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Ovalbumin: A Potent Allergen for Inducing Systemic Inflammatory Response Causing Asthma

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ABSTRACT:
Background: Asthma is a chronic inflammatory disease of the airways characterized by airway hyperresponsiveness, inflammation, and remodeling. The present study aims to investigate the histopathological changes observed in the lungs of Wistar albino rats sensitized and challenged with ovalbumin (OVA), providing insights into the effects at the tissue level and potential mechanisms underlying these conditions.
Methods: Wistar albino rats were sensitized with intraperitoneal injections of OVA combined with aluminum hydroxide and subsequently challenged with OVA aerosols. After the OVA challenge, rats were euthanized, and blood and lung tissues were collected. IgE, total, and differential leukocytes were measured in the blood samples. Lung tissues were fixed in 10% formalin and thin sections of the paraffin-embedded lungs were prepared and stained with hematoxylin and eosin to assess the histopathological alterations.
Results: Histopathological analysis of lung tissues revealed marked inflammatory cell infiltration, predominantly eosinophils, and lymphocytes, in the peri-bronchial and peri-vascular regions. Interstitial edema was also observed. Vascular changes, such as increased permeability and dilation, were noted. The study demonstrates that OVA sensitization and challenge in Wistar albino rats induce significant histopathological changes in the lungs, resembling the pathological features of human asthma. These findings highlight the utility of this model in understanding disease mechanisms and evaluating potential therapeutic interventions for airway inflammatory diseases.

Keywords:
Asthma, Allergic rhinitis, Histopathological, Ovalbumin

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1. INTRODUCTION

Asthma is a chronic inflammatory disease of the respiratory tract characterized by airway hyperresponsiveness, mucus hypersecretion, and structural remodeling of the airways (Holgate, 2012). The prevalence of these conditions has been increasing globally, necessitating further investigation into their underlying pathophysiology and potential therapeutic interventions. Animal models, particularly those using rodents, have proven invaluable in understanding these disease mechanisms and evaluating the efficacy of new treatments (Kumar *et al.,* 2014).

One of the most widely used animal models for studying allergic asthma is the ovalbumin (OVA)-induced model in Wistar albino rats. This model mimics many of the key features of human asthma, including eosinophilic inflammation, increased levels of IgE, and structural changes in the lungs and airways (Lambrecht & Hammad, 2015). Ovalbumin, a glycoprotein found in egg whites, acts as an antigen that triggers an allergic response when administered to sensitized animals. Upon exposure, it leads to the activation of T-helper 2 (ThH2) cells and the subsequent release of cytokines such as IL-4, IL-5, and IL-13, which are central to the development of asthma-like symptoms (Elias *et al.,* 2003). Histopathological examination of the lungs provides critical insights into the extent and nature of tissue damage caused by OVA-induced inflammation. Studies have shown that repeated exposure to OVA can result in airway remodeling, including epithelial cell hyperplasia, goblet cell metaplasia, subepithelial fibrosis, and smooth muscle hypertrophy (Roche *et al.,* 2004). These changes contribute to the characteristic symptoms of asthma, such as airway narrowing and obstruction, leading to breathing difficulties and reduced lung function. The present study aims to evaluate the histopathological effects of ovalbumin on the lungs of Wistar albino rats. By examining the structural changes in these tissues, we seek to enhance our understanding of the pathological processes involved in asthma which could inform the development of more effective therapeutic strategies.

2. MATERIALS AND METHOD

2.1 **Experimental Animals:** Six to seven-week-old, male and female Wistar albino rats (200±50 g) were procured from the Department of Pharmacology, Datta Meghe College of Pharmacy, Salod (H), Wardha, Maharashtra (India) (571/Po/Re/S/02/CPCSEA) for the experiment. The protocol (Proposal N0.06, Date: 16/02/2021) of the experiment was approved by the Institutional Animal Ethical Committee (IAEC), and all the experiments were conducted as per the guidance of the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Animal Welfare Division, New Delhi, India. All rats were kept in the animal house with free access to water ad libitum and a standard pellet diet. The housing environment was maintained at constant temperature of 22 ± 1 °C relative humidity of 55 ± 5%, operated on a 12 h light /12 h dark-hour light/dark cycle.

