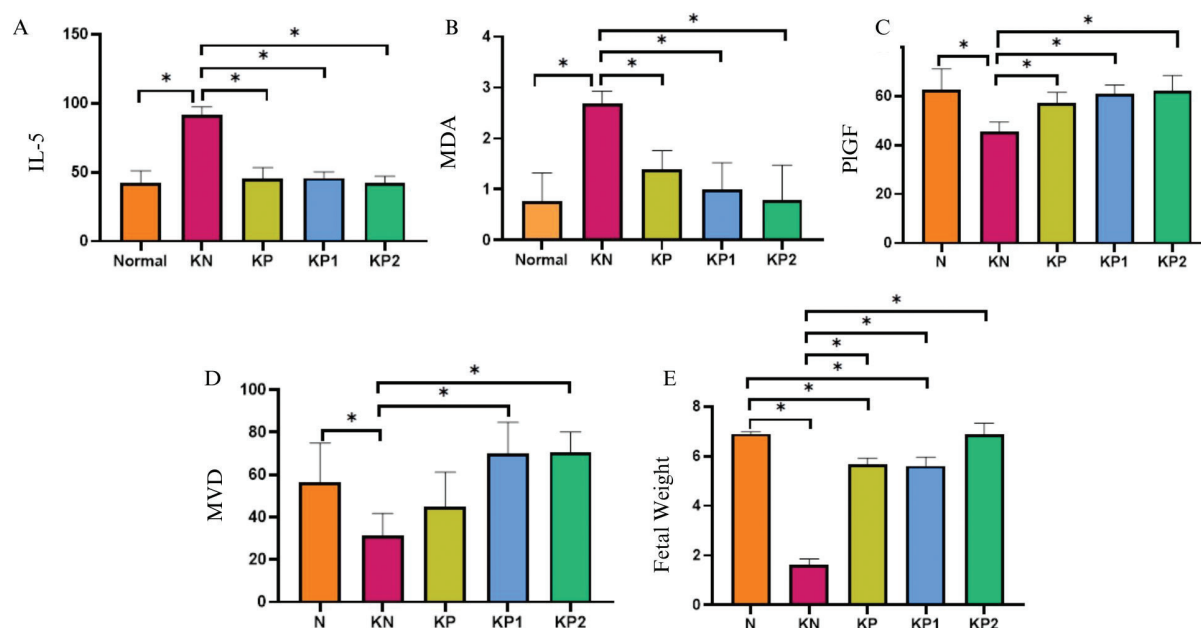


Figure 3. Combination of *A. galanga* extract and prednisone significantly reduced IL-5 (A) and MDA (B) levels while increasing PIGF (C), placental angiogenesis (MVD) (D), and fetal weight (E) compared to the control group.

Histopathological placenta

OVA sensitization and challenge induced an asthma model in pregnant rats. Treatment with *Alpinia galanga* extract and prednisone, alone or in combination, was administered to evaluate its effects on asthma-related inflammation and fetal outcomes. Negative controls were included for CD34 staining, and microvessel density (MVD) was quantified in five high-power fields using ImageJ. Slides were interpreted by a blinded pathologist to prevent observational bias. Histological images were captured with scale bars and a total magnification of 200× (20× objective lens × 10× ocular lens).

The histopathological analysis showed that in healthy rats the placental picture is close to normal, with well-organized trophoblasts and clearly defined placental villi (Fig. 4); no signs of tissue degeneration or disorganization were observed. In contrast, the asthmatic pregnant rat group exhibited structural disorganization, poorly structured placental villi, and signs of cellular degeneration. The KP group (asthmatic rats treated with prednisone) showed a more organized placental structure, indicating a protective effect of prednisone; however, the placental villi remained suboptimal. In the KP1 group (asthmatic rats treated with EAG), the placental tissue appeared more organized, with evidence of villous regeneration due to the protective effects of *A. galanga*. In the KP2 group (asthmatic rats treated with a combination of prednisone and EAG), the placental structure closely resembled normal conditions, with well-organized placental villi and no signs of degeneration or disorganization. As illustrated in Fig. 4, the combination of *A. galanga* extract and prednisone provided the most effective protection for placental structure, supporting optimal angiogenesis and significantly reducing inflammation.

