MEDICAL SCIENCE MONITOR BASIC RESEARCH

ANIMAL STUDIES

eISSN 2325-4416
© Med Sci Monit Basic Res, 2017; 23: 368-372
DOI: 10.12659/MSMBR.904077

Received: 2017.02.28
Accepted: 2017.04.19
Published: 2017.11.27

Pantethine Down-Regulates Leukocyte Recruitment and Inflammatory Parameters in a Mouse Model of Allergic Airway Inflammation

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

B 1 Mhamad Abou-Hamdan*
DEF 1 Bouchra Gharib
AD 2 Marc Bajenoff
ACD 3 Valérie Julia
ACDEF 1 Max de Reggi

1 CNRS, NICN UMR 7259, Aix Marseille University, Marseille, France
2 CIML, Aix Marseille University, Marseille, France
3 IPMC – CNRS UMR7275 – INSERM ERL1080, University of Nice-Sophia Antipolis, Nice, France

* Present affiliation: Laboratory of Immunology, EDST-PRASE, Faculty of Sciences, Lebanese University, Beirut, Lebanon

Corresponding Author: Max de Reggi, e-mail: max.dereggi@univ-amu.fr
Source of support: This work was supported by the CNRS, Aix-Marseille University (AMU) and Nice-Sophia Antipolis University

Background: Migration of leukocytes into airways is the hallmark of allergic asthma. The aim of this study was to target the pathological process using pantethine, a pleiotropic natural compound which has been recently shown to down-regulate chemokine-driven T cell migration.

Material/Methods: Mice were sensitized to the Leishmania LACK antigen, then treated or not treated with pantethine and exposed to LACK or saline aerosol. After sacrifice of the animals, cells in the bronchoalveolar lavage were analyzed and inflammatory parameters were determined to evaluate inflammation seriousness.

Results: As compared to untreated animals, pantethine-treated animals displayed a moderated response to the allergen, as documented by decreased infiltration of inflammatory cells (all types), in addition to reduced levels of lung Th2 cytokines and circulating LACK-specific IgE.

Conclusions: These data reveal the potential therapeutic importance of pantethine to moderate allergic asthma pathology. The compound has been previously shown to exert a broad range of protective activity in animals and in humans, with few or no adverse effects.

MeSH Keywords: Anti-Allergic Agents • Asthma • Cell Migration Inhibition • Mice • Pantetheine

Full-text PDF: https://www.basic.medscimonit.com/abstract/index/idArt/904077

Indexed in: [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Background

Allergic asthma is a chronic inflammatory lung disease characterized by airway hyper-responsiveness, lung eosinophilia, and airway remodeling [1]. The upstream pathological event is the infiltration of T lymphocytes into airways, followed by recruitment of eosinophils, leading to Th2 cytokine production, with ultimate secretion of allergen-specific IgE by B lymphocytes, as well as mastocyte activation and release of histamine and pro-inflammatory factors. The recruitment of inflammatory cells is under the control of the chemokine/chemokine receptor CXCL12/CXCR4 axis, which therefore plays a pivotal role in the development of lung inflammation [2]; drugs that alter the chemokine/receptor complex block experimental allergic asthma [3].

In vitro experiments allowed us to show that the natural compound pantethine impairs CXCL12-driven mouse or human T lymphocytes chemotaxis and transendothelial migration [4], in connection with its known hypolipidemic properties [5] and its stabilizing effect on cell membrane lipids [6]. Pantethine consists of 2 pantetheine residues linked by a disulfide bond (Figure 1A). In the intestinal tract, the –SS– group is reduced, yielding pantetheine, which is phosphorylated by pantothenate kinase (PanK; 2.7.1.33) [7] to generate 4'-phosphopantetheine (4'-PP). The latter forms the active moiety of coenzyme A (CoA), an essential cofactor of lipid synthesis and degradation. The aim of this study was to investigate *in vivo* impediment of T cell migration by pantethine, using a mouse model of allergic airway inflammation. We determined airway invasion by inflammatory cells as well as the immunological response to the allergen, namely inflammatory cytokines and allergen-specific antibodies known to be related with severity of airways diseases [8].

