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Modulatory role of gabapentin against ovalbumin-induced asthma, bronchial and airway inflammation in mice

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ABSTRACT

Allergic asthma is a type of chronic immune-mediated inflammatory lung disorders with constantly increased worldwide prevalence. Gabapentin is an L-type calcium channel blocker used essentially as antiepileptic and recently has been indicated for management of post-operative and neuropathic pains as an anti-inflammatory. The current study was conducted to evaluate the anti-inflammatory and anti-allergic properties of gabapentin in a mouse-model of Ovalbumin-induced allergic asthma. Mice received OVA (10 mg) adsorbed on $Al(OH)_3$ on days 0 and 7 and were challenged by exposure to nebulized OVA solution (1%) form days 14–16. Asthma induction was associated with significant biochemical, oxidative and inflammatory imbalance. Daily oral gabapentin (50 mg/kg), significantly reduced lung inflammatory cells counts', serum LDH and catalase activities and lung/body weight index. Moreover, gabapentin significantly increased lung GSH concentration and enhanced SOD activity. Lung contents of TNFα, IL-4 and IL-13 significantly declined as well. IL-13; is the major contributor to airway hyper-responsiveness; the charetrestic hallmark of asthma and IL-4; a major chemoattractant cytokine. Lung histopathology significantly improved parallel to the biochemical improvements. In conclusion; Gabapentin's modulatory effect on IL-4, IL-13 and TNFα activities accounts for the observed anti-inflammatory and anti-allergic properties.

1. Introduction

Allergic asthma is a chronic respiratory inflammatory disorder associated with elevated serum levels of Th2 derived cytokines; IL-4, IL-13 with subsequent bronchial hyperactivity and airway infiltration with numerous types of inflammatory cells including; eosinophils and mast cells, goblet cells and smooth muscle hyperplasia and increased serum levels of IgE (Wills-Karp, 1999; Ci et al., 2012).

Tissue and bronchial inflammation are induced by activation of mast cells, eosinophils, and T lymphocytes (Busse and Lemanske, 2001). Long term persistent inflammation induces structural changes in the airway components and alveolar walls. The airway smooth muscle (ASM) mediates airway hyper-responsiveness (AHR); the charetrestic hallmark of asthma which is also believed to conduce to airway inflammation and remodeling with increased sensitivity to different broncho-constrictor stimuli (Zuyderduyn et al., 2008). Airway remodeling, chronic airway inflammation and thickening of all compartments of the airway wall, subsequently supervenes. Such events have profoundly affects airway narrowing and further contributes to the chronicity of asthma (Kudo et al., 2013).

Interleukins (IL); IL-4, IL-5 and IL-13 amongst various other cytokines were reported to significantly contribute to both phases of allergic asthma attack; initiation and challenge phases (Schnyder-Candrian et al., 2006). Continuous bronchial inflammation and release of various inflammatory mediators are believed to initiate and trigger asthma symptoms either directly or indirectly by stimulating ASM constriction, AHR to various stimuli and inducing structural changes in the airway components' wall and eventually airway remodeling (Ozier et al., 2011).

There is increasing proof that inflammation and increased oxidative stress in the airways interplay in the pathogenesis of allergic asthma. Alveolar macrophages isolated from asthmatic patients revealed enhanced $O_2^{\cdot -}$ generation alongside other ROS. Alveolar macrophages, eosinophils and neutrophils from asthmatic subjects were also reported to produce more ROS compared to normal individuals (Dworski, 2000).

Understanding pathogenic pathway underlying allergen-induced modification of both body and immune responses can effectively contribute to exploration and introduction of new and effective asthma therapies. The search for new and effective medications for management of asthma is a dynamic process. Interestingly, exploring already in

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use medications for other pathological conditions that might co-exist with asthma can be an attractive therapeutic approach for certain patients.

Gabapentin is a structural analog of γ-aminobutyric acid. It was initially markted in 1994 for management of partial seizures (Morris, 1999; Rose and Kam, 2002) and later proved pharmacological value as an analgesic (Ho et al., 2006; Gordh et al., 2008). It has been used for management of neuropathic and post-operative pain. Its therapeutic action is mediated *via* voltage-gated calcium ion channels blockade. It binds to the α2*δ* subunit of voltage-dependent calcium channel in the central nervous system (Rose and Kam, 2002). Moreover, gabapentin has been reported to interact with protein kinase C, NMDA receptors and to affect activity of various inflammatory cytokines (Kukkar et al., 2013).

Scientific literatures referred to the antioxidant and neuro-protective properties of gabapentin in different pathological conditions (Baydas et al., 2005). New insights were reported regarding mechanisms of actions of gabapentin, including its anti-inflammatory properties in experimental models of paw edema induced by various agents, including; dextran, carrageenan, histamine, PGE2, compound 48/80, serotonin and bradykinin, with down-regulation of levels of the pro-inflammatory cytokines and attenuation of neutrophil infiltration. Moreover, its anti-oxidant properties have been reported (Dias et al., 2014).

The current study was conducted to evaluate anti-allergic and anti-inflammatory properties of gabapentin (50 mg/kg) against Ovalbumin-induced allergic asthma in mice. Antioxidant, anti-inflammatory and immunomodulatory properties of gabapentin were investigated.

