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Original article

Inhibitory effects of sildenafil and tadalafil on inflammation, oxidative stress and nitrosative stress in animal model of bronchial asthma

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ABSTRACT

Background: Cyclic neucleotides are involved in many cellular functions including smooth muscle relaxation, inflammation, and signal transduction. Sildenafil and tadalafil are phosphodiesterase-5 (PDE-5) inhibitors which prevent the degradation of cyclic neucleotide *i.e.* guanosine 3′,5′ cyclic monophosphate (cGMP) and increase the levels of cGMP. In this study sildenafil and tadalafil were evaluated for their anti-inflammatory, anti-oxidative and anti-nitrosative stress potential in animal model of bronchial asthma.

Methods: Wistar rats were sensitized with 10 mg intraperitoneal (*ip*) ovalbumin adsorbed to 10 μg of aluminum hydroxide on day 0. Animals were given sildenafil (1 and 3 mg/kg *ip*) and tadalafil (1 and 3 mg/kg *ip*) from day 1 to day 14. Also, on day 14 animals were challenged with ovalbumin (1 mg *ip*). After 24 h, samples were collected to analyze interleukin-4 (IL-4) and tumour necrosis factor-α (TNF-α), in serum and bronchoalveolar lavage fluid (BALF). The oxidative stress markers malondialdehyde (MDA), reduced glutathione (GSH) and nitric oxide metabolites (NO_x) were also measured in serum.

Results: Pre-treatment with sildenafil (1 and 3 mg/kg *ip*) and tadalafil (1 and 3 mg/kg *ip*) significantly reduced the levels of pro-inflammatory cytokines IL-4 and TNF-α in rat serum and BALF. In addition, pre-treatment with both the drugs decreased the levels of MDA and NO_x and increased the levels of GSH in serum.

Conclusions: Sildenafil and tadalafil decreased pro-inflammatory cytokines in serum and BALF. Both drugs inhibit oxidative and nitrosative stress in animal model of bronchial asthma and could have a therapeutic potential in bronchial asthma.

Introduction

Bronchial asthma is a syndrome characterized by inflammation followed by airflow obstruction and bronchial hyper-responsiveness to a variety of stimuli; resulting in episodic bronchoconstriction and increased mucus secretion, which are the hallmarks of the disease. Airway inflammation is central to the pathogenesis of bronchial asthma. Besides, disturbances in pro/anti-oxidant balance also have a key role to play. Asthma is associated with increased oxidative stress as activated inflammatory cells such as macrophages and eosinophils give rise to generation of reactive oxygen species [1]. The principal therapeutic objective in asthma is to reduce airway inflammation and to reverse bronchoconstriction.

Phosphodiesterases (PDEs), a family of enzymes controls degradation of intracellular cyclic neucleotides cAMP/cGMP that are integral to variety of cellular functions like smooth muscle relaxation, inflammation, and signal transduction [2,3]. Pharmacotherapy of various inflammatory airway diseases like asthma and chronic obstructive pulmonary disease (COPD) is now being targeted at increasing the levels of cyclic adenosine monophosphate (cAMP) or cyclic guanosine monophosphate (cGMP). Increased cAMP or cGMP levels cause bronchodilatory, anti-inflammatory and pulmonary vasodilatory effects [4–6]. There are eleven known isoforms of PDEs, of which PDE-5 is widely expressed in the airway epithelium and preferentially hydrolyses cGMP [7,8]. Sildenafil and tadalafil are specific PDE-5 inhibitors, which have been used in airway diseases like COPD and pulmonary artery hypertension. Sildenafil/tadalafil cause bronchodilation by protein-kinase G dependent mechanism resulting from rise in cGMP levels [9].

Zaprinast (a PDE-5 inhibitor) has produced bronchodilator effect in patients with asthma [10,11]. Also, vardenafil (another selective PDE-5 inhibitor) has shown an anti-allergic effect by inhibiting immunologic and non-immunologic mast-cell-

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mediated allergic reactions in murine models [12]. Sildenafil is more selective for PDE-5 and is more potent than zaprinast. Various studies have shown anti-inflammatory, anti-oxidative and anti-nitrosative stress potential of sildenafil [13,14] but the same remains unexplored in a model of bronchial asthma. Therefore, in the present study we investigate the effect of two PDE-5 inhibitors *i.e.* sildenafil and tadalafil on inflammation, oxidative stress and nitrosative stress in experimentally induced bronchial asthma. An animal model of allergic asthma was developed in rats as described earlier [15–17]. The effects of sildenafil and tadalafil were evaluated on markers of inflammation – interleukin-4 (IL-4) and tumour necrosis factor-α (TNF-α) in serum and bronchoalveolar lavage fluid (BALF). Additionally, oxidative stress markers malondialdehyde (MDA), reduced glutathione (GSH), and nitrosative stress markers were measured in serum.

