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Saxagliptin mitigates airway inflammation in a mouse model of acute asthma via modulation of NF-kB and TLR4

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ABSTRACT

Saxagliptin (***Saxa***), a dipeptidyl dipeptidase-4 (DPP-4) inhibitor, is widely used for the treatment of type 2 diabetes mellitus. It has been documented to have immunomodulatory and anti-inflammatory actions. Our objective was to delineate the protective effect and the underlying mechanism of ***Saxa***-in comparison with Dexamethasone (***Dexa***) – in airway inflammation induced by ovalbumin (***OVA***) in mice.

Methods: Mice were ***OVA***-sensitized and challenged for the induction of acute asthma. Mice were orally administrated ***Saxa*** or ***Dexa***. Total and differential cell counts, lactate dehydrogenase (LDH) and total protein concentrations were assessed in bronchoalveolar lavage fluid (BALF). The toll-like receptor 4 (TLR4), nuclear factor-kappa B (NF-kB), reduced glutathione (GSH), and total nitrate/nitrite products (NOx) levels as well as myeloperoxidase (MPO) activity in lung tissues were measured. Histopathological examination of the lung specimens was carried out using the hematoxylin and eosin (H & E) staining.

Results: Histopathological examination revealed that both ***Saxa*** and ***Dexa*** ameliorated ***OVA***-induced inflammatory changes and significantly reduced total and differential leukocyte counts, LDH and total protein level in BALF upon comparison with ***OVA*** group. In addition, both treatments significantly mitigated ***OVA***-induced oxidative stress as evidenced by diminished lung NOx level and MPO activity and elevated GSH level. The elevation of TLR4 and NF-kB levels in lung tissue were ameliorated by ***Saxa*** and ***Dexa*** administration.

Conclusion: *Saxa* had marked antiasthmatic effect in ***OVA***-induced allergic asthma through modulation of TLR4 and NF-κB signaling. Also, ***Saxa*** may represent a promising therapeutic agent for acute allergic asthma.

1. Introduction

Bronchial asthma is a prevalent airway inflammatory disease, identified by recurrent obstruction of airways and associated with excessive mucus secretion and airway infiltration of inflammatory cells. Type 2 (Th2) lymphocytes play a crucial role in the pathogenesis of airway inflammatory diseases through the induction of the release of Th2-derived cytokines especially IL-4, IL-5, and IL-13. These cytokines promote IgE release and infiltration of inflammatory cells into the lungs that result in the pathogenesis of allergic asthma [1]. In addition, these cytokines and inflammatory cells induce the formation of the high levels of reactive oxygen species (ROS) and consequential loss of antioxidant defenses [2].

Toll-like receptors (TLRs) are a pattern recognition receptor [3]. New studies have been reported that ***OVA*** activates TLR4 pathway and its downstream target (NF-κB) causing exacerbation of Th2 associated inflammatory responses and promotion of the gene expression of inflammatory cytokines [4,5]. NF-κB – a multicellular transcription factor – also has a main role in modulating inflammatory and immune responses through regulation of Th2 cytokines and gene expression [3]. Moreover, previous reports showed that suppression of NF-kB could mitigate ***OVA***-induced allergic asthma [4–6].

On the other hand, a great attention should be paid to the pharmacological management of allergic asthma in type 2 diabetic patients who received antidiabetic drugs because of the increased coincidence of both diseases in the population. In addition, some antidiabetic drugs were reported to mitigate the allergic inflammation and oxidative stress, main characteristics of asthma, besides controlling serum glucose level. Dipeptidyl peptidase-4 (DPP-4) inhibitors are a class of drugs used for the management of type 2 diabetes mellitus. Up till now, the anti-inflammatory effects of DPP-4 inhibitors have been evaluated in different organ pathologies, such as non-alcoholic fatty liver disease [7], and gentamicin-induced nephrotoxicity [8].

In the field of respiratory diseases, DPP-4 is recently recognized as

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an important factor in immunity and inflammation responses [9]. It has been reported that DPP-4 plays a potential role in many respiratory diseases and it is linked to airway inflammation in animal models of asthma [10,11]. It has been found that DPP-4 expression was significantly elevated in bronchial epithelial cells from asthma patients [11]. Moreover, diverse members of DPP-4 inhibitors have been reported to ameliorate experimentally-induced lipopolysaccharide-induced pulmonary fibrosis [12] and to mitigate pulmonary arterial remodeling in pulmonary hypertension [13].

Saxagliptin - a newer DPP-4 inhibitor – has been declared to have antioxidant activities which may have beneficial effects on various body tissues. Moreover, ***Saxa*** has displayed obvious anti-inflammatory effect in the subacute and acute models of inflammation [14,15]. In addition, Helal *et al*, [8] also have demonstrated a significant nephroprotective effect against gentamicin-induced nephrotoxicity via mainly its anti-inflammatory, antiapoptotic and antioxidant properties. Our previous research demonstrates that ***Saxa*** mitigates ***OVA***-induced allergic asthma via suppression of Th2-associated IL-13 [16]. However, the underlying mechanisms of antiasthmatic and anti-inflammatory activities of ***Saxa*** in ***OVA***-induced asthma have not been fully studied yet.