Material and Methods

Mice and induction of allergic airway inflammation

Female BALB/cAnN mice were purchased from Janvier, France. All animals were raised under specific pathogen-free conditions at the animal facility of IPMC (University of Nice) and used at 6 weeks of age. At days 0 and 7, all the animals were submitted to a sensitization procedure which consisted of 2 i.p. injections of 10 µg of LACK protein precipitated in 2 mg of aluminum hydroxide (alum) [9] (Figure 1B). Then, the mice were randomly distributed into 4 groups, each of 6 animals. Starting from day 17, treated animals received 8 daily i.p. injections of 5 or 7.5 mg of pantethine, whereas untreated animals received a saline solution. Then, starting at day 19, treated and shame-treated mice were exposed to a LACK (1 mg/ml) aerosol challenge for 30 min on 5 consecutive days. Control animals were injected and challenged with saline. Aerosolization was performed using a Passport aerosol compressor (Invacare Corporation, Elyria, OH) connected to a 6500 cm^3 box that served as the deposition chamber for the mice. The animals were anesthetized and sacrificed on day 25. The effect of the treatment was evaluated using the cellular content of the bronchoalveolar lavage (BAL) as well as the levels of lung cytokines and circulating LACK-specific IgE and IgG1. Experiments were conducted following protocols approved by the DNAX Animal Care and Use Committee.

Analysis of bronchoalveolar lavage cells

After mice were sacrificed and blood samples were collected, a canula was placed into the trachea, and the lung was washed 4 times with 1 ml of pyrogen-free saline warmed to 37°C. For differential cell count, cells were stained with monoclonal antibodies to CCR3 (R&D Systems), Gr1, CD3, and CD19 (Becton Dickinson) and analyzed using a FACSCalibur flow cytometer equipped with CellQuest software. Eosinophils were defined as $CCR3^+CD3^-CD19^-$, neutrophils as $Gr\text{-}1^{hi}CD3^-CD19^-$, and lymphocytes as $CD3^+CD19^+$.

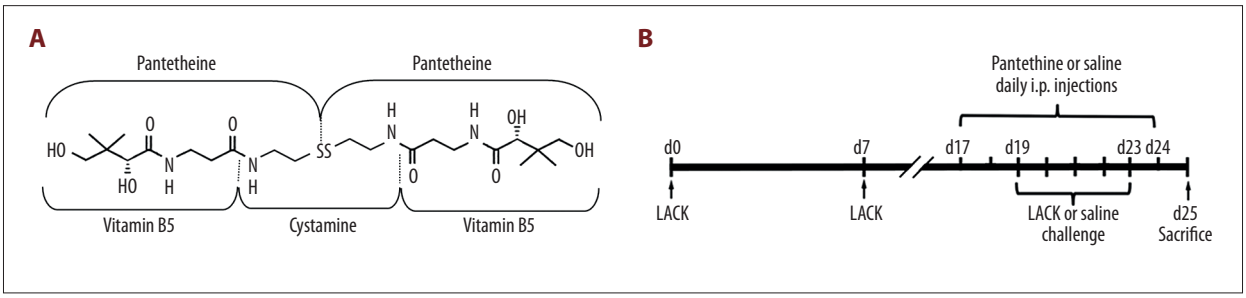

Figure 1. Mouse sensitization and pantethine treatment. (**A**) The pantethine molecule with its constituents; vitamin B5 is pantothenic acid. (**B**) Schematic view of the protocol used. Mice were sensitized with 2 i.p. injections of LACK protein in alum. The animals (6 per group) then received 8 daily i.p. injections of pantethine (5 or 7.5 mg) or saline and they were challenged with LACK aerosol. The control group was injected and challenged with saline.

