

# Withaferin A Effects on House Dust Mite Allergen-Induced Airway Inflammation

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## Introduction

Lung inflammation is a prevalent feature of respiratory illnesses such as asthma, characterized by immune cell infiltration resulting in airway hyperresponsiveness, mucus hypersecretion, and tissue remodeling (3, 5). Withaferin A (WFA), a natural compound extracted from *Withania somnifera*, or Ashwagandha plant, exhibits immunomodulatory and anti-inflammatory properties (2). Studies have suggested that WFA could serve as a promising treatment for asthma partly due to its ability to suppress *Mmp-9* expression, which contributes to airway inflammation and remodeling in asthma (4).

**Hypothesis:** We hypothesize that WFA suppresses airway inflammatory cell infiltration in allergic asthma by reducing *Mmp-2* and *Mmp-9* expression and activity.

## Methods

House dust mite (HDM) extract or phosphate buffered saline (PBS) was intranasally administered 3 times/ week, while WFA or PBS was intraperitoneally injected once/day. Blood and bronchoalveolar lavage (BAL) fluid were collected at the endpoint, with lung tissue harvested for ribonucleic acid (RNA) and protein extraction. Enzyme-linked immunosorbent assay (ELISA) was used to measure IL-33 levels, and quantitative reverse-transcription polymerase chain reaction (qRT-PCR) was used to measure *Mmp-2*, *Mmp-9*, *Ccl11*, *Tgf1*, and *Il17ra* levels in the BAL and lung tissue.

