



Nicorandil alleviates ovalbumin-induced airway inflammation in a mouse model of asthma

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ABSTRACT

Nicorandil is an antianginal drug that has anti-inflammatory property. This study aimed to investigate the effects of nicorandil on allergic asthma induced by ovalbumin (OVA) in mice in comparison with dexamethasone. Mice were sensitized to OVA (on days 0 and 7) and challenged with OVA three times (on days 14, 15 and 16). Nicorandil was given orally for 5 days 1 h before OVA treatment in days of challenge. Progression of asthma was accompanied by significant elevation in the lung/body weight index, LDH, total protein, IL-13 and NF-κB levels besides inflammatory cell counts in BALF; Also pulmonary MDA and NO contents were significantly increased but GSH and SOD levels were decreased. Histopathological alterations in lung tissues were also observed. In contrast, nicorandil treatment significantly alleviated OVA-induced lung injury. In conclusion, our results proposed that nicorandil is equivalent to dexamethasone in ameliorating allergic asthma by restoring oxidant/antioxidant balance and reducing inflammation.

1. Introduction

Allergic asthma is an inflammatory disorder associated with obstruction of airway, airway hyperresponsiveness (AHR) and overproduction of mucus (Holgate, 2012). T-helper type 2 (Th2) cells produce cytokines, such as IL-13, IL-5 and IL-4. These cytokines have a critical role in the pathogenesis of asthma via stimulating production of immunoglobulin (Ig)E by B cells and infiltration of inflammatory cells (Wei et al., 2016). Consequently, inhibition of these cytokines may offer prospective therapeutic approach for allergic asthma treatment.

NF-κB, a vital transcription factor, plays a crucial role in inflammatory disorders such as chronic obstructive pulmonary disease as well as asthma. This is attributed to its involvement in regulating the production of various mediators of inflammation (Suzuki et al., 2011).

Corticosteroids are usually used in relieving the undesired manifestations of asthma. However, adverse effects such as hypertension, cataract, and osteoporosis and growth retardation in children might result from long run treatment (Durrani et al., 2011). Therefore, many attempts have been made to find more safe alternatives.

Nicorandil is an antianginal drug characterized by coupling of a nitric oxide (NO) donor with nicotinamide. Also, it acts as ATP-dependent K⁺ channels opener and activates guanylyl cyclase. It has potent beneficial effects in the vascular system through reducing preload and afterload besides increasing coronary perfusion (Lefer and Lefer, 1988). Many studies reported protective effects of nicorandil in myocardial infarction (Wu et al., 2015, Yamada et al., 2016).

Furthermore, nicorandil was identified to increase angiogenesis and reduce isoproterenol-induced cardiac hypertrophy, fibrosis and inflammation in combination with mesenchymal stem cells (Mohamed et al., 2015). Nicorandil was also shown to inhibit inflammation and alleviate chronic renal injury (Tamura et al., 2012).

This work was carried out to explore the mechanisms and beneficial effects of nicorandil against ova-induced allergic airway inflammation in a mouse model in comparison to dexamethasone treatment.

2. Materials and methods

2.1. Chemicals

Nicorandil was purchased as tablets (Adancor®, Merck, Egypt) and was suspended in CMC (0.5%). Dexamethasone was purchased as a pharmaceutical drug (Fortecortin ampoules 8 mg/2 ml). Ova albumin (OVA) was obtained as pure powder (Loba Chemie PVT. Ltd. (Bombay, India). All chemicals used were of high quality.

2.2. Animals

Forty male Swiss Albino mice (weighing 25 ± 5 g) were used. Mice were placed in plastic cages at temperature 25 °C, allowed free food and water and sustained on a 12 h light/dark cycle. Whole experiments fulfilled the requirements of Mansoura University Animal Care and Ethics Committee.

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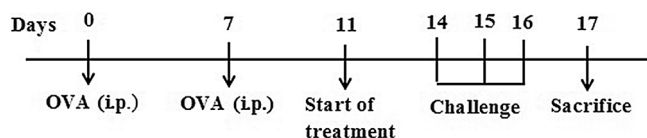


Fig. 1. The protocol used to induce asthma in mice. OVA: ovalbumin.