Material and methods

Wistar rats (220–250 g) of either sex were used in the study. The study protocol was approved by the Institutional animal ethics committee. Animal experiments and procedures were conducted as per CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines for the use and care of laboratory animals. The animals were housed in standard laboratory conditions. The animals had free access to standard pellet diet and water.

The drugs and chemical used in the study were: Sildenafil [Silagra 50™, Cipla India (with calcium hydrogen phosphate anhydrous, cellulose microcrystalline, croscarmellose sodium, silica colloidal anhydrous, magnesium stearate, lactose monohydrate, hypromellose, titanium dioxide (E171), triacetin as excipients)], tadalafil [Tadacip 20™, Cipla India (with lactose monohydrate, hypromellose, triacetin, titanium dioxide (E171), iron oxide yellow (E172), talc, lactose monohydrate, croscarmellose sodium, hydroxypropylcellulose, microcrystalline cellulose, sodium laurilsulfate, magnesium stearate as excipients) and prednisolone (Wysolone 5™ Wyeth India), Ovalbumin (Sigma-Aldrich, USA) and aluminum hydroxide (Sigma-Aldrich, USA). All drugs/chemical were of high analytical grade. Drug/chemical solutions were freshly prepared using physiological saline.

Sensitization and challenge schedule in animals [18]

Wistar rats were sensitized with ovalbumin (10 mg per rat, *ip*) adsorbed to 10 μg of aluminum hydroxide on day 0. After sensitization, rats were divided into six groups (n = 12):

Group 1 (disease-control) normal saline (2 ml/kg *ip*) was given to disease-control group from day 1 to day 14.

Group 2 received sildenafil (1 mg/kg/day, *ip*) from day 1 to day 14 and group 3 received sildenafil in the doses of 3 mg/kg/day *ip* from day 1 to day 14. Group 4 received tadalafil (1 mg/kg/day *ip*) from day 1 to day 14 and group 5 received tadalafil in the doses of 3 mg/kg/day *ip* from day 1 to day 14.

Dose selection was based on available literature using sildenafil [19,20] and tadalafil [21,22] in animal models.

Group 6 received prednisolone 5 mg/kg *ip* which was taken as positive control for cytokines in serum and BALF.

On day 14, all animals were challenged with ovalbumin (1 mg *ip*) in 0.5 ml of isotonic saline. After 24 h (on day 15) of challenge all animals were anaesthetized for terminal sacrifice and blood and BALF were collected.

Serum separation and BALF collection

The collected blood from each rat was centrifuged (4000 rpm, for 10 min at 4 °C) to remove serum and the yielded serum samples stored at −80 °C for analysis of TNF-α, IL-4, MDA, GSH, and nitric oxide metabolites (NO_x). BALF was obtained after cannulating the trachea of the rats. BALF sample were centrifuged at 1000 rpm for 10 min at 4 °C, and the resultant cell-free supernatant was used for estimation of cytokines (TNF-α and IL-4).

Cytokines (TNF-α, IL-4) measurement in serum and BALF

TNF-α and IL-4 levels were estimated in serum and BALF sample using ELISA kits. The detection limits of kits were 20 pg/ml and 15 pg/ml for TNF-α and IL-4, respectively. Microtiter plates were pre-coated with antibody against the rat cytokines being measured. Standards of the analyte and serum/BALF samples (50 μl) were added to the wells and incubated for 3 h (4 h for IL-4). After washings 100 μl of antibody solution was added and incubated for 1 h. Then, 100 μL of avidin-horseradish peroxidase solution was added and after 30 min, 100 μl of tetramethylbenzidine was added. After 15–20 minutes, 100 μl of 1 M sulphuric acid was added. The absorbance was read at 450 nm and the standard curves were plotted.