Indexed in: [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

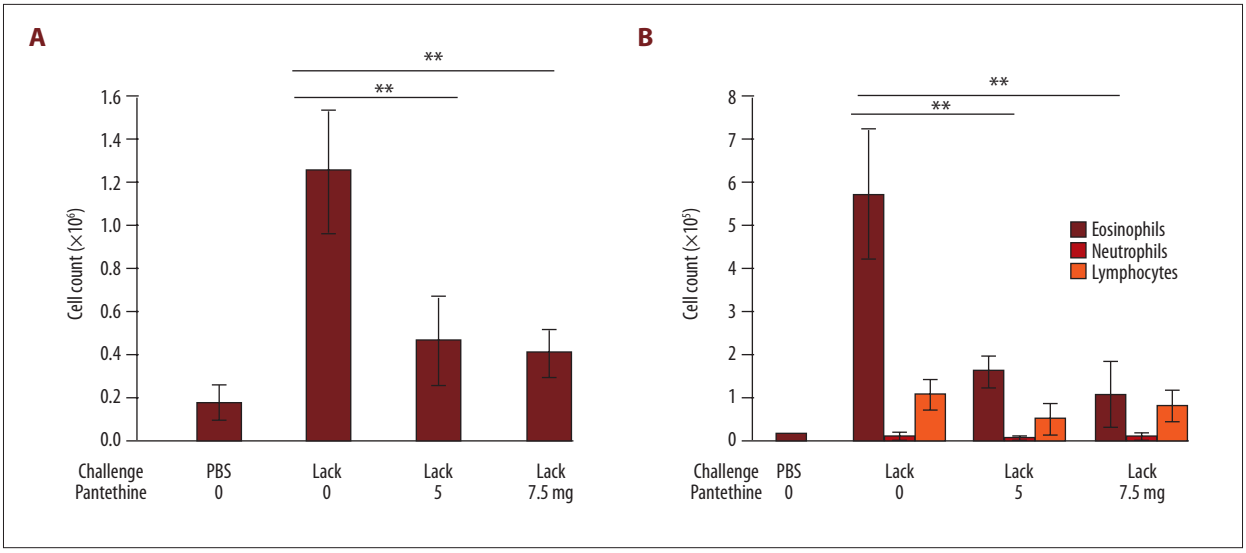

Figure 2. Analysis of alveolar infiltrating leukocytes. Cells in the bronchoalveolar lavages, collected from the 4 experimental groups of LACK-sensitized mice, were determined using FACS analysis. (**A**) Total cell count. LACK challenge induced a massive infiltration of leukocytes into the airways. The infiltration was, however, significantly reduced in pantethine-treated mice compared to untreated ones. (**B**) Cell type analysis showed that infiltrating leukocytes were mainly eosinophils, which were drastically reduced in pantethine-treated animals compared to untreated ones (n=6 per group). Data are mean values ± confidence intervals of the mean; significant difference between treated and untreated animals, ** $p<0.01$.

Determination of circulating LACK-specific IgE and IgG1 levels

For IgE, plates coated with anti-IgE antibody were incubated with serum dilutions and then biotinylated LACK antigen was added. For IgG1, plates coated with the LACK protein were incubated with serum dilutions and then biotinylated anti-IgG1 antibody was added. In both cases, horseradish peroxidase-conjugated streptavidin and its substrate tetramethylbenzidine (TMB) were used for detection. The $_{450\ nm}$OD was determined by spectrometry.

Cytokine assays

Lung tissue was homogenized on ice using a tissue-tearer (Biospec Products, Racine, WI) in 350 µl of extraction buffer (HEPES 50 mM, NaCl 0.5 M, NP40 0.2%, and protease inhibitor). Samples were centrifuged at 13 000 rpm for 10 min at 4°C. Supernatants were analyzed for the determination of IL-4, IL-5, IL-10, and IL-13 levels using the Becton Dickinson Multiplex Immunoassays system.

Statistical analysis

Following Wang [10], statistical analyses were performed using ANOVA with SPSS. Data are expressed as means ± standard deviations (SD) or represent individual values (n=6 animals per group); * $p<0.05$, ** $p<0.01$ or *** $p<0.001$ indicated statistical significance.

Results

Effect of pantethine treatment on lung leukocyte recruitment

The number of cells in the bronchoalveolar lavage (BAL) was 1.2×10^6 in LACK-challenged mice, in contrast to 1.8×10^5 in saline-challenged ones ($p<0.0001$) (Figure 2A). Following pantethine treatment, BAL count was reduced by more than 60% ($p<0.007$). Eosinophils, a major cellular effector of the pathology, formed the main body of infiltrating leukocytes in LACK-challenged mice, whereas they were absent in PBS-challenged ones. Pantethine treatment drastically reduced their number by more than 70% ($p<0.009$) (Figure 2B).

Effect on circulating LACK-specific IgE and IgG1

LACK-specific IgE levels were almost undetectable in the serum of saline-challenge animals, while they were high in LACK-challenged ones. The levels were reduced by 64% in pantethine-treated mice compared to untreated mice ($p=0.001$). Similar changes were found for LACK-specific IgG1 levels. In the latter, pantethine reduced the antibody levels by 53% ($p<0.022$) (Figure 3).