2**.2 Chemicals:** The prime allergen ovalbumin, from chicken egg white (MW~45 kDa; 80% purity; RM7331, HiMedia Labs, India) was adjuvanted using aluminum hydroxide ($Al(OH)_3$, HiMedia, India) which was gelated under alkaline conditions as per standard procedures. Ovalbumin is a globular protein. It folds into a compact three-dimensional structure. It is made up of a single polypeptide chain consisting of 385 amino acids. It is soluble in water and can form stable solutions (LiChan *et al.,* 2012, Békésiová *et al.,* 2017). Prolonged skin exposure to OVA can lead to significantly increased levels of total and OVA-specific IgE in the serum and the infiltration of CD3 (+) T-cells, eosinophils, and neutrophils. Additionally, there is an increase in mRNA levels of interleukin (IL)-4, IL-5, and IFNγ (Spergel *et al.,* 1998).

2.3 Experimental design: Wistar albino rats were divided into four groups (n = 6) receiving the following treatments: Group-1, CM: Normal Control Male; treated with normal saline. Group-2, CF: Normal Control female; treated with normal saline. Group-3, OvaM: Male rats were sensitized and challenged with

Ovalbumin. Group-4, OvaF: Female rats were sensitized and challenged with Ovalbumin.

Wistar albino rats were sensitized with ovalbumin (OVA) at a dose of 100 µg/kg, adsorbed in 100 mg/mL aluminium hydroxide, and were administered intraperitoneally (i.p.), on the 7th, 14th, and 21st days. They were then challenged with OVA (1% w/v, adsorbed in 100 mg/mL Al $(OH)_3$ aluminum hydroxide), for 30 minutes, three times per week for three weeks in the OVA. For the challenge, rats were placed into a plastic chamber (70 cm x12 cm x12 cm) attached to a nebulizer (Dr. Trust, Nureca Inc, New York, USA). In the Normal control (CM and CF) group, rats were sensitized and challenged with intraperitoneal injection and aerosolized saline.

2.4 Measurement of leucocyte count and serum IgE concentration in blood

Total leukocyte, and differential leukocytes (neutrophils, eosinophils, basophils lymphocytes, and monocytes) count in the blood were measured using an automated hematological analyzer (Nihon Kohden Celltac alpha). Serum IgE was also measured. from control and treated groups in blood serum using VIDAS total IgE detection kit based on Enzyme-Linked Fluorescence Assay (ELFA).

2.5 Histological study of lungs

All animal experiments were conducted in accordance with institutional guidelines for the care and use of laboratory animals. Wistar albino rats were anesthetized using chloroform to minimize discomfort, and following adequate anesthesia, the rats were euthanized by exsanguination. The lungs were carefully dissected and immediately rinsed in ice-cold phosphate-buffered saline (PBS) pH=7.0 to remove excess blood. The dissected tissues were then fixed in Bouin's solution for 48 hours at room temperature to preserve cellular structure. For histological processing, fixed tissues were dehydrated through a graded series of ethanol (30%, 70%, 80%, 90%, and 100%), cleared in xylene, and embedded in paraffin wax. Tissue blocks were sectioned into 5µm thick slices using a microtome. Sections were mounted onto glass slides and dried overnight at 37°C. The tissue sections were then deparaffinized in xylene and rehydrated through a descending ethanol series (100%, 90%, 80%, and 70%). Double Staining was performed using hematoxylin & eosin (H&E) for 10 minutes, followed by washing in running tap water to remove excess stain. Stained sections were mounted with coverslips using a permanent mounting medium (DPX). Microscopic examination was carried out using a light microscope (ZEISS Primo Star), and images were captured with imaging software for further analysis.