2.3. Experimental design

Mice were divided arbitrarily into 4 experimental groups ($n = 10$): Control group; OVA group; OVA + dexamethasone (2 mg/kg) (Wu et al., 2017) (DEX) group; and OVA + nicorandil (100 mg/kg) (NICO) group. In all groups except the control one, mice were exposed to sensitization on days 0 and 7 by intraperitoneal (i.p.) injection of 25 μ g OVA emulsified with 1 mg $\text{Al}(\text{OH})_3$ in 200 μ l phosphate-buffered saline (PBS) (pH 7.4). On days 14, 15 and 16, mice were challenged with inhalation of 1% OVA solution in saline, for 30 min by nebulizer (da Cunha et al., 2016). DEX was injected i.p. for 6 days starting from day 11 till the end of experiment. Nicorandil was given orally for 6 days starting from day 11 till the end day. Mice were sacrificed 24 h after the last challenge. The protocol is illustrated in Fig. 1. After sacrifice, each group was divided into 2 subgroups. Lungs from the first subgroup were used for collection of BALF, while lungs of the other subgroup were used for estimating lung/body weight index (calculated by dividing the weight of whole lung by mouse body weight) and then used for preparation of homogenates (left lungs) and histopathology examination (right lungs).

2.4. Collection of BALF and cell counting

After sacrifice, the chest was opened, and trachea was cannulated in the first subgroup. The lungs were then washed with 1.0 ml aliquots of ice cold saline for 3 times to collect BALF. Afterwards, samples were centrifuged and the supernatants were stored at -80°C for ELISA assay. Cold PBS was used to resuspend the cell pellets for determination of inflammatory cell counts in BALF.

2.5. Estimation of lactate dehydrogenase

LDH activity was evaluated in BALF using Human assay kit (Germany). Assay was performed according to the given procedures.

2.6. Determination of total protein

Total protein content was estimated in BALF by means of SPINRE-ACT kit (Spain) according to the instructions provided.

2.7. Preparation of lung homogenates

Left lungs of the second subgroup were harvested, weighed then homogenized in chilled 1.15% KCl (pH 7.4) to yield 10% w/v tissue homogenates (Daba et al., 2002) for evaluation of MDA, GSH, SOD and NO contents.

2.8. Determination of malondialdehyde (MDA)

MDA levels were estimated in lung homogenates according to (Ohkawa et al., 1979).

2.9. Estimation of reduced glutathione (GSH)

Levels of GSH were evaluated in lung homogenates according to (Ellman, 1959).

2.10. Determination of superoxide dismutase (SOD)

Pulmonary SOD activity was measured spectrophotometrically by monitoring pyrogallol auto-oxidation (Marklund, 1985).

2.11. Measurement of nitric oxide (NO)

NO content was determined in lung as indication of NO production according to (Miranda et al., 2001). Samples were spectrophotometrically measured at 540 nm.

2.12. Cytokine assay

Levels of IL-13 were measured in BALF by ELISA kit purchased from Cusabio Biotech Co, USA according to the given protocol.

2.13. Estimation of NF- κ B

NF- κ B levels were measured in BALF by mouse NF- κ B ELISA kit, purchased from Cusabio Biotech Co, USA.

2.14. Lung tissue preparation for histopathology

Right lungs of the second subgroup were excised, weighed, immersed in 10% buffered formalin and embedded in paraffin wax before being sliced. The slices were then stained with H&E staining to reveal the structural features.

2.15. Statistical analysis

Results are presented as means $\pm$ SEM. Statistical comparisons were carried out using one-way ANOVA, followed by Tukey Kramer test. Statistical significance was calculated at $P < 0.05$.

3. Results

3.1. General animal data

Animals were weighed at the beginning and at the end of the experiment. Changes in body weight were recorded. OVA produced a significant loss in body weight when compared with control group. Nicorandil or dexamethasone administration significantly increased body weight when compared with OVA-challenged group (Table 1). OVA caused a death of 10% of mice in OVA-challenged group.