2. Materials and methods

2.1. Animals

Thirty adult male 8 weeks old Swiss Albino mice, purchased from the "Egyptian Organization for Biological Products and Vaccines" (VACSERA, Egypt), weighing approximately (20–25) g, were used to conduct the study. Mice were kept under standardized environmental and nutritional conditions throughout the experimental period. Experimentation procedures were in accordance to the ethical guidelines for animals use in experimental studies adopted by "Research Ethics Committee", Faculty of Pharmacy, Mansoura University, Egypt.

2.2. Drugs and chemicals

Gabapentin, as a fluffy white powder, it was a generous gift from EVA pharmaceuticals (Giza, Egypt). It was dissolved in normal saline for oral administration. **Ovalbumin,** as a creamish white powder, (Loba Chemie Ltd., India).

2.3. Allergic asthma induction

2.3.1. Sensitization and treatment phase

Mice received (10 mg) OVA adsorbed on 1 mg $Al(OH)_3$ by intraperitoneal (IP) injection on days 0 and 7; sensitization phase (Hsu et al., 2013). The mixture was vigorously vortexed prior to sampling to ensure homogenous and effective OVA adsorption on the surface of $Al(OH)_3$. Mice were randomly divided into three experimental groups, each comprising 10 mice: Normal control, mice received 0.2 mL of normal saline, IP and the vehicle orally once daily, Allergic asthma control, mice were sensitized with OVA as previously described and received the vehicle orally once daily; and Gabapentin-treated group, mice were sensitized with OVA as previously described and received gabapentin (50 mg/kg, orally) (Radulovic et al., 1995) once daily. Both gabapentin and the vehicle were administered once daily by oral route starting from day 0 and for further 16 days.

2.3.2. Challenge phase

OVA challenge was performed by exposure to aerosolized 1% OVA in normal saline. Aerosolized OVA was provided using ultrasonic nebulizer in a large exposure compartment system. All the mice in the same experimental group inhaled 1% OVA nebulized solution for 1 h/day at an adjusted constant flow rate for 3 days; days 14–16 (Beigelman et al., 2009; Foster et al., 2011). Normal control mice inhaled nebulized normal saline aerosol instead.

2.4. Sacrification and specimen collection

Twenty four hours post OVA challenge; mice were sacrificed by an overdose of thiopental sodium (40 mg/kg, I.P.). Blood samples were collected *via* puncture of the retro-orbital plexus, left to coagulate for 30 min, centrifuged (3000 rpm for 15 min) and sera were separated and used for biochemical analyses.

2.5. Collection Bronchoalveolar lavage fluid (BALF) and quantification of pulmonary inflammatory cells

After exsanguination, mice were placed on their dorsal side, the thoracic cavities were opened, and the tracheas were cannulated. One ml of ice-cold saline was infused and extracted and the procedure was repeated twice to perform BALF. About 0.70 ± 0.30 ml/mouse were retrieved and were centrifuged (4000 rpm, 4 °C, 10 min) (Dhami et al., 2001). The cell pellets were re-suspended in 200 µL of fresh ice cold saline. Lymphocytes, neutrophils, eosinophils and monocytes were counted (Said et al., 2018; Locke et al., 2007). Then, the lungs were isolated, washed in ice-cold saline, and weighed for calculation of lung/body weight index.

2.6. Preparation of Lung homogenate and determination of lung total protein and malondialdehyde (MDA) contents, catalase and superoxide dismutase (SOD) activities and reduced glutathione (GSH) concentration

Five hundred mg of lung samples from the right lung lobes were homogenized as described by (Buege and Aust, 1978; Abdelaziz et al., 2015) and used for quantification of lung total protein and malondialdehyde (MDA) contents, superoxide dismutase (SOD) activity, reduced glutathione (GSH) concentration, and catalase activity using Biodiagnostic assay kits (Giza, Egypt) as instructed by the manufacturer. Total lung protein contents were quantified using BCA Protein Assay kit (Thermo Scientific, Rockford, IL, USA) as instructed by the manufacturer.

2.7. Determination of lactate dehydrogenase (LDH) and catalase activities in serum samples

Lactate dehydrogenase (LDH) and catalase activities were quantified in serum samples as instructed by the manufacturer; Biodiagnostic Co. (Giza, Egypt).

2.8. Determination of lung tumor necrosis factor–α (TNF-α), lung and BALF's interleukin-4 (IL-4), and IL-13 contents

Lung contents of TNF-α,IL-4 and IL-13 were quantified as instructed by the manufacturer using ELISA kits; Bender MedSystems GmnH, (Vienna, Austria).

2.9. Histopathological examination

The isolated left lung lobes were fixed in 10% buffered formalin. Five µm thick lung apecimen were prepared and stained with hematoxylin and eosin (H&E). Specimens were examined randomly, and the pathologist was masked to the experimental groups. Semi quantitative assessment of inflammation was performed as described by (Zeldin

et al., 2001; Elekes et al., 2007). The following parameters were scored and the individual scores of each parameter were summed to yield an inflammatory score of 0–10:

- Perivascular/peribronchial acute inflammation,
- Perivascular edema and,
- Number of macrophages/mononuclear cells in the alveolar spaces.

2.10. Statistical analyses

Data are expressed as mean ± SEM. Data were statistically analyzed using ANOVA followed by Tukey-Kramer's post-hoc test for comparison between parametric data and Kruskal-Wallis followed by Dunn's post-hoc test for comparisons between non-parametric data. $p < 0.05$ were considered statistically significant. Instat-3 software was used for statistical analyses.