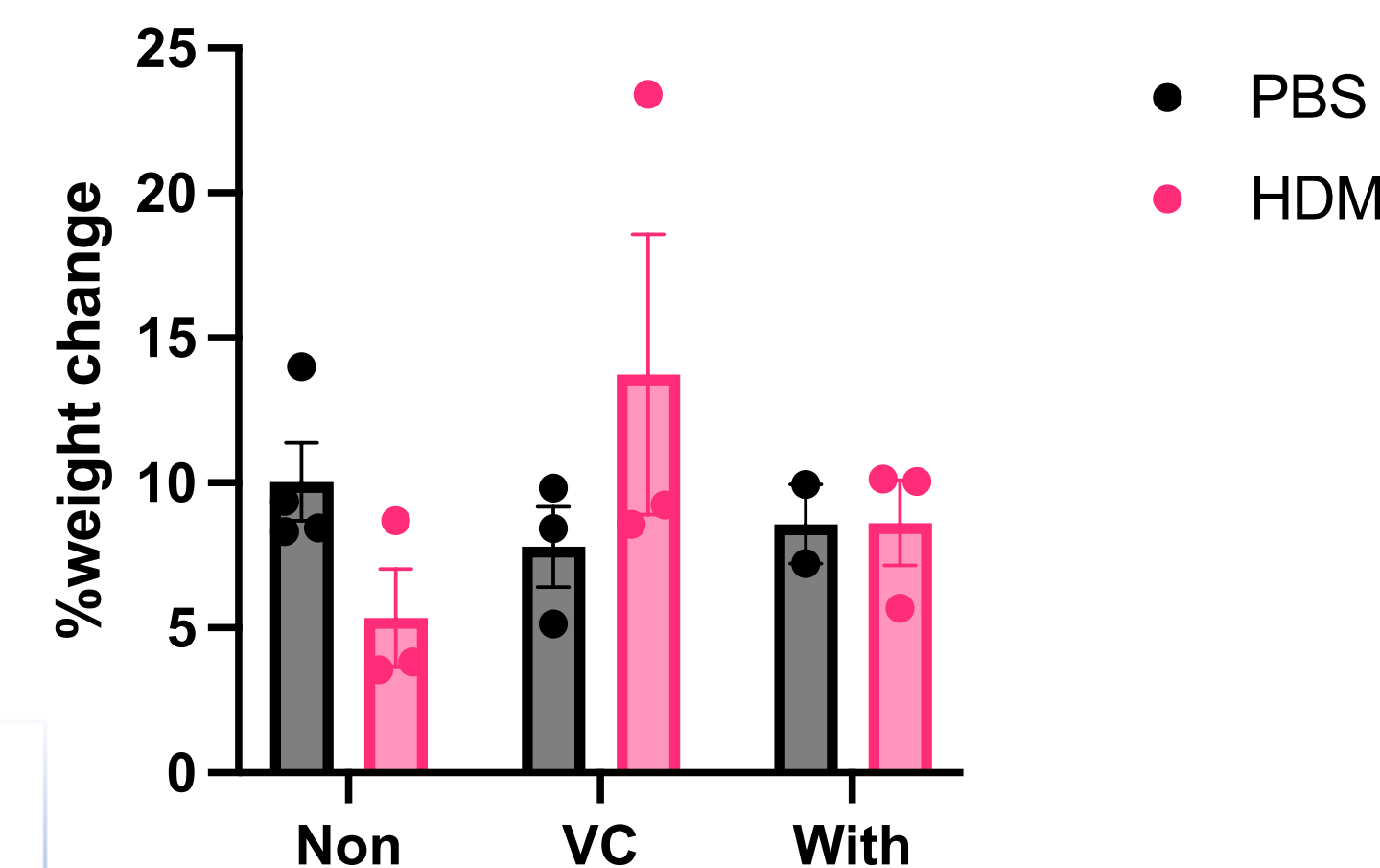


Figure 2: Percent body weight change/group over 2-wk period

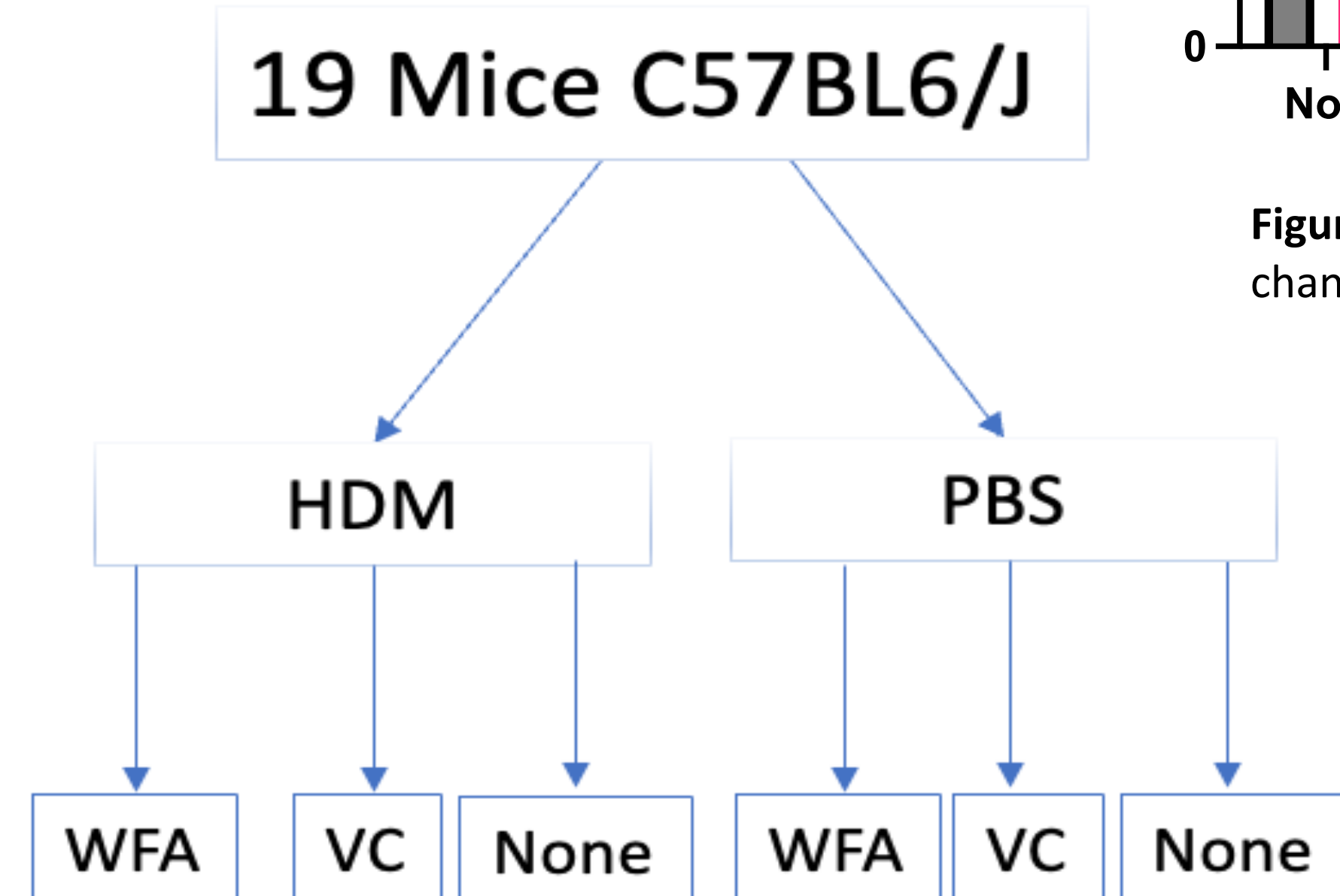


Figure 1. 19 total mice: PBS/Non- n=4; PBS/VC- n=3; PBS/WFA- n=2; HDM/Non- n=3; HDM/VC- n=3; HDM/WFA- n=3  
\*Outlier removed in PBS/WFA; Non= non-injection; VC= vehicle control; WFA= Withaferin A=