Discussion

To our knowledge, this is the first study demonstrating that combination therapy (prednisone + EAG) significantly improved angiogenic markers and fetal weight compared to monotherapies in pregnant rats with an asthma model.

Elevated IL-5 levels play a crucial role in asthma pathogenesis, contributing to airway inflammation, obstruction, and hyperresponsiveness. These effects are particularly relevant in pregnancy and impact fetal development. Furthermore, IL-5 as a proinflammatory mediator produced by Th2 cells causes anatomical changes resulting in clinical manifestations of asthma that enter the body until they reach the placenta (Goldstein et al. 2020). The reduction in IL-5 levels was associated with the anti-allergic properties of *A. galanga* rhizome extract, which has been shown to inhibit beta-hexosaminidase release, a marker of IgE antigen-mediated degranulation. The anti-inflammatory properties of the ethanol extract of *A. galanga* rhizome were evaluated in a mouse model of asthma, inhibiting the expression of Th1 and Th2 cytokines. Seo et al. (2013) reported that the active compound 1'-acetoxychavicol acetate (ACA) in *A. galanga* rhizome extract showed comparable anti-inflammatory activity to corticosteroids in previous models. This aligns with corticosteroids in suppressing cytotoxic T cells. ACA reduced eosinophil and IgE infiltration in the lungs of mice and mitigated histopathological changes, including airway remodeling, goblet cell hyperplasia, and glycoprotein secretion (Seo et al. 2013; Kusmardi et al. 2021). Because asthma is mediated by various inflammatory pathways, *A. galanga* rhizome extract is a promising candidate for asthma drugs. The beneficial effects of corticosteroids in asthma are related to their ability to reduce the activation of the airway