Estimation of GSH in serum

GSH was estimated by the method described by Ellman [23]. Briefly, 1 ml TCA (5%) was added to 0.5 ml of serum sample and the mixture centrifuged to remove the proteins. To 0.1 ml of this homogenate, 4 ml of phosphate buffer (pH 8.4), 0.5 ml of DTNB and 0.4 ml double distilled water were added. The mixture was vortexed and absorbance read at 412 nm within 15 min. Also, the same above steps were subjected with concentrations of standard glutathione (1–100 μg). The readings of absorbance were plotted against the concentration of GSH to produce standard curve. The concentration of GSH was determined by linear standard graph.

Estimation of malondialdehyde (MDA) in serum

MDA (indicator of lipid peroxidation) was estimated as described by Okhawa et al. [24]. In serum sample (0.5 ml), acetic acid (20%, pH 3.5) 1.5 ml, thiobarbituric acid (0.8%), sodium lauryl sulfate (8.1%) 0.2 ml were added. The mixture was heated at 100 °C for 1 h in boiling water bath and cooled with tap water. Five ml of butanol: pyridine (15:1% v/v) and 1 ml of distilled water were added in the mixture. The mixture was centrifuged at 4000 rpm for 10 min. Thereafter, absorbance measured at 532 nm using a spectrophotometer.

Estimation of nitrates and nitrites (NO_x) in serum

NO_x were estimated by as described by Tracey [25]. Serum NO_x contents were determined in which aspergillus nitrate reductase was coupled with NADPH and FAD to convert all nitrates present in sample into nitrites. Assay mixture contained 50 μl serum, 10 μl of 0.86 mM/l NADPH, 10 μl of 0.11 mM/l FAD, 10 μl nitrate reductase (2 U/ml) and 20 μl of 310 mM/l potassium phosphate buffer in a total assay volume of 100 μl. Samples were incubated at 37 °C for one hour in the dark, followed by addition of 5 μl of 1 M/l zinc sulphate to precipitate proteins. After centrifugation of microtubes, 50 μl of supernatant from each microtube was transferred into individual wells of 96 well microplate followed by addition of 100 μl Griess reagent, for color development. Readings were taken after 10 min in at 540 nm. Standard curves were generated using known concentration of sodium nitrate and converted to NO_x content by using a nitrate standard curve. Data was expressed as nmol/ml of serum.

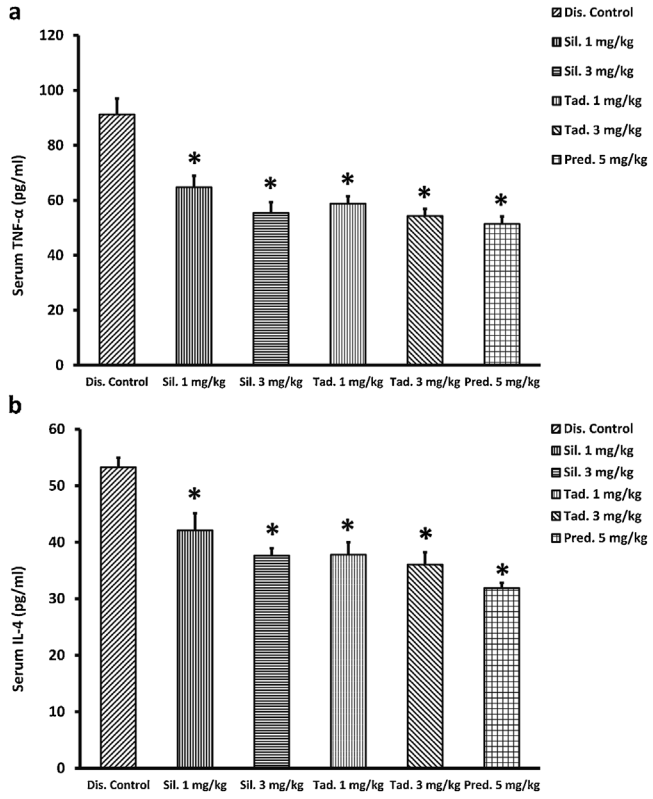

Fig. 1. Changes in serum TNF-α (a) and IL-4 (b) levels in ovalbumin challenged Wistar rats at day 14. Animals were first sensitized (day 0) and then divided into six treatment groups (n = 12/group). They received normal saline 2 ml/kg (disease-control), sildenafil 1 mg/kg, sildenafil 3 mg/kg, tadalafil 1 mg/kg, tadalafil 3 mg/kg and prednisolone 5 mg/kg, *ip* for 14 days. Results are expressed as mean ± SE. $^{*}p<0.05$ was considered significant as determined by one-way analysis of variance followed by Dunnett's t-test.