## Results

### Hematoxylin and Eosin (H&E) Staining

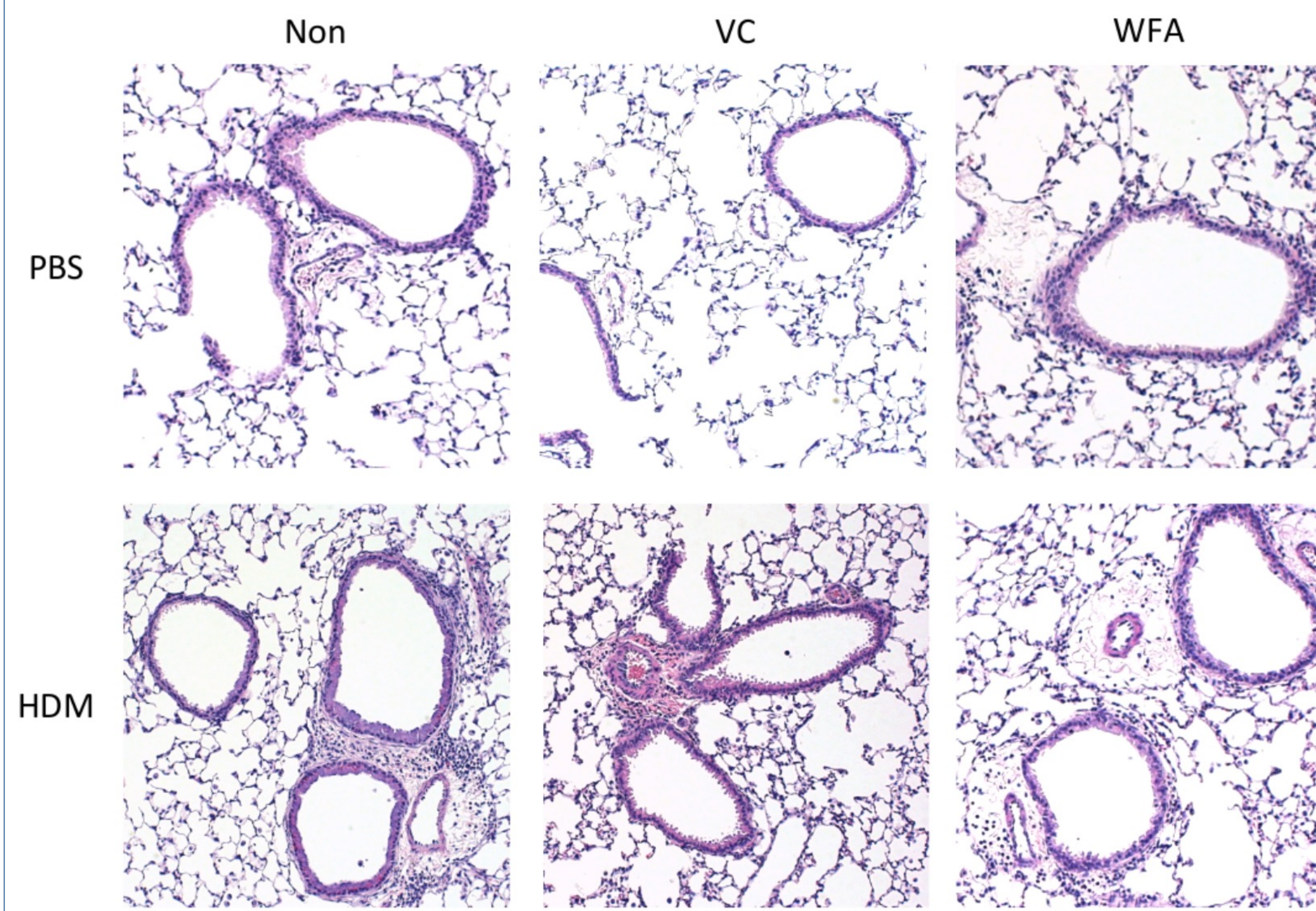


Figure 3. H&E staining visualizes structures and inflammatory cells in lung tissue. Withaferin A visually reduced airway inflammation, as seen by similar staining for eosinophils and neutrophils as compared to the control