Tyrosol improves ovalbumin (OVA)-induced asthma in rat model through prevention of airway inflammation

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Abstract

Purpose

Asthma is an inflammatory disease that affects many people around the world, especially individuals of pediatric age. The effectiveness of tyrosol, a natural phenolic compound, was examined in the asthma model induced by ovalbumin (OVA).

Methods

For this purpose, 4 groups, each consisting of 8 rats, were formed. Serum physiological was given to the control group for 21 days. OVA was given to OVA, OVA + Dexamethasone (Dexa) and OVA + tyrosol groups intraperitoneally and by inhalation. Additionally, 0.25 mg/kg Dexa was administered to the OVA + Dexa group and 20 mg/kg tyrosol to the OVA + Tyrosol group by oral gavage. Serum, blood, BALF fluid and lung tissues of the rats were examined.

Results

it was observed that the MDA level decreased, GSH level and GPx activity increased, and there was no change in CAT activity in the tyrosol treatment groups. It was also observed that NF-κB, TNF-α, IL-4, IL-5, IL-13, IFN-γ, and IgE levels decreased compared to the OVA group. However, no effect on IL-1 β level was observed. In addition, it was determined that tyrosol treatment increased the IL-10 level. The results of the histopathological investigation of lung tissue showed that tyrosol significantly ameliorated OVA-induced histopathological lesions. Additionally, PAS staining showed that mucus hypersecretion was significantly reduced with the use of tyrosol. In addition, it was determined that the number of eosinophils decreased significantly.

Conclusions

The obtained results showed that tyrosol presented antioxidant and anti-inflammatory features on OVA-induced rats and preserved tissue architecture.

Introduction

Allergic asthma has been described as a complex inflammatory disease characterized by immune-mediated hypersensitivity reaction and chronic airway inflammation (Dogan et al. 2020). Airflow limitation is seen common in patients with asthma, and its clinical symptoms include stertorous respiration, shortness of breath, chest tightness and cough. It has been reported that the incidence of asthma has increased greatly in the last decade and that it can be fatal if not treated in a timely manner, that it will affect approximately 400 million people by 2025 (Masoli et al. 2004; Nguyen et al. 2012; Kao et

al. 2018). Airway hyperresponsiveness (AHR) is one of the most prominent features of asthma. In addition to inflammatory cells such as neutrophils and eosinophils, various cytokines and mast cells and T cells are involved in the hypersensitivity of the airways (Regele 2000; Elaidy et al. 2018). Mast cells play important roles not only in inflammation and immediate allergic reactions, but also in the development of chronic airway inflammation and airway remodelling, and in the clinical symptoms of asthma (Chiappara et al. 2001; Jehangir et al. 2019). Asthma is a chronic inflammatory disorder of the airways with a change in the T helper (Th) 1/Th2 balance and an imbalance in the ratio between regulatory T cells (Chan et al. 2016). It has been reported that while Th2 cells cytokines cause inflammatory responses in the asthmatic airways, Th1 cells can inhibit the development of Th2 cells and reduce the asthma response caused by Th2 (Yang et al. 2019). Currently, the protocols used in the treatment of asthma include leukotriene modifiers, glucocorticoids, leukotriene and mast cell stabilizers and besides their serious side effects, they show only symptomatic effects (Salama et al. 2012). Corticosteroids have been reported to be the most effective nonspecific anti-inflammatory drugs used extensively in the treatment of asthma, but it has been stated that they cause systemic immunosuppression and this leads to an increase in the possibility of infection (Migliorati et al. 1994). For this reason, the issue of determining safe and effective alternatives for the treatment of this disease has become interesting for researchers and it has been stated that especially natural prospective drugs with minimal side effects have come to the fore (Ezz-Eldin et al. 2020).

Tyrosol, 2- (4-hydroxyphenyl) -ethanol, is a phenolic compound found mainly in olive oil and white wine, and whose many biological and physiological activities have been reported. Together with hydroxytyrosole, it is one of the main phenolic compounds of olive oil (Lee et al. 2016). In some studies conducted to determine the biological activities of tyrosol, it has been reported that it has antidiabetic effect by regulating carbohydrate metabolism (Chandramohan et al. 2015) and, it has neuroprotective effect in cerebral ischemia thanks to antioxidant feature in rats (Bu et al. 2007). It has been stated that the daily consumption of olive oil in the Mediterranean diet may have an anticancer effect and that the tyrosol in its content contributes to this. However, anti-depressant, anti-stress, anti-inflammatory and anti-apoptotic effects of tyrosol have also been reported in previous studies (Pacifici et al. 2020; Qi et al. 2020). The protective efficacy of tyrosol, which has been reported to have many biological activities up to date, in an experimental animal asthma model was investigated in the study. For this purpose, due to the effectiveness of T cells on immune response changes, an asthma model was created in rats with OVA, which is universally used as a protein allergen in asthma models (Sun et al. 2010). On the other hand, the protective effects of tyrosol were determined in the light of the analysis of oxidation and inflammation markers and histopathological findings. The obtained findings were compared with the standard reference, Dexa.

Material And Method

Animals used in the experiment

In the experiment, 10 weeks old 32 Wistar albino rats weighing 220-250 g were used. The animals used in the experiment were obtained from Hatay Mustafa Kemal University Experimental Research Application and Research Center. The working environment was conducted in the manner as rats be in a daily cycle in accordance with the conditions of the care and use of laboratory animals (12 hours of light-12 hours of dark and at 21±1°C).

Chemical and reagent kits

IL-1β, IL-4, IL-5, IL-10, IL-13, TNF-α, IFN-γ, NF-κB and IgE ELISA kits used in the study were purchased from Bioassay Technology Laboratory, China Ovalbumin (OVA; grade II), aluminium hydroxide ($Al(OH)_3$) and tyrosol were purchased from Sigma Aldrich Chemical Co., USA. Decort (Deva) commercial preparate was used as dexamethasone. Other chemicals and solutions used in the study were purchased from Sigma Aldrich Chemical Co., USA in analytical purity.

