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Research Article

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Investigation of Possible Immunomodulatory Effects of Resveratrolas an Add-on Therapy in a Murine Model of Ovalbumin-Induced Bronchial Asthma

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Abstract

Background: *Bronchial asthma is a cause of significant morbidity and mortality with increasing prevalence worldwide..Although corticosteroids are routinely used in management of bronchial asthma either for long term therapy or control of acute attacks, but unfortunately they have limitations due to their diverse side effects. Resveratrol, a natural polyphenol, exhibits a wide range of biological and pharmacological activities, such as anticarcinogenesis, cardiovascular protection, and anti-inflammatory effects. Thus it is of much interest to investigate possible immunomodulatory effects of resveratrol in bronchial asthma representing a new mechanism that has not yet been fully elucidated.*

Methods: *This experiment was performed on50 albino mice divided in 5 equal groups. The normal control group (group I) received a veheicle of saline. The Ova-untreated group (group II); received ovalbumin (OVA) via twoI.P.injections in days 0 and 14 in a dose of 100 µg and challenged with an intranasal (I.N.) dose of 50 µg OVA in days 14, 25, 26&27. The OVA- challenged group treated by Resveratrol (group III) in a dose of 30 mg/kg given by oral gavage1 h. before challenge in days 14, 25, 26 & 27. The OVA- challenged group treated by Dexamethasone (group IV) in a dose of 2.5 mg/kgby intraperitoneal injection (I.P) 1 h. before challenge in days 14, 25, 26&27. OVA- challenged group treated by dexamethasone and resveratrol (group V) in the same dose regimen. At the end of experiment in the 28th day (24 hours after the last challenge in the 27thday), serum was analyzed for total IgE(TIgE)levels and bronchoalveolar lavage (BAL) was investigated for inflammatory cell count. The lung tissues were examined for histopathology score and immunohistochemistry for TLR2, CD4 and CD8.*

Results: *The untreated group of asthma model showed a significant increase in serum level of TIgE, the number of total inflammatory cells, inflammatory score and the number of positive TLR2, CD4+ and CD8+ when compared to normal group. The treatment by both of resveratrol and dexamethasone was better than treatment with resveratrol alone as it showed significant decrease in the whole studied parameters.*

Conclusion:*We demonstrated the immunomodulatory effect of resveratrol preclinical in bronchial asthma induced model via suppression of TLR2, CD4 and CD8 expression. We could suggest using resveratrol as add- on therapy with dexamethasone to achieve more efficacies in management of bronchial asthma. However, this should be verified in further clinical studies.*

Keywords: Bronchial asthma,Resveratrol (RES),Dexamethasone (DEXA), Ovalbumin (OVA)

Introduction

Bronchial asthma is defined as chronic inflammation with reversible airway obstruction and airway hyperresponsiveness (AHR) as cardinal features that presented clinically by variable symptoms, namely wheeze, breathlessness, chest tightness or cough. The most prevalent form of asthma is atopic asthma which is initiated by the exposure to inhaled allergens and resultant allergen- specific immune responses. It is a cause of significant morbidity and mortality and is estimated to affect 300 million people worldwide, with increasing prevalence [1].

At an immunological level; Th2 inflammation and the presence of activated eosinophils and mast cells are key features of asthma. Toll-like receptors (TLRs) have recently emerged as key receptors of the innate immune system. They recognize specific pathogen-associated molecular patterns initiating a host defense response. TLR2 signaling may coordinate the development of a Th1 phenotype in some individuals and where this function is reduced there could be an increased risk of atopy and asthma [2]. However, there is also the possibility that in adults in whom asthma has developed, activation of TLR2 may lead to exacerbations in inflammation and symptoms. Recent evidence suggests that bacterial infection, which will result in activation of TLR2, contributes to disease severity. Activation of TLR2 on pulmonary mast cells results in the release of various

mediators associated with asthma including leukotriene's [3].

Routinely, management of asthma either long-term therapy or control of acute attacks includes use of adrenergic receptor agonists as bronchodilators and glucocorticosteroids to control the inflammatory response [4]. However, corticosteroids have a number of important limitations. Firstly, steroid resistance, where a subpopulation of patients with asthma shows poor response to the drugs, is a concern. Secondly, corticosteroids cannot be used at the highest and optimal concentrations in young children. Thirdly, corticosteroids have only limited efficacy in preventing and reversing airway remodeling changes [5].

Resveratrol (RES) is a natural non flavonoid polyphenols compound found in the skin of red grapes. RES has been proven in variety of medical conditions including; regulation of the stress, inflammation, autophagy, metabolism and mitochondrial biogenesis. The protective mechanisms of RES are mainly due to its ability to effectively scavenge reactive oxygen species and suppress its associated oxidative stress (Sun et al., 2008) [6].In addition to various biochemical, biological, and pharmacological activities, resveratrol has been found to exhibit numerous immunomodulatory effects such as suppression of lymphocyte proliferation, changes in cell-mediated cytotoxicity, cytokine production, and induction of apoptosis. Resveratrol is also able to modulate innate immune response by inhibiting expression of costimulatory molecules (CD80 and CD86) and major histocompatibility complex classes I and II in bone marrow-derived dendritic cells [7].

Materials and methods

Drugs and chemicals:

- **Ovalbumin:** Ovalbumin(Ova)(Chicken egg albumin), a phosphorylated-glycoprotein in the form of lyophilized powder, was purchased from New Jersy, USA (Catalog no. : 1-800-ACROS-01).
- **Carboxy Methyl Cellulose (CMC) 0.5%:** It was in the form of powder, a product of Al-Gomhoria pharmaceutical company, Egypt. It was prepared as 0.5 g /100 ml distilled water.
- **Resveratrol:** Trans-Resveratrol micronized capsules (100 mg/ capsule) a product of Pure and Health Co., USA. It was suspended in CMC 0.5% in a concentration of 2.5 mg / ml.
- **Dexamethasone:** Dexamethasone sodium phosphate ampoules of 2ml each ampoule contain 8 mg, obtained from AMRIVA PHARM.IND. It was diluted in isotonic saline (0.9%) to a final concentration of 0.25 mg/ml.

