Chapter 3
Print ISBN: 978-93-49473-37-9, eBook ISBN: 978-93-49473-58-4

Therapeutic Effects of *Asparagus officinalis* Extract on Carrageenan-Induced Allergic Asthma in Swiss Albino Mice

Doaa M Elnagar [a,b*], Sarah A Banaeem [a], Wejdan S AL-Qahtani [a], Norah M Al- Qahtani [a], Ahmed M Rady [a], Khalid E Ibrahim [a], Aisha H Alqarni [a], Shaden A Alshareef [a], Samiah A Almalki [a] and Nuha M Alabdulhadi [a]

DOI: https://doi.org/10.9734/bpi/psnid/v3/4112

Peer-Review History:
This chapter was reviewed by following the Advanced Open Peer Review policy. This chapter was thoroughly checked to prevent plagiarism. As per editorial policy, a minimum of two peer-reviewers reviewed the manuscript. After review and revision of the manuscript, the Book Editor approved the manuscript for final publication. Peer review comments, comments of the editor(s), etc. are available here: https://peerreviewarchive.com/review-history/4112

ABSTRACT

Background: Allergic asthma is a chronic inflammatory alteration of lung airways stimulated by immune-mediated reactions. The most represented symptoms of asthma are cough, chest tightness, shortness of breath and wheezing.
Purpose: To determine the inhibitory effect of Asparagus officinalis extract (AOE) on allergic asthma induced by carrageenan (CGN).
Methods: Forty male mice were divided into 4 groups: the 1st group was untreated control; the 2nd group was treated orally and daily with 500 mg/kg AOE for one week; the 3rd group was treated with a single dose of CGN 2 % w/v (200 µL/mice) intraperitoneally and left for one week; while the 4th group was treated first with CGN as in the 3rd group and treated with AOE as in the 2nd group after CGN injection for one week. After treatment, the animals were sacrificed, and blood samples were subjected to white blood cell count. IL-6 and TNF-α was measured in the lung homogenate while histopathological analysis was performed for lung samples.
Results: A single dose of CGN resulted in a significant increase in white blood cell (WBC) count and pro-inflammatory cytokines IL-6 (490) and TNF-α (980) pg/ml; p = 0). Histopathological analysis showed severe lung alterations such as accumulation of infiltration cells that blocked alveolar sacs, and over-secretion of collagenous fibers, extracellular matrix, and hyaline membranes. Moreover,

[a] Zoology Department, College of Science, King Saud University, Saudi Arabia.
[b] Zoology Department, Faculty of Women, Ain Shams University, Egypt.
**Corresponding author: E-mail: elnagard1@yahoo.com;*

treatment with AOE after CGN injection significantly reduced WBC count (14x109) (p = 0) and pro-inflammatory cytokines (IL-6 (300) and TNF-α (830)pg/ml) that were raised by CGN (p = 0). Furthermore, AOE reduced the pathological signs that were induced by the matrix, and to the improvement of lung function and reduction of collagenous fibers and hyaline membranes.
Conclusion: Carrageenan is an allergen-induced inflammatory agent and causes marked pathological alterations, thereby increasing pulmonary pathological scarring in addition to increasing the WBC count and pro-inflammatory cytokines. Asparagus officinalis extract reduces the allergic effect and lung pathological signs induced by CGN. Since AOE inhibits the production of inflammatory cytokines, it has the potential to be developed as a source of active pharmaceutical ingredients for the management of lung and airway injury.

Keywords: Carrageenan; asparagus officinalis extract; lung; allergy; cytokines.