Experimental protocol

Study groups consisted of 4 groups in total: Group 1 (Control group), Group 2 (OVA), Group 3 (Asthma + Dexa 0.25 mg/kg) and Group 4 (Asthma + Tyrosol 20 mg/kg). Establishing an experimental allergic asthma model and usage doses of dexamethasone were determined according to the literature (Boskabady et al. 2019). The dosage of tyrosol was made in accordance with the study of Güvenç et al. (Güvenç et al. 2019). The rats in the control group who underwent the practices of 1 ml serum physiological by oral gavage method once a day from the 1st to the 22nd day of the study, serum physiological intraperitoneally at a volume of 1 ml once a day on days 1, 2 and 3, physiological serum physiological as aerosol with the help of a nebulizer (Omron) in a closed box with a diameter of 35 - 25 - 15 cm on the 6th, 9th, 12th, 15th, 18th and 21st days for a total of 6 days. To create sensitivity in rats in Group 2, Group 3 and Group 4, 1 ml allergen suspension containing 1 mg ovalbumin + 100 mg aluminium hydroxide in serum physiological intraperitoneally on the 1st, 2nd, and 3rd days of the study, 1% ovalbumin solution in serum physiological on days 6, 9, 12, 15, 18 and 21 once a day for 20 minutes were applied as aerosol with the help of a nebulizer. In addition, the rats in Group 2 were administered 1 ml of serum physiological by oral gavage method once a day from the 1st to the 22nd day, the rats in Group 3 were administered Dexa once a day from the 1st to the 22nd day of the study in 1 ml physiological serum physiological by oral gavage method at a dose of 0.25 mg/kg. In Group 4, tyrosol was administered once a day from the 1st to the 22nd day of the study in 1 ml serum physiological at a dose of 20 mg/kg by oral gavage method. All applications within the scope of the experiment were carried out within the framework of the permit of Hatay Mustafa Kemal University Animal Experiments Local Ethics Committee, numbered 2019/09-3.

Bronchoalveolar lavage fluid (BALF) output

On the 22nd day of the experiment, rats were anesthetized (xylazine 10 mg/kg + Ketamine 60 mg/kg) and tracheostomy was performed by using a 12-gauge cannula (2 mm inner diameter). After tracheostomy, heparinized saline 0.1 ml was injected intravenously through the femoral vein to prevent blood clotting. It

was discharged by using a small polyethylene tube in case of excessive bronchial secretions. The lungs were washed three times (3 x 5 ml) through a cannulated tracheal tube with 5.0 ml of serum physiological. BALF fluid was collected (approximately 11-12 ml/rat) and centrifuged at 1500 rpm, 4°C for 10 minutes. It was stored at -80°C until analysis was done.

Determination of total and differential leukocyte count in BALF fluid

The cell pellet was resuspended in 1 ml of serum physiological to determine the total and differential leukocyte count. Froti prepared from BALF fluid was stained with giemsa stain and the percentage of leukocytes was determined.

Taking and analysing blood samples and determination of blood parameters

After the BALF and blood were collected, the anesthetized rats were decapitated. Hemogram parameters were measured in Mindray 2800 BC brand automatic blood count device on fresh blood samples taken into blood tubes with EDTA. In addition, smears were prepared from these fresh blood samples and leukocyte percentages were determined by giemsa staining method. Serums were prepared from blood samples taken into serum tubes and stored at -80 ºC for oxidative damage and inflammation parameters analysis.

Determination of lipid peroxidation, GSH level, CAT and GPx activities

Samples taken from lung tissues were homogenized with 1.15% KCl at a ratio of 1/10 and MDA analysis was performed in half of the homogenate. The other half was centrifuged at 5000 g for 1 hour (at +4°C) and its supernatants were separated, GSH, GPx and CAT analyses were performed. In the study, the MDA level was determined according to the method of Ohkawa et al. (Ohkawa et al. 1979). The method of Beutler et al. was used to determine the reduced glutathione (GSH) level (Beutler et al. 1963). MDA and GSH levels are given in nmol/ml. Catalase (CAT) enzyme activity was determined according to Aebi's method (Aebi 1984), and glutathione peroxidase (GPx) activity was determined according to Beutler's method (Beutler 1975). Protein analyses in the homogenate and supernatant were performed according to the method of Lowry et al. (Lowry et al. 1951).

Determination of inflammation and allergic reaction markers

Inflammation markers in lung tissue and serum samples were determined by enzyme-linked immunosorbent assay (ELISA) by using commercial kits. The measurements in determining the amounts of interleukin-1 beta (IL-1β), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-10 (IL-10), interleukin-13 (IL-13), tumour necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), nuclear factor kappa B (NF-κB) which are the inflammation markers and immunoglobulin E (IgE) protein, which is an allergic reaction marker, were made by using an ELISA plate reader.

Histopathological evaluation

At end of the experiment, all rats were sacrificed for histological examination. The lobe of the left lung tissue was removed and fixed in 10% neutral buffer formalin for 48 hours the tissue. The lung tissue samples were blocked in paraffin after they were dehydrated by passing through an ascending alcohol series and became transparent by passing through a series of xylene. Serial sections of 5 micron thick were taken from these paraffin blocks by microtome (Leica RM 2135) and then stained with hematoxylin and eosin (H&E) and Periodic Acid Schiff (PAS). The slides were examined (Olympus CX21, Olympus Corporation. Tokyo, Japan)1 and photographed (Olympus DP12)1 using a light microscope. To determine the severity of inflammatory cell infiltration, peribronchial cell counts were performed blind based on a 5-point scoring system as previously described (Myou et al. 2003). Briefly, the scoring system was: 0, no cells; 1, a few cells; 2, a ring of cells 1 cell layer deep; 3, a ring of cells 2–4 cells deep; 4, a ring of cells >4 cells deep. To determine the extent of mucus production, goblet cell hyperplasia in the airway epithelium was quantified blind using a 5-point grading system described by Tanaka colleagues (Tanaka et al. 2001). The adopted grading system for Mucus Production Scores (MPS) was: 0: < 0.5% PAS positive cells, 1: < 25%, 2: 25-50%, 3: 50-75% and 4: > 75%. Scoring of inflammatory cells and goblet cells was performed in at least five different fields for each lung section. Additionally, the lung injury scores were based on categories: inflammation, congestion, haemorrhage, thickened interalveolar septa according to the previously described method with minor changes (Smith et al. 1997). Those score indexes were graded as follows semi- quantitatively: no injury=score of 0; injury in 25% of the field=score of 1 (slight); injury in 50% of the field=score of 2 (medium); injury in 75% of the field=score of 3 (medium-severe); and injury throughout the field=score of 4 (severe). Each sample was investigated in five microscopic fields and scoring of inflammatory cells and goblet cells and the severity of lung injury was evaluated by the average score.