Ovalbumin (OVA) (Chicken egg albumin), a phosphorylated-glycoprotein in the form of lyophilized powder, was purchased from New Jersy, USA (Catalog no: 1-800-ACROS-01). Carboxy Methyl Cellulose (CMC) 0.5%: It was in the form of powder, a product of Al-Gomhoria pharmaceutical company, Egypt. It was prepared as 0.5 g /100 ml distilled water. Resveratrol (RES): Trans-Resveratrol micronized capsules (100 mg/ capsule) a product of Pure and Health Co., USA. It was suspended in CMC 0.5% in a concentration of 2.5 mg / ml. Dexamethasone sodium phosphate (DEXA) ampoule of8 mg / 2ml, obtained from AMRIVA PHARM.IND. It was diluted in isotonic saline (0.9%) to a final concentration of 0.25 mg/ml.

At the end of experiment in the 28th day(24 hours after the last challenge in the 27th day), the mice were anaesthetized with ether The heart was exposed and blood withdrawn using a 1 ml syringe and 23G needle in 1.5 ml centrifuge tube for 2-3 hours allowed to clot centrifuged at 3,000 rpm for 30 min to obtain serum that stored at -20°C until analyzed for total IgE (TIgE).

Animals

Fifty adult male albino mice weighing 20-25 g were used in this study .They were maintained in cages under hygienic conditions at room temperature of 20-25 C with food and water ad libitum all over the period of experiment. This research work was approved by the Institutional Research Ethics Committee, Faculty of medicine, Tanta University (Approval code 1995/07/13).

The mice were divided into 5 equal groups:Group 1, Served as normal group, received a veheicle of 0.2 ml saline intraperitoneal injection (I.P.) in days 0 & 14 and 50 µl saline intranasal (I.N.) instillation in days 14, 25, 26 & 27. Group 2,OVA- challenged untreated group (group 2) received ovalbumin .The mice received two I.P. injections , in days 0 and 14 in a dose of 100 µg ovalbumin in 0.2 ml saline adsorbed to 1 mg aluminum hydroxide ($Al(OH)_3$(Alum) as adjuvant. In days14, 25, 26 & 27, mice were anesthetized with 0.2 ml ketamine (0.44 mg/ml) before challenged with an intranasal (I.N.) dose of 50 µg in 50 µl saline. The group received a vehicle of CMC 0.5% (1 h before challenge in days of challenge) (Handerson et al., 1996).Group 3, OVA- challenged group, treated by resveratrol, in a dose of 30 mg/kg given by oral gavage in days 14, 25, 26 & 27 (1 h before challenge in days of challenges) (Zang et al., 2011). Group 4, OVA- challenged group treated by dexamethasone in a dose of 2.5 mg/kg by intraperitonealinjection (I.P) in days 14, 25, 26&27 (1 h before challenge in days of challenges)(Kim et al., 2004b).Group 5, OVA- challenged group treated by resveratrol and dexamethasone in the same dose regimen (Figure1).

Assay of Serum total Ig E (TIgE).

Principle and procedure

The kit uses a double- antibody sandwich enzyme- linked immunosorbent assay (ELISA) to assay the level of (Mouse total Immunoglobulin's E (TIg E) in samples (Sunredbio, Catalouge No. 201-02-0450). The procedure was performed according to manufacturer's protocol. In brief, Immunoglobulin E (Ig E) was added and incubated in a well which is pre-coated with Mouse Immunoglobulin E (Ig E) monoclonal antibodythen adding Ig E antibodies labeled with biotin and combined with Streptavidin-HRP to form immune complex; then incubation and washing were carried out again to remove the uncombined enzyme. After adding chromogen solution A,B the colour of the liquid changes into blue,and at the effect of acid, the colour finally becomes yellow. The Chroma of colour and the concentration of the mouse substance TIgE of sample were positively correlated and optical density (OD) was measured at wave length 450 nm.

Collection, processing and analysis of Bronchoalveolar lavage (BAL)

After termination by cardiac puncture, Bronchoalveolar lavage(BAL) was performed by tying the left lung and the trachea was exposed and a small incision made at the proximal end to allow cannulation with a 1 ml syringe and 23G needle sheathed with polythene tubing. The needle was held in place and a seal formed using forceps for BAL that was performed by instillation of 0.5 mL of saline. After instillation, the thorax was gently massaged then the BAL fluid (BALF) was withdrawn. The process was repeated 4 times. All the aspirated fluid was pooled in a 1.5 ml centrifuge tube and stored on

ice for rapid processing within 1 hour. One ml of the retrieved BAL fluid was centrifuged at 1500 g for 10 min. The cell free supernatant was removed and the cell pellet was resuspended in 200 µL Saline and used for total inflammatory cell count. Live cell counts were performed in a Neubauerhaemocytometer using a 1:2 dilution with 0.4% trypan blue solution. The total inflammatory cell count was expressed as $\times 10^5$ / ml BALF.

Light microscope histopathological and inflammatory score examination of lung tissue

Left lobe lung tissues were fixed in 10 % formalin, cut into 5 µm sections, stained with hematoxylin and eosin (H&E) and examined under light microscope on a morphologic basis, the inflammatory infiltrates per section was scored using the method described by [8]. Lungs that showed no focal inflammation were scored as grade 0. Those that showed one or two centrally located microscopic foci of inflammatory infiltrate were graded as 1. In grade 2, a dense inflammatory infiltrate was seen in a perivascular and peribronchial distribution originating in the center of the lung. In grade 3, the perivascular and peribronchial infiltrates extended to the periphery of the lung.

Immunohistochemistry of TLR2,CD4+ and CD8+

Four-micrometer sections were obtained from the paraffin embedded specimens from Left lobe lung tissues and stained as following:

Deparaffinize and rehydrate tissue section. Then, incubate side in hydrogen peroxide for 10-15 minutes to reduce the nonspecific background staining due to endogenous peroxidase). Wash 2 times in buffer. If required, incubate tissue in digestive enzyme. Then wash 4 times in buffer.(Optional) apply Ultra V block and incubate for 5 minutes at room temperature to block nonspecific background staining. Rinse (optional). Then apply primary antibody (CD4 Ab1: MS-237-PABX,-PIABX, CD8 RM-9116-PCS,TLR2) and incubate according to manufacture recommendations. Wash 4 times in buffer.Then apply biotinylation Goat Anti-Polyvalent and incubate for 10 minutes at room temperature. Then wash 4 times in buffer. Apply Streptavidin Peroxidase and incubate for 10 minutes at room temperature. Rinse 4 times in buffer. Incubate with peroxidase-compatible chromogen of choice according to manufacture recommendations.