1. INTRODUCTION

Asthma is a complex, partially heritable disease with a marked heterogeneity. Its development is influenced both by genetic and environmental factors (Komlósi et al., 2022). Allergic asthma is a chronic inflammatory alteration of lung airways stimulated by immune-mediated reactions (Périz et al., 2020). The most represented symptoms of asthma are cough, chest tightness, shortness of breath and wheezing (Papi et al., 2018). Many common triggers for allergic asthma are environmental, and some others look chemical in nature. Natural triggers include pollen, dust mites, mold, and insects, while chemical triggers may be fumes, cigarettes, and smog (Bates et al., 2009). Bronchial inflammation, smooth muscle spasm, and mucus production in allergic asthma are triggered by IL-4, IL-5, and IL-13, which are released by Th2 cells (Athari, 2019; Attia et al., 2021). Chronic inflammatory allergic asthma is accompanied by histopathological signs such as metaplasia and hyperplasia of bronchioles epithelial and goblet cells and mucus hypersecretion (Elnagar et al., 2024). Additionally, allergic asthma activates T helper cells (Th2) as a result of allergens which in turn produces extreme secretions of many cytokines such as interleukins-4, 5, 6, 9, 13 (IL-4, IL-5, IL-6, IL-9, IL-13) (Shinagawa & Kojima, 2003).

Nevertheless, many experimental allergens have been used in various animal models of allergic asthma in laboratory settings, such as ovalbumin, mite allergens, cockroach extracts, and cotton dust, the choice depending on the condition (Zosky & Sly, 2007; Elnagar et al., 2024). The present study used carrageenan (CGN) as an asthmatic allergen. Many studies have used CGN in animal models to induce inflammation, edema, hyperalgesia, and erythema following subcutaneous paw injection. CGN is red seaweed extract; it has been used in many industries as a gelling and thickening factor (Elnagar et al., 2024). CGN consists mainly of polysaccharide macromolecules that are composed of d-galactose and 3,6-anhydro-d-galactose units with a binding sulfate group (Hagimori et al., 2009).

Asparagus officinalis belongs to the Asparagaceae family and is a medicinal vegetable cultivated in Europe and Asia. It is used in everyday life as a component

of soups, salads, and vegetable dishes (Huang et al., 2008). *Asparagus officinalis,* or sparrow grass as it is known in folk medicine, is a herbaceous and perennial climbing plant (Elnagar et al., 2024). Its height may reach 100-150 cm, and it is used as a spring vegetable (Mfengwana & Mashele, 2019). Different parts of the plant contain bioactive components such as oligosaccharides, steroidal saponins, flavonoids, and amino acid derivatives. Findings have reported that *Asparagus officinalis* rhizome has an impact on inflammatory activities via the reduction of arthritic activity induced in rat paws by CGN (Kumar et al., 2023). *Asparagus officinalis* also reduces liver inflammation induced by CGN and pro-inflammatory cytokines (Elnagar, 2023; Elnagar et al., 2024). The present work aimed to investigate the effect of *Asparagus officinalis* extract on asthma induced by CGN in male albino mice.

2. EXPERIMENTAL

2.1 Materials

Powder of Carrageenan was obtained from Fit Lane Nutrition (USA) (Elnagar et al., 2024). Extract of *Asparagus officinalis* rhizome was purchased from Solaray Company (USA).

2.2 Animals

Male albino mice (40) aged 14 weeks with an average weight of 30 ± 5 g were used in the study. The Animal House of Zoology, Department of Science College, King Saud University, Riyadh provided the animals for this research (Elnagar et al., 2024). The animals were housed under controlled temperature (23 ± 5 ℃) and 12 / 12 h light-dark cycle and had free access to clean water and a commercial diet (Elnagar et al., 2024). The study was approved by the Institutional Review Board (IRB), Committee of Ethics, King Saud University, Riyadh, Saudi Arabia (approval no. KSU-SE-22-02), and followed international guidelines for animal studies.

2.3 Study Protocol

Male Swiss albino mice were divided randomly into four groups, 10 in each group (Elnagar et al., 2024). The first group was untreated control; 2nd group was treated orally and daily with 500 mg/kg AO extract for one week; the 3rd group was treated with a single dose of carrageenan 2 % w/v (200 µL/mice) intraperitoneally and left for one week; 4th group was treated firstly with CGN as in the 3rd group and treated with AOE as in the 2nd group after CGN injection for one week (Elnagar, 2023).