3. Results

3.1. Effect of gabapentin (50 mg/kg) on lung/body weight index

As presented in (Fig. 1), allergic asthma progression in asthmatic control was associated with a significant escalation in lung/body weight index in comparison to normal control by approximately 1.5 folds. Oral gabapentin (50 mg/kg) did not induce a significant decrease in lung/body weight index in comparison to the asthmatic control ($p < 0.05$).

3.2. Effect of gabapentin (50 mg/kg) on BALF's total and differential inflammatory cell content

Asthmatic control group revealed a significant increase in total inflammatory cell count in BALF in comparison to normal control by approximately 5 folds with about 12 folds increase in eosinophil count. Oral gabapentin (50 mg/kg) induced a significant decrease in total inflammatory cell count by about 55% compared to asthmatic control and induced a significant decrease in differential cell count by an average; monocytes 68%, neutrophils and eosinophils 100%, in comparison to asthmatic control ($p < 0.05$), Fig. 2.

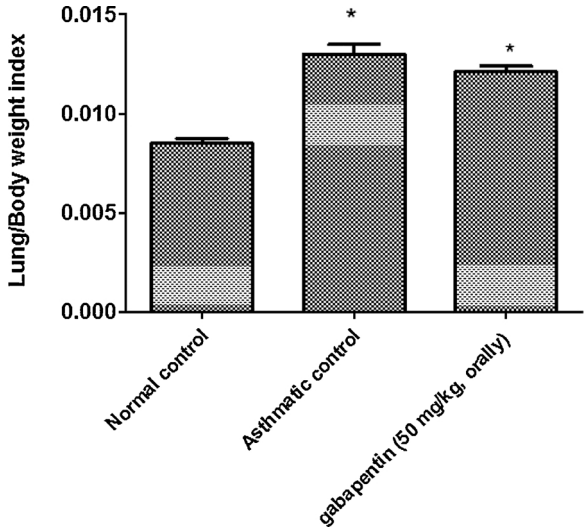

Fig. 1. Effect of daily oral gabapentin (50 mg/kg) for 16 days on lung/ body weight index in asthmatic mice:
Data are expressed as mean ± SEM of (10 mice/group).
Allergic airway inflammation was induced by I.P injection of 10 mg Ovalbumin +1 mg $Al(OH)_3$, on days 0 and 7, gabapentin was administered orally from day 0 to day 16.
Statistical analysis was performed using (ANOVA) followed by Tukey-Kramer's multiple comparisons test.
*Significantly different *Vs* normal control, ($p < 0.05$).
#Significantly different *Vs* asthmatic control, ($p < 0.05$).

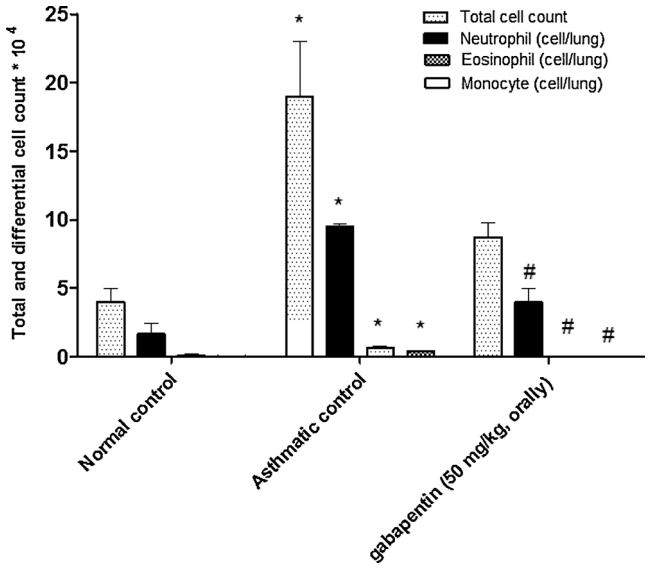

Fig. 2. Effect of daily oral gabapentin (50 mg/kg) for 16 days total and differential cell counts in asthmatic mice.
Data are expressed as mean ± SEM of (10 mice/group).
Allergic airway inflammation was induced by I.P injection of 10 mg Ovalbumin +1 mg $Al(OH)_3$, on days 0 and 7, gabapentin was administered orally from day 0 to day 16.
Statistical analysis was performed using (ANOVA) followed by Tukey-Kramer's multiple comparisons test.
*Significantly different *Vs* normal control, ($p < 0.05$).
#Significantly different *Vs* asthmatic control, ($p < 0.05$).

3.3. Effect of gabapentin (50 mg/kg on total lung protein content and serum LDH activity

Asthmatic control mice revealed a significantly increased total lung protein content by about 2 folds compared to normal control. Oral gabapentin (50 mg/kg) induced a decrease in total lung protein content compared to asthmatic control but in a non-significant way, Table 1. Meanwhile, asthmatic control had a significantly increased serum LDH activity compared to normal control by about 3 folds, daily oral gabapentin (50 mg/kg) significantly reduced serum LDH activity by 55% compared to asthmatic control group ($p < 0.05$), Table 1.

Table 1
Effect of daily oral gabapentin (50 mg/kg) for 16 days on total lung protein content and serum LDH activity in asthmatic mice.