Immunostaining cells in the airway submucosa were counted at a magnification of 400. The final result was expressed as the number of positive cells/mm^2 [9,10].

Statistical analysis

All collected data were tabulated and statistically analyzed using the Statistical programme for Social Science (SPSS); version 16 for window. The data were subjected to one way ANOVA and Post-hoc Turkey's multiple comparison tests. Data were presented as mean ± standard error of mean (SEM).

Results

Serum totalIgE (TIgE) levels (ng/ml) Figure2:

Treatment by RES showed a significant decrease in serum TIgElevel as compared to the untreated group 2. Treatment by DEXA showed a significant decrease in serum TIgE level as compared to the untreated group 2 and group 3 that treated by RES;respectively. Treatment by both RES and DEXA showed a significant decrease in serum TIgE level as compared to the untreated group 2, group 3 that treated by RES and group 4 that treated by DEXA; respectively.

Total inflammatory cell count (105 / ml BALF) Figure 3:

Treatment by RES showed a significant decrease in total inflammatory cell count as compared to the untreated group 2. Treatment by DEXA showed a significant decrease in total inflammatory cell count as compared to the untreated group 2 and group 3 that treated by resveratrol; respectively. Treatment by both RES and DEXA showed a significant decrease in total inflammatory cell count as compared to the untreated group 2 and group 3 that treated by RES.However, it showed non-significant decrease in total inflammatory cell count as compared to group 4 that treated by DEXA alone.

Light microscopeHistopathological examination of lung tissues Figure 8:

Light microscopic study of the Ova untreated asthma group 2, showed perivascular and peribronchiolar eosinophilic infiltration. It also showed perivascular mast cell and lymphocytic infiltration. In theRES treated group 3, showed nearly normal lung tissues with mild peribronchiolareosinophilic and lymphocytic infiltration. DEXA treated group 4, showed the preservation of the normal histological structure of the lung tissues, with prevention of the histopathological changes induced by Ova bronchial asthma model. RES and DEXA treated group 5, showed nearly normal lung tissues with mild peribronchiolareosinophilic and lymphocytic infiltration.

The inflammatory score in the different studied groups Figure 4:

Treatment by RES showed a significant decrease in the inflammatory score as compared to the untreated group. Treatment by DEXA showed a significant decrease in the inflammatory score as compared to the untreated group 2 and group 3 that treated by RES Treatment by combination of RES and DEXA showed a significant decrease in the inflammatory score as compared to the untreated group 2 and group 3 that treated by RES.However, it showed a non-significant change in the inflammatory score as compared to group 4 that treated by DEXA alone.

Immunohistochemical Analysis of TLR2, CD4+ and CD8+ in lung tissue:

The number of positive TLR2 /mm^2 Figure 5,9:

Treatment by RES showed a significant decrease in the number of TLR2 as compared to the untreated group 2. Treatment by DEXA showed a significant decrease in the number of TLR2 as compared to the untreated group 2. But, when compared to group 3 that treated by resveratrol, the number of TLR2 was still significantly high. Treatment by both RES and DEXA showed a significant decrease in the number of TLR2 as compared to the untreated group 2 , group 3 that treated by RES and group 4 that treated by DEXA; respectively.

The number of positive CD4+cells/mm^2Figure 6,10:

Treatment by RES showed a significant decrease in the number of CD4+ cells as compared to the untreated group 2 .Treatment by DEXA showed a significant decrease in the number of CD4+ cells as compared to the untreated group 2 and group 3 that treated by RES. Treatment by both RES and DEXA showed a significant decrease in the number of CD4+ cells as compared to the untreated group 2, group 3 that treated by RES and group 4 that treated byDEXA; respectively.

The number ofpositive CD8+ cells/mm^2Figure 7,11:

Treatment by RES showed a significant decrease in the number

of CD8+ cells as compared to the untreated group 2.Treatment by DEXA showed a significant decrease in the number of CD8+ cells as compared to the untreated group 2 and group 3 that treated by RES. Treatment by both RES and DEXA showed a significant decrease in the number of CD8+ cells as compared to the untreated group 2, group 3 that treated by RES and group 4 that treated by DEXA; respectively.

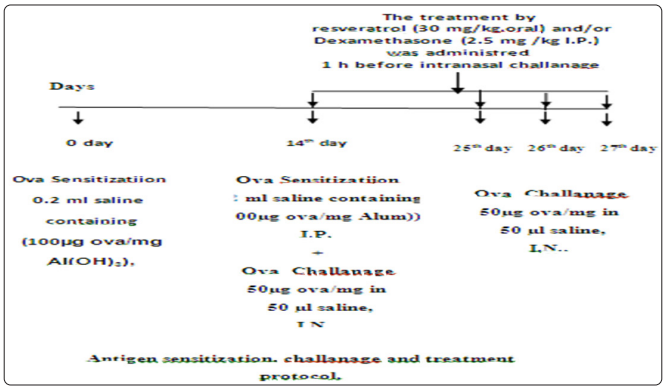


Figure 1: Antigen sensitization, challenge and treatment protocol

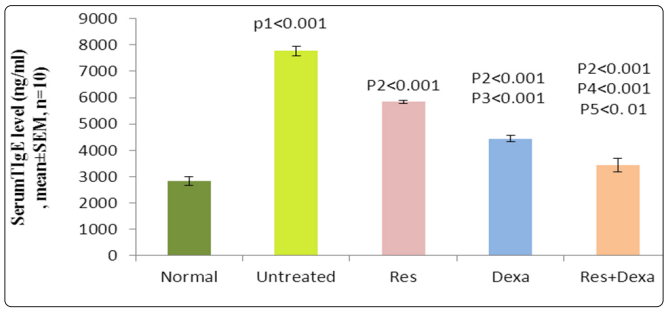


Figure 2: Serum TIgE levels (ng/ml) in the different studied groups

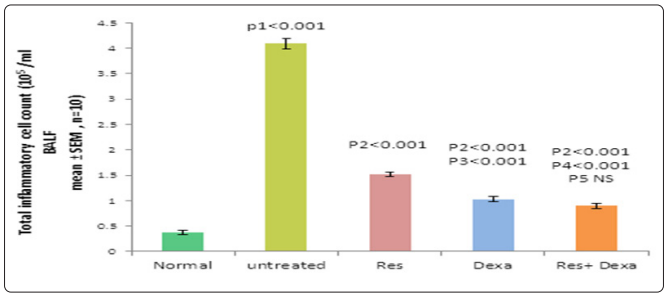


Figure 3: Total inflammatory cell count,(105 / ml BALF) in the different studied groups