Statistical analysis

Statistical evaluation of the data obtained at the end of the study was made by using IBM SPSS Statistics 23 package program and $P<0.05$ value was considered statistically significant. Kruskal-Wallis test was used to determine the difference between groups obtained semi-quantitatively. The determination of different groups was determined by the Mann-Whitney U test. Statistical significance was considered at a p-value of <0.05. One-way ANOVA (Tukey) and SPSS (version 12.0; SPSS, Chicago, IL) statistics program were used for biochemical analysis. While all values are given as mean ± standard error (± S.E.M.), results at $p <0.05$ were considered significant.

Results

Tyrosol treatment showed a regulatory effect on antioxidant parameters

Within the scope of the study, in order to determine the effects of tyrosol administration on antioxidant parameters in experimental asthma animal model, MDA, GSH levels and, GPx and CAT enzyme activities were determined in lung tissue. According to the results, it was observed that the MDA level increased significantly in the OVA group compared to the other groups. On the other hand, it was observed that

tyrosol treatment reduced the MDA level to levels close to the control group. In the Dexa group, it reduced the MDA level to lower levels compared to the control group. When the MDA levels between the groups were compared, there was no statistical difference between the control and OVA + Tyrosol groups, but it was significantly higher in the OVA group ($p<0.001$). When GSH levels were compared between the groups, a significant decrease was observed in the OVA group compared to the control group. On the other hand, the decrease in GSH level due to OVA was significantly prevented in the group in which OVA and tyrosol were administered ($p<0.001$). This effect of tyrosol was slightly greater than that of the group which was administered Dexa. When evaluated in terms of GPx enzyme activities, it was observed that the enzyme activity decreased significantly in the OVA group compared to the control group. On the other hand, it was determined that tyrosol treatment significantly increased the GPx enzyme activity compared to the OVA group ($p<0.001$). Except for the OVA group, there was no significant difference between the other groups in terms of GPx enzyme activity ($p<0.001$). When the lung tissue CAT enzyme activities were compared between the groups, there was no statistically significant difference ($p<0.05$). The effect of tyrosol on antioxidant parameters is given in Table 1.

The effect of tyrosol treatment on IgE, IFN-γ, IL-5, IL-10, and IL-13 levels

IgE, IFN-γ, IL-5, IL-10 and IL-13 cytokine levels were analysed in serum and lung tissue samples in all groups. According to the results, a significant increase was observed in the IgE level in both serum and lung samples in the OVA group compared to the control group. On the other hand, it was determined that IgE levels were significantly decreased in the group where OVA and tyrosol were administered together, both in serum and lung tissues compared to OVA group. Similar effects were seen in the group treated with Dexa. It was observed that there was no statistically significant difference in IgE levels between the control group and in the groups where tyrosol and Dexa were administered together ($p<0.01$). When the IFN-γ level was examined in serum and lung tissue samples, a significant increase was observed in both tissues in the OVA group compared to the control group ($p<0.01$, $p<0.001$, respectively). The IFN-γ level in the OVA + Tyrosol group in both serum and lung tissue was found to be close to the control group values. When the effects of tyrosol and Dexa were compared, no statistically significant difference was observed in serum and lung tissues ($p<0.01$, $p<0.001$, respectively). When IL-5 levels were examined, it was determined that there was a significant increase in serum and lung samples of the OVA group. It was found that OVA-induced increase was prevented in both tissues in the groups where tyrosol and Dexa were administered. IL-5 levels in both serum and lung tissue in these groups were found to be close to the control group ($p<0.001$). When IL-10 levels were examined within the scope of the study, a statistically significant increase was observed in the groups which underwent Tyrosol and Dexa treatment compared to the OVA group ($p<0.05$). On the other hand, when the lung tissues were examined, it was seen that the IL-10 level in the OVA group decreased slightly, although not statistically significant. It was determined that the tyrosol treatment significantly increased the IL-10 level compared to both the control and the OVA group. When the control and Dexa groups were compared, it was found that IL-10 level increased significantly in the Dexa group ($p<0.001$). When IL-13 levels were examined in serum and lung tissue samples, it was determined that there was a significant increase in the OVA group compared to the control group, and that Tyrosol treatment prevented this increase significantly ($p<0.01$, $p<0.001$,

respectively). While IL-13 level in the group which underwent tyrosol in serum tissue was found to be close to the control group ($p<0.01$) this difference was statistically significant in lung tissue (p 0.001). The effect of Dexa was similar to tyrosol in both tissues. The effects of tyrosol treatment on IgE, IFN-γ, IL-5, IL-10 and IL-13 levels for serum and lung tissues are summarized in Tables 2 and 3, respectively.