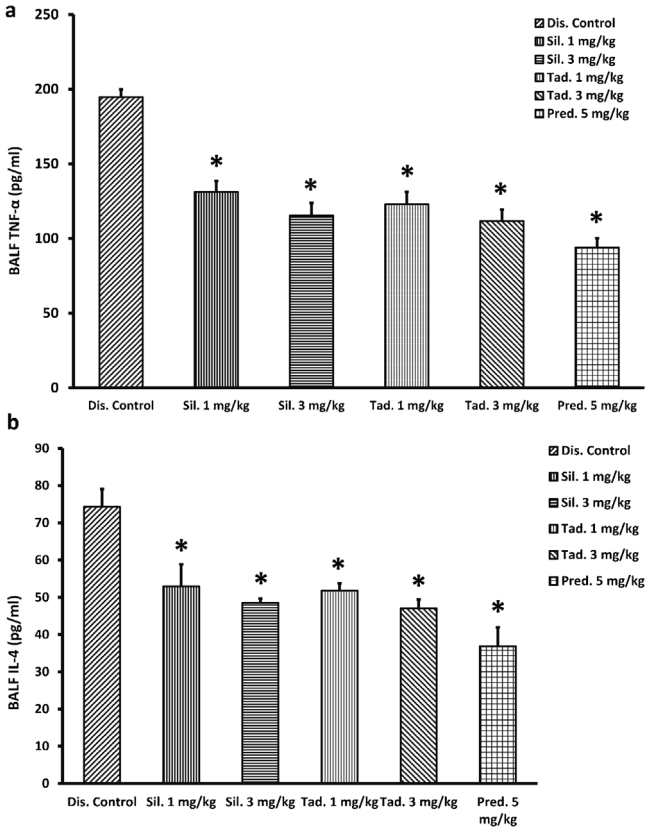

Fig. 2. Changes in serum TNF-α (a) and IL-4 (b) levels BALF of ovalbumin challenged Wistar rats at day 14. Animals were first sensitized (day 0) and then divided into six treatment groups (n = 12/group). They received normal saline 2 ml/kg (disease-control), sildenafil 1 mg/kg, sildenafil 3 mg/kg, tadalafil 1 mg/kg tadalafil 3 mg/kg and prednisolone 5 mg/kg, *ip* for 14 days. Results are expressed as mean ± SE. $^{*}p<0.05$ was considered significant as determined by one-way analysis of variance followed by Dunnett's t-test.

Statistical analysis

All results are expressed as Mean ± SE. Statistical calculations were performed by GraphPad Prism (version 5.00, open source). The results obtained were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's t-test. Values of $p<0.05$ were considered significant.

Results

Effect of sildenafil and tadalafil on TNF-α and IL-4 levels in serum and BALF

Sildenafil (1 and 3 mg/kg) dose dependently reduced the levels of serum TNF-α as compared to disease-control ($p < 0.05$) (Fig. 1a). The reductions were statistically significant. These changes were comparable to the effects of the standard drug, prednisolone (5 mg/kg) which also caused statistically significant decrements in serum TNF-α.

Similarly, tadalafil (1 and 3 mg/kg) also caused statistically significant reductions in serum TNF-α levels as compared to disease-control ($p < 0.05$) (Fig. 1a). These results were comparable to the standard drug, prednisolone 5 mg/kg which also caused a statistically significant reduction in serum TNF-α levels (Fig. 1a).

Sildenafil (1 and 3 mg/kg) significantly reduced the levels of serum IL-4 as compared to disease-control ($p < 0.05$) (Fig. 1b). These changes were comparable to the effects of the standard drug, prednisolone (5 mg/kg) which also caused statistically significant decrements in serum IL-4. Tadalafil (1 and 3 mg/kg) also exhibited similar reductions in serum IL-4 as compared to disease-control ($p < 0.05$, Fig.1b). These results were comparable to the standard drug, prednisolone 5 mg/kg which also caused a statistically significant reduction in serum TNF-α levels (Fig. 1b).

Sildenafil (1and 3 mg/kg) caused statistically significant decrements in the levels of BALF TNF-α as compared to disease-control ($p < 0.05$) (Fig. 2a). The results were comparable to standard drug prednisolone (5 mg/kg), which caused statistically significant decrement in BALF TNF-α (Fig. 2a).