3.2. Effect of nicorandil (100 mg/kg) or dexamethasone (2 mg/kg) on lung/body weight index in asthmatic mice

As shown in Fig. 2, OVA-challenged group produced significant increase in the lung/body weight index in comparison with the control group. Treatment with nicorandil or dexamethasone significantly reduced lung/body weight index when compared to OVA group.

Table 1

Effect of nicorandil (100 mg/kg) on % mortality and changes in body weight in asthmatic mice.

Groups	% Mortality	Change in body weight (gm)
Control	0	11.1 $\pm$ 0.9
OVA	10	-7 $\pm$ 0.4*
DEX	0	12.2 $\pm$ 1.1 [#]
NICO	0	11.9 $\pm$ 1.1 [#]

Data expressed as means $\pm$ SEM ($n = 10$). Analyses performed using one-way ANOVA followed by Tukey- Kramer multiple comparisons post hoc test. * $p < 0.05$ vs. control; # $p < 0.05$ vs. OVA.

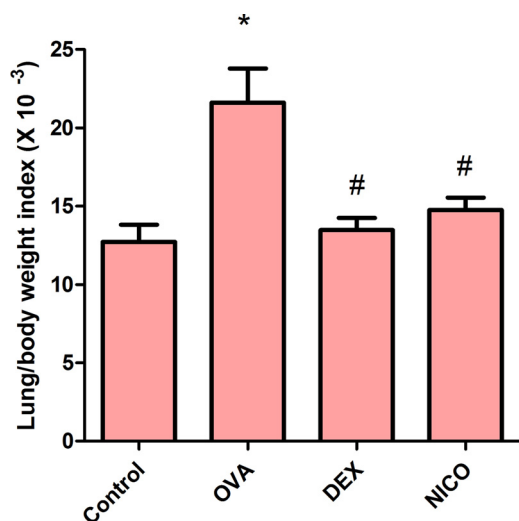


Fig. 2. Effect of nicorandil (100 mg/kg) or dexamethasone (2 mg/kg) on lung/body weight index in asthmatic mice. Values are expressed as means $\pm$ SEM ($n = 5$). Comparisons performed using one-way ANOVA followed by Tukey–Kramer's multiple comparisons post hoc test. OVA ova-albumin, DEX dexamethasone NICO nicorandil, * $p < 0.05$ vs. control; # $p < 0.05$ vs. OVA-treated group.

3.3. Effect of nicorandil (100 mg/kg) or dexamethasone (2 mg/kg) on total and differential cell counts in BALF of asthmatic mice

OVA-challenged group significantly elevated total cell counts, neutrophils, lymphocytes, monocytes, eosinophils and basophils in BALF when compared to the control group. However, treatment with nicorandil markedly decreased the inflammatory cells exerting suppressive effects like that of DEX group (Table 2).

3.4. Effect of nicorandil (100 mg/kg) or dexamethasone (2 mg/kg) on LDH and total protein levels in BALF of asthmatic mice

OVA significantly increased both LDH and total protein levels in comparison with control mice. In NICO and DEX groups, LDH and total protein levels were significantly decreased when compared to OVA group (Fig. 3A & B).

3.5. Effect of nicorandil (100 mg/kg) or dexamethasone (2 mg/kg) on the antioxidant status in lung homogenates of asthmatic mice

OVA significantly increased MDA and decreased GSH and SOD levels in comparison with control group. However, treatment with nicorandil or dexamethasone reversed OVA-induced alterations (Table 3).

3.6. Effect of nicorandil (100 mg/kg) or dexamethasone (2 mg/kg) on NO content in lung homogenate of asthmatic mice

Production of NO in lung tissues was significantly greater in OVA group compared to the control one, however this OVA-exacerbated NO production was significantly reduced by treatment with either

nicorandil or dexamethasone (Fig. 4).

3.7. Effect of nicorandil (100 mg/kg) or dexamethasone (2 mg/kg) on levels of IL-13 and NF- κ B in BALF of asthmatic mice

The results indicated that IL-13 and NF- κ B levels were elevated in the BALF of OVA group when compared to the control group. In contrast, administration of nicorandil or dexamethasone reduced OVA-induced IL-13 and NF- κ B elevation to the normal levels (Fig. 5A & B).