Effect of Tyrosol treatment on TNF-α, IL-1β, IL-4 and NF-κB levels

Serum and lung tissue TNF-α, IL-1β, IL-4 and NF-κ*B* levels were examined. Accordingly, while a significant increase in the level of TNF-α was observed in both tissues in the OVA group, it was determined that Tyrosol significantly prevented this increase ($p<0.01$). When the Tyrosol and Dexa groups were compared, it was observed that TNF-α levels were not statistically significant ($p> 0.01$). When serum and lung tissue IL-1*β* levels were analysed, no statistically significant difference was observed between the groups ($p> 0.05$). When the IL-4 level was examined, no statistically significant difference was found between the groups in serum samples ($p> 0.05$). On the other hand, it was determined that IL-4 level of the lung tissue increased significantly in the OVA group compared to the control group, and this increase was significantly prevented in the tyrosol treatment group ($p<0.01$). IL-4 levels were similar in Tyrosol, Dexa and control groups. When the serum NF-κB level was examined, a statistically significant increase was found in the OVA group compared to the control group ($p<0.01$). Although some decrease in NF-κB level was observed in the groups treated with Tyrosol, it was not statistically significant compared to the OVA group. The effect of Dexa and Tyrosol on the level of NF-κB was similar. When the lung tissue NF-κB levels were compared between the groups, it was seen that the NF-κB level in the OVA group was significantly higher than the other groups. However, NF-κB levels decreased significantly in the tyrosol and Dexa treatment groups compared to the OVA group. NF-κB levels of control, Tyrosol and Dexa groups were close to each other. The effect of tyrosol on TNF-α, IL-1*β*, IL-4 and NF-κB levels for serum and lung tissues are summarized in Tables 4 and 5, respectively.

Effects of tyrosol treatment on inflammatory cell numbers

Within the scope of the study, the neutrophil, eosinophil, lymphocyte, and monocyte percentage counts were determined in blood and BALF fluid samples. According to the data obtained, it was determined that the percentage neutrophil count was not statistically significant between the groups in BALF fluid ($p> 0.05$). As for the blood neutrophil count value in percent, while a significant increase was observed in the group which underwent Dexa, no significant difference was observed between the other groups ($p<0.001$). When the percentage of eosinophils in blood samples was examined, a significant increase was observed in the OVA group compared to the control group. On the other hand, this value decreased in the Tyrosol group almost to the same level as the control group. The reducing effect of tyrosol on the percentage eosinophil count was statistically significantly higher than Dexa ($p<0.001$). When the percentage of eosinophils in BALF fluid was examined, it was determined that there was a statistically significant difference between all groups. Accordingly, an increase was observed in the OVA group compared to the control group. It was determined that the percentage of eosinophils decreased significantly in the groups which underwent tyrosol and Dexa compared to the OVA group. When these groups were compared

among themselves, it was seen that the percentage of eosinophils in the Dexa group was lower than the Tyrosol group ($p<0.001$). When the percentage lymphocyte counts were examined, it was found that there was no significant difference in blood tissue between the control, OVA and Tyrosol groups. However, this value was found to be significantly decreased in the Dexa group compared to the other groups. When the BALF fluid percentage lymphocyte counts were examined among the groups, it was observed that there was a significant decrease in the OVA group. It was seen that the tyrosol treatment eliminated this effect and brought the percent lymphocyte levels to similar levels with the control group. It was observed that the percentage of lymphocytes increased in the Dexa group compared to the control group ($p<0.001$). When blood tissue percentage monocyte values were examined, there was no significant difference between the OVA group, the control and Tyrosol groups. When the Dexa and OVA groups were compared, this value was found to be significantly higher in the Dexa group ($p<0.05$). When the BALF fluid percent monocyte counts were examined, there was a statistically significant decrease in the other groups compared to the control group ($p<0.001$). It was observed that tyrosol and Dexa treatment had no effect on percent monocyte values. The obtained data are given in Tables 6 and 7.

Histopathological evaluation

The H&E and PAS staining findings of histopathological sections of rat pulmonary tissues in each group, inflammation scores, MPS and histopathological findings were shown in Fig. 1, Fig. 2, Fig. 3, Fig. 4, and Table 8, respectively. In order to determine the histological feature of lung tissue, H&E and PAS staining were performed. There were statistically significant differences between groups in terms of Inflammation score and MPS ($p<0.0001$). Inflammation score was 0.250 ± 0.16 in the control group, 3.125 ± 0.227 in the OVA group, 1.375 ± 0.183 in the OVA + Tyrosol group, and 1.125 ± 0.227 in the OVA + Dexa group. MPS was 0.250 ± 0.16 in the control group, 3.250 ± 0.250 in the OVA group, 1.250 ± 0.16 in the OVA + Tyrosol group and 1.000 ± 0.189 in the OVA + Dexa group. Microscopical examination of H&E sections from the rats of control group showed the normal histological structure (Fig. 1 A). The marked typical pathological features were observed in OVA-induced groups as compared to control groups. From moderate to severe alveolar and bronchiolar damage with thickened interalveolar septa, massive inflammatory cell infiltrations, perivascular and peribronchiolar edema, hemorrhage, emphysema of some alveoli and vascular congestion were observed in histology of lung tissue from OVA induced rats. Proliferation (epithelization) was detected in type II epithelial cells in some alveoli. In addition, inflammatory cell infiltration situated locally in bronchial and bronchiolar propria mucosa and lymphoid cell hyperplasia in the manner of peribronchiolar follicle were noted (Fig. 1 B, C and D). Treatment with tyrosol and Dexa markedly reduced pulmonary injury. Lung tissue from OVA+Tyrosol (Fig. 1 E) and OVA+Dexa (Fig. 1 F) group rats showed slight infiltration of inflammatory cells. In these groups, mild enlargement of the interalveolar septa and rarely peribronchiolar lymphoid cell hyperplasia were observed. In the histopathological examinations of the sections stained with PAS, no increase in goblet cells and mucus secretion was found in the control group (Fig. 2 A). The lung tissue of OVA-induced rats showed mucus hypersecretion and goblet cell hyperplasia (Fig. 2 B). However, co-treatment with tyrosol (Fig. 2 C) and Dexa (Fig. 2 D) gave rise to a reduction goblet cell hyperplasia and mucus overproduction in the lung tissue.