Since identifying the anti-inflammatory effects of ***Saxa*** may provide a therapeutic agent that can be valuable in the management of type 2 diabetic patient with allergic asthma, this study is complementary to our previous work [16] and aims to assess the antiasthmatic effect of ***Saxa*** – in comparison with ***Dexa***-on ***OVA***-induced acute asthma in mice with emphasis on oxidative stress, inflammation.

2. Methods

2.1. Chemicals and drugs

Ovalbumin (***OVA***) was purchased from Loba Chemie PVT. Ltd. (Bombay, India). **Dexamethasone (*Dexa*)** was purchased as **Epidron 4 mg/ml ampoules** from *Eipico Pharmaceutical Co.*, Egypt. **Saxagliptin monohydrate (*Saxa*), Onglyza 5 mg tablets,** *Bristol Myers Squibb* (Pennington, NJ, USA), was purchased from the market. It was prepared as 0.25% suspension in 0.5% carboxymethylcellulose (CMC) immediately before use.

2.2. Animals

Adult male Swiss albino mice (7 weeks old, n = 40) were used for this experiment (obtained from VACSERA, Giza, Egypt). The animals were fed with a balanced chow diet with free access to water *ad libitum*, housed in cages under pathogen-free conditions, and kept on a 12/12-hr light/dark cycle. All experimental procedures presented in this study were carried out in compliance with guiding principles for the manipulation of laboratory animals approved by "Research Ethics Committee", Faculty of Pharmacy, Mansoura University, Egypt.

2.3. OVA-induced acute allergic asthma protocol and treatment

To evaluate the preventive potential of ***Saxa*** on ***OVA***-induced acute allergic asthma, mice were randomly separated into four equal groups (n = 10/group) as follows: control (***CTRL***) group, ***OVA*** group (acute asthma group), ***OVA/Dexa*** group (**Dexa**, 3 mg/kg/day, orally) and ***OVA/Saxa*** group (***Saxa***, 10 mg/kg/day, orally) [14,16]. ***Dexa*** group was considered as a standard control for comparison with ***Saxa*** group. The ***Saxa*** dose was chosen depending on our preliminary experiments and prior reports [16].

The protocol of the induction of acute asthma and drug treatments was delineated in Fig. 1. In brief, sensitization of mice was carried out with the injection of 20 μg ***OVA*** and 1 mg aluminum hydroxide [$Al(OH)_3$], intraperitoneally, on days zero and 7. After that, the challenge of mice was carried out on days 14, 15 and 16 through nebulization of 1% (w/v) ***OVA*** in normal saline, 1 h after administration of ***Dexa*** and ***Saxa***, for 30 min with an output rate of 1ml/min [17]. Nebulization was done in groups (10 mice per each) in a whole-body inhalation exposure system connected to an ultrasonic nebulizer (NE-U17, Omron Co., Tokyo, Japan).

On days 11–16, *mice in* ***OVA/Dexa*** and ***OVA/Saxa*** groups received ***Dexa*** and ***Saxa*** at oral doses of 3 and 10 mg/kg, respectively [18,19]; while ***CTRL*** and ***OVA*** groups receive the vehicle only. On Day 17, 24 h after the last ***OVA*** challenge, mice were sacrificed and BALF in each group was collected, and the lung tissues were also isolated and rinsed with ice-cold 1.15% potassium chloride (pH 7.45).

2.4. Preparation of BALF and counting of total and differential leukocytes

For the preparation of BALF, 0.5 ml of cold saline was infused through the lung and withdrawn 3 times via the tracheal cannula. The BALF was then centrifuged at 112 RCF at 4°C for 10 min. The supernatants were, then, stocked at -80 °C for biochemical measures and cytokines detections. Pellets containing BALF cells were resuspended in cold saline for total and differential leukocytes count. The total and the differential number of inflammatory cells in BALF were detected by Wright–Giemsa staining [6].

2.5. Determination of LDH activity and total protein content in BALF

The levels of LDH activity and total protein in BALF were detected using assay kits purchased from Spinreact (Girona, Spain) following the previously described procedures [20,21].

2.6. Preparation of lung homogenate and determination of total nitric oxide (NOx) contents, reduced glutathione (GSH) concentration and myeloperoxidase (MPO) activities in lung tissues

Left lung lobe was homogenized as previously described [22]. Briefly, left lung sections were weighed and homogenized in 1.15% potassium chloride solution to prepare 10% w/v lung homogenate. Lung homogenate was centrifuged and the supernatant was collected after centrifugation to measure the oxidative stress markers. GSH content was detected in lung homogenate according to the method of Moron *et al*, [23] with a slight modification. Briefly, the protein in 0.45 ml of lung homogenate was precipitated with 0.05 ml of 50% (w/v) trichloroacetic acid and then centrifuged at 1000 g for 5 min. The reaction mixture containing 0.25 ml of supernatant, 1 ml of 0.2 M Tris–HCl (containing 1 mM EDTA, pH 8.9) and 0.05 ml of 10 mM 5,5′-dithiobis-(2-nitrobenzoic acid) in absolute methanol was kept at room temperature for 5 min, and the yellow color developed was measured spectrophotometrically at 412 nm.