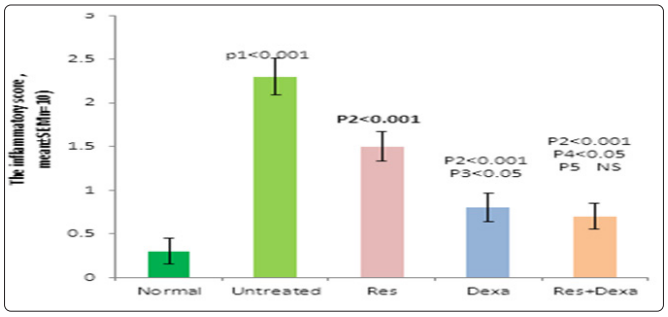


Figure 4: The inflammatory score of lung tissue in the different studied groups

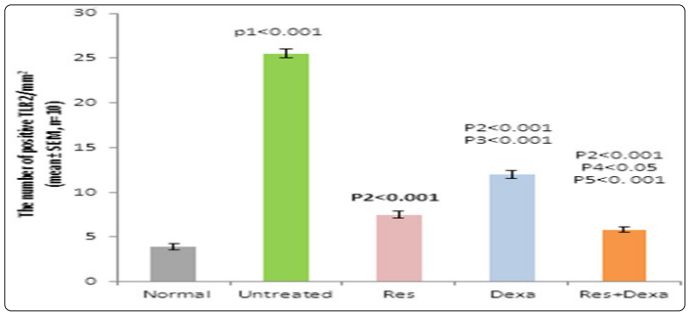


Figure 5: The number of positive TLR2 /mm2 in the lung tissue of the different studied groups

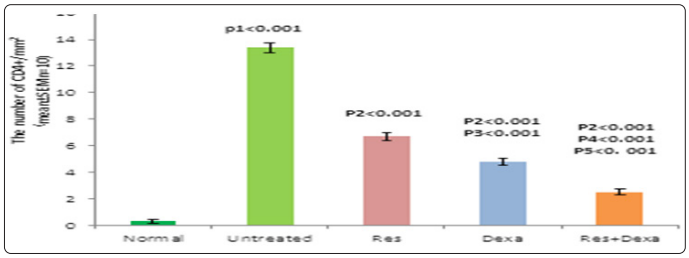


Figure 6: The number of CD4+ cells/mm2 in lung tissue of the different studied groups

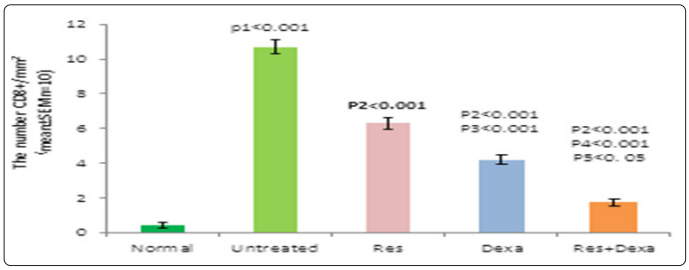
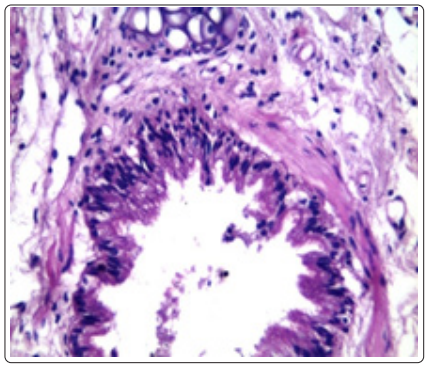


Figure 7: The number of CD8+ cells/mm2 in lung tissue of the different studied groups

Values expressed as mean± SEM, n= number.
Tukey test
p1= Group 2 (Untreated) Vs group1 (Normal)
P2 = Group3 (Resveratrol), group 4 (Dexamethasone) and group 5 (Resveratrol + Dexamethasone)Vs group2 (Untreated).
P3 = Group 4 (Dexamethasone) Vs group3 (Resveratrol)
P4= Group 5 (Resveratrol + Dexamethasone)Vs group3 (Resveratrol)
P5= Group 5 (Resveratrol + Dexamethasone) Vs group 4 (Dexamethasone)
Res=Resveratrol, Dexa=Dexamethasone



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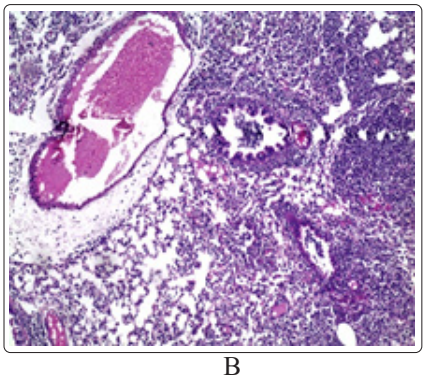

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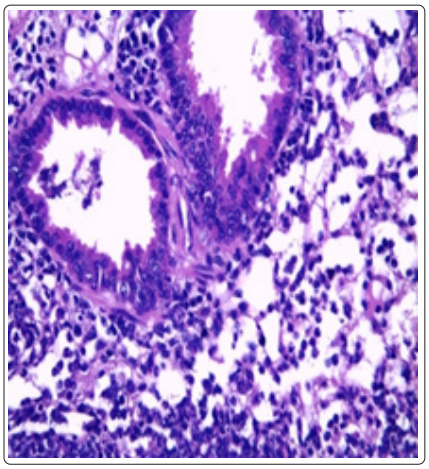

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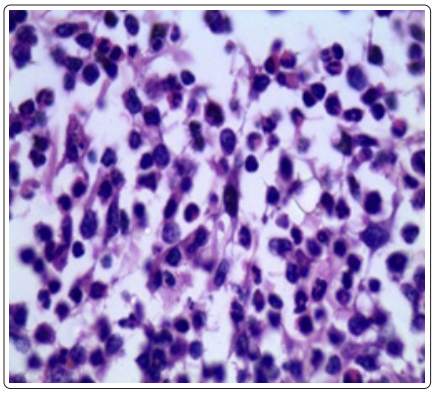