Discussion

Asthma, one of the most common chronic and non-communicable diseases, causes quality of life and lifestyle disruptions and puts a burden on families, communities and countries (Périz et al. 2020). It has been reported that asthma is difficult to treat due to the complexity of its etiology, and that elucidation of the underlying mechanisms is of great importance in order to develop highly effective drugs with low side effects (Ma et al. 2019). The OVA-induced airway inflammation model has many similarities with human allergic asthma symptoms (Maslan and Mims 2014). In the study, the protective effects of tyrosol in the OVA-induced asthma model were examined. In this context, tyrosol was given to rats together with OVA and to another group, Dexa was given to compare the efficacy of tyrosol. At the end of the experimental application, biochemical and histopathological examinations were performed on serum, blood, BALF and lung tissue. The effects of tyrosol on antioxidant parameters, IgE, inflammation markers, inflammation cell numbers and histopathological changes were examined in the samples.

It has been stated in previous studies that the airways of asthmatic patients are stimulated in a manner to cause excessive oxidative stress, this increased mucus and sputum production and also damaged lung cells (Sussan et al. 2015). One of the formation mechanisms of MDA is lipid peroxidation which is induced by oxygen radical. Lipid peroxidation causes decrease in membrane fluidity and impairment of membrane function (Kuzu et al. 2019). Formation of MDA is considered as one of the main markers of oxidative stress (Türk et al. 2020). In the study, it was observed that the MDA level increased significantly in the OVA treated group compared to the control group. It has been reported that OVA exposure alters redox homeostasis and reduces antioxidant defence mechanism, resulting in induction of the production of highly reactive hydroxyl radicals, stimulation of lipid peroxidation, and cellular damage (Tiwari et al. 2014). However, it was determined that MDA in the Tyrosol treated group decreased to the levels of the control group. In the study conducted by Güvenç et al., it was reported that MDA increased in rats treated with $AlCl_3$, whereas tyrosol treatment significantly decreased the MDA level (Güvenç et al. 2020). GSH is an important cellular antioxidant that prevents the redox cycle and free radical formation (Martínez-Martos et al. 2014). In general, the increase in reactive oxygen species can be expressed by a decrease in the GSH level or the GSH/GSSG (oxidized glutathione) ratio (Kuzu et al. 2018). In the study, it was observed that the GSH level decreased significantly in the OVA group compared to the control group, whereas the GSH level in both the Dexa and tyrosol treatment groups came close the control group values. This curative effect of tyrosol was slightly higher than that of Dexa. In previous studies, it was reported that OVA exposure caused a decrease in GSH level and this was associated with increase in their consumption due to lipid peroxidation, and it was found that Dexa treatment prevented this situation (Hanna et al. 2019). The effect of tyrosol on GSH level was investigated in the dextran sulfate sodium-induced colitis model, and its curative effect on decreasing GSH level was reported (Güvenç et al. 2019). GPx has been reported as one of the main antioxidants in the lungs, and its activity has been shown to be reduced in asthma (Rahman et al. 2006). Decrease in GPx levels inhibits the production of Th1-dependent cytokines and increases Th2-related responses (Peterson et al. 1998). Within the scope of the study, when lung GPx activities were compared between the groups, it was seen that GPx activity was significantly

decreased in the OVA group compared to the control group, and that the GPx activity in the tyrosol and Dexa treatment groups was similar to the control group. In the diabetes model created experimentally in rats, it has been reported that tyrosol prevents the decrease in GPx enzyme activity in the liver and pancreatic tissue and reduces the formation of free oxygen species thanks to its antioxidant properties (Chandramohan and Pari 2016). It is stated that tyrosol can accumulate in the cell over time and reach useful concentrations in order to exert its protective effects. Different studies performed before show that biophenols such as tyrosol activate endogenous defence systems and especially they preserve intracellular GSH content and activate related enzymes, which are glutathione reductase, glutathione peroxidase and gamma glutamylcysteine synthetase and provide an indirect protection against oxidative stress (Di Benedetto et al. 2007). In this study, CAT enzyme activity was investigated in order to specify the effect of tyrosol on antioxidant parameters. However, it was determined that there was no statistically significant difference between the groups in terms of CAT activity. In previous studies, it was reported that CAT activity was decreased (Dalouchi et al. 2021) or no change was observed in OVA-induced experimental animals (Pourmehdi et al. 2020) It is thought that the different results obtained from the studies are due to the different administration of OVA treatment to different experimental animals. It has been reported that OVA exposure alters redox homeostasis and decreases the antioxidant defence mechanism, causing an increase in reactive species, consequentially resulted in lipid peroxidation and cellular damage (Yosri et al. 2017). Considering the effect of tyrosol on antioxidant parameters within the scope of the study, it can be said that it helps to protect the intracellular redox balance and prevents lipid peroxidation by increasing GSH level and GPx activity. In a previous study, Tyrosol was reported to have an *in vitro* radical scavenging effect (Chandramohan et al. 2017). Therefore, it can be said that tyrosol shows its antioxidant effect determined in the study both directly and by supporting the endogenous antioxidant defence system.

IgE, produced by B cells and binding to specific FcεRI receptors found on mast cells, is the main agent released in allergic asthma and which stimulates the degranulation of these cells and the release of allergic bronchoconstrictive factors including leukotrienes, prostaglandins and histamine (Barnes 2008a). Within the scope of the study, IgE levels were measured in both serum and lung tissue in order to investigate the effect of tyrosol treatment on allergic reactions. According to the results, it was observed that the IgE levels in both serum samples and lung tissues of rats increased in the OVA-induced asthma model compared to the control group, whereas this increase was largely prevented in the groups treated with tyrosol and Dexa. In accordance with this study, it has been stated in previous studies that OVA administration increases the IgE level (Eftekhar et al. 2019). In the study conducted by Je et al., they reported that tyrosol had a curative effect on IgE levels induced by OVA in mice, and found that this effect was more than Dexa (Je et al. 2015). Lin and colleagues stated that OVA exposure increased the IgE level in experimental animals and the treatment they applied decreased the IgE level and this effect could occur by suppressing the IL-4 level (Lin et al. 2020). It was determined also in our study that IL-4 level decreased with tyrosol treatment. Therefore, it can be said that tyrosol shows its inhibitory effect on IgE by suppressing IL-4 level. Due to the role of IgE in the pathogenesis of asthma, interruption of IgE synthesis or suppression of IgE function by various molecules has been evaluated as a new approach for

asthma treatment (Lin et al. 2012). Therefore, the prevention of the increase in OVA-induced IgE level of tyrosol treatment becomes important in this respect.