Total nitrate/nitrite (NOx) products were evaluated as an indicator of nitric oxide (NO) production following the previously described procedure [24]. Lung homogenate (0.5 ml) was added to 0.25 ml of 0.3 N NaOH. After incubation for 5 min at room temperature, 0.25 ml of 5% (w/v) ZnSO4 was added for deproteinization. This mixture was then centrifuged at 3000g for 20 min at 4 °C, and 0.3 ml of the resultant supernatant was added to 0.3 ml VCl3 (8 mg/ml) in 1 M HCl and 0.3 ml Griess reagent [0.15 ml of 2% (w/v) sulfanilamide in 5% (v/v) HCl and 0.15 ml of 0.1% (w/v) N-(1-naphthyl)-ethylendiamine dihydrochloride in distilled water]. After incubation for 45 min at 37 °C, samples were measured spectrophotometrically at 540 nm.

The pulmonary neutrophil infiltration was examined by evaluation of MPO activity as previously described [25,26].

2.7. Determination of TLR4 and NF-κB concentrations in lung homogenates

The concentrations of TLR4 and NF-κB in lung homogenates were quantified using ELISA kits (MyBioSource, Inc. San Diego, USA) following the manufacturer instructions.

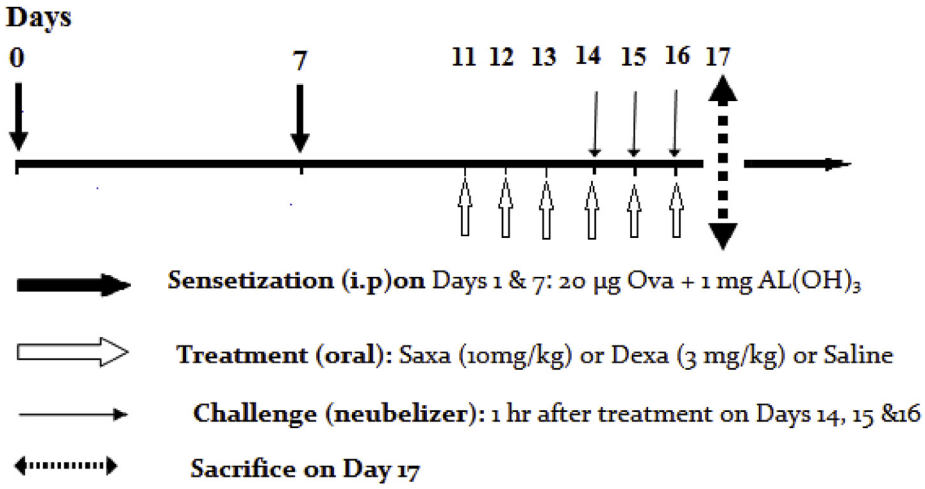

Fig. 1. The protocol of the induction of acute allergic asthma in mice and *Saxa* (10 mg/kg) *and Dexa* (3mg/kg) treatmentsMice were sensitized on days 1 and 7; challenged on days 14, 15 & 16 with ovalbumin (***OVA***). Mice in the ***CTRL*** group were saline-treated. Mice in the ***OVA***/***Dexa*** or **OVA**/***Saxa*** groups were orally treated with ***Dexa*** and ***Saxa*** at doses of 3 mg/kg/day and 10 mg/kg/day, respectively, on days 11-16.

2.8. Lung histology examination

The right lung lobe from each animal was isolated and fixed in 10% (v/v) neutral buffered formalin. The specimens were dehydrated, embedded in paraffin, and cut into sections (5 mm thickness) that then were stained with H & E stains. The histopathological changes of the lung tissues were observed by a treatment-blinded pathologist.

2.9. Statistical analysis

All results were expressed as means ± standard error of the mean (SEM). Statistical analysis was performed by using GraphPad Prism V. Statistical significance ($p < 0.05$) was assessed by one-way analysis of variance followed by Tukey-Kramer's test.

3. Results

3.1. Effect of Saxa and Dexa on total and differential cell counts in BALF

The infiltration and the accumulation of both total cells and neutrophils in BALF of ***OVA*** group was significantly elevated by about 4-fold, lymphocytes was significantly elevated by 5-fold while both monocytes and eosinophils was significantly elevated by 3-fold, respectively, when compared to the ***CTRL*** group. However, ***Dexa*** *or* ***Saxa*** treatment demonstrated a significant decrease in the accumulated cells in comparison with ***OVA*** group. ***Dexa*** treatment significantly decreased total inflammatory cell content by approximately 76% and lung content of neutrophils, lymphocytes, monocytes, and eosinophils significantly declined by about 70, 39, 76 & 82%, respectively, when compared with that of the ***OVA*** group. ***Saxa*** treatment significantly decreased total inflammatory cell content by approximately 68% and lung content of neutrophils, lymphocytes, monocytes, and eosinophils significantly declined by about 69, 54, 52 & 68%, respectively, when compared with that of the ***OVA*** group. Thus, ***Dexa*** and ***Saxa*** could attenuate infiltration of inflammatory cells, total and differential cell count in BALF to the same level (Fig. 2).