Experimental groups	Total lung protein content (μg/mg tissue)	Serum LDH (U/L)
Normal control	7.991 ± 0.732	50.12 ± 2.890
Asthmatic control	14.002 ± 0.619 *	160.55 ± 13.037*
Gabapentin (50 mg/kg, orally)/ Ovalbumin (I.P)	11.524 ± 0.913	72.87 ± 3.413#

Data are expressed as mean ± SEM of (10 mice/group).
Allergic airway inflammation was induced by I.P injection of 10 mg Ovalbumin +1 mg $Al(OH)_3$, on days 0 and 7, gabapentin was administered orally from day 0 to day 16.
Statistical analysis was performed using (ANOVA) followed by Tukey-Kramer's multiple comparisons test.
* Significantly different *vs* normal control, ($p < 0.05$).
\# Significantly different Vsasthmatic control, ($p < 0.05$).

Table 2
Effect of daily oral gabapentin (50 mg/kg) for 16 days on lung malondialdehyde (MDA) content, superoxide dismutase (SOD) activity and reduced glutathione (GSH) content in asthmatic mice.

Experimental groups	MDA (nmol/mg.tissue)	SOD (U/gm tissue)	GSH (mg/mg tissue)
Normal control	0.48 ± 0.031	44.230 ± 2.282	0.869 ± 0.074
Asthmatic control	1.15 ± 1.106*	12.019 ± 1.500*	0.543 ± 0.013*
Gabapentin (50 mg/kg, orally) / Ovalbumin (I.P)	1.07 ± 0.064	65.580 ± 2.518 *,#	0.682 ± 0.005 *,#

Data are expressed as mean ± SEM of (10 mice/group).
Allergic airway inflammation was induced by I.P injection of 10 mg Ovalbumin + 1 mg $Al(OH)_3$, on days 0 and 7, gabapentin was administered orally from day 0 to day 16.
Statistical analysis was performed using (ANOVA) followed by Tukey-Kramer's multiple comparisons test.
* Significantly different *vs* normal control, ($p < 0.05$).
# Significantly different Vsasthmatic control, ($p < 0.05$).

3.4. Effect of gabapentin (50 mg/kg) on oxidative/anti-oxidative stress hemostasis

3.4.1. Lung MDA content

As presented in Table 2, asthmatic control revealed a significant augmentation in lung MDA content compared to normal control by about 2.5 folds. Daily oral gabapentin (50 mg/kg) failed to induce a significant decrease in lung MDA content compared to asthmatic control group ($p < 0.05$).

3.4.2. Lung SOD activity

Lung SOD activity in asthmatic control mice significantly declined in comparison to normal control by approximately 3 folds. Oral gabapentin (50 mg/kg) induced a significant increase in lung SOD activity by about 78% compared to asthmatic control, Table 2

3.4.3. Lung GSH concentration

As presented in Table 2, allergic asthma control revealed a significant decrease in lung GSH content compared to normal control by approximately 2 folds. Oral gabapentin (50 mg/kg) significantly increased lung GSH content compared to normal control group by about 22% ($p < 0.05$).

3.5. Effect of gabapentin (50 mg/kg) on serum and lung catalase activities

3.5.1. Serum catalase activity

As shown in Table 3 induction of allergic asthma in asthmatic control decreased serum catalase activity compared to normal control by about 2 folds. Oral gabapentin (50 mg/kg) resulted in a significant enhancement in serum catalase activity by about 45% compared to asthmatic control. Gabapentin treatment succeeded to restore serum catalase activity to almost normal levels observed in the normal control ($p < 0.05$).

3.5.2. Lung catalase activity

In context, allergic asthma progression induced a significant decrease in lung catalase activity by about 2 folds compared to normal control. On the other hand, daily oral gabapentin (50 mg/kg) significantly boosted lung catalase activity by about 66% compared to asthmatic control ($p < 0.05$), Table 3.

3.6. Effect of gabapentin (50 mg/kg) on lung tumor necrosis factor-α (TNF-α) content

As shown in Table 4 lung TNF-α content in asthmatic control significantly escalated in comparison to normal control by about 3 folds. Daily oral gabapentin (50 mg/kg significantly lessened lung TNF-α content by about 40% in comparison to asthmatic control ($p < 0.05$).

3.7. Effect of gabapentin (50 mg/kg) on lung and BALF interleukin-4 (IL-4) contents

As shown in Table 5, allergic asthma progression in asthmatic control significantly increased lung IL-4 content compared to normal control by about 2 folds. Oral gabapentin (50 mg/kg) forced a significant retraction in lung IL-4 content by approximately 50% compared to asthmatic control. Almost basal IL-4 levels were restored with gabapentin treatment compared to normal control. Meanwhile, allergic asthma induced a non significant elevation in BALF IL-4 content compared to normal control group ($p < 0.05$).

Table 3
Effect of daily oral gabapentin (50 mg/kg) for 16 days on serum and lung catalase activities in asthmatic mice.

Experimental groups	Serum catalase (U/L)	Lung catalase (U/gm)
Normal control	580.920 ± 18.268	567.237 ± 33.883
Asthmatic control	351.206 ± 37.384*	344.817 ± 48.279*
Gabapentin (50 mg/kg, orally) / Ovalbumin (I.P)	641.100 ± 43.769#	1023.444 ± 55.328*,#

Data are expressed as mean ± SEM of (10 mice/group).
Allergic airway inflammation was induced by I.P injection of 10 mg Ovalbumin + 1 mg $Al(OH)_3$, on days 0 and 7, gabapentin was administered orally from day 0 to day 16.
Statistical analysis was performed using (ANOVA) followed by Tukey-Kramer's multiple comparisons test.
* Significantly different *Vs* normal control, ($p < 0.05$).
# Significantly different Vsasthmatic control, ($p < 0.05$).

