World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Available online at: http://www.wjpsonline.org/
Original Article

Antiasthmatic Effect of Saxagliptin on Ovalbumin-Induced Allergic Asthma in Mice

Ahmed G. Abd Elhameed[1], Nermeen A. Megahed[2] and Manar G. Helal[1]*

[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Mansoura University, Egypt
[2]Department of Pathology, Faculty of Medicine, Mansoura University, Egypt

Received: 23-03-2018 / Revised Accepted: 25-04-2018 / Published: 01-05-2018

ABSTRACT

In the current study, the protective activity of saxagliptin (*SAX)* on ovalbumin (*OVA*)-induced allergic asthma in mice was evaluated.
Methods: Mice with *OVA*-induced allergic asthma were orally administered SAX at a dose of 10 mg/kg/day for 6 days. We determined the count of total cells, differential inflammatory cells and interleukin (IL)-13 concentration in bronchoalveolar lavage fluid (BAL). The concentration of lipid peroxidation marker, malondialdehyde (MDA), was also examined in lung homogenate. Histopathological alterations in lung tissues were also detected by Hematoxylin and Eosin staining.
Results: *SAX* inhibited *OVA*-induced airway inflammation and oxidative stress in the lung. *OVA*-induced allergic asthma was suppressed by *SAX* as evidenced by decreased lung body weight ratio and leukocyte infiltration in the lungs, and also suppressed *OVA*-induced elevation of level of IL-13 in BAL. In addition, SAX decreased the MDA concentration in lung homogenate.
Conclusion: Our study demonstrated that *SAX* attenuated *OVA*-induced allergic asthma and it may represent a promising agent for the control of allergic asthma.

Keywords: Saxagliptin, interleukin, ovalbumin, asthma, inflammation

Address for Correspondence: Manar G. Helal, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Mansoura University, Mansoura, 35516, Egypt; Email: manargamal2008@gmail.com

How to Cite this Article: Ahmed G. Abd Elhameed, Nermeen A. Megahed and Manar G. Helal. Antiasthmatic Effect of Saxagliptin on Ovalbumin-Induced Allergic Asthma in Mice. World J Pharm Sci 2018; 6(5): 54-60.

INTRODUCTION

Allergic asthma is one of the most prevalent chronic diseases and affects peoples in all ages. Moreover, the prevalence of asthma has augmented in the last 20 years [1]. Allergic asthma is identified by airway occlusion, increased mucus secretion, and bronchial infiltration by eosinophils, neutrophils, mast cells, and T lymphocytes. T lymphocytes play major roles in airway inflammation and remodeling through cytokines. T helper 2 (Th2) cytokines, including IL-13, prompt allergen-specific immunoglobulin (Ig) E production and inflammatory mediator release from mast cells.

Dipeptidyl peptidase-4 (DPP-4) inhibitors are broadly used therapeutic approach for type 2 diabetes. They inhibit the enzyme DPP-4, there for extending the physiological actions of the incretin hormones, with a clinically relevant attenuation in serum glucose level[2]. However, DPP-4 are universal enzymes that are produced in different types of cells and tissues [3]. Hence, DPP-4 inhibitors may valuably attenuate chronic inflammation and oxidative stress in different diseases. Among DPP-4 inhibitors, *SAX* has anti-inflammatory properties, antioxidant and immunomodulatory properties [2, 4, 5]. The therapeutic approaches for asthma are composed of several bronchodilators and anti-inflammatory drugs. Because lung and systemic oxidative stress augment inflammatory processes related to asthma[6], the antioxidant and anti-inflammatory activities may propose *SAX* as a treatment strategy to attenuate allergic asthma manifestation.
As long as, this study was performed to test whether *SAX* attenuates airway alterations in *OVA*-induced allergic asthma as well as to elucidate that the protective effect of SAX in this study is related to the anti-inflammatory and antioxidant activity.

MATERIALS AND METHODS

Animals: Adult male Swiss albino mice (7 weeks old, n=30) 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 conserved on a 12/12-h light/dark cycle. All experimental procedures used in this study were designed in compliance with guiding principles for the care and use of laboratory animals approved by "The research Ethics Committee", Faculty of Pharmacy, Mansoura University, Egypt.