2.6 Statistical analysis

M.S. Excel was used for statistical analysis of haematological (Total WBCs count and Differential Count) and biochemical parameters (IgE). Mean values were assessed for significance by ANOVA. The probability value (p-value) <0.05 was considered statistically significant.

3. RESULTS

3.1 Ovalbumin induces lung inflammation and increases Pulmo-Somatic Index

The Pulmo-Somatic Index (PSI) was measured after dissection to evaluate the extent of inflammation and edema induced by ovalbumin in the experimental group compared to the control group. Rats in the experimental group (Group:3 to Group:4) showed a significant increase in lung weight compared to the control group ($p < 0.05$). The significant increase in lung weight in the experimental group indicates the presence of asthma-like symptoms characterized by lung inflammation and edema (Figure 1 & Table 1).

Table 1: Pulmo-Somatic Index

S.N.	Animal Group	Mean Body Weight (MBW) in gm	Mean Lung Weight (MLW) in gm	Pulmo-Somatic Index (PSI)
1	Group:1, Control Male	158.16 ± 4.75	1.55 ± 0.21	0.97 ± 0.08
2	Group:2, Control Female	161.33 ± 7.94	1.55 ± 0.42	0.95 ± 0.10
3	Group:3, Male + Ova	144.16 ± 14.44	1.6 ± 0.18	1.11 ± 0.12
4	Group:4, Female + Ova	150.16 ± 7.62	2.75 ± 0.64	1.82 ± 0.40

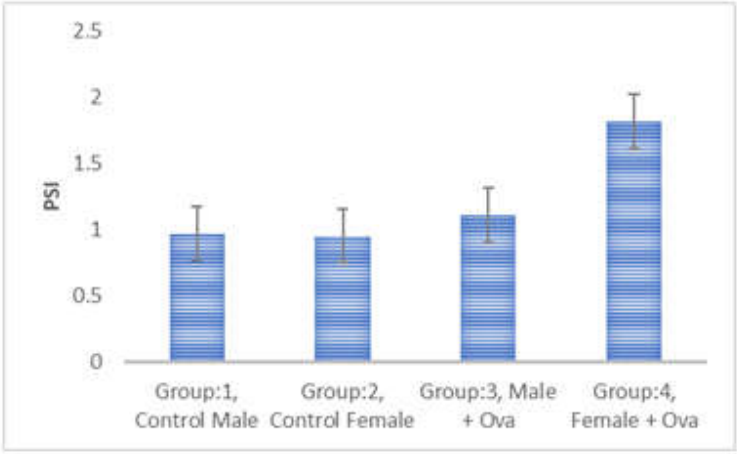

Figure 1: Pulmo-Somatic Index (PSI)

3.2 Ovalbumin provokes immune response and elevates serum IgE levels

The experimental group exhibited significant changes in differential WBC count compared to the control group, indicating a systemic inflammatory response and potential alterations in erythropoiesis. The mean WBC count in different experimental and control groups is summarized in Table 2 and Figure 2. The experimental group showed significant changes in lymphocyte, neutrophil, and eosinophil count compared to the control group (Figure 3). No significant changes were found in the monocyte count (Figure: 4). Changes in lymphocyte, neutrophil, and eosinophil count indicate a systemic inflammatory response and potential alterations in erythropoiesis. Serum IgE in different experimental groups and control groups is summarized in Table 3 and Figure 5. The maximum serum IgE level of 10.83 ng/ml was found in the Female experimental group IV treated with ovalbumin followed by group III (8.83 ng/ml).