group, while the PBS/Non and VC groups had more inflammation and increased staining for these cells around the airways.

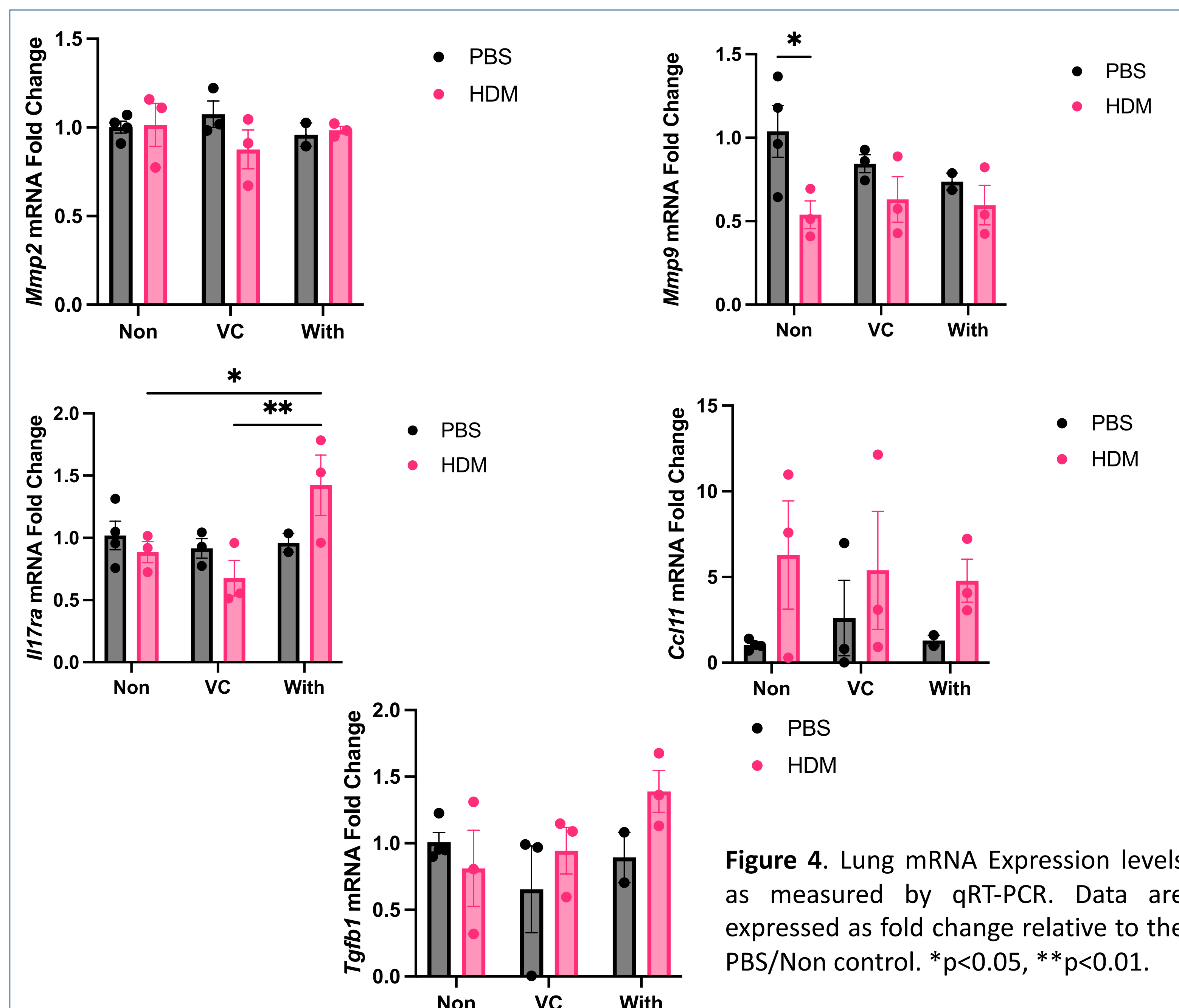


Figure 4. Lung mRNA Expression levels as measured by qRT-PCR. Data are expressed as fold change relative to the PBS/Non control. \*p<0.05, \*\*p<0.01.