Tadalafil (1 and 3 mg/kg) also exhibited similar reductions and the results were statistically significant when compared to disease-control ($p < 0.05$). The results of tadalafil on BALF TNF-α levels were comparable to standard drug prednisolone (5 mg/kg), which caused statistically significant decrement in BALF TNF-α (Fig. 2a).

Treatment with sildenafil (1 and 3 mg/kg) significantly reduced BALF IL-4 levels as compared to disease-control ($p < 0.05$). The results were comparable to standard drug prednisolone (5 mg/kg), which caused statistically significant decrement in BALF IL-4 (Fig. 2b).

Tadalafil in the doses of 1 and 3 mg/kg also significantly reduced BALF IL-4 levels ($p < 0.05$). The standard drug prednisolone in the doses of 5 mg/kg resulted in a statistically significant decrement in IL-4 level in BALF. The results were comparable to standard drug prednisolone (5 mg/kg), which caused statistically significant decrement in BALF IL-4 (Fig. 2b).

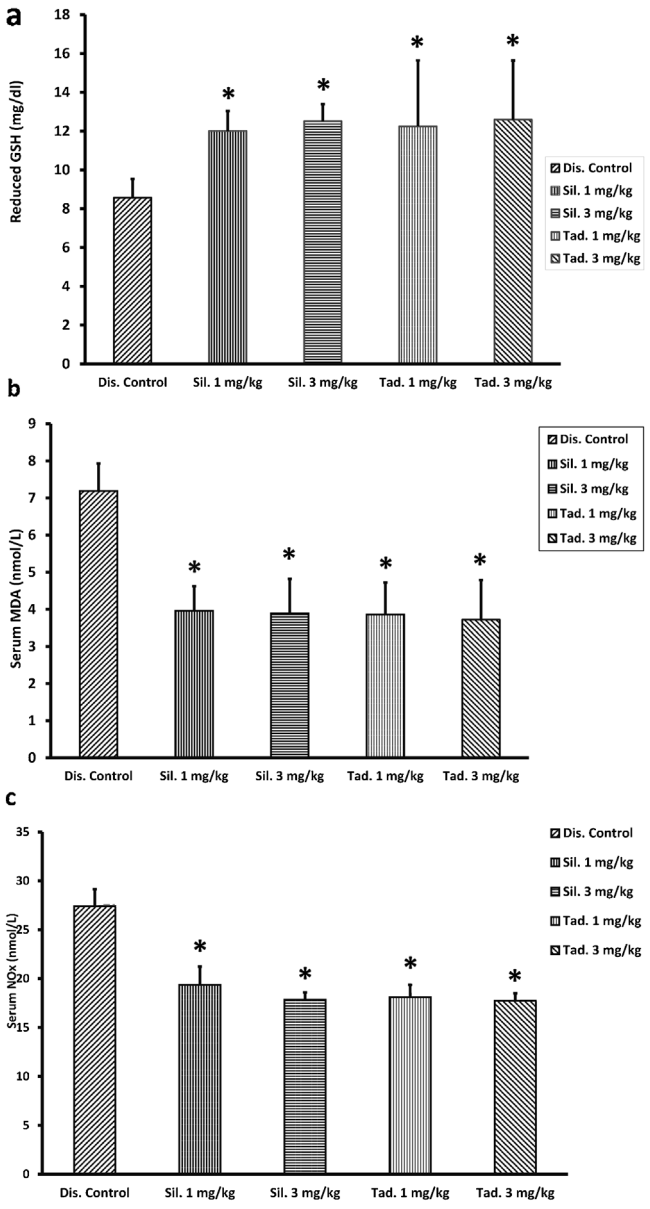

Fig. 3. Changes in reduced GSH (a), MDA (b) and nitric oxide metabolites'(c) levels in ovalbumin challenged Wistar rats' serum at day 14. Animals were first sensitized (day 0) and then divided into treatment groups (n = 12/group). They received normal saline 2 ml/kg (disease-control), sildenafil 1 mg/kg, sildenafil 3 mg/kg, tadalafil 1 mg/kg and tadalafil 3 mg/kg, *ip* for 14 days. Results are expressed as mean ± SE. *$p<0.05$ was considered significant as determined by one-way analysis of variance followed by Dunnett's t-test.