Chemicals and drugs: Ovalbumin (*OVA*) was purchased from Loba Chemie PVT. Ltd. (Bombay, india). **Saxagliptin monohydrate (*SAX*), Onglyza 5mg tablets,** manufactured by *Bristol Myers Squibb* (Pennington,NJ,USA), was purchased from the market. It was prepared as 0.25% suspension in 0.5% CMC immediately before use. Other chemical reagents used in the experiment were analytical grade.

***OVA*-induced allergic asthma protocol and treatment:** In order to evaluate the preventive potential of *SAX* on *OVA*-induced allergic asthma in mice, mice were randomly allocated into three equal groups (n =10/group) as follows: Control (*CTL*) group, allergic asthma group (*OVA* group), and treatment group(*SAX* group) [7].

Briefly, mice were sensitized with an IP injection of 20 µg *OVA* and 1 mg aluminum hydroxide [$AL(OH)_3$] on days 0 & 7. Then they were challenged, on days 14, 15 and 16 with nebulization of 1% (w/v) *OVA* in normal saline,1 hr after administration of *SAX* for 30 minutes[8].
Mice in the *CTL* group were sensitized and challenged with saline. On days 11–16, mice in the *SAX* group were administered *SAX* orally at dose of10mg/kg/day[9, 10]; mice in *CTL* group and *OVA* group were given saline. On Day 17, mice were sacrificed 24 hrs after the last *OVA* challenge and bronchoalveolar lavage fluid (BAL) in each group was collected, and the lung tissues were also isolated and washed with ice cold 1.15% potassium chloride (KCL) (pH 7.45) then weighed for calculation of lung per body weight ratio[11].

Collection of BAL and counting of total and differential leukocytes: For preparation of BAL, 0.5 ml of cold saline was infused through the lung and withdrawn for 3 times via tracheal cannula. The BALF was then centrifuged at 1000 rpm at 4ºC for 10 minutes. The supernatants were stored at-80ºC for biochemical measures and cytokines detections. Pellets containing BAL cells were resuspended in cold saline for total and differential leukocytes count. The total number of inflammatory cells was counted, then the samples were centrifuged onto glass slides and the numbers of eosinophils, neutrophils, macrophages and lymphocytes in BALF were detected by Wright–Giemsa staining[12].

Determination of IL-13 level in BAL: The level of IL-13 in BAL was quantified using ELISA kits according to the manufactory instructions.

Preparation of lung homogenate and determination of lipid peroxidation marker in the lung tissues: Left lung specimen was homogenized as previously described[13].Briefly, left lung sections were weighed and homogenized in 1.15% KCL solution to prepare 10% w/v lung homogenate. Lung homogenate was centrifuged and the supernatant was collected after centrifugation. The level of malondialdehyde (MDA), lipid peroxidation indicator, was measured in lung tissues following the procedure of Gerard-Monnier *et al.*, (1998) [14].

Lung histology examination: The right lung lobe from each animal was removed and fixed in 10% (v/v) neutral buffered formalin. The tissue was dehydrated, embedded in paraffin, sectioned at 5 mm thickness. Then the sections were stained with hematoxylin and eosin (H&E) stains. The histopathological alterations of the lung tissues were observed by treatment-blinded pathologist.

Statistical analysis: All results were expressed as means ± standard error of mean (SEM). Statistical analysis was performed by using Graph Pad Prism V. Statistical significance (P <0.05) was assessed by one-way analysis of variance (ANOVA) followed by Tukey test.

RESULTS

Effect of SAX on lung /body weight ratio in OVA-induced allergic asthmatic mice: The lung/body weight ratio of *OVA* group was significantly increased by 31% compared to the *CTL* group. In *SAX* group, the lung/body weight ratio significantly lowered by 22% when compared to that of the *OVA* group as shown in Figure (1).

Effect of SAX ontotal and differential cell countin BAL of OVA-induced allergic asthmatic mice: To assess whatever *SAX* could attenuate infiltration of inflammatory cells, total and differential cell count in BAL was determined. Mice in *OVA* group demonstrated significant elevation in the infiltration and the accumulation of total cells, macrophages, neutrophils, eosinophils, and lymphocytes compared to *CTL* group. However, *SAX* treatment significantly decreased the accumulation of these cells in comparison with *OVA* group (table 1).