Table 2: Mean WBCs Count

Animal Group	Control Male	Control Female	Male + Ova	Female + Ova
Mean WBCs Count (/ml)	5166.667	5616.667	5716.667	7450
SD	1209.408	1527.634	1238.413	1116.692

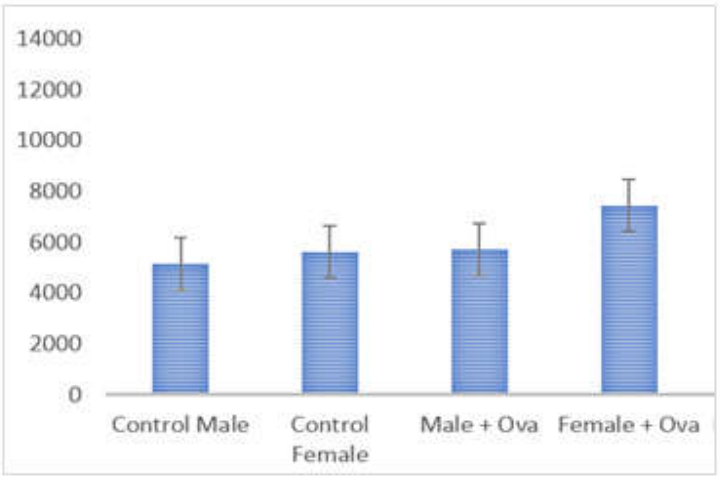

Figure: 2 Mean WBCs Count (/mm³)