3.2. Effect of Saxa and Dexa on LDH activity and total protein content in BALF

OVA-induced allergic asthmatic mice demonstrated a significant elevation on LDH activity and total protein content in BALF by about 6 and 1.5 fold, respectively, upon comparing with those in the ***CTRL*** group. Oral treatment with ***Dexa*** or ***Saxa*** significantly decreased LDH activity in BALF by 69% and 58%, respectively, compared with those of the ***OVA*** group as shown in Fig. 3A. Moreover, oral treatment with ***Dexa*** or ***Saxa*** significantly reduced total protein content in BALF by 56% and 52%, respectively, compared with those of the ***OVA*** group as shown in Fig. 3B.

3.3. Effect of Saxa and Dexa on NOx contents, GSH concentration and MPO activities in the lung tissues

OVA-sensitized and challenged mice showed a significant elevation in NOx content by 50% and MPO activity by about 3.8 fold with a marked reduction in GSH level by 32% in lung specimens compared to *the* ***CTRL*** group. The increased NOx content in the lung of mice was significantly attenuated by ***Dexa*** and ***Saxa*** treatment by 48% and 36%, respectively. ***Dexa*** and ***Saxa*** significantly decreased lung MPO activity by 1.7 and 1.7 fold, respectively, compared to ***OVA***-treated mice. Moreover, a significant elevation in the lung GSH level by 41% and 37% was noticed in ***Dexa***-and ***Saxa***-treated mice, respectively, compared to ***OVA***-treated mice as presented in Table (1).

3.4. Effect of Saxa and Dexa on TLR4 content in lung homogenates

The ***OVA*** group exhibited a marked elevation in the lung homogenate content of TLR4 by approximately 4.1 folds compared to the ***CTRL*** group. ***Dexa*** or ***Saxa*** administration significantly attenuated this ***OVA***-induced exacerbation of TLR4 by 29% and 24%, respectively, in comparison with the ***OVA*** group (Fig. 4).

3.5. Effect of Saxa and Dexa on NF-κB content in lung homogenates

Lung NF-κB content significantly escalated by approximately 3.5 folds in the ***OVA*** group in comparison to ***CTRL*** group upon asthma progression. ***Dexa*** and ***Saxa*** significantly reduced lung NF-κB content by 59% and 42%, respectively, compared to the ***OVA*** group (Fig. 5).

3.6. Effect of Saxa and Dexa on histopathological alterations in the lung specimens

To explore the effects of ***Dexa*** and ***Saxa*** on ***OVA***-induced allergic asthma, the histopathological changes in lung tissues from mice in each group were observed using H & E staining. As shown in Fig. 6, ***OVA***-sensitized and challenged mice demonstrated extensive infiltrated inflammatory cells surrounding the bronchi, thickened walls of bronchial epithelium and congested blood vessels, indicating airway inflammation of the ***OVA***-treated mice. ***Dexa*** and ***Saxa*** treatment attenuated bronchial airway inflammation and infiltration of inflammatory cells.