2.4 Sample Collection

At the end of the experiment, animals were anesthetized using carbon dioxide (CO_2) flow (Elnagar, 2023). Blood samples were collected via cardiac puncture, then drained into EDTA tubes and subjected to leucocytic count (Elnagar et al., 2024). Lung samples were removed and cut into small pieces, fixed in 10 % neutral buffered formalin (NBF) for histopathological investigation.

2.5 Biochemical Analysis

Lungs were homogenized in cold PBS with a ratio of 1:3 for 3 min, then centrifuged for 15 min at 4^{o}C twice, and then filtered (Elnagar et al., 2024). The supernatant was separated and stored at -80^{o}C till assay.

2.6 Determination of Inflammatory Cytokines

Tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6) were determined in the lung homogenate using commercial kits of enzyme-linked immunosorbent assay (ELISA) (Lukacst al., 1995).

2.7 Histopathological Analysis

Lung samples were fixed in 10 % formalin and then dehydrated with ethanol; they were embedded in paraffin wax, then sectioned into 6-µm size samples, and stained using hematoxylin and eosin, Masson's trichrome, and PAS stain. Photomicrographs of the sections were taken using Nikon-Japan (Elnagar et al., 2024). Stained lung sections were subjected to the pulmonary histopathological scoring system (Passmore et al., 2018).

2.8 Statistical Analysis

Data was represented as mean ± standard error of the mean (SEM), and the differences between the treated and control mice were evaluated using one-way ANOVA, and the differences were statistically significant when $p \leq 0.05$ (Elnagar et al., 2024).

3. RESULTS

3.1 AOE Lowered WBCs Count Raised by CGN

Mice group treated with AOE showed insignificant change in WBC count compared to the control group. Whereas the group treated with CGN revealed a significant ($p < 0.05$) WBC increase compared to the control group (Elnagar et al., 2024). However, pre-treatment with AOE before CGN treatment resulted in a significant WBC decrease ($p < 0.05$) compared to the group treated with CGN only (Fig. 1) (Elnagar et al., 2024).

AOE treatment revealed no significant change in cytokines (TNF-α and IL6) levels compared to control levels. Meanwhile, treatment with single-dose CGN resulted in significant ($p < 0.05$) increases in TNF-α and IL6 levels compared to control levels (Elnagar et al., 2024). Furthermore, treatment with AOE before CGN caused a significant decrease ($p < 0.05$) in TNF-α and IL6 cytokine levels compared to the group treated with CGN alone.

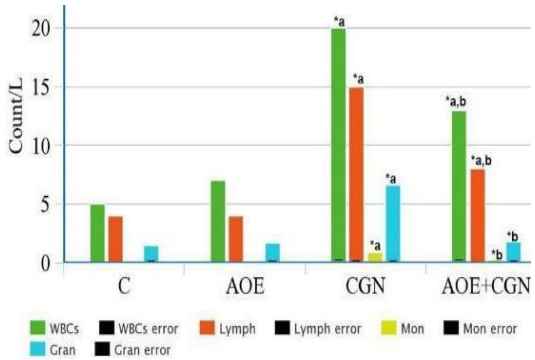

Fig. 1. WBC count in all the groups

Key: The Bar graph showed a significant decrease ($p < 0.05$) in WBC count following treatment with AOE previously increased by CGN