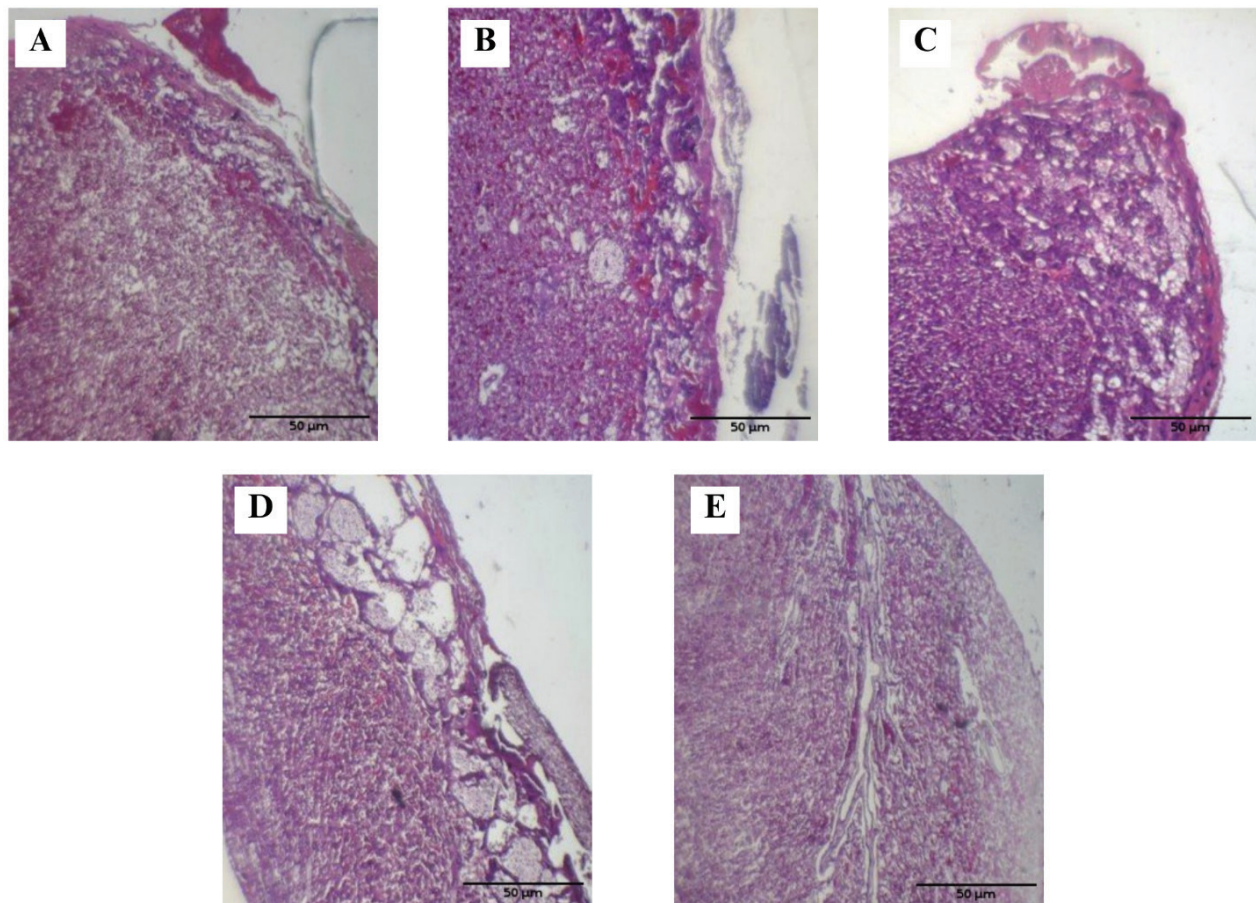


Figure 4. The histopathological analysis revealed that combination group (E) was more effective in reducing inflammations. These findings suggest that the combination of prednisone and EAG provided the most effective protection, restoring placental structure to improved villous architecture resembling healthy controls with well-organized villi, reduced inflammation, and optimal angiogenesis, compared to other treatment groups. A. Pregnant control rats; B. Pregnant with asthma; C. Prednisone treatment; D. EAG treatment; E. Prednisone + EAG treatment. Scale bars: 50 µm (A–E).

epithelium by inflammatory cells and cytokines. Previous studies have explained that the administration of prednisone at a maintenance dose of 5 mg orally during 4–6 years of treatment in severe asthma leads to the conclusion that corticosteroids improve airway damage due to asthma (Seo et al. 2013). The observed decrease in MDA levels supports the oxidative stress theory in asthma pathophysiology. Reduced MDA suggests lowered lipid peroxidation, indicating that EAG attenuates oxidative stress-mediated placental dysfunction. EAG's flavonoids (ACA) may mitigate oxidative damage, thereby improving placental nutrient transport and contributing to improved placental health and fetal development (Goldstein et al. 2020; Haitamy et al. 2024).

Allergen exposure during pregnancy disrupts oxygen and nutrient delivery to the placenta, potentially leading to fetal hypoxia and growth restriction (To et al. 2018). Abnormalities in the reoxygenation process underlie low oxygen intake to the placenta. As pregnancy progresses, there is a decrease in placental perfusion followed by reperfusion. The placenta expresses increased oxidative stress, increasing the risk of cell damage (Fazel et al. 2021). Investigations on the placenta from pregnancies complicated by asthma identified reduced central capillary placental volume, cap-

illary length, and decreased capacity of blood vessels to dilate and narrow due to asthma (Fazel et al. 2021). Together, these data indicate that the development of the fetal cardiovascular system is affected, resulting in placental dysfunction from the mother to the fetus. Antioxidant activity is shown by *A. galanga* extract. The immunomodulatory efficacy of *A. galanga* rhizome is related to the phenolic antioxidants of *A. galanga*, especially ACA, which increase nitric oxide and endothelial function with a working mechanism of maintaining nitric oxide signals that are disrupted by allergen exposure and weakening vascular damage caused by oxidative stress (Fitriana et al. 2024).

In addition, biomarker analysis provides insight into the molecular mechanism of the potential combination therapy of prednisone and EAG, showing the occurrence of a balance of angiogenic factors with markers of increased PlGF levels in pregnancy asthma due to the mechanism of action of EAG from the potential of flavonoids that suppress the failure of the vascular remodeling process so that there is no narrowing of the intraluminal diameter of the decidual spiral artery and maintains a thin and elastic wall so that the function of the placenta as a pathway for transporting oxygen and nutrients to the fetus is good.

Microvessel density (MVD) assessment is a quantitative calculation of blood vessels that reflects the angiogenesis process (Mayhew 2009). As evidence of the potential for combination therapy of prednisone and EAG in this study, placental angiogenesis can be seen from the microvessel density (MVD). The increase in angiogenesis in the placental mucosal endothelium in pregnancy asthma indicates growth and development as well as healing of wounds due to asthma. This shows that combination intervention has the potential to increase angiogenesis in pregnancy asthma.