Table 4
Effect of daily oral gabapentin (50 mg/kg) for 16 days on lung tumor necrosis factor- α (TNF-α) content in asthmatic mice.

Experimental groups	Lung TNF- α (pg/mg tissue)
Normal control	3.845 ± 0.376
Asthmatic control	10.420 ± 1.020*
Gabapentin (50 mg/kg, orally) / Ovalbumin (I.P)	6.220 ± 0.606#

Data are expressed as mean ± SEM of (10 mice/group).
Allergic airway inflammation was induced by I.P injection of 10 mg Ovalbumin + 1 mg $Al(OH)_3$, on days 0 and 7, gabapentin was administered orally from day 0 to day 16.
Statistical analysis was performed using (ANOVA) followed by Tukey-Kramer's multiple comparisons test.
* Significantly different *Vs* normal control, ($p < 0.05$).
# Significantly different Vsasthmatic control, ($p < 0.05$).

Table 5
Effect of daily oral administration of gabapentin (50 mg/kg) for 16 days on lung and BALF interleukin-4 (IL-4) contents in asthmatic mice.

Experimental groups	Lung IL-4 (*pg*/mg tissue)	BALF IL-4 (*pg*/ml)
Normal control	1.022 ± 0.102	1.82 ± 0.07
Asthmatic control	2.240 ± 0.108*	1.835 ± 0.07
Gabapentin (50 mg/kg, orally) / Ovalbumin (I.P)	1.114 ± 0.102#	1.80 ± 0.015

Data are expressed as mean ± SEM of (10 mice/group).
Allergic airway inflammation was induced by I.P injection of 10 mg Ovalbumin +1 mg Al(OH)3, on days 0 and 7, gabapentin was administered orally from day 0 to day 16.
Statistical analysis was performed using (ANOVA) followed by Tukey-Kramer's multiple comparisons test.
* Significantly different *Vs* normal control, ($p < 0.05$).
# Significantly different Vsasthmatic control, ($p < 0.05$).

Table 6
Effect of daily oral gabapentin (50 mg/kg) for 16 days on lung and BALF interleukin-13 (IL-13) content in asthmatic mice.

Experimental groups	Lung IL-13 (*pg*/mg tissue)	BALF IL-13 (*pg*/ml)
Normal control	4.197 ± 0.396	15.57 ± 1.12
Asthmatic control	14.643 ± 1.228*	15.60 ± 0.60
Gabapentin (50 mg/kg, orally) / Ovalbumin (I.P)	7.773 ± 0.719#	13.95 ± 0.20

Data are expressed as mean ± SEM of (10 mice/group).
Allergic airway inflammation was induced by I.P injection of 10 mg Ovalbumin +1 mg $Al(OH)_3$, on days 0 and 7, gabapentin was administered orally from day 0 to day 16.
Statistical analysis was performed using (ANOVA) followed by Tukey-Kramer's multiple comparisons test.
* Significantly different *Vs* normal control, ($p < 0.05$).
# Significantly different Vsasthmatic control, ($p < 0.05$).

3.8. Effect of gabapentin (50 mg/kg) on lung and BALF interleukin-13 (IL-13) contents

As shown in Table 6, lung IL-13 content in asthmatic control significantly increased in comparison to normal control by about 4 folds. Daily oral gabapentin (50 mg/kg), significantly suppressed lung IL-13 content by about 57% compared to asthmatic control. Basal lung IL-13 content in gabapentin treated group was restored compared to normal control. Allergic asthma induction resulted in non significant increase in BALF IL-13 content in comparison to normal control ($p < 0.05$).

3.9. Effect of gabapentin (50 mg/kg) on histopathological examination of lung specimen stained with H&E stain

Histopathological inspection of lung sections stained with H&E stain of normal control mice showed uniform lung histopathology with no evidence of either perivascular edema or peribronchial inflammation (Fig. 3A), while specimen from asthmatic control revealed significant build up of peribronchial and macrophage infiltration in the alveolar space by 100% in comparison to normal control and significant perivascular edema, (Table 7; Fig. 1; B, C, D). In context, lung specimen examination of asthmatic control mice revealed severe inflammation. Perivascular edema infiltrated more than 70% of perivascular circumference with evident peribronchial mononuclear infiltration, congestion of alveolar spaces. Alveolar inflammatory cells and macrophages infiltrated more than 25% of alveolar spaces, (Fig. 3B, C, D).

On the other hand, daily oral gabapentin (50 mg/kg) significantly reduced perivascular edema by about 35% but failed to induce a decrease in peribronchial inflammation. Gabapentin treatment decreased macrophages infilteration in alveolar space by about 18% compared to the asthmatic control (Table 7; Fig. 2E, F, G).

4. Discussion

Allergic asthma is a disease of increased worldwide prevalence with immense cost on patients' quality of life. The off-label use of certain therapeutic agent appears to be attractive approach. The observations of the current study sheds light on the protective efficacy of gabapentin; an anticonvulsant used for management of neuropathic and post-operative pain due to its anti-inflammatory properties in amelioration of oxidative, biochemical and inflammatory aberrations associated with allergic asthma progression in an Ovalbumin-induced allergic asthma in mice.