3.8. Effect of nicorandil (100 mg/kg) or dexamethasone (2 mg/kg) on histopathological examination

Lung histopathological alterations were detected by staining with H & E. The observations showed that sections of the lung from the control group revealed normal lung architecture, without any evidence of inflammation (Fig. 6A), while sections from asthmatic mice showed a significant increase in perivascular edema, peribronchial infiltration and macrophages in the alveolar space, in addition to thickened walls of alveoli when compared to control group (Fig. 6B). However, treatment with nicorandil or dexamethasone markedly ameliorated OVA-induced lung injury (Fig. 6C & D).

4. Discussion

Allergic asthma is a common respiratory disease so its management is of serious concern. The results of the present study proposed the beneficial effect of nicorandil in alleviating the allergic airway inflammation and identified the possible mechanisms by which nicorandil exerts its protective effect against OVA-induced asthma.

The present study showed that OVA significantly decreased the body weight of challenged mice when compared to normal mice. This effect may be attributed to increased inflammation in adipose tissue (Batista et al., 2014). Treatment with nicorandil or dexamethasone significantly restored the body weight to the normal levels. This action may be due to their anti-inflammatory property. OVA-challenged mice showed significant elevation in lung/body weight index when compared to the control group, indicating pulmonary edema with accumulation of serous fluids within the lungs. Administration of nicorandil or dexamethasone significantly reduced lung/body weight index when compared to OVA group.

This study revealed that OVA-induced asthma was associated with infiltration of inflammatory cells (neutrophils, lymphocytes, monocytes, eosinophils and basophils) in the lung. The infiltration of inflammatory cells might have a role in the development of allergic asthma via releasing cytotoxic proteins (Hamid et al., 2003; Wei et al., 2016). Nicorandil as well as dexamethasone treated mice showed significant reduction in OVA-induced inflammatory cells infiltration. Moreover, histological examination illustrated that nicorandil and dexamethasone alleviated OVA-induced lung tissue inflammation.

Eosinophils, neutrophils and macrophages of asthmatic patients produce reactive oxygen species (ROS) higher than those of normal ones (Teramoto et al., 1996). ROS overproduction or suppression of defense mechanisms results in bronchial hyper reactivity. These ROS

Table 2
Effect of nicorandil (100 mg/kg) on total and differential cell counts in BALF of asthmatic mice.

Groups	Total cell count ($\times 10^4$)	Neutrophils ($\times 10^4$)	Lymphocytes ($\times 10^4$)	Monocytes ($\times 10^4$)	Eosinophils ($\times 10^4$)	Basophils ($\times 10^4$)
Control	243 $\pm$ 17.2	149.9 $\pm$ 11.6	82.9 $\pm$ 6.2	4.3 $\pm$ 0.4	3.42 $\pm$ 0.6	2.42 $\pm$ 0.1
OVA	3240 $\pm$ 87.1*	2653 $\pm$ 106.5*	363 $\pm$ 6*	120 $\pm$ 16.9*	56 $\pm$ 5.4*	47.8 $\pm$ 6.3*
DEX	638 $\pm$ 25.7*#	397.6 $\pm$ 14.5*#	206.8 $\pm$ 11.6*#	16.7 $\pm$ 2.9#	11.4 $\pm$ 1.3#	3.4 $\pm$ 0.16#
NICO	735 $\pm$ 29*#	463.2 $\pm$ 20.6*#	233.7 $\pm$ 12.8*#	19.7 $\pm$ 1.8#	16.4 $\pm$ 1.2*#	6.5 $\pm$ 0.16#

Data expressed as means $\pm$ SEM ($n = 5$). Analyses performed using one-way ANOVA followed by Tukey–Kramer multiple comparisons post hoc test. * $p < 0.05$ vs. control; # $p < 0.05$ vs. OVA.