Effect on lung cytokines

LACK-challenged mice displayed significantly higher lung levels of IL-4, IL-5, IL-10, and IL-13 as compared to saline-challenged

Indexed in: [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

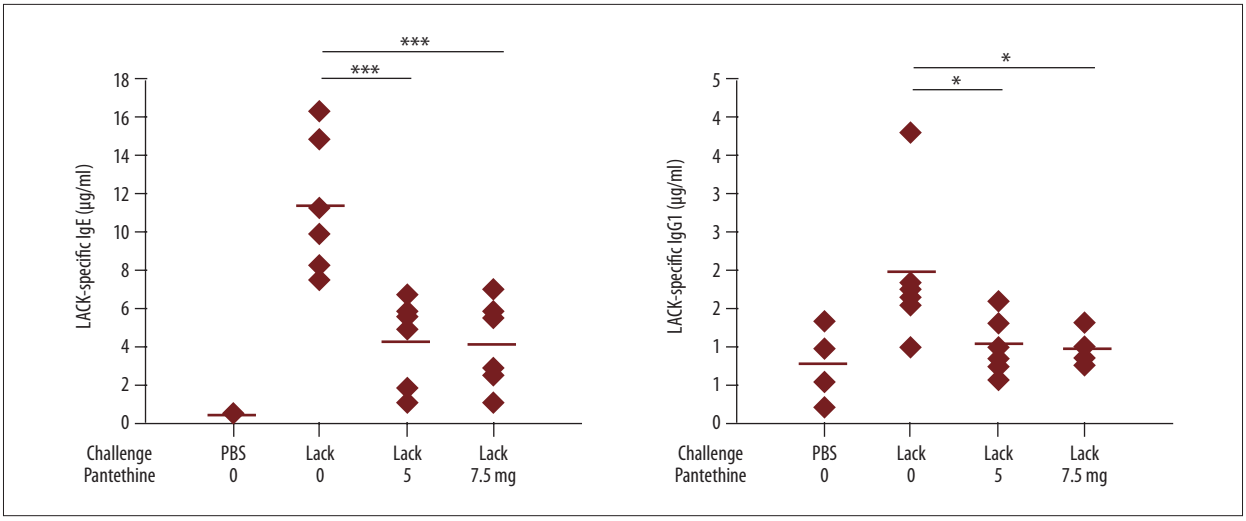

Figure 3. Lung levels of LACK-specific IgE and IgG1. Each dot represents a single mouse and the bar represents the mean value of each experimental group (n=6 per group). Significant difference of the mean between treated and untreated animals, * $p<0.05$; *** $p<0.001$.