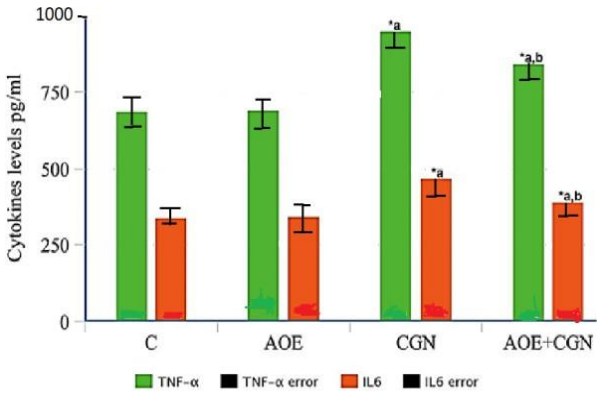

Fig. 2. Estimation of pro-inflammatory cytokines

Key: Bar graph shows a significant decrease of pro-inflammatory cytokines by pre-treatment with AOE that was increased by CGN

Untreated control mice lungs showed the normal structure of well-opened bronchioles and alveolar sacs besides thin interalveolar septa, with no depositions of collagenous fibers (Figs. 3A, 4A) with less percentage and optical density of Masson's trichrome stain (Fig. 5) (Elnagar et al., 2024); also, it revealed normal content of extracellular matrix in the interalveolar septa and around alveoli but no hyaline membranes (Fig. 6 A) with less percentage and optical density of PAS stain distribution (Fig. 7), in addition to having the lowest pathological score (Table 1) (Elnagar et al., 2024). Additionally, the lungs of the mice group treated with AOE resembled the same results as the untreated control (Fig. 3 B, Fig. 4 B, and Fig. 6 B) (Elnagar et al., 2024). Whereas the lungs of mice treated with a single dose of CGN exhibited severe pathological alterations manifested by the accumulation of

inflammatory great aggregations leading to wide thickness of interalveolar septa and blockade of alveolar sacs (Fig. 3C) (Elnagar et al., 2024).

Pulmonary histopathological scoring system criteria:

Score	Vascular alterations	Vascular and alveolar changes	Bronchiole alterations
0	Few	Few	None
1	Hemorrhage and mild RBC obstruction	Patchy edema, mild inflammation	Mild infiltration
2	Moderate hemorrhage and obstruction	Moderate alveolar septa thickening, moderate infiltration	Moderate infiltration, dysplasia of lining epithelia, hyaline membranes
3	Diffuse hemorrhage and intense obstruction	Severe alveolar septa thickening, severe infiltration exudate, amorphous materials	Severe infiltration exudate, dysplasia a and hyperplasia, destruction of bronchiole, hyaline membranes

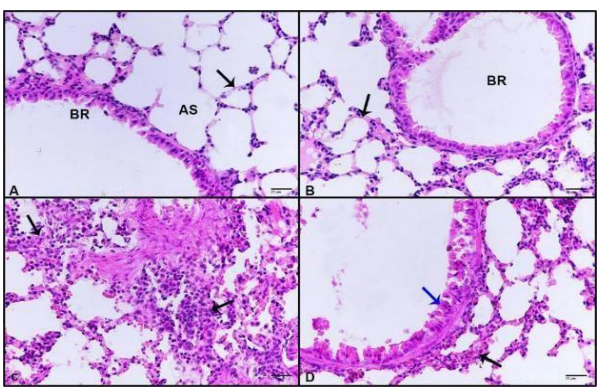

Fig. 3. Photomicrographs of mice lung stained by H&E

Key: (A) Untreated control lung showing normal pulmonary view, bronchiole (BR), alveolar sac (AS), interalveolar (black arrow); (B) lung treated with AOE revealing healthy structure; (C) lung treated with CGN displayed thickened interalveolar septa due to accumulation of infiltrative cells (black arrows); (D) lung treated with AOE + CGN showing less pathological signs, less thickened interalveolar septum (black arrow); less distorted columnar bronchiolar epithelia (blue arrow). (H & E-400x)