In the experimental model of OVA-induced allergic asthma, OVA sensitization then challenge is sufficient to stimulate the hallmark characteristics of clinical asthma, including; elevated levels of IgE, airways inflammation, and exacerbations of bronchial remodeling (Nials and Uddin, 2008; Manni et al., 2014).

Total lung protein has been reported to be a significant index of lung permeability, (Ge et al., 2010). Lung/body weight index and BAL content of inflammatory cells, lung and histopathology impairments significantly increased in allergic asthma control, confirming incidence of pulmonary edema and enhanced extravasation of serous fluids into lung tissue. Observations of Zhang et al. (2009) and Guo et al. (2016) give credence to the current observations.

Neutrophils and macrophages are involved in conversion of molecular oxygen to ROS (Koyama et al., 2001). The release of a wide array of mediators, including TNF-α, ROS and proteolytic enzymes propagate following neutrophils activation, further recruiting other inflammatory cells contributing to both local pulmonary injury and edema development in the airspaces (Zhai et al., 2004; Rimal et al., 2005), which may account for the increase in lung/body weight index in asthmatic control group observed in the current study.

Asthma progression in the current study was associated with a significant increase in serum lactate dehydrogenase (LDH) activity. Lung contents of TNF-α, IL-4 and IL-13 significantly increased as well confirming incidence of pulmonary inflammation and development of AHR. Indeed, the recruitment and chemoattraction of inflammatory cells to the airways, challenged by OVA which is associated with increased airway hyper-reactivity and increased levels of OVA-specific IgE, IL-4 and IL-13, (Park et al., 2009; Xiong et al., 2015),

Nevertheless, active metabolites production and reactive free radicals generation have been reported to contribute to pulmonary damaging effect of Ovalbumin (Tewtrakul et al., 2003). In agreement, Ovalbumin induced a significant oxidants load with exhaustion of antioxidants defenses. Lung malondialdehyde (MDA) content significantly escalated, while, lung reduced glutathione (GSH) concentration, superoxide dismutase (SOD) activity and serum and lung catalase activities significantly declined.

Oxidative stress contributes to asthma pathogenesis (Yosri et al., 2017). Moreover, oxidative stress's deleterious impacts have been reported to be significantly augmented inflammation is prominent. Accumulated lines of evidence confirm that implication and interplay of both of inflammation and increased oxidative within the airways of asthmatic experimental models to be strongly correlated (Paredi et al., 2002; Jung et al., 2011).

Animal models have confirmed ROS to add to airway hyper-responsiveness (AHR). ROS increase vagal tone due to damage of oxidant-sensitive β-adrenergic receptors and suppression of mucociliary clearance. H_2O_2 enhanced airway smooth muscle (ASM) contractility involved in AHR in animal models, (Lee et al., 2006). Moreover, ROS were reported to directly stimulate histamine release from mast cells and enhance mucus secretion from airway epithelial cells (Krishna et al., 1998). Increased generation of ROS directly induces oxidative damage and sheds epithelial cells. Nevertheless, previous studies have