Allergen exposure in pregnancy asthma causes placental damage (Grieger et al. 2013). Often associated with low birth weight because it interferes with the binding of blood and oxygen that is circulated throughout the body, one of which is in the placenta so that the fetus experiences a lack of oxygen and nutrient intake (To et al. 2018). Lack of oxygen can cause low birth weight, premature birth, and even death. The mechanism of action of *A. galanga* rhizome extract balances Th1 and Th2 activity and minimizes the production of inflammatory mediators. In this study, the combination of prednisone and *A. galanga* rhizome extract demonstrated a significant therapeutic effect in preventing fetal growth retardation, as evidenced by increased fetal birth weight approaching the normal range. This study has some limitations; the present study used a small animal model with short-term outcomes. Further research should explore long-term neonatal effects and dose-response relationships.

Conclusion

This study highlights the potential of *A. galanga* extract as a complementary therapy in pregnancy asthma, either alone or in combination with prednisone. The results of this study indicate that the combination therapy of prednisone and EAG has a synergistic effect. EAG demonstrated potential as a lower-risk adjunct pending further safety studies. In the development of new treatment strategies in humans, future studies should assess safety and efficacy in non-rodent preclinical models prior to human trials. These findings warrant deeper mechanistic exploration and validation in larger-scale studies.

Acknowledgments

The author would like to thank the supervisors of this research from the Medical Science Study Program, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia, and Universitas Muhammadiyah Purwokerto, Banyumas, Central Java, Indonesia. The authors also extend their gratitude to the Indonesian Education Scholarship Program (BPI), the Center for Higher Education Funding and Assessment (PPAPT), the Center for Higher Education Funding and Assessment Ministry of Higher Education, Science, and Technology of the Republic of Indonesia, and the Indonesia Endowment Fund for Education Agency (LPDP).

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

The authors declared that no clinical trials were used in the present study.

The authors declared that no experiments on humans or human tissues were performed for the present study.

The authors declared that no informed consent was obtained from the humans, donors or donors' representatives participating in the study.

Experiments on animals: This research was approved by the Research Ethics Committee of Universitas Sebelas Maret, Surakarta, Indonesia (30/UN27.06.11/KEP/EC/2024). All treatment of animals was carried out according to the ethical guidelines and regulations for the care and use of laboratory animals.

The authors declared that no commercially available immortalised human and animal cell lines were used in the present study.

Funding

This research was funded by the Indonesian Education Scholarship Program (BPI), the Center for Higher Education Funding and Assessment (PPAPT), the Center for Higher Education Funding and Assessment Ministry of Higher Education, Science, and Technology of the Republic of Indonesia, and the Indonesia Endowment Fund for Education Agency (LPDP).

Author contributions

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Data availability

All of the data that support the findings of this study are available in the main text.