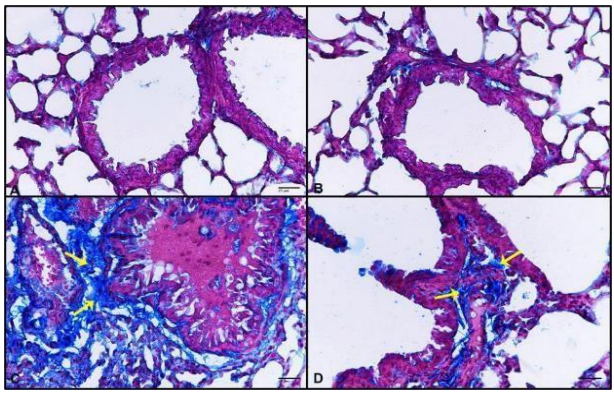

Fig. 4. Photomicrographs of mice lung stained by Masson's trichrome.
Key: (A) Untreated control lung showing no collagenous depositions, (B) lung treated with AOE revealing no collagenous depositions, (C) lung treated with CGN displayed depositions of collagenous fibers and extracellular matrix (yellow arrows), distorted epithelia of bronchioles that is congested with edema (D) lung treated with AOE+CGN showing less depositions (yellow arrow). (Masson's trichrome-400x)

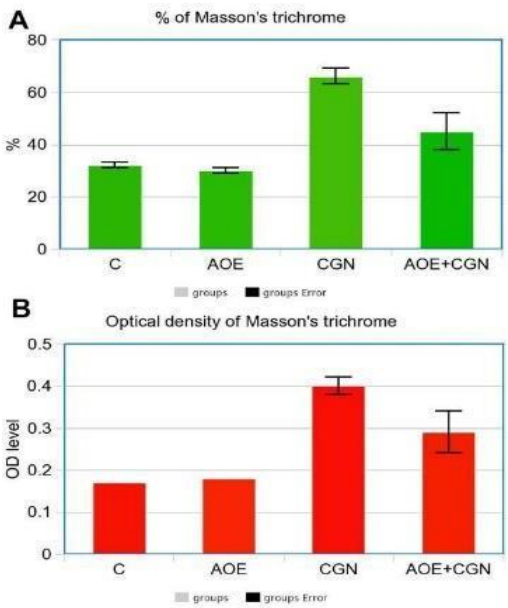

Fig. 5. Masson's trichrome stain percentage and optical density
Key: Treatment with AOE before CGN single dose lowered Masson's trichrome stain percentage and optical density, (A) Masson's trichrome stain percentage (%) (B) Masson's trichrome stain optical density (OD)

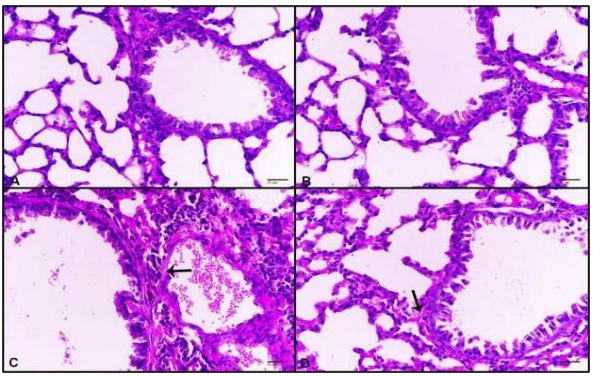

Fig. 6. Photomicrographs of mice lung stained by Periodic acid Schiff
Key: (A) untreated control lung showing no hyaline membranes, (B) lung treated with AOE revealing no hyaline membranes, (C) lung treated with CGN displayed hyaline membranes (black membrane), (D) lung treated with AOE+CGN showing less hyaline membranes (black arrow). (PAS-400x)

Table 1. Effect of AOE on the pathological scoring of lung raised by CGN

Group	Vascular alterations	vascular and alveolar changes	Bronchiole alterations	Total score
C	0	0	0	0
AOE	0	0	0	0
CGN	2	3	3	8
AOE+CGN	1	1	1	3