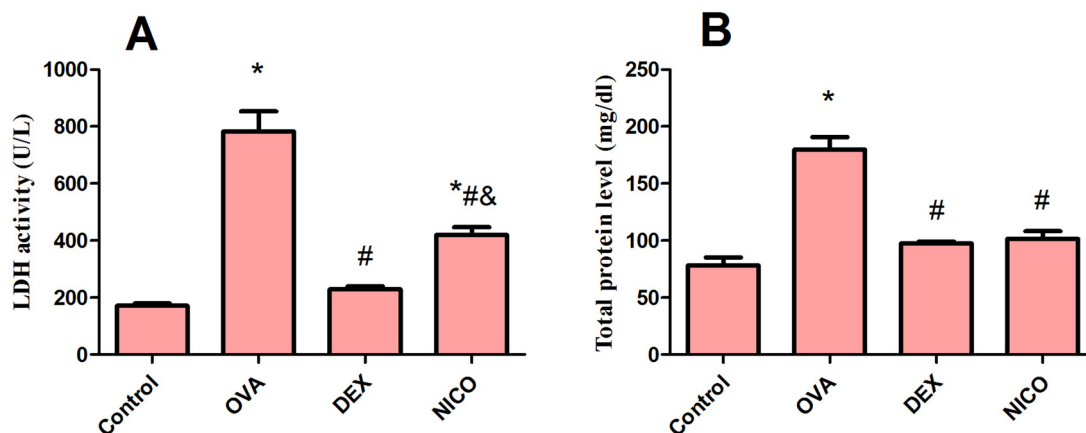


Fig. 3. Effect of nicorandil (100 mg/kg) or dexamethasone (2 mg/kg) on LDH and total protein levels in BALF of asthmatic mice. Values are expressed as means $\pm$ SEM (n = 5). Comparisons performed using one-way ANOVA followed by Tukey–Kramer's multiple comparisons post hoc test. OVA ova-albumin, DEX dexamethasone NICO nicorandil, *p < 0.05 vs. control; #p < 0.05 vs. OVA-treated group; & p < 0.05 vs. DEX group.

Table 3

Effect of nicorandil (100 mg/kg) on antioxidant status in lung homogenates of asthmatic mice.

Groups	MDA (nmol/g)	GSH (μ mol/g)	SOD (U/mg)
Control	4.7 $\pm$ 0.5	0.44 $\pm$ 0.01	48 $\pm$ 0.7
OVA	14.2 $\pm$ 1.6*	0.27 $\pm$ 0.004*	32.6 $\pm$ 2.8*
DEX	7 $\pm$ 0.7#	0.39 $\pm$ 0.02#	45.4 $\pm$ 1.3#
NICO	5.8 $\pm$ 0.6#	0.4 $\pm$ 0.01#	45.7 $\pm$ 1.1#

Data expressed as means $\pm$ SEM (n = 5). Analyses performed using one-way ANOVA followed by Tukey–Kramer multiple comparisons post hoc test. *p < 0.05 vs. control; #p < 0.05 vs. Ova.

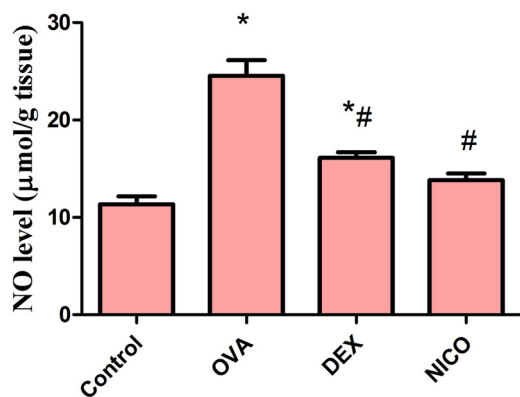


Fig. 4. Effect of nicorandil (100 mg/kg) or dexamethasone (2 mg/kg) on NO level in lung homogenate of asthmatic mice. Values are expressed as means $\pm$ SEM (n = 5). Comparisons performed using one-way ANOVA followed by Tukey–Kramer's multiple comparisons post hoc test. OVA ova-albumin, DEX dexamethasone NICO nicorandil, *p < 0.05 vs. control; #p < 0.05 vs. OVA-treated group.