References

- Ahlina FN, Nugraheni N, Salsabila IA, Haryanti S, Da'i M, Meiyanto E (2020) Revealing the reversal effect of galangal (*Alpinia galanga* L.) extract against oxidative stress in metastatic breast cancer cells and normal fibroblast cells intended as a co- chemotherapeutic and anti-ageing agent. *Asian Pacific Journal of Cancer Prevention* 21: 107–117. <https://doi.org/10.31557/APJCP.2020.21.1.107>
- Bobic S, Seys S, De Vooght V, Callebaut I, Hox V, Doms C, Vinckier S, Jonckx B, Saint-Remy J-M, Stassen J-M, Bullens DMA, Ceuppens JL, Carmeliet P, Hellings PW (2012) Placental growth factor contributes to bronchial neutrophilic inflammation and edema in allergic asthma. *American Journal of Respiratory Cell and Molecular Biology* 46: 781–789. <https://doi.org/10.1165/rcmb.2011-0152OC>
- Cahyono B (2020) Penentuan aktivitas antioksidan senyawa kuersetin dan ekstrak lengkuas menggunakan HPLC dan UV-Vis . *Alchemy: Journal Of Chemistry* 8: 24–32. <https://doi.org/10.18860/al.v8i2.10594>
- Chaiyana W, Sriyab S, Okonogi S (2022) Enhancement of cholinesterase inhibition of *Alpinia galanga* (L.) Willd. essential oil by microemulsions. *Molecules* 27: 3275. <https://doi.org/10.3390/molecules27103275>
- Chouni A, Paul S, Paul S, Paul S (2018) A review on phytochemical and pharmacological potential of *Alpinia galanga*. *Pharmacognosy Journal* 10: 9–15. <https://doi.org/10.5530/pj.2018.1.2>
- Dewyea VA, Nelson MR (2005) Asthma in pregnancy. *Allergy and Asthma Proceedings* 26: 323–325. <https://pubmed.ncbi.nlm.nih.gov/16270728/>.
- Eram S, Mujahid M, Bagga P, Ansari VA, Ahmad MA, Kumar A, Ahsan F, Akhter MS (2019) A review on phytopharmacological activity of *Alpinia galanga*. *International Journal of Pharmacy and Pharmaceutical Sciences: 6–11*. <https://doi.org/10.22159/ijpps.2019v11i3.31352>
- Fazel N, Kundi M, Jensen-Jarolim E, Pali-Schöll IM, Kazemzadeh A, Esmaily H, Abdizadeh MF, Akbarzadeh R, Ahmadi R, Jabbari H (2021) Quality of life and asthma control in pregnant women with asthma. *BMC Pulmonary Medicine* 21: 415. <https://doi.org/10.1186/s12890-021-01797-9>
- Fitriana F, Sulistyowati S, Indarto D, Soetrisno S, Nurwati I, Widyaningsih V (2024) Effect of kopyor coconut water on early-onset preeclampsia-like impairments in rats induced by L-nitro-arginine methyl ester. *Pharmacia* 71: 1–11. <https://doi.org/10.3897/pharmacia.71.e127575>
- Gao Y, Nanan R, Macia L, Tan J, Sominsky L, Quinn TP, O'Hely M, Ponsonby A-L, Tang MLK, Collier F, Strickland DH, Dhar P, Brix S, Phipps S, Sly PD, Ranganathan S, Stokholm J, Kristiansen K, Gray LEK, Vuillermin P (2021) The maternal gut microbiome during pregnancy and offspring allergy and asthma. *Journal of Allergy and Clinical Immunology* 148: 669–678. <https://doi.org/10.1016/j.jaci.2021.07.011>
- Goldstein JA, Gallagher K, Beck C, Kumar R, Gernand AD (2020) Maternal-fetal inflammation in the placenta and the developmental origins of health and disease. *Frontiers in Immunology* 11. <https://doi.org/10.3389/fimmu.2020.531543>
- Grieger J, Wood L, Clifton V (2013) Improving asthma during pregnancy with dietary antioxidants: The current evidence. *Nutrients* 5: 3212–3234. <https://doi.org/10.3390/nu5083212>
- Haitamy MN, Sentono HK, Prayitno A, Soetrisno S, Setiamika M, Nurwati I, Cimiatty ARR (2024) Revealing the therapeutic potential of *Nigella sativa* extract in *Aspergillus niger*-induced otitis externa: Anti-inflammatory and antioxidant properties. *Trends in Sciences* 21(10). <https://doi.org/10.48048/tis.2024.8145>
- Jones AR, Tuckwell C, Wright IMR, Morrison JL, Kandasamy Y, Wittwer MR, Arstall MA, Stark MJ, Davies M, Hurst C, Okano S, Clifton VL (2020) The impact of maternal asthma during pregnancy on offspring retinal microvascular structure and its relationship to placental growth factor production in utero. *Microcirculation* 27(6): e12622. <https://doi.org/10.1111/micc.12622>