C: Control, AOE: Asparagus officinalis extract, CGN: Carrageenan

Moreover, CGN-induced dysplasia and destruction of bronchioles columnar epithelia, heavy depositions of collagenous fibers and extracellular matrix stained blue by Masson's trichrome (Fig. 4 C) that sections registered a high score of Masson's trichrome percentage and optical density (Fig. 5), PAS staining for lungs of mice treated with CGN revealed intense existence of hyaline membranes (Fig. 6 C) with the highest levels of PAS stain percentage and optical density (Fig. 7), also pathological score reached the highest value (Table 1) (Elnagar et al., 2024).

However, mice lungs treated with AOE prior to CGN administration showed great improvement represented by recovery of lung tissue except for some alterations of bronchioles epithelia (Fig. 3 D). Lung sections stained by Masson's trichrome showed less content of fibers (Fig. 4 D) and less percentage and optical density (Fig. 5) (Elnagar et al., 2024). Staining by PAS also revealed less hyaline membrane content (Fig. 6 D) besides, there was a lower percentage and optical density (Fig. 7), in addition to lowering the pathological score (Table 1) (Elnagar et al., 2024).

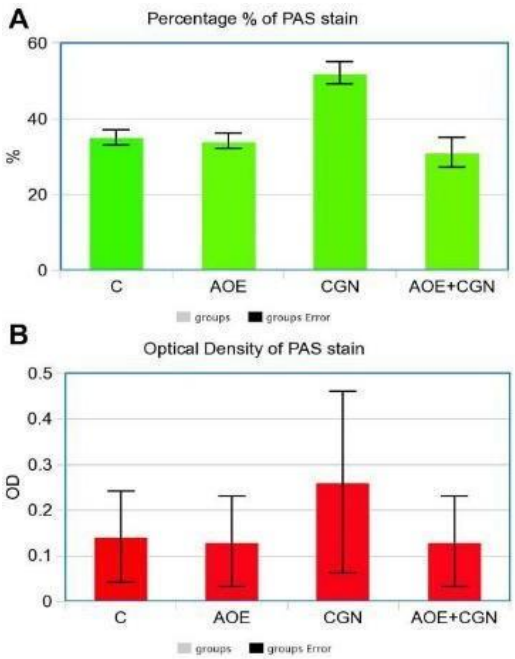

Fig. 7. PASs stain percentage and optical density
Key: Treatment with AOE before CGN single dose lowered pas stain percentage and optical density, (A) PAS stain percentage % (B) PAS stain optical density (OD)

4. DISCUSSION

In the present study, the effect of *Asparagus officinalis* on allergic asthma was investigated by using carrageenan (CGN) as the allergen trigger (Elnagar et al., 2024). Many studies have used CGN as a trigger of inflammation an inducer of edema, and a hypersensitivity in rats (Lukacs et al., 1995); another study used CGN experimentally for screening anti-inflammatory drugs (Fehrenbacher et al., 2012). Therefore, the present study used CGN as a trigger for asthma to study the effect of AOE on asthma induced experimentally by CGN (Elnagar et al., 2024).

Previous studies reported that exposure to CGN increased WBC counts, especially neutrophils and eosinophils (Patil et al., 2019). In addition, another study revealed that injection with a single dose of CGN significantly raised WBC count (Elnagar, 2023).

These findings were compatible with the present results that showed CGN administration increased WBC count, lymphocytes, and monocytes. However, the present work revealed that AOE administration significantly decreased the total count of WBCs, lymphocytes, monocytes, and neutrophils that were raised due to

CGN treatment, and this is in tandem with the study by Elnagar, (2023) (Elnagar et al., 2024).