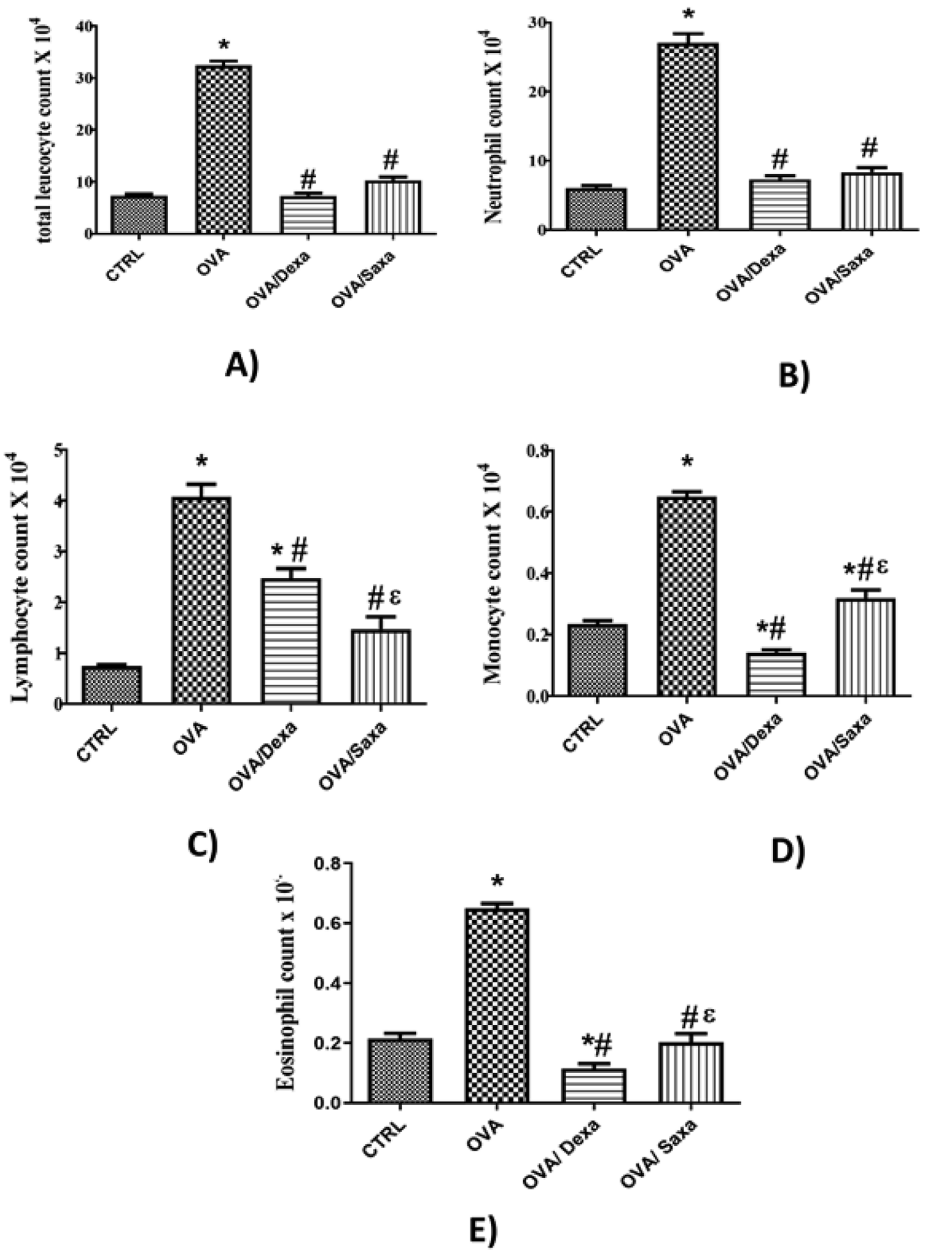

Fig. 2. Effect of *Saxa* (10 mg/kg) *and Dexa* (3mg/kg) on OVA-induced alteration in BALF A) total leukocyte count, B) neutrophils, C) lymphocytes, D) monocytes and E) eosinophils

Acute asthma was induced by *OVA* sensitization on days 1 and 7 followed by *OVA* challenge on days 14, 15 & 16. *Dexa* (3 mg/kg, orally) and *Saxa* (10 mg/kg, orally) were administered once daily 1 hr before *OVA* challenge from days 11 to 16. The infiltration and the accumulation of total cells, neutrophils, lymphocytes, monocytes and eosinophils in BALF of *OVA* group was significantly elevated when compared to the *CTRL* group. However, *Saxa or Dexa* treatment demonstrated a significant decrease in the accumulated cells in comparison with *OVA* group.

Values represent the mean ± SEM, n = 10.

Data were statistically evaluated using one-way analysis of variance followed by Tukey-Kramer's test.

*$p^{<}$0.05, compared with ***CTRL***

#$p^{<}$0.05, compared with ***OVA***

€ $P^{<}$0.05, compared with ***Dexa***.

4. Discussion

The present study delineates the therapeutic potential of ***Saxa***, in comparison with ***Dexa****, in* ***OVA****-induced allergic asthma.* The results of the current study demonstrates that DPP-4 inhibition with ***Saxa*** ameliorates ***OVA***-induced allergic asthma in mice and there is a non-significant discrepancy between the effect of ***Saxa*** and ***Dexa*** treatment. ***Saxa*** was previously reported to attenuate asthma-associated lung injury by interfering with the IL-13 production and suppressing lipid peroxidation in lung tissues [16]. Herein, a novel evidence and delineated confirmation of the previously published findings by the same research team prove that ***Saxa*** could mitigate airway inflammation and protect against allergic asthma induced by ***OVA*** *via* modulation of the TLR4 and NF-kB as well as enhancement of antioxidant activity.

Asthma is allergic disorder characterized by airway inflammation, airway occlusion, and mucus hypersecretion. Exposure to an allergen triggers airway inflammation that is associated with Th2 responses such as induction of eosinophil, neutrophil and macrophage infiltration which further enhances the release of various inflammatory mediators and cytokines [1]. TLR4 is an important immune pattern recognition receptor that controls innate and adaptive immune responses and promotes the synthesis and release of cytokines, contributing to the production of inflammatory response [27].

Recently, several studies have revealed that lipopolysaccharide-aggravated allergic airway inflammation in mast cells is mediated through activation of TLR4 in mast cells which in turn stimulates Th2 responses [28,29]. It was proven that TLR4/ROS/NF-κB downstream pathway regulates the production of Th2 cytokines in lipopolysaccharide-stimulated mast cells [27]. TLR4 signaling initiates the activation of NF-κB that induces stimulation of gene transcription and expression of numerous inflammatory mediators. NF-κB also has a fundamental role in regulating the immune response as well as cell proliferation and differentiation [30]. Consequently, the TLR4/NF-κB signaling pathway is essential for immunoregulation processes and is an important pathological mechanism underlying asthma.

TLR4, and NF-κB expression were found to be significantly elevated in lung tissues of ***OVA***-induced asthma in mice [5]. Moreover, ***Saxa*** was also reported to attenuate NF-κB-mediated macrophage infiltration