Effect of SAXon IL-13 levelin BAL of OVA-induced allergic asthmatic mice: *As presented in figure 2,* micesensitized and challenged with *OVA* showed signification increase in the IL-13concentration in BAL when compared to mice in *CTL* group. This *OVA*-exacerbation of IL-13was significantly decreased by treatment with *SAX* compared to *OVA*-sensitized and challenged mice.

***Effect of SAX on* lipid peroxidation marker in the lung tissues *of OVA-induced allergic asthmatic mice*:** Mice in *OVA* group showed marked increase in MDA concentration in lung tissues. The increased MDA levels in the lung of mice were significantly attenuated upon *SAX* administration in comparison with *OVA*-treated mice (Figure 3).

***Effect of SAX on* histopathological alterations in the lung tissues *of OVA-induced allergic asthmatic mice*:** To explore the effect of *SAX* 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 Figure 4, mice sensitized and challenged by *OVA* demonstrated extensive infiltration of inflammatory cells surrounding the bronchi, thickened walls of bronchial epithelium and congested blood vessels, indicating airway inflammation of the *OVA*-treated mice.*SAX* treatment attenuates bronchial airway inflammation and accumulation of inflammatory cells.

DISCUSSION

In the current study, we explored the antiasthmatic effect of *SAX* on *OVA*-induced allergic asthma. We demonstrated for the first time that oral administration of *SAX* attenuated asthma-associated lung injury by decreasing the infiltration of inflammatory cells around bronchial airway and vessels, interfering with IL-13production in BAL and suppressing lipid peroxidation occurred in the lung tissues following sensitization and challenge with *OVA*.

In recent years, several studies have demonstrated that DPP4 could become a therapeutic approach for management of allergic asthma [15]. Moreover, *SAX*, a DPP-4 inhibitor, was reported to have antioxidant, antifibrotic and anti-inflammatory activities which may be beneficial in treatment of asthma and other inflammatory diseases [7, 16, 17] . In agreement with these studies, in the current work, we provide novel evidence that *SAX* has antiasthmatic activity and could mitigate allergic airway inflammation.

Th-2 immune response is considered as the hallmark of *OVA*-induced allergic asthma through infiltration of inflammatory cells and expression of Th2 cytokines, such as IL-4, IL-5 and IL-13 [18, 19]. IL-4, and IL-5 control the maturation, survival and differentiation of eosinophils [20]. IL-13, another key cytokine in asthma, promotes B-cell differentiation and plays a dominant role in airway inflammation and hyper responsiveness and mucus secretion [21]. Recent studies provided evidence that inhibition of Th2 immune response could mitigate allergic asthma [12].

Results of our study showed that notable increase in the total and differential inflammatory cell counts in BALF of *OVA*- challenged mice was abolished by *SAX*. In addition, *SAX* also decreased the level of Th2-associated IL-13 and the number of eosinophils and basophils, suggesting that antiasthmatic effect of *SAX* may be related to interference with Th2 immune response. Furthermore, histological analysis showed that *SAX* mitigated *OVA*-induced lung tissue injury; providing evidence for the protective effect of *SAX* against asthma.

Oxidative stress has been participated in the development of *OVA*-induced allergic asthma. In patients with allergic inflammatory airway, recruited inflammatory cells release excess reactive oxygen species (ROS) with a shift toward massive level of oxidative stress [22, 23].MDA, one of several by-products of lipid peroxidation process, was measured in lung homogenate to reflect the redox imbalance in asthmatic airways. In the present study, mice in *SAX* group had decreased level of MDA in lung homogenate as compared to *CTL* mice. This apparent *SAX* antioxidant activity is consistent with some previous findings reporting antioxidant activity of *SAX* in various tissues [4, 5, 24].

CONCLUSION

We demonstrated that *SAX* mitigated the symptoms of asthma in *OVA*-induced allergic asthmatic mice. Further, this study showed that *SAX* inhibited leukocyte infiltration, cytokine production and oxidative stress in the allergic asthmatic lung, which may support the anti-asthmatic effect of *SAX*.

Table 1: Table 1: Effect of oral administration of SAX (10 mg/kg/day) on total and differential inflammatory cells counts in bronchoalveolar lavage fluid (BAL)of OVA-induced allergic asthmatic mice.