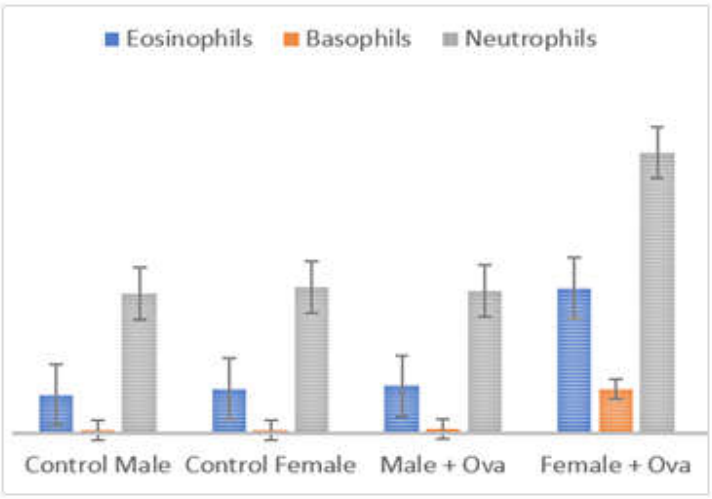

Figure: 3 Granulocyte counts in different experimental groups

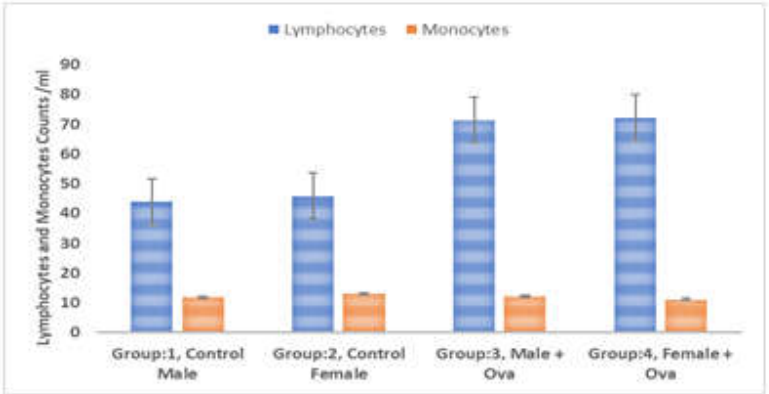

Figure: 4 Agranulocyte counts in different experimental groups

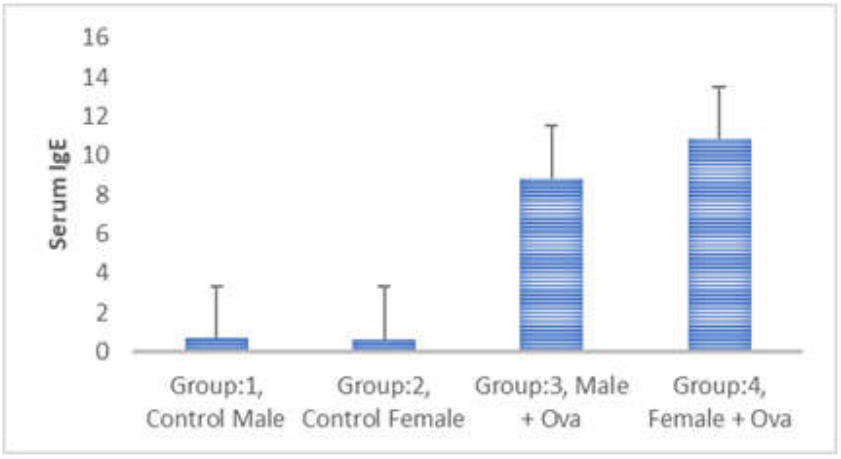

Figure 5: Mean Serum IgE level in different experimental groups.

Table 3: Mean Serum IgE level in different experimental groups.

Animal Group	Control Male	Control Female	Male + Ova	Female + Ova
Mean Serum IgE Level (ng/ml)	0.666667	0.633333	8.833333	10.83333
SD	0.163299	0.136626	4.070217	8.13429

3.3 Ovalbumin induces histopathological alterations in lung tissue

Control groups (I & II) show normal architecture with clear alveolar spaces with no signs of inflammation or cellular infiltration. Ovalbumin groups (III) showed the presence of inflammatory cells, primarily eosinophils and neutrophils, thickening of the bronchial walls with, and increased mucus production and perivascular and peri-bronchial inflammation. Ovalbumin Groups (IV) showed a higher number of inflammatory cells, pronounced bronchial wall thickening, mucus production, and high peri-vascular and peri-bronchial inflammation in comparison with the Ovalbumin Group (III).

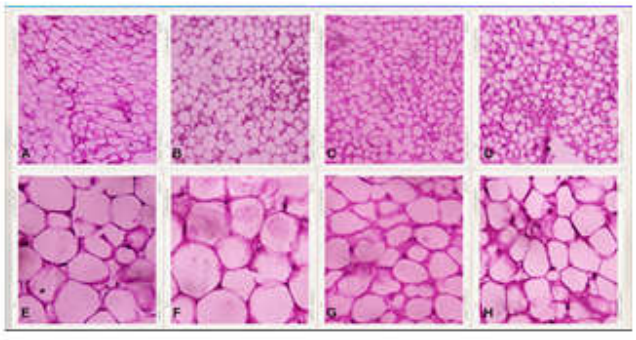

Figure 6: T.S. of Lungs (100X) Alveoli A. Control Male, B. Control Female, C. Ova Male, D. Ova Female. T.S. of Lungs (400X) Alveoli E. Control Male, F. Control Female, G. Ova Male, H. Ova Female

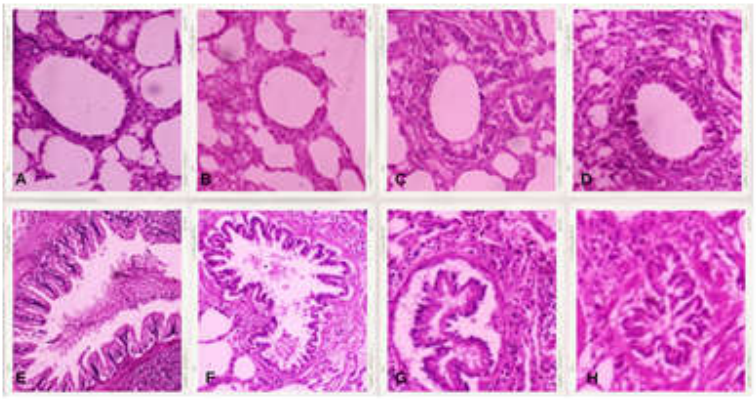

Figure 7: T.S. of Lungs (400X) Alveoli A. Control Male, B. Control Female, C. Ova Male, D. Ova Female. T.S. of Lungs (400X) Bronchioles E. Control Male, F. Control Female, G. Ova Male, H. Ova Female