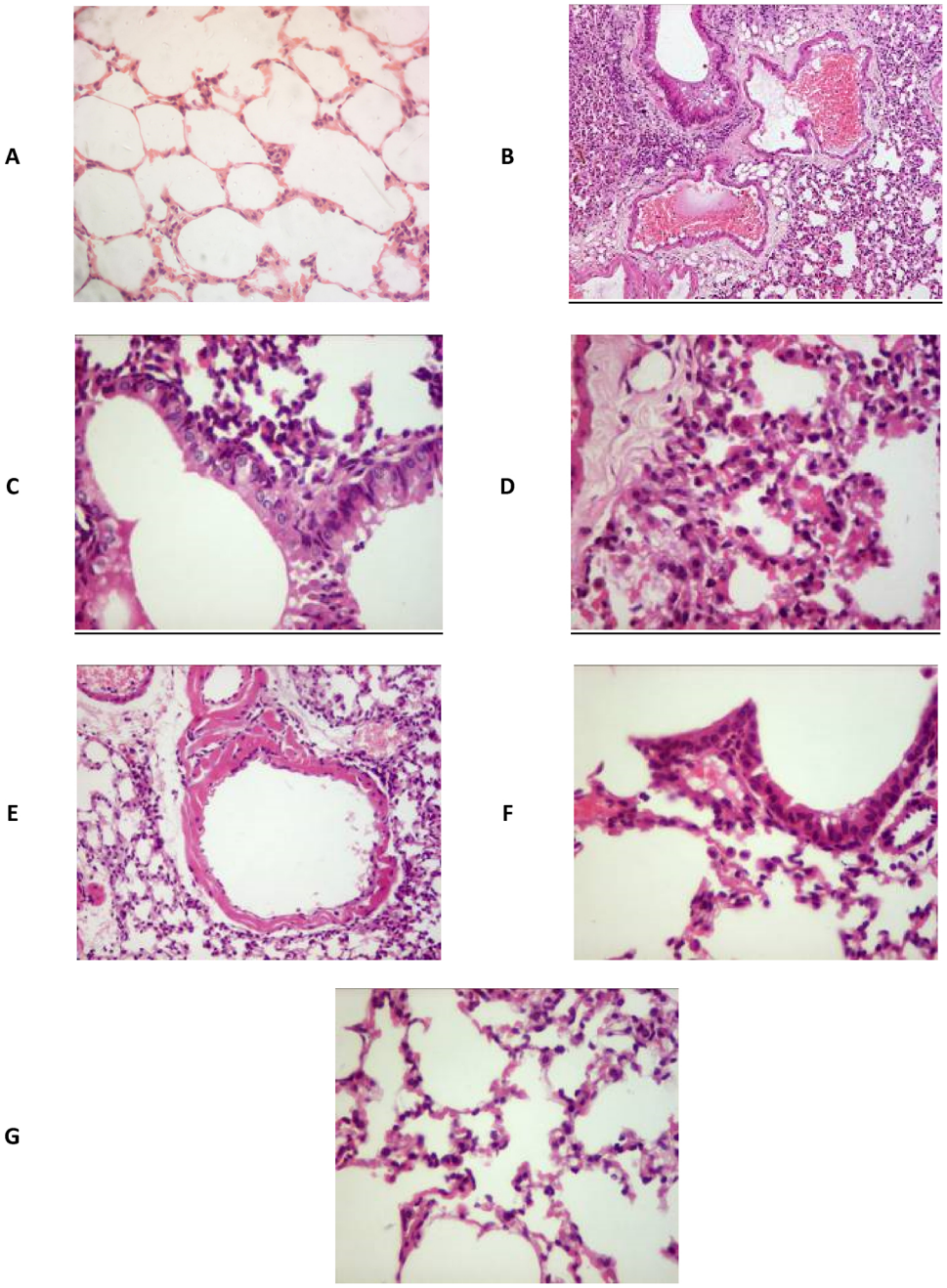

Fig. 3. Effect of daily oral gabapentin (50 mg/kg) for 16 days on histopathological examination in asthmatic mice:
A; Normal control: no inflammation, edema, H&E (400x). B, C, D; Asthmatic control: perivascular edema (100x), peribronchial inflammation (400x) and macrophages in alveolar spaces (400x) respectively. E, F, G; Gabapentin (50 mg/kg): Specimens showing moderate perivascular edema, peribronchial inflammation and decrease in macrophages in alveolar space, H&E (100x), (400x) & (400x) respectively.

reported ROS to contribute to endothelial barrier dysfunction and to increase fluid permeability, macromolecules and inflammatory cells recruitment (Park et al., 2009).

TNF-α is a cytokine with a variety of effects including stimulation and inhibition of cell growth, cytotoxicity, angiogenesis, inflammation and immune-modulation. Its role in many inflammatory and respiratory diseases of which; asthma has been well documented (Mukhopadhyay et al., 2006; Menzella et al., 2015). TNFα is primarily produced by immune cells such as macrophages and monocytes but B-lymphocytes, T-cells, eosinophils, mast cells and glial cells; all of which implicated in pathogenesis of asthma, have been also reported to release TNFα upon stimulation (Esposito and Cuzzocrea, 2009). High level of TNF-α is linked directly to sever asthma and asthmatic complications. Moreover, TNF-α has been reported to be involved in potentiation of histamine release in association with decreased antigen concentration (Mukhopadhyay et al., 2006).

TNF-α is a major contributor to induction of inflammatory cascade and severity of tissue injury (Giebelen et al., 2007), neutrophils' adherence to pulmonary capillaries, extravasation into the alveolar space and subsequent activation (Ware, 2006). Moreover, TNF-α further augments oxidative stress by sensitizing infiltrating leukocytes and increasing oxidant production (Said et al., 2018; Manna et al., 1998).

In the current study, asthma development was associated with a significant increase in lung contents of both IL-4 and IL-13 respectively, which escalated by 2 and 4 folds respectively. On the other hand, BALF's content of both mediators did not demonstrate any significant

Table 7
Effect of daily oral gabapentin (50 mg/kg) for 16 days on histopathological examination of lung specimen stained with H&E stain of asthmatic mice.

Experimental groups	Perivascular edema	Peribronchial inflammation	Macrophage and mononuclear cells in alveolar space
Normal control	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Asthmatic control	1.7 ± 0.300*	1.50 ± 0.100*	2.0 ± 0.00*
Gabapentin (50 mg/kg, orally) / Ovalbumin (I.P)	1.0 ± 0.288#	1.00 ± 0.057 *,#	1.3 ± 0.173#

Data are expressed as mean ± SEM of (10 mice/group).
Allergic airway inflammation was induced by I.P injection of 10 mg Ovalbumin +1 mg $Al(OH)_3$, on days 0 and 7, gabapentin was administered orally from day 0 to day 16.
Statistical analysis was performed using (ANOVA) followed by Tukey-Kramer's multiple comparisons test.
* Significantly different *vs* normal control, ($p < 0.05$).
# Significantly different Vsasthmatic control, ($p < 0.05$).

change. Th_2 derived cytokines IL-4 and IL-13 which are well documented to play pivotal roles in allergic inflammation and the development of AHR (Hansbro et al., 2013). Both IL-4 and IL-13 are crucial for inducing B lymphocytes switching for towards IgE production (Lemanske and Busse, 2010). The IL-4Rα/IL-13Rα1 complex mediates Signal transducer and activator of transcription 6 (Stat6) signaling, proven to be essential for development of AHR in experimental models of asthma (Bellini et al., 2012). IL-4 is a crucial effector for Th_2 lymphocyte differentiation (Barnes, 2008), where, IL-4 deficient mice have been reported to be resistant to asthma progression (Yosri et al., 2017).