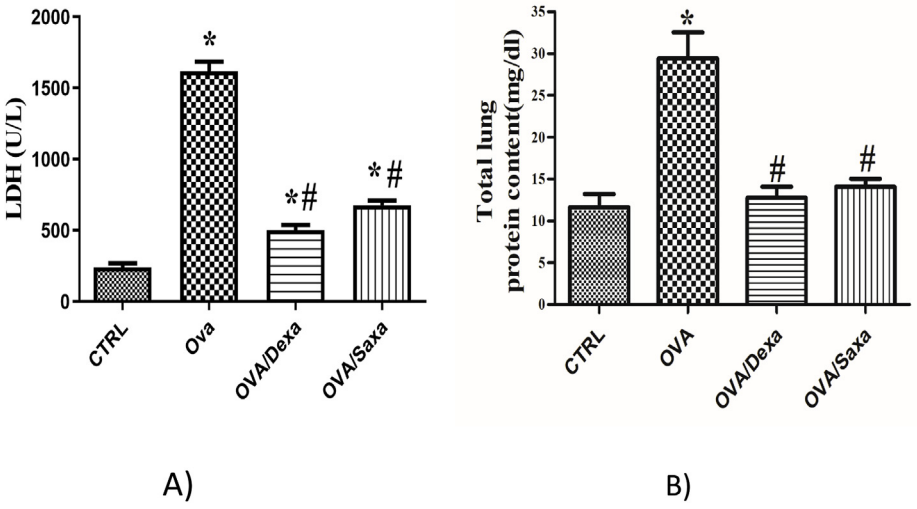

Fig. 3. Effect of Saxa (10 mg/kg) and Dexa (3mg/kg) on A) LDH activity and B) total protein content in BALF of OVA-induced allergic asthmatic mice Acute asthma was induced by ***OVA*** sensitization on days 1 and 7 followed by ***OVA*** challenge on days 14, 15 & 16. ***Dexa*** (3 mg/kg, orally) and ***Saxa*** (10 mg/kg, orally) were administered once daily 1 hr before ***OVA*** challenge from days 11 to 16. Mice in ***OVA***-group demonstrated a significant elevation on LDH activity and total protein content in BALF upon comparing with those in the ***CTRL*** group. Oral treatment with ***Dexa*** or ***Saxa*** significantly decreased LDH activity and total protein content in BALF, compared with ***OVA*** group.
Values represent the mean ± SEM, n = 10.
Data were statistically evaluated using one-way analysis of variance followed by Tukey-Kramer's test.
*$p^{<}$0.05, compared with ***CTRL***
#$p^{<}$0.05, compared with ***OVA***.

in a streptozotocin-induced diabetic nephropathy model independent of glucose lowering action [14]. The phenomenon that ***Saxa*** suppressed the levels of NF-κB and TLR4 in the lung tissues was also reported in ***OVA***-induced allergic asthma model of our study. Therefore, it appears that ***Saxa*** mitigated ***OVA***-induced allergic asthma changes by mediated suppression of TLR4/NF-κB downstream signaling pathway.

The current observations are parallel to the results of a previous report, which demonstrated that DPP-4 inhibition with ***Saxa*** prevented angiotensin II-induced cardiac fibrosis and inflammation mainly through suppression of angiotensin II–induced upregulation of TLR4 gene expression and activation of cardiac NF-κB in male mice [19]. In addition, DPP-4 inhibition with linagliptin, a DPP-4 inhibitor, was reported to significantly attenuate NF-κB activation in human kidney proximal tubular cells [31].

Interestingly, the activation of NF- κB has been shown to stimulate infiltration of Th2 lymphocytes into the lungs and to increase the accumulation of inflammatory cells, and the release of inflammatory cytokines such asIL-4, IL-5, and IL-13 [32,33]. These inflammatory cytokines, IL-4, IL-5, and IL-13, are key contributors in the pathogenesis of allergic asthma.

IL-4, and IL-5 regulate the maturation, survival and differentiation of eosinophils [34]. IL-13, another key cytokine in asthma, promotes B-cell differentiation and plays a dominant role in airway inflammation and hyperresponsiveness and mucus secretion [35]. Recent studies provided evidence that inhibition of Th2 immune response could mitigate allergic asthma [6]. In our previous work, ***Saxa*** was found to decrease the Th2-associated IL-13 content and the number of eosinophils and basophils in BALF, suggesting that the antiasthmatic effect of ***Saxa*** may be related to interference with Th2 immune response [16].

In the present work, the observed increment in MPO activity in the ***OVA*** group reveals neutrophil infiltration which was additionally affirmed by measurement of BALF's inflammatory cells' count and histopathological examination. Macrophages and neutrophils are phagocytes that assume real roles in the pathogenesis of numerous inflammatory diseases. They belong to the innate immune system and can switch between different modes of activation upon cues received from their immediate microenvironment. Once activated, they secrete numerous mediators that shape and regulate the microenvironment [36,37].

In the current study, the mice in the ***OVA*** group demonstrate severe inflammatory response with a significant inflammatory cell infiltration in BALF. Treatment with ***Saxa*** and ***Dexa*** remarkably attenuated the increase in leukocyte infiltration in the airway as evidenced in BALF'S inflammatory cells quantification, histopathological examination and MPO activity. Consecutive histopathological examination showed that ***Saxa*** or ***Dexa*** administration attenuated the airway inflammation and the accumulation of infiltrated inflammatory cells as well as the mucus that result in airway obstruction. These findings describe a strong anti-inflammatory effect of ***Saxa*** on the ***OVA***-induced inflammatory airway in mice and the potential of ***Saxa*** as a therapy for allergic asthma.