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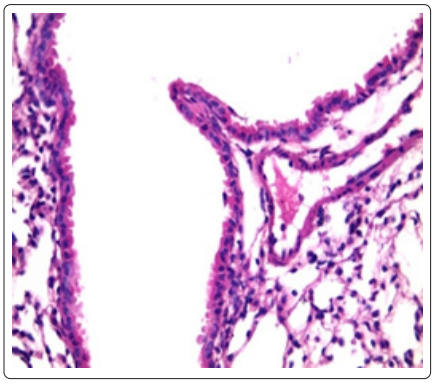

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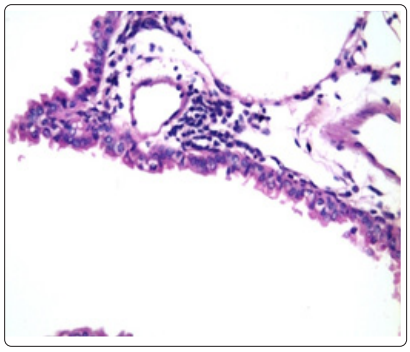

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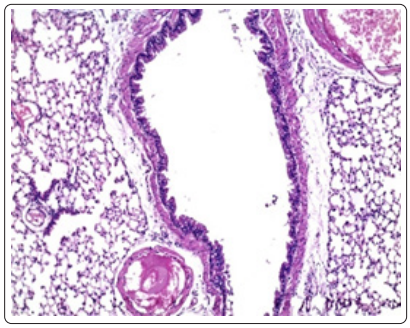
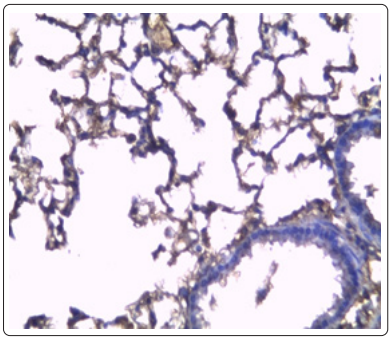


G

Figure 8: Histopathology of lung tissue of A: normal Control group of bronchial asthma model (H&E, × 400) ,B: OVA- untreated group of bronchial asthma model (H&E, × 125) C: OVA - untreated group of bronchial asthma model (H&E, × 400),D: OVA - untreated group of bronchial asthma model (H&E, × 1000),E: RES treated group of bronchial asthma model (H&E × 400), F: DEXA treated group of bronchial asthma model (H&E × 400) and G: RES and DEXA treated group of bronchial asthma model (H&E × 400).



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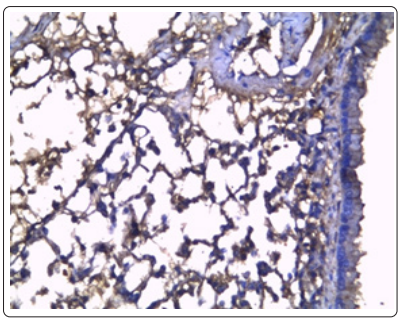

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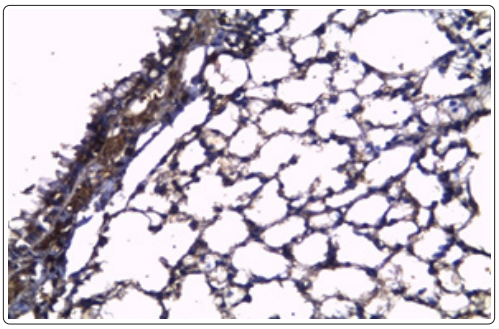

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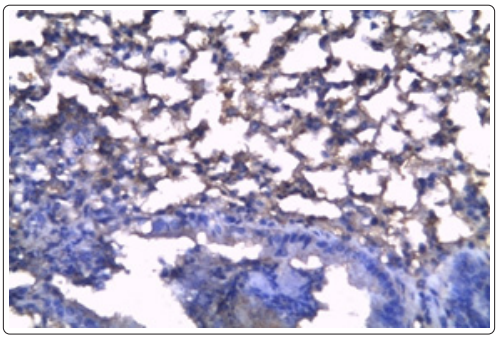

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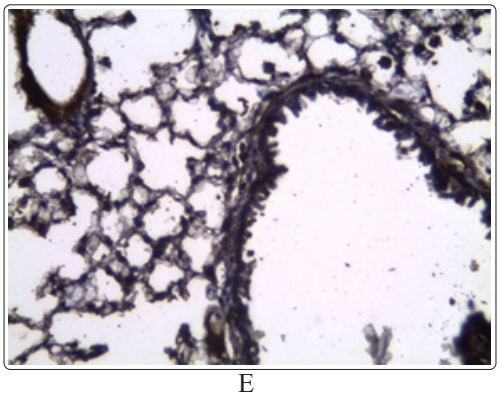
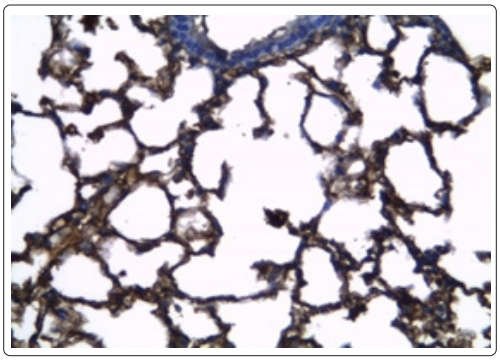


E

Figure 9: Immunohistochemical staining for TLR2 in lung tissue ofA: normal Control group of bronchial asthma Model, B: OVA-untreated group of bronchial asthma model, C: RES treated group of bronchial asthma model, D: DEXA treated group of bronchial asthma model and E: RES and DEXA treated group of bronchial asthma model(PAP, ×400).