oxidize phospholipids of cell membrane and produce lipid peroxides which impair functions of membrane and leads to tissue injury (Ciencewicki et al., 2008). Thus, any attempt to attenuate the intracellular ROS may help in the treatment of inflammatory lung diseases. In the current study, MDA content in the lung, an index of lipid peroxidation, was significantly increased after OVA exposure. This observation agreed with previous study that reported increased lipid peroxidation following OVA challenge (Yosri et al., 2017). Nicorandil treatment significantly reduced lung MDA content, indicating decreased lipid peroxidation secondary to decreased ROS generation. This reduction in MDA levels induced by nicorandil was greater than that produced by dexamethasone, so this result highlights the role of nicorandil as a potent antioxidant.

In this study, OVA-challenged mice showed significant decrease in lung SOD activity and GSH levels. On contrast, nicorandil and dexamethasone-treated mice markedly reduced oxidative stress as it increased SOD and GSH levels suggesting that nicorandil has a vital role in the modulation of anti-oxidative response and improve oxidant/antioxidant balance.

Our results showed that OVA increased level of total protein and LDH activity in BALF. This result was confirmed by earlier study that reported elevation in serum LDH and lung total protein content (Yosri et al., 2017). Nicorandil as well as dexamethasone-treated mice exhibited significant reduction in total protein level and LDH activity, suggesting the cytoprotective effect of nicorandil.

NO is an important mediator implicated in the progression of asthma. In asthma, extensive production of NO might be cytotoxic and produce marked pathological alterations (e.g., inflammatory cellular infiltration, mucus production and airway edema) observed during asthma exacerbations (Ricciardolo, 2003). Many reports stated that massive amounts of NO may recruit eosinophils in the airway and shift the balance toward Th2 cells, thus triggering the airway inflammation (Moon et al., 2008, Nader et al., 2012). There was a significant elevation in NO levels in OVA group compared to the control one. Nicorandil or dexamethasone treatment significantly reduced the OVA-triggered NO levels.

It has been reported that Th2 cytokines (IL-13, IL-5 and IL-4) play key roles in inducing inflammation in the lungs as they enhance IgE production. IL-13 directly stimulates airway hyperreactivity and mucus hypersecretion (Nader et al., 2012). Elevated levels were observed in asthmatic patients (Wills-Karp et al., 1998, Webb et al., 2000). In this study, allergic airway induction was associated with significant increase in IL-13 level, while treatment with nicorandil or dexamethasone attenuated OVA-induced IL-13 production.

NF- κ B plays a role in regulating cytokines production (Huang et al., 2015). Normally, NF- κ B is in the cytoplasm and connected to I κ B protein (Rai et al., 2015), on stimulation, I κ B separates from NF- κ B then NF- κ B translocated in the nucleus for regulation of gene transcription of inflammatory cytokines (Zhao et al., 2014). NF- κ B activation is implicated in asthma models of humans or animals (Bureau et al., 2000). Treatment with nicorandil or dexamethasone inhibited the OVA-induced increase in NF- κ B production.

Collectively, the present data provides evidence that oral administration of nicorandil conferred significant therapeutic effect against OVA-induced allergic asthma. This effect might be due to its ability to improve the antioxidant status, decrease nitric oxide production, and modulate IL-13 and NF- κ B signaling. Further clinical studies are required to verify this effect.