It is known that IFN-γ, a Th1 cytokine, plays a dominant role in many inflammatory diseases and immune disorders (Barnes 2008b). It has been experimentally and clinically proven that changes in IFN-γ levels are associated with the severity and duration of asthma (Rajizadeh et al. 2019). In the conducted study, it was observed that IFN-γ level in both serum and lung tissue increased in the OVA treatment groups compared to the control group, and it was almost the same as the control group in the tyrosol and Dexa treatment groups. In the study conducted by Hanna and colleagues, it was observed that the change in IFN-γ level in rats treated with OVA and Dexa was similar to the results obtained from our study (Hanna et al. 2019). However, in our study, the effect of tyrosol on IFN-γ level was determined for the first time. It has been reported that the NF-κB signalling pathway is activated to regulate the inflammatory reaction and immune response, which are important pathological features of bronchial asthma (Zhong et al. 2016). Activation of NF-κB is important for the expression of various inflammatory cytokines, including TNF-α, IL-1β and IL-4 (Je et al. 2015). It has been reported that while TNF-α and IL-1β contribute to the inflammatory response and airway constriction, IL-4 plays a role in IgE production and eosinophil growth by B cells. IL-5 affects eosinophil maturation and supports IgE production (Kim et al. 2020). IL-13 contributes to mucus secretion in this process (Nader et al. 2012). In the study, it was determined that NF-κB and TNF-α levels increased in both serum and lung tissue in the OVA treatment group compared to the control group. On the other hand, in the groups treated with Dexa and Tyrosol, NF-κB in the lung decreased to the control group levels and TNF-α in serum and lung tissue decreased to control group levels. When IL-1β levels were examined, it was seen that there was no difference between the groups in both tissues. Previous studies have shown that tyrosol decreases NF-κB activation and alleviates mast cell-mediated allergic inflammation (Je et al. 2015). In another study, it was reported that significant increases in TNF-α level were observed in lung tissue and BALF in experimental animals induced by lipopolysaccharide and tyrosol treatment caused reductions in both protein level and transcription level of TNF-α in these tissues. In the same study, different from the result we obtained, it was reported that tyrosol decreased the increasing IL-1β expression (Kim et al. 2017). The difference here is thought to be due to the determination of the amount of IL-1β at the protein level in our study. The NF-κB signalling pathway may play an important role in mucus secretion in rats with bronchial asthma. Therefore, it has been emphasized that due to its roles in inflammation and mucus secretion, the NF-κB signalling pathway may be an effective therapeutic target of bronchial asthma (Liu et al. 2020). In the study, IL-4, IL-5, and IL-13 levels as Th2 cytokines were examined in serum and lung tissues. It was determined that cytokines in serum except IL-4 increased in OVA treatment groups compared to the control group and all cytokine levels increased in lung tissue compared to the control group. Yan et al. reported that Th2 cells in the lungs of asthmatic patients secrete large amounts of IL-4, IL-5 and IL-13 (Yan et al. 2011). However, tyrosol treatment was found to reduce increasing cytokine levels and this effect was similar to Dexa. It has been stated that Dexa, a glucocorticoid drug, exerts its powerful anti-inflammatory effect against bronchial asthma by inhibiting the production of IL-4, IL-5 and IL-13, which are important cytokines in asthma (Westergaard et al. 2015). However, there is no study investigating the effect of tyrosol on these

cytokines in the literature. In a previous study, it was found that carvacrol, which is a phytochemical having molecular structure similar to tyrosol, reduces the levels of IL-4, IL-5 and IL-13 (Ezz-Eldin et al. 2020). Studies have shown that Th2-mediated cytokines such as IL-4, IL-5 and IL-13 play a role in regulating, prolonging and increasing the inflammatory response in asthma (Thakur et al. 2019; Menzella et al. 2020). In another study, it was reported that asthmatic mice lacking the IL-13 receptor had significantly less airway remodelling than mice with wild-type asthma (Chen et al. 2013). According to the results we obtained from the study, it can be said that tyrosol decreased these cytokine levels in the OVA-induced asthma model in rats and showed an anti-inflammatory effect and presented a protective effect. As a cytokine with anti-inflammatory properties, IL-10 limits the immune response by inhibiting the production of various cytokines and chemokines (Bolandi et al. 2021). It has been reported that IL-10 can have beneficial effects in controlling airway remodelling and may reduce type 1 collagen synthesis and smooth muscle cell proliferation (Selzman et al. 1998). In the study, it was determined that IL-10 levels in the serum and lung tissues increased significantly in the tyrosol and Dexa treatment groups compared to the OVA group. It has been reported that IL-10 can be a target in asthma treatment due to its anti-inflammatory effect (Mäkelä et al. 2000). Therefore, it is thought that tyrosol can act against the formation of asthma by increasing the level of IL-10. Excessive activation of Th2 cell is considered to be the main factor playing an important role in pathological symptoms in the lung during the development of asthma (Liou et al. 2020). It has been stated that various allergens play a role in the formation of Th1 and Th2 mediated immune response in the underlying pathogenesis of asthma (Thakur et al. 2019). Therefore, when the results obtained from the study are evaluated together, it can be stated that tyrosol may affect the NF-κB level and Th1/Th2 cytokine levels induced by OVA and prevent the development of asthma in rats.

Th1/Th2 imbalance is associated with changes in total serum IgE and allergen-specific serum IgE levels, airway response and eosinophilia (Guan et al. 2019). It has also been reported that the number of eosinophils in the peripheral blood and bronchial lavage of asthmatic patients is associated with the severity of the disease (Louis et al. 2000). In the study, it was determined that the number of eosinophils in percent increased significantly in both blood and BALF fluid in the OVA treatment group, however, this increase was prevented in the groups treated with Dexa and tyrosol. While this effect was greater in the BALF fluid in the Dexa group, the effect of Tyrosol in the blood was greater than that of Dexa. In a previous study, it was shown to increase the number of eosinophils in both BALF and serum, similar to the findings related to OVA treatment. It has been stated that this situation is related to increased IL-4, IgE and TNF-α production (Parlar and Arslan 2020). In another study, it was reported that vitamin E treatment reduces the level of IgE indirectly by suppressing the production of IL-4, which increases IgE production, and of IL-5, which plays a role in eosinophil migration (Jiang et al. 2021). Therefore, it can be said that tyrosol treatment shows its effect on the eosinophil count in percent by decreasing the levels of IgE, TNF-α, IL-4 and IL-5.