Effects of sildenafil and tadalafil on serum oxidative and nitrosative stress markers

Sildenafil (1 and 3 mg/kg) caused dose dependent and statistically significant increments in serum GSH as compared to the disease-control ($p < 0.05$) (Fig. 3a). Similarly, tadalafil (1 and 3 mg/kg) also caused statistically significant increments in serum GSH levels as compared to the disease-control ($p < 0.05$, Fig. 3a).

Sildenafil (1 and 3 mg/kg) resulted in statistically significant reductions in serum MDA levels as compared to disease-control ($p < 0.05$) (Fig. 3b).

Tadalafil (1 and 3 mg/kg) also brought about statistically significant decrease in serum MDA levels as compared to disease-control ($p < 0.05$, Fig. 3b)

Sildenafil (1and 3 mg/kg) caused statistically significant reductions in the serum nitric oxide metabolites' levels in comparison to disease-control ($p < 0.05$) (Fig. 3c). Tadalafil (1 and 3 mg) also exhibited similar inhibitory responses which was statistically significant as compared to disease-control ($p < 0.05$, Fig. 3c).

Discussion

In the present study, animal model of allergic asthma in rats was developed and the effects of sildenafil and tadalafil were evaluated on standard markers of inflammation, oxidative and nitrosative stress. Inflammatory cytokines levels (TNF-α and IL-4) were measured in rat serum and BALF. GSH, MDA and nitric oxide metabolites were estimated in serum.

Sildenafil and tadalafil, at two doses of 1 and 3 mg/kg *ip* each, significantly reduced levels of inflammatory cytokines TNF-α and IL-4 in both serum and BALF, in a dose-dependent manner. Our results are in concurrence with other studies. One study has shown that sildenafil reduced airway inflammation through restoring the cGMP pathway in acrolein-induced airway inflammation in rats [13]. In another study, sildenafil treatment reduced airway hyper-reactivity, leukocyte influx and NO generation in bronchial asthma model in guinea pigs by causing elevated cGMP [20].

Recent studies have indicated that disturbance in pro-oxidant/anti-oxidant balance may result in oxidative stress which could play a key role in the genesis of bronchial asthma [26].

In the present study, sildenafil and tadalafil have reduced oxidative and nitrosative stress. Both the drugs decreased serum MDA level, an index of lipid peroxidation, dose dependently. Significant increments in the level of reduced glutathione (GSH), a key anti-oxidant, were observed in both the treatment groups. Additionally, statistically significant reductions in the levels of serum NO_x were also observed in sildenafil and tadalafil treated groups.

Our results are in agreement with other studies showing anti-inflammatory and anti-oxidative potential of sildenafil and tadalafil. In one study the anti-oxidative and anti-inflammatory properties of sildenafil were suggested to protect against scald burn related acute lung injury in a rat model [14]. Another study has shown that tadalafil ameliorated silica-induced pulmonary damage in experimentally-induced pulmonary silicosis in rats and reduced BALF content of inflammatory cells, MDA, nitrite/nitrate, and TNF-α [27]. Similarly, a study has shown that sildenafil exerted antioxidant effect by increasing GSH and decreasing MDA levels in colon in experimental model of colitis in rats. Also, sildenafil reduced the levels of serum TNF-α in this model [28]. The beneficial role of sildenafil against oxidative damage has also been demonstrated in patients of pulmonary artery hypertension [29].

The anti-asthmatic properties of PDE-5 inhibitors might be related to both, inhibition of secretion of mediators of anaphylaxis and relaxation of lung smooth muscle, both of which are cyclic nucleotide dependent phenomena [30]. In addition, cAMP and cGMP control many cellular processes, such as inflammation, neuroendocrine signals and degranulation of neutrophils. cGMP is synthesized by soluble guanylate cyclases (sGCs). Sildenafil and tadalafil increase the concentration of cAMP and cGMP by inhibiting PDE-5 and enhance NO-cGMP signalization. Activation of sGC and generation of cGMP mediates the effects of NO. Further, NO synthase (NOS) is responsible for forming NO. NOS exists as endothelial (eNOS), neuronal (nNOS), and inducible (iNOS). Sildenafil also induces mRNA expression of eNOS [31]. eNOS produces NO at nanomolar concentrations, which has been documented to have anti-inflammatory actions [32], and appears to be related directly or indirectly to the inhibition of the key transcription factor – Nuclear Factor κB (NF-κB) [33,34]. Also, intracellular accumulation of cGMP activates cGMP dependent

protein kinase with subsequent phosphorylation of specific substrate proteins through activation of sGC, and activation of PKG (cGK), which in turn phosphorylates several proteins [35].