Group	Total leucocyte count X 10^4	Neutrophil count X 10^4	Lymphocyte count X 10^4	Monocyte count X 10^4	Eosinophil count x 10^4
CTL group	7.35 ±0.2	6.07 ± 0.3	0.7 ± 0.02	0.23 ± 0.01	0.21 ± 0.01
OVA group	32.4 ± 0.8*	27.02 ± 1.3 *	4.07 ± 0.2 *	0.65 ± 0.01 *	0.65 ± 0.01*
SAX group	10.28 ± 0.6 *#	8.3 ± 0.7#	1.46 ± 0.24 #	0.32 ± 0.02 *#	0.20 ± 0.02#

OVA challenge was performed for 30 min after *OVA* sensitization on day 0 & 7 and *SAX* was administered once daily 1 hr before *OVA* challenge from days 11 to 16. *CTL* (control group), *OVA* (Ovalbumin group) and *SAX* (Saxagliptin group). Values represent the mean ± SEM, n=10. Data were statistically evaluated by means of ***one way analysis of variance*** followed by ***Tukey-Kramer*** multiple comparisons test.
* *P<0.05,* compared with *CTL* group
# *P<0.05,* compared with *OVA* group

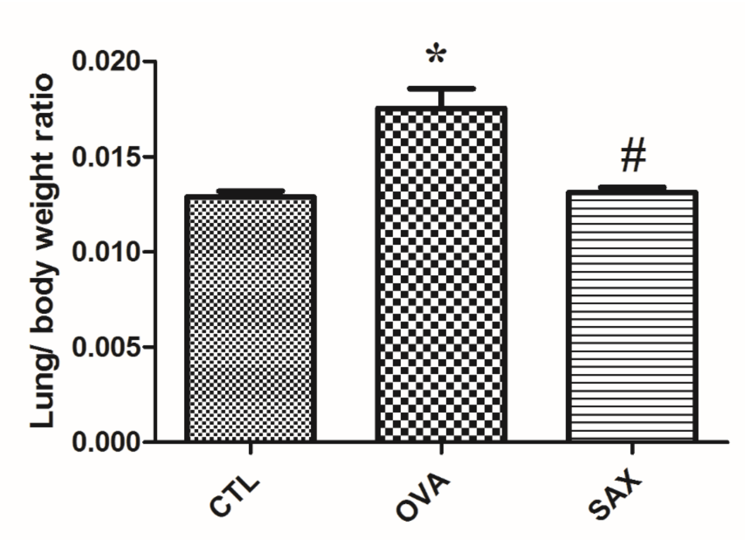


Figure 1: Effect of oral administration of *SAX* (10 mg/kg/day) on lung /body weight ratio in *OVA*-induced allergic asthmatic mice.

OVA challenge was performed for 30 min after *OVA* sensitization on day 0 & 7 and *SAX* was administered once daily 1 hr before OVA challenge from days 11 to 16. *CTL* (control group), *OVA* (Ovalbumin group) and *SAX* (Saxagliptin group). Values represent the mean ± SEM, n=10. Data were statistically evaluated by means of *one way analysis of variance* followed by *Tukey-Kramer* multiple comparisons test.
* *P<0.05,* compared with *CTL* group; # *P<0.05,* compared with *OVA* group

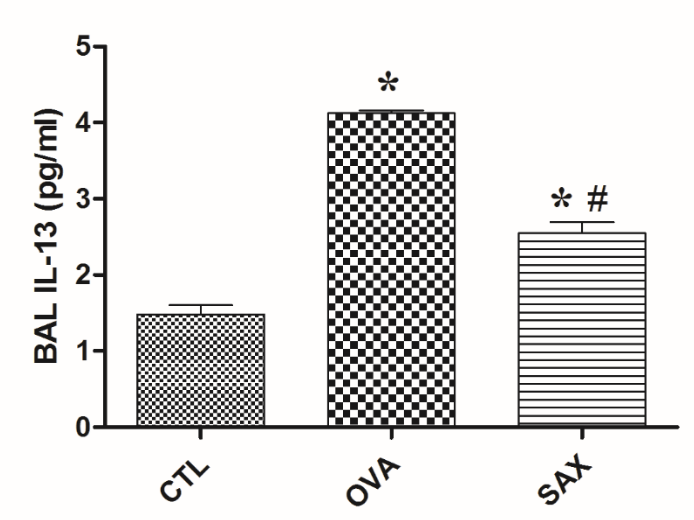


Figure 2: Effect of oral administration of *SAX* (10 mg/kg/day) on IL-13 level in BAL of *OVA*-induced allergic asthmatic mice

OVA challenge was performed for 30 min after *OVA* sensitization on day 0 & 7 and *SAX* was administered once daily 1 hr before *OVA* challenge from days 11 to 16. *CTL* (control group), *OVA* (Ovalbumin group) and *SAX* (Saxagliptin group). Values represent the mean ± SEM, n=10. Data were statistically evaluated by means of ***one way analysis of variance*** followed by ***Tukey-Kramer*** multiple comparisons test.