The histopathological data obtained within the scope of the study support the results obtained from the examination of antioxidant and inflammation markers. Accordingly, it was observed that inflammation and mucus production scores increased in the OVA group compared to the control group and

inflammation, congestion, haemorrhage, thickened interalveolar septa, perivasculitis and peribronhiolitis were detected in the lung tissue. It was determined that inflammation and mucus production scores decreased in the groups treated with tyrosol and Dexa and, histopathological lesions in the lung tissue were prevented. Previous studies have shown that OVA exposure has caused interstitial inflammation and fibrosis, emphysema, and epithelial damage in the lungs of animals and this confirms the induction of sensitivity (Boskabady et al. 2019). In another study, the presence of severe inflammation was shown in histopathological analysis of lung tissue of asthma-induced rats (Zhu et al. 2019). It has been reported that tyrosol exerts its protective effects on cell structure by suppressing inflammatory cell infiltration and pulmonary edema in animals induced by lipopolysaccharide (Kim et al. 2017). In another study, it was reported that tyrosol can improve the survival rate of mice in acute lung injury induced by lipopolysaccharide and reduce lung damage by suppressing the inflammatory reaction and oxidative stress (Wang et al. 2017). In our study, it is thought that tyrosol contributes to the preservation of tissue architecture with its regulating effect on antioxidant parameters and inflammation-reducing effect.

Conclusion

Asthma is characterized by bronchial inflammation, oxidative stress, and an imbalance in antioxidant defence mechanisms. According to the results obtained from the study, it was determined that tyrosol treatment reinforced the antioxidant defence system, reduced allergic response, prevented airway inflammation by regulating proinflammatory and anti-inflammatory cytokine levels and by reducing inflammatory cell numbers and, improved asthma symptoms in the OVA-induced asthma model. However, it is considered necessary to investigate different doses of tyrosol and its effects on the different pathways that cause inflammation.

Declarations

Ethics approval All applications within the scope of the experiment were carried out within the framework of the permit of Hatay Mustafa Kemal University Animal Experiments Local Ethics Committee, numbered 2019/09-3.

Consent to Participate Not applicable

Consent to Publish Not applicable

Author contributions MC conceived and designed research. MK, CTİ, ME, ND, AU, İG, ET, MG conducted experiments. MG analysed data. MK, MC and CTİ wrote the manuscript. All authors read and approved the manuscript and all data were generated in-house and that no paper mill was used.

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Tables

Group/Parameter	MDA (nmol/mL)	GSH (nmol/mL)	GPx (U/gr prot)	CAT (U/ml)
Control	4.227± 0.34^{b}	4.825± 0.12^{c}	176.271± 2.70^{b}	35.458± 2.05
OVA	7.391± 0.34^{c}	2.940± 0.23^{a}	148.496± 3.66^{a}	34.463± 1.21
OVA + Dexa	2.845± 0.25^{a}	3.978± 0.14^{b}	174.420± 2.77^{b}	32.337± 1.40
OVA + Tyrosol	5.267± 0.30^{b}	4.106± 0.09^{b}	171.889± 1.34^{b}	31.742± 1.77
P Value	P:0.000	P:0.000	P:0.000	P:0.355

Table 1 Effects of tyrosol treatment on antioxidant parameters in lung tissue

Values are expressed as mean ± S.E.M. of eight rats in each group. The difference between values with different letters (a, b, c) in the same column is statistically significant
Malondialdehyde (MDA), Reduced Glutathione (GSH), Glutathione Peroxidase (GPx), and Catalase (CAT)

Table 2 The effects of tyrosol treatment on IgE, IFNγ, IL-5, IL-10, IL-13 levels in serum

Group/Parameter	IgE (pg/ml)	IFN-γ (pg/ml)	IL-5 (pg/ml)	IL-10 (pg/ml)	IL-13 (pg/ml)
Control	10.840± 0.54^{a}	30.792± 1.39^{a}	8.072± 0.20^{a}	98.015± 3.67^{a,b}	15.061± 0.37^{a}
OVA	13.325± 0.19^{b}	37.025± 0.33^{b}	9.675± 0.12^{b}	94.717± 1.49^{a}	18.082± 0.51^{b}
OVA + Dexa	11.317± 0.29^{a}	31.794± 0.98^{a}	8.252± 0.15^{a}	107.909± 4.15^{b}	15.821± 0.44^{a}
OVA + Tyrosol	11.765± 0.20^{a}	31.868± 0.99^{a}	8.335± 0.29^{a}	104.795± 1.54^{b}	16.067± 0.40^{a}
P Value	P:0,001	P:0,002	P:0,000	P:0,024	P:0,001

Values are expressed as mean ± S.E.M. of eight rats in each group. The difference between values with different letters (a, b, c) in the same column is statistically significant
Immunoglobulin E (IgE), Interferon gamma (IFN-γ), Interleukin (IL-)

Table 3 The effects of tyrosol treatment on IgE, IFN-γ, IL-5, IL-10, IL-13 levels in lung tissue