In the present work, LDH activity and total protein content of BALF significantly elevated in ***OVA*** group, reflecting the damage of cell membrane and augmented leakage of fluids into the lung tissue. However, ***Saxa*** administration significantly restored these alteration indicating the cytoprotective action of ***Saxa***, which is comparable to that of ***Dexa***.

Asthma pathogenesis is also characterized by the oxidant-antioxidant imbalance. Cellular and tissue damage associated with allergic asthma leads to the release of an excessive amount of ROS from leukocytes. ROS further increase airway sensitivity and mucus production [38]. NOx is a universal molecule responsible for various biological and

Table 1
Effect of *Saxa* (10 mg/kg) and *Dexa* (3 mg/kg) on lung reduced glutathione (GSH), total nitrate/nitrite (NOx) concentrations and MPO activity.

Groups	Lung GSH content (μmol/g wet tissue)	Lung NOx content (mmol/g wet tissue)	Lung MPO activity (U/g tissue)
CTRL	2.6 ± 0.06	23.37 ± 0.78	1.34 ± 0.1
OVA group	1.78 ± 0.04*	35.17 ± 2.1*	5.126 ± 0.5*
OVA/Dexa (3 mg/kg)	2.51 ± 0.06#	18.04 ± 1.28#	2.94 ± 0.1*,#
OVA/Saxa (10 mg/kg)	2.44 ± 0.13#	22.68 ± 1.26#	3 ± 0.2*,#

Acute asthma was induced by OVA sensitization on days 1 and 7 followed by OVA challenge on days 14, 15 & 16. *Saxa* (10 mg/kg, orally) and *Dexa* (3 mg/kg, orally) were administered once daily 1 hr before OVA challenge from days 11 to 16.
Values represent the mean ± SEM, n = 10.
Data were statistically evaluated using one-way analysis of variance followed by Tukey-Kramer's test.
*$p^{<}$0.05, compared with CTRL.
#$p^{<}$0.05, compared with OVA.

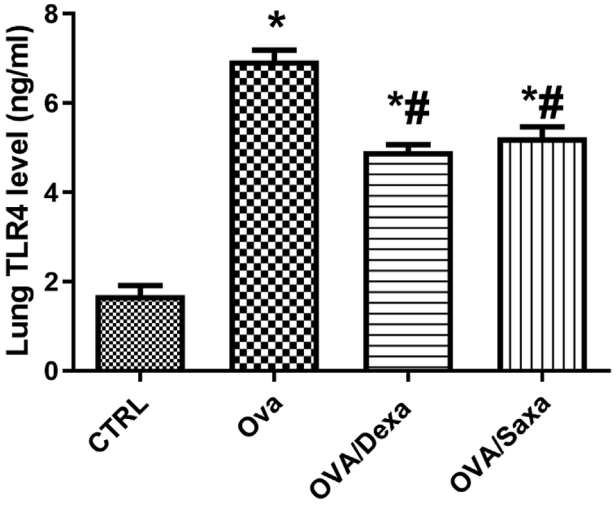

Fig. 4. Effect of Saxa (10 mg/kg) and Dexa (3mg/kg) on TLR4 content in lung homogenates Acute
asthma was induced by ***OVA*** sensitization on days 1 and 7 followed by ***OVA*** challenge on days 14, 15 & 16. ***Dexa*** (3 mg/kg, orally) and ***Saxa*** (10 mg/kg, orally) were administered once daily 1 hr before ***OVA*** challenge from days 11 to 16. ***OVA***-group demonstrated a significant elevation on the lung homogenate content of ***TLR4*** in comparison with ***CTRL*** group. Oral treatment with ***Dexa*** or ***Saxa*** significantly decreased the lung homogenate content of ***TLR4***, compared with ***OVA*** group.
Values represent the mean ± SEM, n = 10.
Data were statistically evaluated using one-way analysis of variance followed by Tukey-Kramer's test.
*p<0.05, compared with ***CTRL***
#p<0.05, compared with ***OVA***.

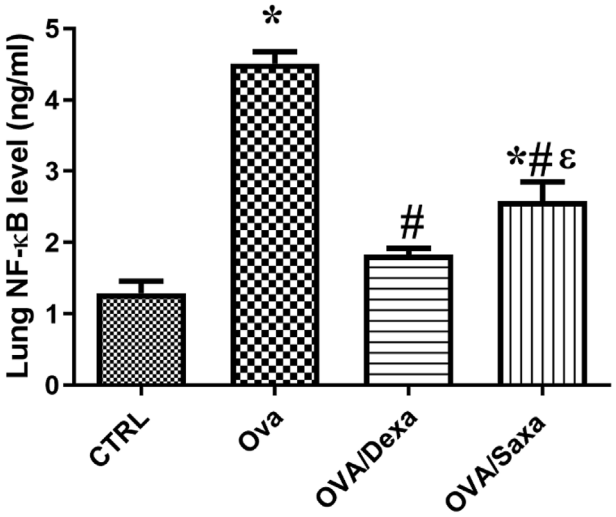

Fig. 5. Effect of Saxa (10 mg/kg) and Dexa (3mg/kg) on NF-κB content in lung homogenates
Acute asthma was induced by ***OVA*** sensitization on days 1 and 7 followed by ***OVA*** challenge on days 14, 15 & 16. ***Dexa*** (3 mg/kg, orally) and ***Saxa*** (10 mg/kg, orally) were administered once daily 1 hr before ***OVA*** challenge from days 11 to 16. ***OVA***-group demonstrated a significant elevation on the lung homogenate content of ***NF-κB*** in comparison with ***CTRL*** group. Oral treatment with ***Dexa*** or ***Saxa*** significantly decreased the lung homogenate content of ***NF-κB,*** compared with ***OVA*** group.
Values represent the mean ± SEM, n = 10.
Data were statistically evaluated using one-way analysis of variance followed by Tukey-Kramer's test.
*p<0.05, compared with ***CTRL***
#p<0.05, compared with ***OVA***
€ P<0.05, compared with ***Dexa***.