It has been reported that induction of inflammation by kappa carrageenan (k-CGN) or lambda carrageenan (ƛ-CGN) resulted in hyper-secretion of inflammatory cytokines such as interleukins (IL-4 and 5) (Duarte et al., 2012). Other studies postulated that not only Th2 cytokines but also pro-inflammatory cytokines such as IL 6 and TNF-α are increased (Wu et al., 2016 Elnagar et al., 2024). The present findings corroborate with previous studies as the results show that the administration of a single dose of CGN resulted in increased proinflammatory cytokines, IL-6 and TNF-α, due to stimulation and increased levels of monocytes and macrophages (Elnagar et al., 2024). AOE administration suppressed the overproduction of IL6 and IL-1β induced by macrophages in the case of SARS-CoV-2 Spike Protein-Induced production of inflammatory cytokines (Ou et al., 2019). The present work also reported that pre-treatment with AOE after CGN lowered levels of pro-inflammatory cytokines.

The current study showed the severe lung pathological alterations induced by CGN, that its single dose caused heavy inflammation in the lung tissue besides the intense accumulation of fibrosis, extracellular matrix, and hyaline membranes (Elnagar et al., 2024). It has been reported that CGN activates neutrophils in the lung, resulting in the accumulation of infiltrative cells leading to lung injury (Shiratoet al., 2021). Since AOE inhibits the production of inflammatory cytokines, it therefore reduces inflammation and lung and airway injury (Shirato et al., 2021).

5. CONCLUSION

Carrageenan is an allergen-induced inflammatory agent and causes marked pathological alterations, thereby increasing pulmonary pathological scarring in addition to increasing the WBC count and pro-inflammatory cytokines (Elnagar et al., 2024). *Asparagus officinalis* extract reduces the allergic effect and lung pathological signs induced by CGN. Since AOE inhibits the production of inflammatory cytokines, it has the potential to be developed as a source of active pharmaceutical ingredients for the management of lung and airway injury.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s).

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Athari, S. S. (2019). Targeting cell signaling in allergic asthma. *Signal Transduction and Targeted Therapy*.

Attia, N. A., Abousada, H. J., Almasoudi, S. S., AlHarbi, A. A., Alghanmi, O. D., Alghamdi, A. J., Almarshud, F. A., Alhazmi, R. K., Haddad, A. H., Alzahrani, A. S., Aljahdali, A. Y., Mahmoud, S. A., Ajjaj, R. G., Mannan, S. M., ALnashri, A. A. (2021). Childhood Asthma and Risk Factors in KSA (Cross Sectional Study). *Journal of Pharmaceutical Research International*.

Bates, J., Rincon, M., & Irvin, C. (2009). Animal models of asthma. *American Journal of Physiology Lung Cell and Molecular Physiology*.

Duarte, D., Vasko, M., & Fehrenbacher, J. (2012). Models of inflammation: Carrageenan air pouch. *Current Protocols in Pharmacology*.

Elnagar, D. M. (2023). Anti-inflammatory activity of *Asparagus officinalis* extract on liver inflammation carrageenan-induced in Swiss albino mice. *Uttar Pradesh Journal of Zoology*.

Elnagar, D. M., Banaeem, S. A., AL-Qahtani, W. S., Al-Qahtani, N. M., Rady, A. M., Ibrahim, K. E., Alqarni, A. H., Alshareef, S. A., Almalki, S. A., Alabdulhadi, N. M. (2024). Inhibitory effect of *Asparagus officinalis* extract on allergic asthma in Swiss albino mice. *Tropical Journal of Pharmaceutical Research*.

Fehrenbacher, J., Vasko, M., & Duarte, D. (2012). Models of inflammation: Carrageenan- or complete Freund's adjuvant (CFA)-induced edema and hypersensitivity in the rat. *Current Protocols in Pharmacology*.

Hagimori, M., Kamiya, S., Yamaguchi, Y., & Arakawa, M. (2009). Improving frequency of thrombosis by altering blood flow in the carrageenan-induced rat tail thrombosis model. *Pharmacological Research*.

Huang, X., Lin, Y., & Kong, L. (2008). Steroids from the roots of *Asparagus officinalis* and their cytotoxic activity. *Journal of Integrative Plant Biology*.