## Results Continued:

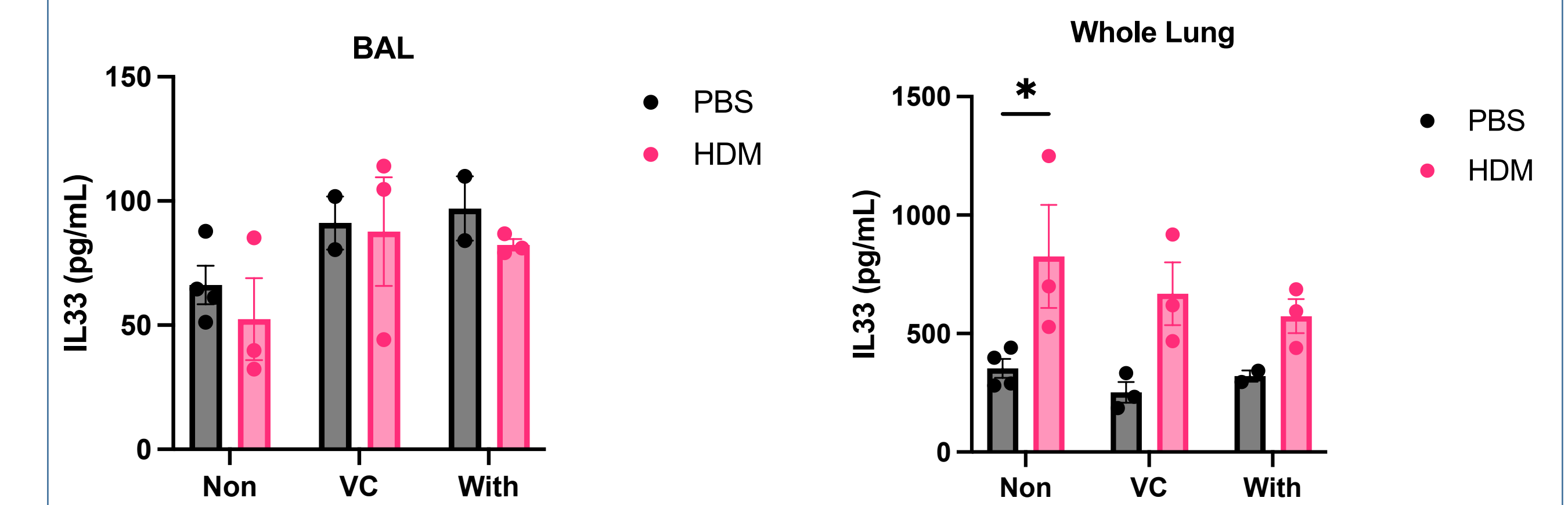


Figure 5. Protein production of IL-33 in BAL fluid and whole lung homogenate as measured by ELISA. IL-33 is a key cytokine that mediates allergen-induced airway inflammation.

## Discussion

- The study investigated the therapeutic potential of WFA on airway inflammation, revealing that it reduced airway inflammation in lung tissue, as evidenced by similar H&E staining patterns of eosinophils and neutrophils between the HDM/WFA group and the control groups.
- Although body weight change was not different between groups, the PBS/WFA groups had the lowest weight gain, suggesting potential benefits for metabolic function.
- Our qPCR results demonstrated varied gene expression, most notably with *Ccl11* having reduced expression in the HDM/WFA group, but respectively *Il17ra* and *Tgf1* having increased expression.
- Furthermore, the WFA-treated groups had the lowest lung production of IL-33, indicating anti-inflammatory effects on the lung.
- To our surprise, we found no effect of WFA on *Mmp2* or *Mmp9* levels in the lung tissue. Further investigation of specific cell types in the airway may reveal effects of WFA on these genes that were not apparent in our study of the whole lung tissue.
- These findings offer insight into the potential therapeutic effects of WFA on allergen-induced airway inflammation. Further investigations using more samples are necessary to elucidate its full therapeutic potential, dosage, and possible side effects.

## References

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