It is important to mention that other PDE-5 inhibitors have also shown their potential in inhibiting asthma symptoms and allergic reactions. Zaprinast (a PDE-5 inhibitor) has produced bronchodilator effect in patients with asthma [10,11].

Also, oral vardenafil (another selective PDE-5 inhibitor) administration ameliorated immunologic and non-immunologic mast-cell-mediated allergic reactions and reduced histamine release in murine models indicating its anti-allergic effect [12].

Our study has some limitations. One limitation is that parameters of oxidative and nitrosative stress assessed in serum samples. They should have been assessed in lung tissue instead. Another major limitation of the study is the absence of any histopathological investigations for the effects of asthma progression. Since limited data regarding the effect of PDE-5 inhibitors on inflammatory markers in experimental models and clinical studies in asthma is available, future studies are warranted to extend our findings by analyzing more markers of inflammation, *e.g.* IL-5, IL-13 and correlate them with histopathologial assessment in asthma models.

To summarize, the results of present study demonstrate that sildenafil and tadalafil decrease the levels of proinflammatory cytokines (IL-4 and TNF-α) in serum and BALF. Both the drugs inhibit oxidative and nitrosative stress in animal model of bronchial asthma. Further clinical studies are recommended to explore the effect of specific PDE-5 inhibitors in airway diseases.

Conflict of interest

The authors declare that they have no conflict of interest.

This research has been carried out using internal resources of the authors' institutes.