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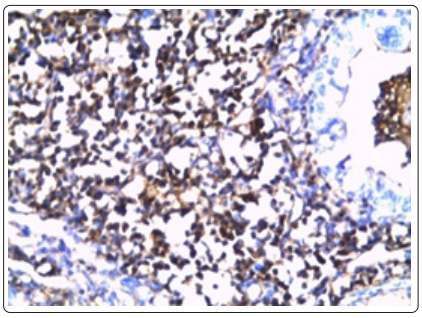

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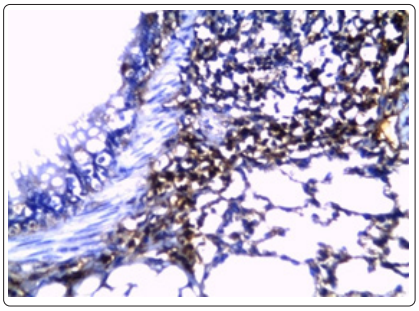

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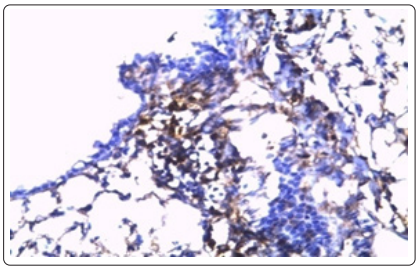

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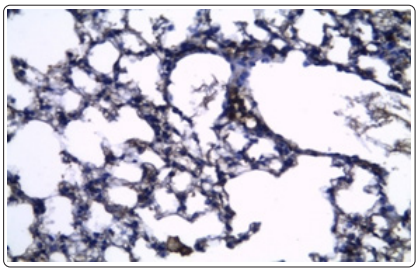

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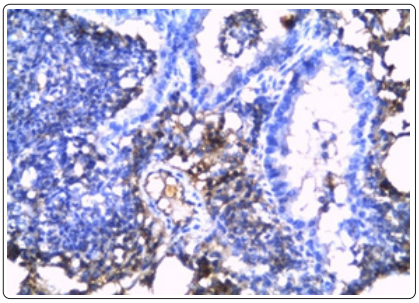
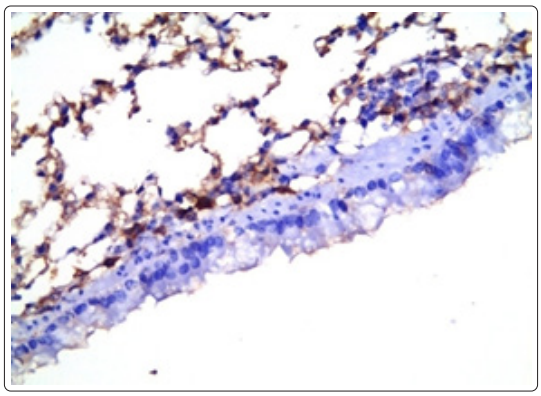


F

Figure 10: Immunohistochemical staining for CD4 in lung tissue of A: normal Control group of bronchial asthma Model, B,C: OVA-untreated group of bronchial asthma model, D: RES treated group

of bronchial asthma model, E: DEXA treated group of bronchial asthma model and F: RES and DEXA treated group of bronchial asthma model (**PAP, ×400**)



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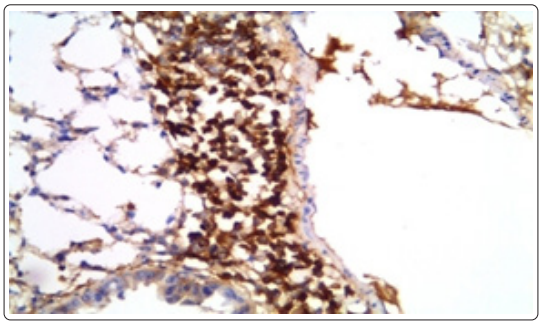

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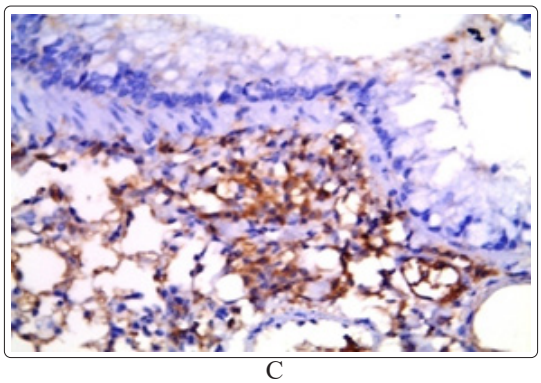

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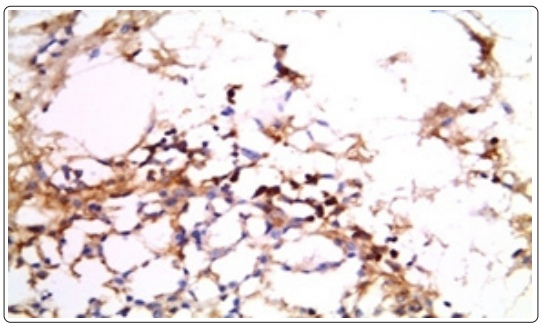

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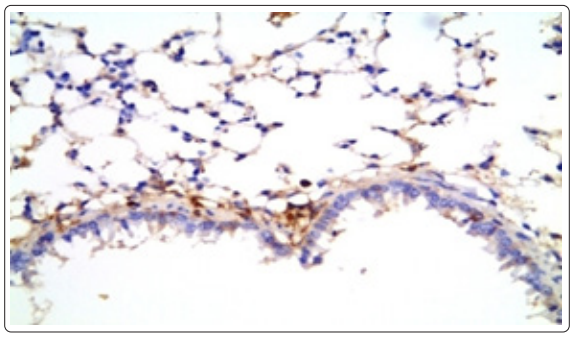

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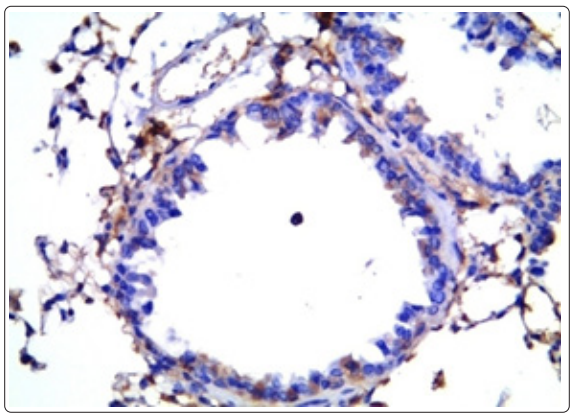

F

Figure 11: Immunohistochemical staining for CD8 in lung tissue ofA: normal Control group of bronchial asthma Model, B, C: OVA-untreated group of bronchial asthma model, D: RES treated group of bronchial asthma model, E: DEXA treated group of bronchial asthma model and F: RES and DEXA treated group of bronchial asthma model (**PAP, ×400**)

Discussion

The present study was designed to investigate the possible immunomodulatory effects of resveratrol and dexamethasone each alone and in combination in a mouse model of Ova- induced bronchial asthma, regarding serum TIgE levels, total inflammatory cell count in bronchoalveolar lavage, histopathological changes and inflammatory score in lung tissues as well as immunohistochemical detection of TLR2 ,CD4 and CD8.