* *P<0.05,* compared with *CTL* group; # *P<0.05,* compared with *OVA* group

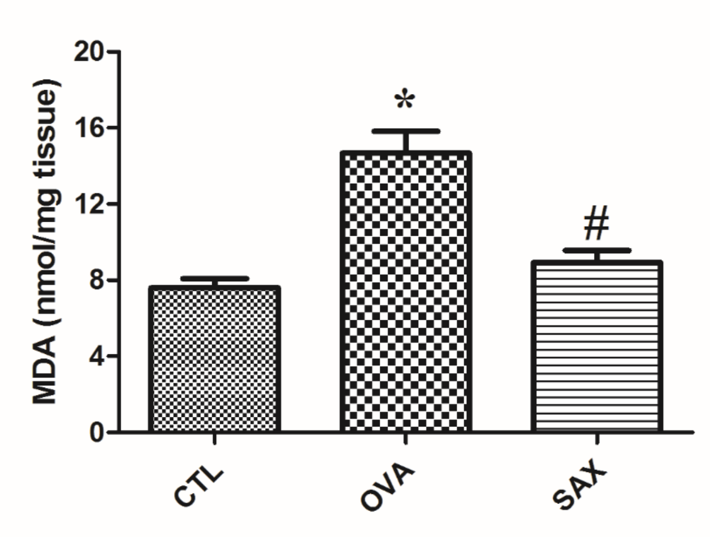


Figure (3): Effect of oral administration of *SAX* (10 mg/kg/day) onMDA, lipid peroxidation marker, in the lung tissues of *OVA*-induced allergic asthmatic mice.

OVA challenge was performed for 30 min after *OVA* sensitization on day 0 & 7 and *SAX* was administered once daily 1 hr before *OVA* challenge from days 11 to 16.

CTL (control group), *OVA* (Ovalbumin group), *SAX* (Saxagliptin group) and MDA (malondialdehyde).

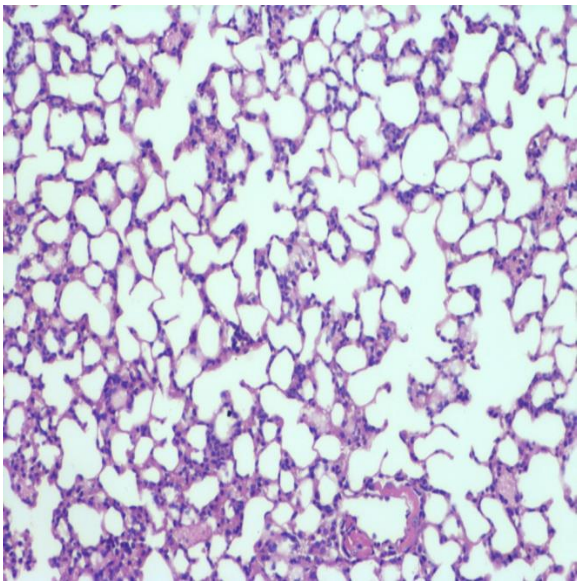
Values represent the mean ± SEM, n=10.

Data were statistically evaluated by means of ***one way analysis of variance*** followed by ***Tukey-Kramer*** multiple comparisons test.