DISCUSSION

In the present study, the Pulmo-Somatic Index (PSI) was measured to assess the extent of inflammation and edema caused by ovalbumin in the experimental group compared to the control group. The highest PSI of 1.82±0.40 was observed in the female experimental group (Group IV) treated with ovalbumin, followed by Group (III) 1.11±0.12. The notable increase in lung weight in the experimental group as compared with control group suggests the presence of asthma-like symptoms, characterized by lung inflammation and edema. In the experimental group airway inflammation can lead to increased lung weight due to the accumulation of inflammatory cells, fluid, and extracellular matrix components. The measurement of serum IgE levels provides critical insights into ovalbumin-induced experimental groups. In this study, the serum IgE level of 10.83333 ng/ml was observed in the female experimental group (Group IV) treated with ovalbumin followed by Group (III) (8.833333ng/ml). The elevated IgE levels in Group III and IV suggest a significant allergic response. IgE is known to mediate allergic inflammation by binding to high-affinity receptors on mast cells and basophils, leading to the release of pro-inflammatory mediators (Gould *et al.,* 2003). The increase in WBC count, particularly neutrophils, suggests an inflammatory response. This finding aligns with previous research indicating that allergic asthma and rhinitis are associated with systemic inflammation characterized by increased WBC counts, especially neutrophils and eosinophils (Busse *et al.,* 2000). The lack of significant changes in monocyte counts suggests that the inflammatory response in this model is primarily driven by granulocytes and lymphocytes. Histological examination of the lungs revealed that control groups (I, II) maintained normal architecture with clear alveolar spaces and no signs of inflammation or cellular infiltration. In contrast, the ovalbumin groups (III, IV) showed the presence of inflammatory cells, primarily eosinophils and neutrophils, thickening of bronchial walls, increased mucus production, and pronounced perivascular and peribronchial inflammation. A study by Melgert *et al.* (2005) reported that female mice exhibited more severe asthma symptoms compared to male mice, attributed to hormonal differences, particularly the influence of estrogen. They observed that estrogen could enhance eosinophil recruitment to the lungs, thereby increasing inflammation and mucus production. Similarly, a study by Fuseini *et al.* (2017) found that estrogen can modulate airway inflammation by influencing the production of cytokines and other inflammatory mediators Their findings indicated that estrogen might exacerbate airway inflammation, leading to increased lung weight due to the accumulation of inflammatory cells and fluid, aligning with the observations of the current study.

Furthermore, in a clinical setting, Farha *et al.* (2009) highlighted that women with asthma often experience exacerbations in relation to hormonal fluctuations during the menstrual cycle.

5. CONCLUSION

The present study successfully demonstrated that sensitization and challenge with ovalbumin in Wistar albino rats induced significant histopathological changes in the lungs, mimicking key features of asthma and allergic rhinitis. The Pulmo-Somatic Index (PSI) was markedly higher in the experimental groups, particularly in females, indicating significant lung inflammation and edema. Histopathological analysis revealed pronounced peri-bronchial and peri-vascular infiltration of inflammatory cells, particularly eosinophils and neutrophils, thickening of bronchial walls, increased mucus production, and epithelial damage. These changes resemble the airway remodeling observed in human asthma, underscoring the relevance of this animal model for studying allergic airway diseases. The elevated serum IgE levels in ovalbumin-treated groups further supported the allergic response, correlating with the histopathological alterations observed. This study also confirmed that systemic inflammation in this model is primarily lymphocytes, with neutrophils and eosinophils playing a central role, while monocytes showed no significant changes. The findings suggest that gender-related differences in asthma severity could be attributed to the modulatory effects of estrogen on airway inflammation, leading to more pronounced pathological changes in female rats. Overall, the ovalbumin-induced asthma model in Wistar albino rats provides valuable insights into the underlying mechanisms of allergic airway diseases and highlights the role of IgE and granulocytic inflammation in asthma pathophysiology. This model could serve as a robust platform for the evaluation of potential therapeutic interventions aimed at mitigating airway inflammation and remodeling in asthma and allergic rhinitis.

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