The function of the distinct TLRs in allergic airway inflammation is in part mediated by the activation of cells of the innate immune system. DCs and mast cells are the major players in allergic asthma. Mast cell activation and subsequent secretion of proinflammatory cytokines and chemokine's is directly induced by TLR ligands [11].

Redecke et al. and Fuchs and Braun have been shown that there was an increased Th2 response and thus allergic asthma aggravation in mice immunized with OVA allergen in combination with a TLR2/1 agonist Conflicting evidence is found on the effect of TLR2/1 agonists in mice models of allergic asthma [12,13]. In contrast, Patel et al. found to reverse established airway inflammation in a murine model of OVA-induced asthma [14].

There is an argument about the dual effect of TLR2. TLR2 agonization bears the potency to both inhibit and promote development of

immune responses. TLR2 agonists show high immunomodulatory and adjuvantic capacity. This makes TLR2 agonization a promising approach for pharmaceutical intervention of allergic disorders [13]. The dual role of TLR2 in asthma seems to depend partly on which TLR2 heterodimer is addressed. Moreover, the agonist dose is likely to play a role in the eventual effect [15].

Li et al. investigated the effect of TLR2 gene deletion in a murine model of ovalbumin (OVA)-challenged asthma [16]. TLR2 wild-type (TLR2(+/+)) and TLR2-deficient (TLR2(-/-)) mice were sensitized to soluble OVA antigens and challenged with OVA, and the extent of allergic airways disease was analyzed in both groups of mice at day 8 after being challenged with OVA aerosol. At day 8 post-OVA, TLR2(-/-) mice exhibited significantly lower airway hyperresponsiveness, airway inflammation, and whole lung T-helper type 2 (Th2) cytokine levels compared with the TLR2(+/+) group. TLR2 is a major contributor to the maintenance of the adaptive Th2-cytokine-driven inflammatory disorder

Warren et al. have shown that TLR2 signaling through myeloid differential primary response protein 88(MyD88) induces complicated signaling events, leading to increased expression of proinflammatory cytokines, chemokine's, and other effector molecules [17].

In the present study, the oral treatment of the bronchial asthma model with resveratrol in mice sensitized and challenged with OVA significantly decreased the serum total IgE (TIgE) and the number of total inflammatory cells in addition to significant decrease in the inflammatory score with preservation of the normal histological structure of the lung tissues and prevention of histopathological changes induced by OVA. Resveratrol also significantly decreased the expression of CD4, CD8 and TLR2 in lung tissues.

Resveratrol, known as trans-3, 5, 4′-trihydroxystilbene has antioxidant and anti-inflammatory activities. Some studies documented different anti-airway inflammation mechanisms [18-21]. But none of these studies investigated its possible immunomodulatory effects that highlighted in the present study.

Lee et al. showed that resveratrol inhibited the increases in total inflammatory cell counts and eosinophil counts in BALF induced by ovalbumin and reduced the levels of total IgE and substantially inhibited OVA-induced eosinophilia and pathological changes in lung tissue [18]. He examined preliminarily the dose effects of resveratrol (20, 30 and 50 mg/kg) on IgE, IgG, and IgG2a in serum to confirm the optimal dosages of resveratrol and used 30 mg/kg (similar to 50 mg/kg) of resveratrol because that dosage was most effective. So, in this study we adopted use of oral resveratrol in a dose of 30 mg/kg.Resveratrol-mediated effects on airway inflammation, BALF inflammatory cell and lymphocyte infiltrates were the result of reduced IFN- γ production that may be related to TLR signaling [22]. In a study about obesity; Kim et al. suggest that resveratrol attenuates cytokines production in the adipose tissue by repressing the TLR2- and TLR4-mediated pro-inflammatory signalling cascades in HFD-fed mice [23].

Park et al. investigated the effects of resveratrol on inflammatory mediator production induced by Heat-Killed Listeria monocytogenes (HKLM), a TLR2 agonist. Resveratrol showed anti-inflammatory effects by inhibiting HKLM-induced Nox-1 expression, ROS production, monocyte chemotactic protein-1 expression (MCP-1 expression), cyclooxygenase-2 (COX-2) expression, metabolite (PGE2 and PGI2) production, and nitric oxide (NO) production after inducible nitric oxide (iNOS) expression [24]. The potent antioxidant and anti-inflammatory properties of resveratrol were linked to inhibition of cytokine stimulated inducible nitric oxide synthase expression and nitrite production, inhibition of granulocyte-macrophage colony stimulating factor release(GM-CSF), IL-8 release, and cyclooxygenase-2 expression [25].

Park et al. have shown that resveratrol in macrophages had a significant protective role in the HKLM-induced inflammatory response through inhibiting the extracellular signal-regulated kinase 1/2 (ERK1/2) and GSK3b pathways.In an animal model for allergic asthma, resveratrol, a well-known flavonoid with anti-inflammatory properties restored inositol polyphosphate 4 phosphatase (INPP4A) activity and this compound interacts with the aryl hydrocarbon receptor (AhR), which can downregulate the expression of pro-inflammatory genes [24,26].

Kim et al. have postulated that the glycogen synthase kinase 3b (GSK3b) and b-catenin pathways are crucial in the balance between pro- and anti-inflammatory cytokine productions [27]. In particular, this pathway plays an essential role in inflammation and immune cells through TLR signaling. For example, GSK3b regulates TLR-mediated cytokine production, and inactivation of GSK3b by LPS has a negative effect on production of the proinflammatory cytokine interferon-β.