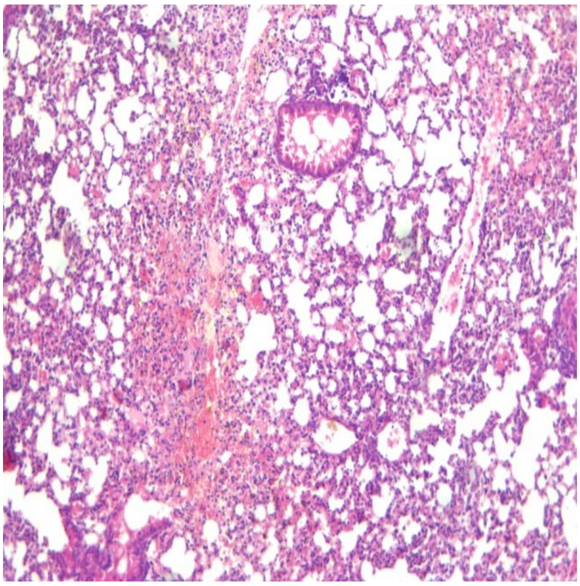
* *P<0.05,* compared with *CTL* group

# *P<0.05,* compared with *OVA* group

CTL



OVA



SAX

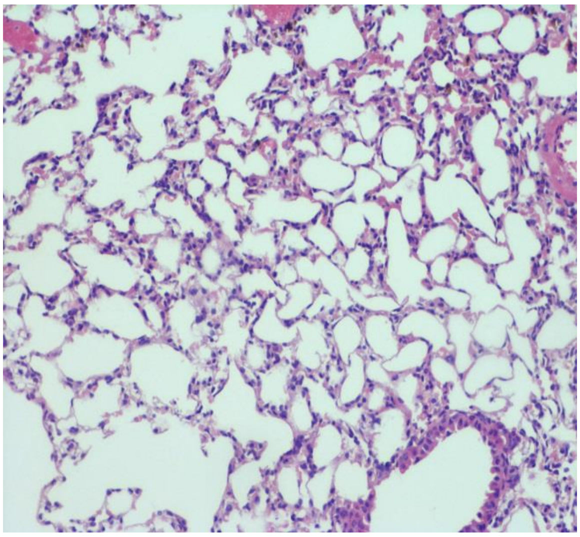


Figure 4: Effect of oral administration of SAX (10 mg/kg/day) on histopathological alterations (hematoxylin-eosin staining, 20x) in the lung tissues of OVA-induced allergic asthmatic mice.
(CTL) control group showed normal bronchial airway. (OVA) OVA group showed extensive infiltration of inflammatory cells surrounding the bronchi, thickened walls of bronchial epithelium and congested blood vessels (SAX) Saxagliptin group showed attenuation of bronchial airway inflammation and infiltration of inflammatory cells.

REFERENCES

1. Belice PJ, Becker EA. Effective education parameters for trigger remediation in underserved children with asthma: A systematic review. The Journal of asthma : official journal of the Association for the Care of Asthma. 2017;54(2):186-201.
2. Deacon CF, Holst JJ. Dipeptidyl peptidase-4 inhibitors for the treatment of type 2 diabetes: comparison, efficacy and safety. Expert Opinion on Pharmacotherapy. 2013;14(15):2047-58.
3. Matheeussen V, Baerts L, De Meyer G, De Keulenaer G, Van der Veken P, Augustyns K, et al. Expression and spatial heterogeneity of dipeptidyl peptidases in endothelial cells of conduct vessels and capillaries. Biological Chemistry2011. p. 189.
4. Chang YP, Sun B, Han Z, Han F, Hu SL, Li XY, et al. Saxagliptin Attenuates Albuminuria by Inhibiting Podocyte Epithelial- to-Mesenchymal Transition via SDF-1alpha in Diabetic Nephropathy. Frontiers in pharmacology. 2017;8:780.
5. Liu Y, Zhang Z, Chen R, Sun J, Chen H. [Therapeutic effect of saxagliptin in rat models of nonalcoholic fatty liver and type 2 diabetes]. Nan fang yi ke da xue xue bao = Journal of Southern Medical University. 2014;34(6):862-8.