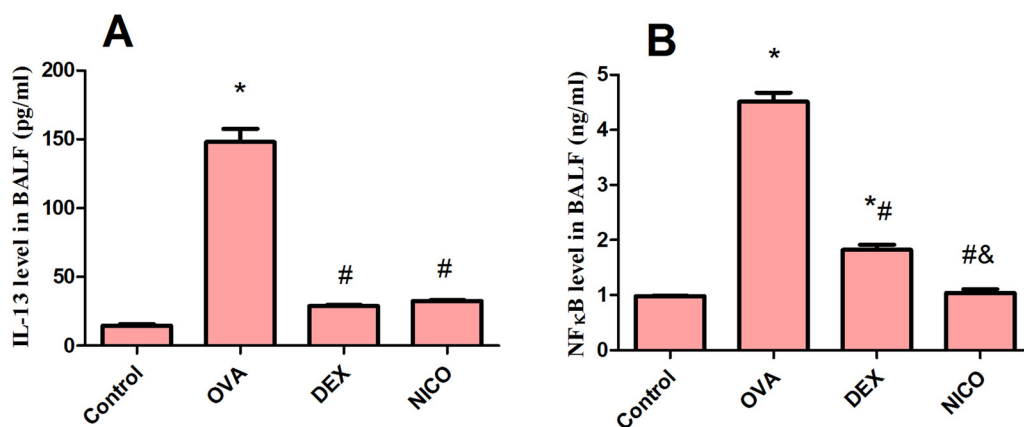


Fig. 5. Effect of nicorandil (100 mg/kg) or dexamethasone (2 mg/kg) on IL-13 and NFκB levels in BALF asthmatic mice. Values are expressed as means $\pm$ SEM (n = 5). Comparisons performed using one-way ANOVA followed by Tuckey–Kramer's multiple comparisons post hoc test. OVA ova-albumin, DEX dexamethasone NICO nicorandil, *p < 0.05 vs. control; #p < 0.05 vs. OVA-treated group; & p < 0.05 vs. DEX group.

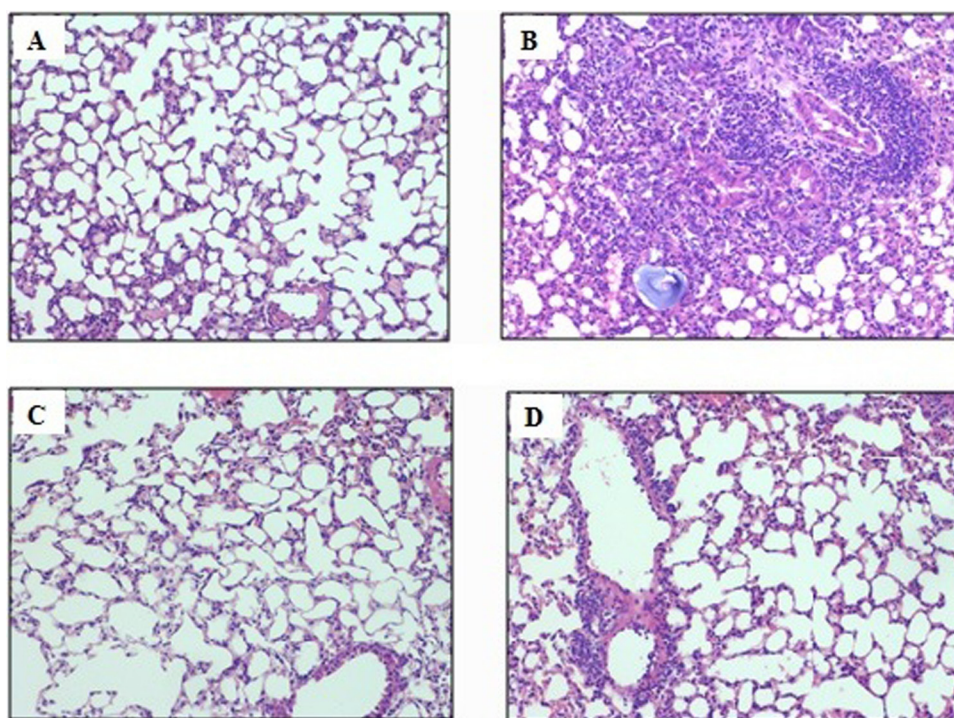


Fig. 6. H & E-stained lung specimens isolated from (A) the control group (100 $\times$); (B) OVA group (100 $\times$), showing perivascular edema, peribronchial inflammation, and alveolar macrophages and inflammatory cells filling alveolar spaces; (C) DEX group (100 $\times$), showing normal lung architecture; (D) NICO group (200 $\times$), showing mild inflammation in perivascular space.

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Conflict of interest statement

The author declares no conflict of interests.

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