In the present study, the treatment of mice with dexamethasone in the bronchial asthma model sensitized and challenged with OVA significantly decreased the serum total IgE (TIgE) and the number of total inflammatory cells in addition to significant decrease in the inflammatory score with preservation of the normal histological structure of the lung tissues and prevention of histopathological changes induced by OVA. Dexamethasone also significantly decreased the expression of TLR2, CD4+ and CD8+ in lung tissues. These results are in agreement with that obtained by Kirkil et al., Lee et al and Mushaben et al [28,18, 29].

Winder et al. concluded that TLR2 is an inducible component of airway epithelial defenses. Enhanced functional TLR2 expression following TNF-α and IFN-γ exposure may serve as a dynamic means to amplify innate immune responses during infectious or inflammatory pulmonary diseases [30]. Under conditions where first response cytokines are present, enhanced TLR2 signaling allows for further amplification of mucosal immunity. They found that glucocorticoids can act as a negative regulator of functional TLR2 expression in well-differentiated human airway epithelia. Since TLR2-mediated responses may occur early in the host response to infection, any factors that negatively impact TLR2 expression or signaling might influence disease outcomes.

Arancibia et al. have tested the effect of dexamethasone, a standard drug for allergic symptoms, and a TLR2/6 agonist alone or in combination with IFN- γ in a model of chronic respiratory sensitization to pollen allergens of Timothy grass [31]. They found that dexamethasone ameliorated inflammatory as well as remodeling parameters. The reduction of CD11c+ cells seems to be the crucial step for these positive results.

Sukkar et al. had shown that TLR expression and function was

regulated by corticosteroids. Overall, dexamethasone had a suppressive effect on cytokine- and ligand-induced TLR2, TLR3, and TLR4 expression and chemokine release [11]. However, dexamethasone increased TLR2 expression induced by combined IFN- γ and TNF- αstimulation.

On comparing the dexamethasone treatment with resveratrol treatment, it showed significant decrease in the serum total IgE (TIgE) and the number of total inflammatory cells in addition to significant decrease in the inflammatory score and the expression of CD4+, CD8+ while resveratrol treatment showed significant decrease in the expression of TLR2 in lung tissues.

In a recent study by, they reported that resveratrol and dexamethasone are equally effective in increasing expression of PTEN that reduced and involved in pathogenesis of ova-induced asthma in mice [20].

In comparison with the untreated mice, the treatment of mice with combination of resveratrol and dexamethasone in the bronchial asthma model sensitized and challenged with OVA significantly decreased the serum total IgE (TIgE) and the total inflammatory cell count in addition to significant decrease in the inflammatory score with preservation of the normal histological structure of the lung tissues and ameliorating of histopathological changes induced by OVA. This combination of resveratrol and dexamethasone also significantly decreased the expression of CD4, CD8 and TLR2 in lung tissues.

The present study showed that treatment by the combination of resveratrol and dexamethasone in the bronchial asthma model was better than treatment with resveratrol alone as it showed significant decrease in the whole studied parameters.

In comparison with dexamethasone treatment alone, although the treatment by both resveratrol and dexamethasone showednon significant change in total inflammatory cell count and the inflammatory score, but this combination caused significant decrease in the serum total IgE (TIgE) in addition to significant decrease in the expression of TLR2, CD4+ and CD8+ in lung tissues.

Asthma is managed with use of long-acting β2-adrenergic receptor agonists as bronchodilators and low-dose inhaled glucocorticosteroids are used to control the inflammatory response. This treatment regime is exceptionally effective for the majority of asthma patients which amount to approximately 300 million people worldwide. However, corticosteroid insensitivity or resistance is a significant clinical problem with approximately 10% of asthma patients requiring the maximum inhaled dose. Further, approximately 1% of patients require regular treatment with oral corticosteroids and a smaller proportion of patients are resistant to corticosteroids [32]. Corticosteroids, particularly at the higher doses, are associated with nontrivial side effects. Although the proportion of patients with corticosteroid insensitivity or resistance is very low, it is an important clinical problem considering the total number of people with asthma [33].

Up to our knowledge; there are no studies about use of resveratrol as an add-on therapy for treatment of bronchial asthma. However, Sadarani and Majumdar, explored the potential of resveratrol to reinstate the effectiveness of dexamethasone when administered as an adjunct in acute lung inflammation induced by cigarette smoke (CS) and lipopolysaccharide (LPS). Combination of resveratrol (50 mg/kg) and dexamethasone (2.5 mg/kg) significantly reduced all inflammatory parameters [34]. The protective effect of the combination was abolished when co-administered with sirtinol, a SIRT1 inhibitor.

Several treatments targeting the TLR pathway have been topics of ongoing research in an attempt to minimize the severity of asthma. Studies relating to asthma mostly show a higher degree of activation of the TLR radical cycle. Thus, antagonists of TLRs, antioxidants and anti-inflammatory drugs may have beneficial effects in patients with asthma [11].

Knobloch et al. suggested that resveratrol is superior to dexamethasone in reducing the release of cytokines/chemokine from human airway smooth muscle cells of patients with chronic obstructive pulmonary disease (COPD). Histone modification by histone deacetylase (HDACs) reverses local chromatin expansion required for transcription of cytokine/ chemokine genes [35]. Besides other mechanisms, resveratrol can suppress gene transcription via activation of SIRT1, a member of the sirtuin class III HDAC family. Activated corticosteroid receptors suppress gene transcription via the recruitment of HDAC2 (a class I/II HDAC) to target genes. HDAC2 is down-regulated in COPD possibly explaining the corticosteroid resistance of airway inflammation [36].

The problem of corticosteroid resistance also raises the question of alternative therapeutic approaches. Resveratrol exhibits anti-oxidative and anti -inflammatory properties could be alternative in COPD therapy 10) [37].

This study demonstrated the immunomodulatory effect of resveratrol preclinically in bronchial asthma induced mice model via suppression of TLR2, CD4+ and CD8+ expression as a possible mechanism. Although treatment by resveratrol with dexamethasone did not provide more anti-inflammatory effect over the treatment with dexamethasone alone based on the non-significant change in total inflammatory cell count and inflammatory score, but this combination exhibited significant synergistic immunomodulatory activity. So, we could suggest using resveratrol as add- on therapy with dexamethasone to achieve more efficacies in management of bronchial asthma. However, this should be verified in further clinical studies.

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