6. Moreno-Macias H, Romieu I. Effects of antioxidant supplements and nutrients on patients with asthma and allergies. The Journal of allergy and clinical immunology. 2014;133(5):1237-44; quiz 45.
7. Gangadharan Komala M, Gross S, Zaky A, Pollock C, Panchapakesan U. Saxagliptin reduces renal tubulointerstitial inflammation, hypertrophy and fibrosis in diabetes. Nephrology (Carlton, Vic). 2016;21(5):423-31.
8. Yosri H, Elkashef WF, Said E, Gameil NM. Crocin modulates IL-4/IL-13 signaling and ameliorates experimentally induced allergic airway asthma in a murine model. International immunopharmacology. 2017;50:305-12.
9. Ryu JH, Xie C, Kim EJ, Park SH, Choi YJ, Kang SS, et al. Reduction of Asthmatic Parameters by Sea Hare Hydrolysates in a Mouse Model of Allergic Asthma. Nutrients. 2017;9(7).
10. Brown SM, Smith CE, Meuth AI, Khan M, Aroor AR, Cleeton HM, et al. Dipeptidyl Peptidase-4 Inhibition With Saxagliptin Ameliorates Angiotensin II-Induced Cardiac Diastolic Dysfunction in Male Mice. Endocrinology. 2017;158(10):3592-604.
11. Pearce ML, Yamashita J, Beazell J. MEASUREMENT OF PULMONARY EDEMA. Circulation research. 1965;16:482-8.
12. Zhang Q, Wang L, Chen B, Zhuo Q, Bao C, Lin L. Propofol inhibits NF-kappaB activation to ameliorate airway inflammation in ovalbumin (OVA)-induced allergic asthma mice. International immunopharmacology. 2017;51:158-64.
13. Yao XJ, Huang KW, Li Y, Zhang Q, Wang JJ, Wang W, et al. Direct comparison of the dynamics of IL-25- and 'allergen'-induced airways inflammation, remodelling and hypersensitivity in a murine asthma model. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2014;44(5):765-77.
14. Gerard-Monnier D, Erdelmeier I, Regnard K, Moze-Henry N, Yadan JC, Chaudiere J. Reactions of 1-methyl-2-phenylindole with malondialdehyde and 4-hydroxyalkenals. Analytical applications to a colorimetric assay of lipid peroxidation. Chemical research in toxicology. 1998;11(10):1176-83.
15. Shiobara T, Chibana K, Watanabe T, Arai R, Horigane Y, Nakamura Y, et al. Dipeptidyl peptidase-4 is highly expressed in bronchial epithelial cells of untreated asthma and it increases cell proliferation along with fibronectin production in airway constitutive cells. Respiratory research. 2016;17:28.
16. Kagal UA, Angadi NB, Matule SM. Effect of dipeptidyl peptidase 4 inhibitors on acute and subacute models of inflammation in male Wistar rats: An experimental study. International journal of applied & basic medical research. 2017;7(1):26-31.
17. Kenawy S, Hegazy R, Hassan A, El-Shenawy S, Gomaa N, Zaki H, et al. Involvement of insulin resistance in D-galactose-induced age-related dementia in rats: Protective role of metformin and saxagliptin. PloS one. 2017;12(8):e0183565.
18. Durrant DM, Metzger DW. Emerging roles of T helper subsets in the pathogenesis of asthma. Immunological investigations. 2010;39(4-5):526-49.
19. Elias JA, Lee CG, Zheng T, Ma B, Homer RJ, Zhu Z. New insights into the pathogenesis of asthma. The Journal of clinical investigation. 2003;111(3):291-7.
20. Hogan SP, Rosenberg HF, Moqbel R, Phipps S, Foster PS, Lacy P, et al. Eosinophils: Biological Properties and Role in Health and Disease. Clinical & Experimental Allergy. 2008;38(5):709-50.
21. Halim TY, Krauss RH, Sun AC, Takei F. Lung natural helper cells are a critical source of Th2 cell-type cytokines in protease allergen-induced airway inflammation. Immunity. 2012;36(3):451-63.
22. Nesi RT, Kennedy-Feitosa E, Lanzetti M, Avila MB, Magalhaes CB, Zin WA, et al. Inflammatory and Oxidative Stress Markers in Experimental Allergic Asthma. Inflammation. 2017;40(4):1166-76.
23. Nesi RT, Barroso MV, Souza Muniz V, de Arantes AC, Martins MA, Brito Gitirana L, et al. Pharmacological modulation of reactive oxygen species (ROS) improves the airway hyperresponsiveness by shifting the Th1 response in allergic inflammation induced by ovalbumin. Free radical research. 2017;51(7-8):708-22.
24. Solini A, Rossi C, Duranti E, Taddei S, Natali A, Virdis A. Saxagliptin prevents vascular remodeling and oxidative stress in db/db mice. Role of endothelial nitric oxide synthase uncoupling and cyclooxygenase. Vascular pharmacology. 2016;76:62-71.