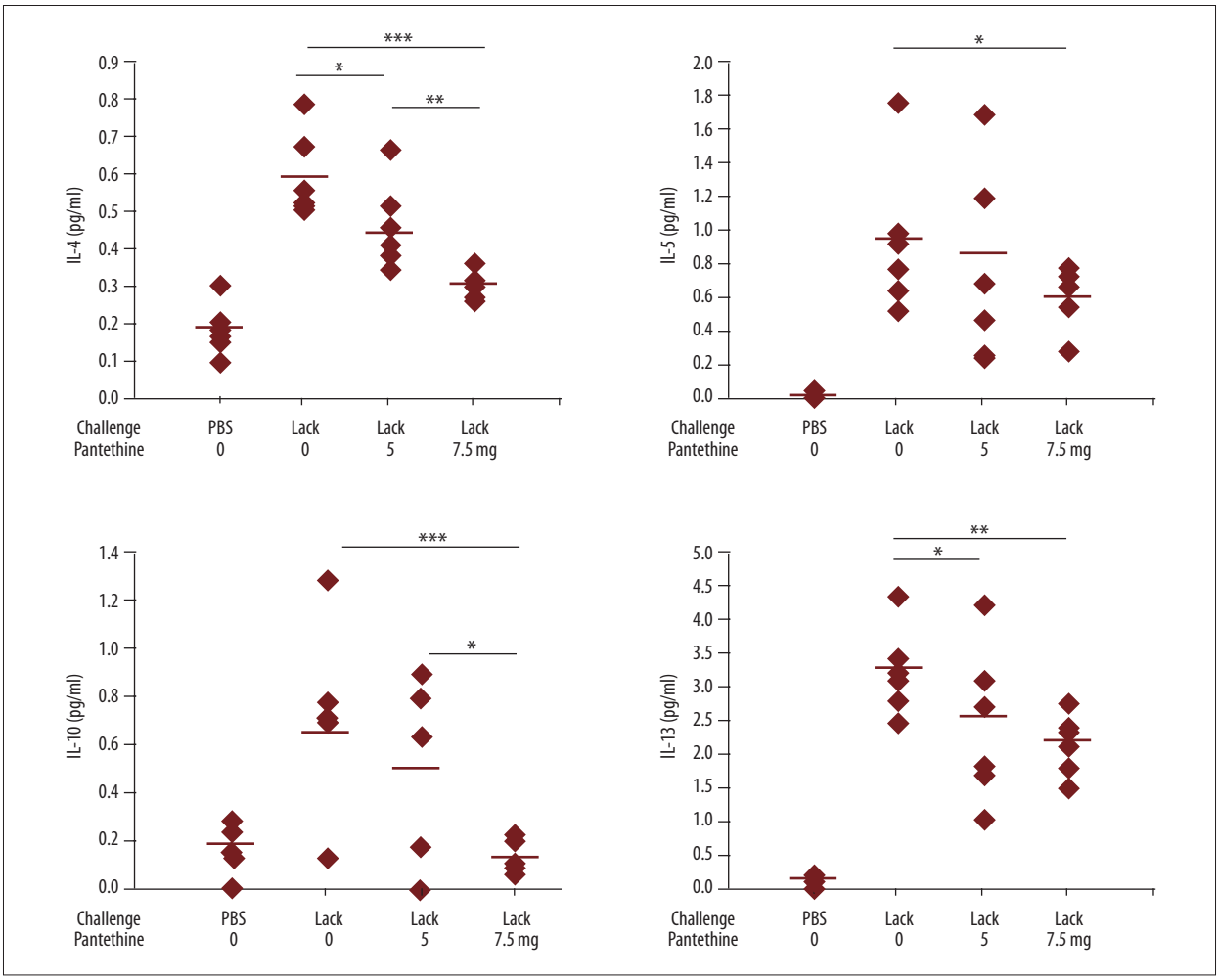

Figure 4. Lung cytokine levels. Each dot represents a single mouse and the bars represent the mean value of each experimental group (n=6 per group). Significant difference of the mean between treated and untreated animals, * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Indexed in: [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

animals (Figure 4). Pantethine treatment markedly reduced the levels of the pro-inflammatory cytokines IL-4 (p=0.0008) and IL-13 (p=0.0082). The treatment was effective also for IL-5 and IL-10, but only at the highest pantethine dose (p=0.041 and p=0.0071, respectively).

Discussion

We explored at the potential protective effect of the low-molecular-weight thiol pantethine against allergic airway inflammation, based on the report that the compound inhibits chemokine-induced migration of T cells [4], a central pathophysiological process in allergic asthma [11–13].

We found that airway infiltration by inflammatory cells was significantly reduced in pantethine-treated mice as compared to saline-treated mice. The event was not limited to lymphocytes, as it involved all inflammatory cell types, including eosinophils, which are assumed to play a broad role in disease development [14]. Accordingly, levels of factors that play a critical role in the disease [15,16] were significantly reduced, as shown by lung levels of Th2 cytokines such as IL-4, IL-5, and IL-13, as well as circulating levels of LACK-specific IgE and IgG1. The protective effect of inhibiting the IgE allergic pathway has been demonstrated in patients with severe persistent asthma, using humanized mAb to IgE [17].

It should be emphasized that pantethine treatment was applied after the LACK injections, meaning that it was efficient in already-sensitized animals. The compound has already been shown to target complex cell pathological pathways, such as the cell response to pro-inflammatory factors associated with cerebral malaria, resulting in the prevention of the cerebral syndrome [6]. It also attenuated the brain energy crisis linked to Parkinson's disease, with subsequent protection against dopaminergic injury [18,19].

Conclusions

Our work shows that pantethine is a potential protective drug against allergic asthma. Among the products known to attenuate the disease, pantethine has the advantage of being a well-tolerated natural compound. It has been already used in humans against various pathologies, with no detectable adverse effects [20]. Based on a previous work showing that it drastically attenuates the diffusion of cancer metastases [21], the present report confirms that pantethine inhibits pathogenic cell migration, meaning that it could provide a distinct appropriate therapy for allergic asthma.