References

[1] Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. World Allergy Organ J 2012;5:9–19.
[2] Francis SH, Blount MA, Corbin JD. Mammalian cyclic nucleotide phosphodiesterases: molecular mechanisms and physiological functions. Physiol Rev 2011;91:651–90.
[3] Azevedo MF, Faucz FR, Bimpaki E, Horvath A, Levy I, de Alexandre RB, et al. Clinical and molecular genetics of the phosphodiesterases (PDEs). Endocr Rev 2014;35:195–233.
[4] Fan Chung K. Phosphodiesterase inhibitors in airways disease. Eur J Pharmacol 2006;533:110–7.
[5] Lugnier C. Cyclic nucleotide phosphodiesterase (PDE) superfamily: a new target for the development of specific therapeutic agents. Pharmacol Ther 2006;109:366–98.
[6] Halpin DM. ABCD of the phosphodiesterase family: interaction and differential activity in COPD. Int J Chron Obstruct Pulmon Dis 2008;3:543–61.
[7] Rotella DP. Phosphodiesterase 5 inhibitors: current status and potential applications. Nat Rev Drug Discov 2002;1:674–82.
[8] Bender AT, Beavo JA. Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use. Pharmacol Rev 2006;58:488–520.
[9] Beavo JA, Conti M, Heaslip RJ. Multiple cyclic nucleotide phosphodiesterases. Mol Pharmacol 1994;46:399–405.
[10] Rudd RM, Gellert AR, Studdy PR, Geddes DM. Inhibition of exercise-induced asthma by an orally absorbed mast cell stabilizer (M & B 22,948). Br J Dis Chest 1983;77:78–86.
[11] Boswell-Smith V, Spina D, Page CP. Phosphodiesterase inhibitors. Br J Pharmacol 2006;147(Suppl 1):S252–7.
[12] El-Awady MS, Said E. Vardenafil ameliorates immunologic- and non-immunologic-induced allergic reactions. Can J Physiol Pharmacol 2014;92:175–80.
[13] Wang T, Liu Y, Chen L, Wang X, Hu XR, Feng YL, et al. Effect of sildenafil on acrolein-induced airway inflammation and mucus production in rats. Eur Respir J 2009;33:1122–32.
[14] Gokakin AK, Deveci K, Kurt A, Karakus BC, Duger C, Tuzcu M, et al. The protective effects of sildenafil in acute lung injury in a rat model of severe scald burn: a biochemical and histopathological study. Burns 2013;39:1193–9.
[15] Bates JH, Rincon M, Irvin CG. Animal models of asthma. Am J Physiol Lung Cell Mol Physiol 2009;297:L401–10.
[16] Mukherjee AA, Kandhare AD, Rojatkar SR, Bodhankar SL. Ameliorative effects of Artemisia pallens in a murine model of ovalbumin-induced allergic asthma via modulation of biochemical perturbations. Biomed Pharmacother 2017;94:880–9.
[17] Dhawale VS, Amara VR, Karpe PA, Malek V, Patel D, Tikoo K. Activation of angiotensin-converting enzyme 2 (ACE2) attenuates allergic airway inflammation in rat asthma model. Toxicol Appl Pharmacol 2016;306:17–26.
[18] Kwasniewski FH, Tavares de Lima W, Bakhle YS, Jancar S. Impairment in connective tissue mast cells degranulation in spontaneously hypertensive rats: stimulus dependent resistance. Br J Pharmacol 1998;124:772–8.
[19] Abdulwaheb M, Makonnen E, Debella A, Abebe D. Effect of Catha edulis foresk (khat) extracts on male rat sexual behavior. J Ethnopharmacol 2007;110:250–6.
[20] Toward TJ, Smith N, Broadley KJ. Effect of phosphodiesterase-5 inhibitor, sildenafil (Viagra), in animal models of airways disease. Am J Respir Crit Care Med 2004;169:227–34.
[21] Gasanov F, Aytac B, Vuruskan H. The effects of tadalafil on renal ischemia reperfusion injury: an experimental study. Bosn J Basic Med Sci 2011;11:158–62.
[22] Koka S, Das A, Salloum FN, Kukreja RC. Phosphodiesterase-5 inhibitor tadalafil attenuates oxidative stress and protects against myocardial ischemia/reperfusion injury in type 2 diabetic mice. Free Radic Biol Med 2013;60:80–8.
[23] Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959;82:70–7.
[24] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351–8.
[25] Tracey WR, Nakane M, Kuk J, Budzik G, Klinghofer V, Harris R, et al. The nitric oxide synthase inhibitor, L-NG-monomethylarginine, reduces carrageenan-induced pleurisy in the rat. J Pharmacol Exp Ther 1995;273:1295–9.
[26] Erzurum SC. New insights in oxidant biology in asthma. Ann Am Thorac Soc 2016;13(Suppl 1):S35–9.
[27] Abdelaziz RR, Elkashef WF, Said E. Tadalafil reduces airway hyperactivity and protects against lung and respiratory airways dysfunction in a rat model of silicosis. Int Immunopharmacol 2016;40:530–41.
[28] Iseri SO, Ersoy Y, Ercan F, Yuksel M, Atukeren P, Gumustas K, et al. The effect of sildenafil, a phosphodiesterase-5 inhibitor, on acetic acid-induced colonic inflammation in the rat. J Gastroenterol Hepatol 2009;24:1142–8.
[29] Semen K, Yelisyeyeva O, Jarocka-Karpowicz I, Kaminskyy D, Solovey L, Skrzydlewska E, et al. Sildenafil reduces signs of oxidative stress in pulmonary arterial hypertension: Evaluation by fatty acid composition, level of hydroxynonenal and heart rate variability. Redox Biol 2016;7:48–57.
[30] Frossard N, Landry Y, Pauli G, Ruckstuhl M. Effects of cyclic AMP- and cyclic GMP- phosphodiesterase inhibitors on immunological release of histamine and on lung contraction. Br J Pharmacol 1981;73:933–8.
[31] Andersson KE. PDE5 inhibitors - pharmacology and clinical applications 20 years after sildenafil discovery. Br J Pharmacol 2018;175:2554–65.
[32] Rizzo M, Kotur-Stevuljevic J, Berneis K, Spinas G, Rini GB, Jelic-Ivanovic Z, et al. Atherogenic dyslipidemia and oxidative stress: a new look. Transl Res 2009;153:217–23.
[33] Aizawa T, Wei H, Miano JM, Abe J, Berk BC, Yan C. Role of phosphodiesterase 3 in NO/cGMP-mediated antiinflammatory effects in vascular smooth muscle cells. Circ Res 2003;93:406–13.
[34] Spiecker M, Peng HB, Liao JK. Inhibition of endothelial vascular cell adhesion molecule-1 expression by nitric oxide involves the induction and nuclear translocation of IkappaBalpha. J Biol Chem 1997;272:30969–74.
[35] Peixoto CA, Gomes FO. The role of phosphodiesterase-5 inhibitors in prostatic inflammation: a review. J Inflamm (Lond) 2015;12:54.