Komlósi, Z. I., van de Veen, W., Kovács, N., Szűcs, G., Sokolowska, M., O'Mahony, L., Akdis, M., & Akdis, C. A. (2022). Cellular and molecular mechanisms of allergic asthma. *Molecular Aspects of Medicine*.

Kumar, S., Srivastava, P., Gupta, S., Dhanawat, M., Rani, S., Ajiboye, B., Gautam, R. (2023). Pharmacological evaluation of different extracts of *Asparagus officinalis* (Asparagaceae) as an analgesic, anti-inflammatory and anti-arthritic agent in rats. *Pharmacognosy Research*.

Lukacs, N., Strieter, R., Chensue, S., Widmer, M., & Kunkel, S. (1995). TNF-alpha mediates recruitment of neutrophils and eosinophils during airway inflammation. *Journal of Immunology*.

Mfengwana, P., & Mashele, S. (2019). Medicinal properties of selected asparagus species: A review. *IntechOpen*.

Ou, Z., Zhao, J., Zhu, L., Huang, L., Ma, Y., Ma, C., Luo, C., Zhu, Z., Yuan, Z., Wu, J., Li, R., & Yi, J. (2019). Anti-inflammatory effect and potential mechanism of betulinic acid on λ-carrageenan-induced paw edema in mice. *Biomedicine and Pharmacotherapy*.

Papi, A., Brightling, C., Pedersen, S., & Reddel, H. (2018). Asthma. *Lancet*.

Passmore, M., Byrne, L., Obonyo, N., See Hoe, L., Boon, A., Diab, S., Dunster, K., Bisht, K., Tung, J., Fauzi, M., et al. (2018). Inflammation and lung injury in an ovine model of fluid resuscitated endotoxemic shock. *Respiratory Research*.

Patil, K. R., Mahajan, U. B., Unger, B. S., Goyal, S. N., Belemkar, S., Surana, S. J., Ojha, S., Patil, C. (2019). Animal models of inflammation for screening of anti-inflammatory drugs: Implications for the discovery and development of phytopharmaceuticals. *International Journal of Molecular Sciences*.

Périz, M., Pérez-Cano, F., Rodríguez-Lagunas, M., Cambras, T., Pastor-Soplin, S., Best, I., Castell, M., & Cladera, M. (2020). Development and characterization of an allergic asthma rat model for interventional studies. *International Journal of Molecular Sciences*.

Shinagawa, K., & Kojima, M. (2003). Mouse model of airway remodeling: Strain differences. *American Journal of Respiratory and Critical Care Medicine*.

Shirato, K., Takanari, J., & Kizaki, T. (2021). Standardized extract of *Asparagus officinalis* stem attenuates SARS-CoV-2 spike protein-induced IL-6 and IL-1β production by suppressing p44/42 MAPK and Akt phosphorylation in murine primary macrophages. *Molecules*.

Wu, W., Wang, F., Gao, X., Niu, T., Zhu, X., Yan, X., & Chen, H. (2016). Synergistic effect of κ-carrageenan on oxazolone-induced inflammation in BALB/c mice. *BMC Gastroenterology*.

Zosky, G., & Sly, P. (2007). Animal models of asthma. *Clinical and Experimental Allergy*.

DISCLAIMER
This chapter is an extended version of the article published by the same author(s) in the following journal.
Tropical Journal of Pharmaceutical Research, 23(5): 825-831, 2024.

Peer-Review History:
This chapter was reviewed by following the Advanced Open Peer Review policy. This chapter was thoroughly checked to prevent plagiarism. As per editorial policy, a minimum of two peer-reviewers reviewed the manuscript. After review and revision of the manuscript, the Book Editor approved the manuscript for final publication. Peer review comments, comments of the editor(s), etc. are available here: https://peerreviewarchive.com/review-history/4112