Acknowledgments

Alexia Lent help us in the experimental work as Polytech Nice's trainee.

References:

1. Wills-Karp M: Immunologic basis of antigen-induced airway hyperresponsiveness. Annu Rev Immunol, 1999; 17: 255–81
2. Gonzalo JA, Lloyd CM, Peled A et al: Critical involvement of the chemotactic axis CXCR4/stromal cell-derived factor-1 alpha in the inflammatory component of allergic airway disease. J Immunol, 2000; 165: 499–508
3. Daubeuf F, Hachet-Haas M, Gizzi P et al: An antedrug of the CXCL12 neutraligand blocks experimental allergic asthma without systemic effect in mice. J Biol Chem, 2013; 288: 11865–76
4. van Gijsel-Bonnello M, Acar N, Molino Y et al: Pantethine alters lipid composition and cholesterol content of membrane rafts, with down-regulation of CXCL12-induced T cell migration. J Cell Physiol, 2015; 230: 2415–25
5. Coronel F, Tornero F, Torrente J et al: Treatment of hyperlipemia in diabetic patients on dialysis with a physiological substance. Am J Nephrol, 1991; 11: 32–26
6. Penet MF, Abou-Hamdan M, Coltel N et al: Protection against cerebral malaria by the low-molecular-weight thiol pantethine. Proc Natl Acad Sci USA, 2008; 105: 1321–26
7. Rock CO, Calder RB, Karim MA, Jackowski S: Pantothenate kinase regulation of the intracellular concentration of coenzyme A. J Biol Chem, 2000; 275: 1377–83
8. Huang AX, Lu LW, Liu WJ, Huang M: Plasma inflammatory cytokine IL-4, IL-8, IL-10, and TNF-alpha levels correlate with pulmonary function in patients with asthma-chronic obstructive pulmonary disease (COPD) overlap syndrome. Med Sci Monit, 2016; 22: 2800–8
9. Julia V, Hessel EM, Malherbe L et al: A restricted subset of dendritic cells captures airborne antigens and remains able to activate specific T cells long after antigen exposure. Immunity, 2002; 16: 271–83
10. Wang C, Liu Y, Wang Y et al: Adenovirus-mediated siRNA targeting CXCR2 attenuates titanium particle-induced osteolysis by suppressing osteoclast formation. Med Sci Monit, 2016; 22: 727–35
11. Gerard C, Rollins BJ: Chemokines and disease. Nat Immunol, 2001; 2: 108–15
12. Moser B, Loetscher P: Lymphocyte traffic control by chemokines. Nat Immunol, 2001; 2: 123–28
13. Zlotnik A, Yoshie O: Chemokines: A new classification system and their role in immunity. Immunity, 2000; 12: 121–27
14. Walsh GM: Antagonism of eosinophil accumulation in asthma. Recent Pat Inflamm Allergy Drug Discov, 2010; 4: 210–13
15. Choi J, Choi BK, Kim JS et al: Picroside II attenuates airway inflammation by downregulating the transcription factor GATA3 and Th2-related cytokines in a mouse model of HDM-induced allergic asthma. PLoS One, 2016; 11: e0167098
16. Oeser K, Maxeiner J, Symowski C et al: T cells are the critical source of IL-4/IL-13 in a mouse model of allergic asthma. Allergy, 2015; 70: 1440–49
17. Yalcin AD: An overview of the effects of anti-IgE therapies. Med Sci Monit, 2014; 20: 1691–99
18. Abou-Hamdan M, Cornille E, Khrestchatisky M et al: The energy crisis in Parkinson's disease: A therapeutic target. In: Rana AQ (ed.). Etiology and pathophysiology of Parkinson's disease. Croatia: Intech, 2011; 273–92
19. Cornille E, Abou-Hamdan M, Khrestchatisky M et al: Enhancement of L-3-hydroxybutyryl-CoA dehydrogenase activity and circulating ketone body levels by pantethine. Relevance to dopaminergic injury. BMC Neurosci, 2010; 11: 51
20. Wittwer CT, Gahl WA, Butler JD et al: Metabolism of pantethine in cystinosis. J Clin Invest, 1985; 76: 1665–72
21. Penet MF, Krishnamachary B, Wildes F et al: Effect of pantethine on ovarian tumor progression and choline metabolism. Front Oncol, 2016; 6: 244

Indexed in: [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]