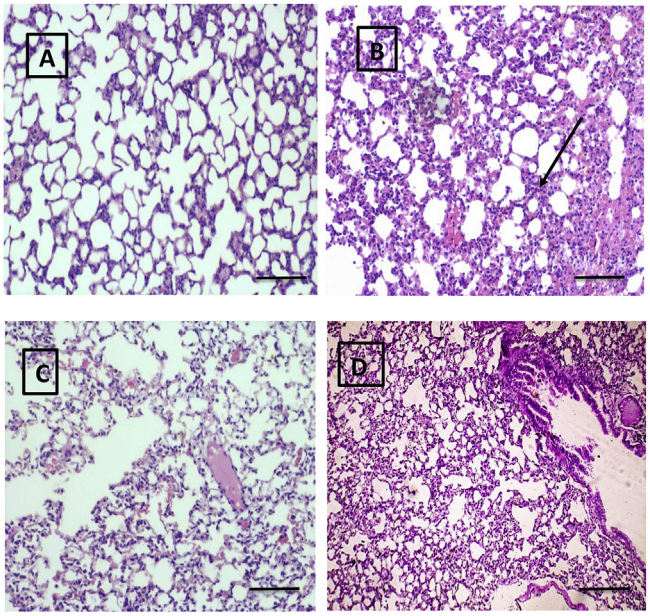

Fig. 6. Effect of Saxa (10 mg/kg) and Dexa (3mg/kg) on OVA-induced histopathological changes in lung tissues stained by H&E stain
Photomicrograph of sections of lung tissues isolated from A) ***CTRL*** (100x, bar 100), showing normal bronchial airway, B) ***OVA*** (100x, bar 100), showing extensive infiltrated inflammatory cells surrounding the bronchi, thickened walls of bronchial epithelium (black arrow) and congested blood vessels, C) ***OVA/Dexa*** (100x, bar 100), showing decrease in the infiltration of inflammatory cells surrounding the bronchi, and D) **OVA/Saxa** (100x, bar 100), ***showing that Saxa*** treatment attenuated bronchial airway inflammation and infiltration of inflammatory cells.
CTRL = control, ***OVA*** = Ovalbumin group, ***OVA/Dexa*** = dexamethasone-treated group, ***OVA/Saxa*** = Saxagliptin-treated group.

pathological effects. It is released in huge quantities in response to ROS-induced upregulation of inducible nitric oxide synthase (iNOS), whose role is well documented in various inflammatory diseases as airways of asthmatic patients [39]. Despite, the role of NOx in the pathogenesis of allergic asthma is not fully understood, it is well defined that NOx are important regulators of airway sensitivity and inflammation in asthma [40]. NOx interacts with superoxide anion to form peroxynitrite, which causes extensive nitrosative stress and cytotoxicity [41,42]. The lungs of ovalbumin (***OVA***) -challenged mice showed a decreased infiltration of inflammatory leukocytes as a result of suppression of iNOS-derived NOx. Therefore, Infiltration of inflammatory leukocytes into the lungs has been positively associated with augmented oxidative stress in allergic asthma [43].

The infiltrated leukocytes produce superoxide anion via the membrane-associated NADPH-dependent complex. Superoxide anion is rapidly converted into H2O2 and hydroxyl radicals (OH•), the latter of which leads to lipid peroxidation [44]. Moreover, antioxidant enzymes, such as GSH; the first line of the cellular defense mechanism against oxidative stress-induced injury, traps superoxide anion and preserves the redox state of the cell, so protecting the lungs from lipid peroxidation [45]. With regard to ***OVA***-induced oxidative stress in allergic asthmatic mice, the current results prove the antioxidant influence of ***Saxa*** as evidenced by restoration of lung tissue antioxidant capacity. ***Saxa*** showed marked suppression of NOx level parallel to the significant elevation of GSH level in lung homogenates. Solini *et al*, [46] agreed with our results and reported that ***Saxa*** improves the anti-oxidant status in an animal model of type 2 diabetes. Also, *Helal et al*, [8] *reported that* ***Saxa*** attenuated gentamicin-induced nephrotoxicity partly through its antioxidant properties and its ability to enhance host anti-oxidant defenses.

In conclusion, our study presented that ***Saxa*** effectively mitigated

airway inflammation in ***OVA***-induced asthma in mice. Moreover, ***Saxa*** decreased the oxidative stress and NF-κB and TLR4 levels in lung tissues suggesting that ***Saxa*** may be nominated as a potential therapy for effective management of allergic asthma through inhibition of the TLR4/ROS/NF-κB signaling pathway.

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Declaration of competing interest

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