- Khairullah AR, Solikhah TI, Ansori ANM, Fadholly A, Ramandinianto SC, Ansharieta R, Widodo A, Riwu KHP, Putri N, Proboningrat A, Kusala MKJ, Rendragraha BW, Putra ARS, Anshori A (2020) A review of an important medicinal plant: *Alpinia galanga* (L.) Eilld. *Systematic Reviews in Pharmacy* 11: 387–395.
- Kojima-Yuasa A, Matsui-Yuasa I (2020) Pharmacological effects of 1'-acetoxychavicol acetate, a major constituent in the rhizomes of *Alpinia galanga* and *Alpinia conchigera*. *Journal of Medicinal Food* 23: 465–475. <https://doi.org/10.1089/jmf.2019.4490>
- Kusmardi K, Wiyarta E, Rusdi NK, Maulana AMuh, Estuningtyas A, Sunaryo H (2021) The potential of lunasin extract for the prevention of breast cancer progression by upregulating E-Cadherin and inhibiting ICAM-1. *F1000Research* 10: 902. <https://doi.org/10.12688/f1000research.55385.1>
- Kwah JH, Stevens WW (2019) Asthma and allergies in pregnancy. *Allergy and Asthma Proceedings*, 414–417. <https://doi.org/10.2500/aap.2019.40.4260>
- Mayhew TM (2009) A stereological perspective on placental morphology in normal and complicated pregnancies. *Journal of Anatomy* 215: 77–90. <https://doi.org/10.1111/j.1469-7580.2008.00994.x>
- Pavón-Romero GF, Serrano-Pérez NH, García-Sánchez L, Ramírez-Jiménez F, Terán LM (2021) Neuroimmune pathophysiology in asthma. *Frontiers in Cell and Developmental Biology* 9. <https://doi.org/10.3389/fcell.2021.663535>
- Popa M, Peltecu G, Gica N, Ciobanu AM, Botezatu R, Gica C, Steriade A, Panaiteanu AM (2021) Asthma in pregnancy. Review of current literature and recommendations. *Maedica (Bucur)* 16(1): 80–87. <https://doi.org/10.26574/maedica.2020.16.1.80>
- Radzikowska U, Golebski K (2023) Sex hormones and asthma: The role of estrogen in asthma development and severity. *European Journal of Allergy* 78: 620–622. <https://doi.org/10.1111/all.15548>
- Raviraja Shetty G, Monisha S (2015) Pharmacology of an endangered medicinal plant *Alpinia galanga* – A review. *Research Journal of Pharmaceutical Biological and Chemical Sciences* 6: 499–511.
- Rey A, Jassem E, Chelminska M (2019) Evaluation of asthma course in pregnancy. *Ginekologia Polska* 90: 464–469. <https://doi.org/10.5603/GP.2019.0080>
- Robijn AL, Bokern MP, Jensen ME, Barker D, Baines KJ, Murphy VE (2022) Risk factors for asthma exacerbations during pregnancy: a systematic review and meta-analysis. *European Respiratory Review* 31: 220039. <https://doi.org/10.1183/16000617.0039-2022>
- Ryu RJ, Easterling TR, Caritis SN, Venkataramanan R, Umans JG, Ahmed MS, Clark S, Kantrowitz- Gordon I, Hays K, Bennett B, Honaker MT, Thummel KE, Shen DD, Hebert MF (2018) Prednisone pharmacokinetics during pregnancy and lactation. *The Journal of Clinical Pharmacology* 58: 1223–1232. <https://doi.org/10.1002/jcph.1122>
- Seo J-W, Cho S-C, Park S-J, Lee E-J, Lee J-H, Han S-S, Pyo BS, Park D-H, Kim B-H (2013) 1'-acetoxychavicol acetate isolated from *Alpinia galanga* ameliorates ovalbumin-induced asthma in mice. *PLoS ONE* 8: e56447. <https://doi.org/10.1371/journal.pone.0056447>
- Sly PD (2019) Maternal asthma, pregnancy complications, and offspring wheeze. *American Journal of Respiratory and Critical Care Medicine* 199: 1–2. <https://doi.org/10.1164/rccm.201808-1584ED>

- Steel RGD (1997) Principles and Procedures of Statistics: A Biometrical Approach. 3rd ed. McGraw-Hill, 631 pp. https://books.google.com/books/about/Principles_and_Procedures_of_Statistics.html?id=XB-bvAAAAMAAJ [February 25, 2025]
- To T, Feldman LY, Zhu J, Gershon AS (2018) Asthma health services utilisation before, during and after pregnancy: a population-based cohort study. *European Respiratory Journal* 51: 1800209. <https://doi.org/10.1183/13993003.00209-2018>
- Wang H, Li N, Huang H (2020) Asthma in pregnancy: pathophysiology, diagnosis, whole-course management, and medication safety. *Canadian Respiratory Journal* 2020: 1–10. <https://doi.org/10.1155/2020/9046842>