Notably, IL-13 was reported to be involved in activation of Stat6 by signal transduction mediated by IL-4, mediating AHR. A time dependent relationship exists between the requirement of both of IL-4 and IL-13 and AHR progression (Yosri et al., 2017). IL-13 signaling blockade, but not IL-4 before allergen challenge attenuated AHR as reported by (Wills-Karp, 1999), which proposes dependence of AHR during allergen exposure on IL-13. Moreover, IL-13 stimulated ASM, up-regulated RhoA protein which stimulated Rho-kinase and enhanced calcium sensitivity. IL-13 has been proven to enhance the production of IgE by plasma, enhance the release of eosinophil chemo-attractants and the contraction of airway smooth muscles cells (Herrick and Bottomly, 2003).

A therapeutic agent that can interfere with the aforementioned sequel and down-regulates oxidative stress can be presumed to offer significant protection against allergic asthma progression. Gabapentin has been reported to demonstrate anti-inflammatory and antioxidant properties (Dias et al., 2014). It binds to the α2δ subunit of voltage-gated Ca^{2+} channels thereby inhibiting Ca^{2+} influx (Oka et al., 2003). Moreover, gabapentin interacts with NMDA receptors and protein kinase C (Kukkar et al., 2013). Gabapentin has also been reported to reduce the activity of Na^+ and K^+ channels, involved in excitotoxicity. Moreover, the anticonvulsant effect of gabapentin is reported to be mediated by blockage of calcium influx into neurons (Baydas et al., 2005).

Gabapentin administration for 16 days (30 mg/kg) alleviated Ovalbumin-induced allergic pathology and demonstrated significant improvement on several scales. The beneficial effect of gabapentin observed in the current study might have arisen during the sensitization phase and outcomes appeared during the challenge phase, gabapentin effectively down-regulated expression of asthma-associated cytokines and attenuated inflammatory changes associating asthmatic attack in the lung tissue. Lung contents of total protein, inflammatory cells, reduced GSH concentration, SOD, catalase activities significantly increased with concomitant histopathological improvement. Interestingly, lung contents of TNFα, IL-4 and IL-13 significantly declined, confirming its modulatory role in pathogenesis of allergic asthma, most significantly airway inflammation and AHR.

According to the observed results, given that gabapentin enhanced antioxidant defenses without successfully significantly reducing lung MDA content, it can be presumed that gabapentin decreased the tissue damage by enhancing the production and action of endogenous antioxidants systematically and within the lung which may outpoint the significant positive impact of gabapentin in the administered dose (50 mg/kg) on the host antioxidant defenses. Kumar et al. (2012), Dias et al. (2014) reported gabapentin to decrease MDA concentration in three different murine models. Interestingly, Abdel-Salam et al. (2012) reported gabapentin to increase brain lipid peroxidation and decrease brain antioxidant enzymes while studying the effect of gabapentin on oxidative stress in a rat model of toxic demyelination in brain. Such observation can be accounted for on the basis of either doses or treatment duration differences.

Results presented in allergic airway model have shown the ability of gabapentin to significantly increase lung content of GSH and SOD, moreover, lung and serum catalase activities were restored and exceeded the normal levels to combat the increase in oxidative stress as well. These results are in accordance with the previous studies of Kumar and Goyal (2008) who reported that treatment with gabapentin in acute hypoxic stress–induced behavioral changes and oxidative damage in mice significantly reduced oxidative damage by increasing GSH concentration and catalase activities.

Alongside the improvement in oxidative stress biomarkers, there was a significant attenuation in serum LDH which is a marker of cell damage in both allergic airway and acute lung injury models, suggesting cytoprotective effect of gabapentin which is in line Oka et al. (2003) who reported gabapentin to preferentially inhibit LDH leakage induced by mild hypoxic insults.

The α2δ-1 dependent analgesic actions of gabapentin in neuropathic pain were correlated with inhibition of inflammatory cytokines and inflammation, as demonstrated by Wodarski et al. (2019) who studied the effectiveness of gabapentin in attenuating allodynia in STZ-induced diabetic neuropathic pain. Gabapentin significantly reduced IL-4 and IL-13 in lung tissue. It can be inferred that the anti-inflammatory effects of gabapentin are mediated through the inhibition of cytokines involved in OVA-induced allergic asthma, and this result is in agreement with Dias et al. (2014) who reported gabapentin to exhibit anti-inflammatory activity in mice by decreasing the action of inflammatory mediators, neutrophil migration and pro-inflammatory cytokine levels. These results are also in accordance with the previous studies of Rosa et al. (2017) who reported gabapentin to impair the T-cell receptor–-driven calcium response and cytokine production associated with the loss of α2δ2 protein in TH2 cells.

In conclusion, gabapentin demonstrated significant anti-inflammatory and antioxidant properties associated with significant attenuation of allergic asthma-induced damage. The observed effect could be mediated primarily by enhancing the production and action of endogenous antioxidants, suppression of inflammatory cytokine secretion; TNF-α, its modulatory effect on IL-4 and IL-13; the charetrestic hallmarks for asthma and its associated AHR. Gabapentin's Ca^{+2} channel blocking action can be presumed to contribute to the observed effect with attenuation of oxidative stress and inflammatory-induced tissue damage. Further experimental and clinical studies investigating dose and time-dependent effect of gabapentin are required to validate its anti-asthmatic properties and optimize therapeutic outcomes.

Conflict of interest

None

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