Group/Parameter	IgE (pg/ml)	IFN-γ (pg/ml)	IL-5 (pg/ml)	IL-10 (pg/ml)	IL-13 (pg/ml)
Control	16.592±0.44^{a}	30.145±1.40^{a}	9.207±0.51^{a}	91.015±2.37^{a}	14.771±0.21^{a}
OVA	20.799±0.82^{b}	44.647±2.22^{b}	13.361±0.47^{b}	83.059±2.31^{a}	20.316±0.80^{c}
OVA + Dexa	15.615±0.64^{a}	33.907±1.03^{a}	9.893±0.20^{a}	122.317±3.80^{c}	16.714±0.12^{b}
OVA + Tyrosol	15.627±1.42^{a}	30.917±1.30^{a}	9.314±0.30^{a}	104.537±1.84^{b}	16.969±0.29^{b}
P Value	P:0.003	P:0.000	P:0.000	P:0.000	P:0.000

Values are expressed as mean ± S.E.M. of eight rats in each group. The difference between values with different letters (a, b, c) in the same column is statistically significant

Table 4 The effects of tyrosol treatment on TNF-α, IL-1β, IL-4 ve Nf-κB levels in serum

Group/Parameter	TNF-α (pg/ml)	IL-1β (pg/ml)	IL-4 (pg/ml)	Nf-κB (pg/ml)
Control	133.383± 2.12^{a}	916.100± 71.45	52.498± 1.54	2.283± 0.448^{a}
Astım	149.372± 3.72^{b}	912.567± 49.35	57.574± 1.58	3.762± 0.14^{b}
Dexamethazon	134.248± 1.65^{a}	931.000± 47.62	52.211± 2.15	2.869± 0.09^{ab}
Tyrosol+Astım	138.713± 0.95^{a}	908.500± 29.563	52.440± 0.56	2.802± 0.21^{ab}
P Value	P:0.001	P:0.990	P:0.077	P:0.009

Values are expressed as mean ± S.E.M. of eight rats in each group. The difference between values with different letters (a, b) in the same column is statistically significant
Tumor necrosis factor alpha (TNF-α), Interleukin 1 beta (IL-1β), Interleukin-4 (IL-4), Nuclear factor kappa-light-chain-enhancer of activated B cells (Nf-κB)

Table 5 The effects of tyrosol treatment on TNF-α, IL-1β, IL-4 ve Nf-κB levels in lung tissue

Group/Parameter	TNF-α (pg/ml)	IL-1β (pg/ml)	IL-4 (pg/ml)	Nf-κB (pg/ml)
Control	120.551±9.28^{a}	842.766±33.42	52.371±1.18^{a}	2.697± 0.12^{a}
OVA	151.729±9.57^{b}	880.200±31.74	74.983±5.07^{b}	3.390± 0.14^{b}
OVA + Dexa	108.248±4.88^{a}	920.900±64.49	51.696±1.54^{a}	2.771± 0.27^{a}
OVA + Tyrosol	127.227±2.02^{a}	842.899±41.52	53.303±4.52^{a}	2.666±0.16^{a}
P Value	P:0.004	P:0.570	P:0.001	P:0.045

Values are expressed as mean ± S.E.M. of eight rats in each group. The difference between values with different letters (a, b, c) in the same column is statistically significant

Table 6 The effect of tyrosol treatment on blood leukocyte counts (%)

Group/Parameter	Neutrophil	Eosinophil	Lymphocyte	Monocyte
Control	19.500±1.25^{a}	2.000±0.36^{a}	72.333±1.28^{b}	6.166±0.70^{ab}
OVA	18.666±0.80^{a}	8.833±0.70^{c}	67.166±0.87^{b}	5.166±0.47^{a}
OVA + Dexa	67.000±2.08^{b}	4.166±0.30^{b}	20.000±2.11^{a}	8.833±1.24^{b}
OVA + Tyrosol	21.166±1.44^{a}	1.833±0.30^{a}	71.166±1.30^{b}	5.833±0.65^{ab}
P Value	P:0.000	P:0.000	P:0.000	P:0.026

Values are expressed as mean ± S.E.M. of eight rats in each group. The difference between values with different letters (a, b, c) in the same column is statistically significant

Table 7 The effect of tyrosol treatment on BALF leukocyte counts (%)

Group/Parameter	Neutrophil	Eosinophil	Lymphocyte	Monocyte
Control	0.500± 0.22	0.333± 0.21^{a}	7.166± 0.70^{b}	92.000± 0.81^{b}
OVA	1.166± 0.30	7.166± 0.40^{d}	4.833± 0.30^{a}	86.833± 0.79^{a}
OVA + Dexa	0.833± 0.40	2.666± 0.33^{b}	9.166± 0.30^{c}	87.500± 0.42^{a}
OVA + Tyrosol	0.833± 0.30	5.000± 0.36^{c}	7.833± 0.47^{bc}	86.666± 0.66^{a}
P Value	P:0.540	P:0.000	P:0.000	P:0.000

Values are expressed as mean ± S.E.M. of eight rats in each group. The difference between values with different letters (a, b, c) in the same column is statistically significant

Table 8 The effects of tyrosol threatment on lung histopathology

Changes/Lesions	Control	OVA	OVA+TYR	OVA+DEX
Thickened interalveolar septa	-/8	8/8	8/8	8/8
Slight	-	-	7	7
Moderate	-	5	1	1
Severe	-	3	-	-
Epithelization	-/8	8/8	0/8	0/8
Slight	-	1	-	-
Moderate	-	3	-	-
Severe	-	4	-	-
Haemorrhage	0/8	8/8	0/8	0/8
Slight	-	3	-	-
Moderate	-	4	-	-
Severe	-	1	-	-
Lymphoid cell hyperplasia	-/8	8/8	5/8	4/8
Slight	-	-	4	3
Moderate	-	5	1	1
Severe	-	3	-	-
Congestion	0/8	8/8	0/8	0/8
Slight	-	4	-	-
Moderate	-	3	-	-
Severe	-	1	-	-
Goblet cell hyperplasia	-/8	8/8	6/8	5/8
Slight	-	-	5	5
Moderate	-	2	1	-
Severe	-	6	-	-

Data are numbers of rats showing changes/number of rats examined for each treatment group. All groups, n = 8. No injury=score of 0; injury in 25% of the field=score of 1 (slight); injury in 50% of the field=score of 2 (medium); injury in 75% of the field=score of 3 (medium-severe); and injury throughout the field=score of 4 (severe)

Figures

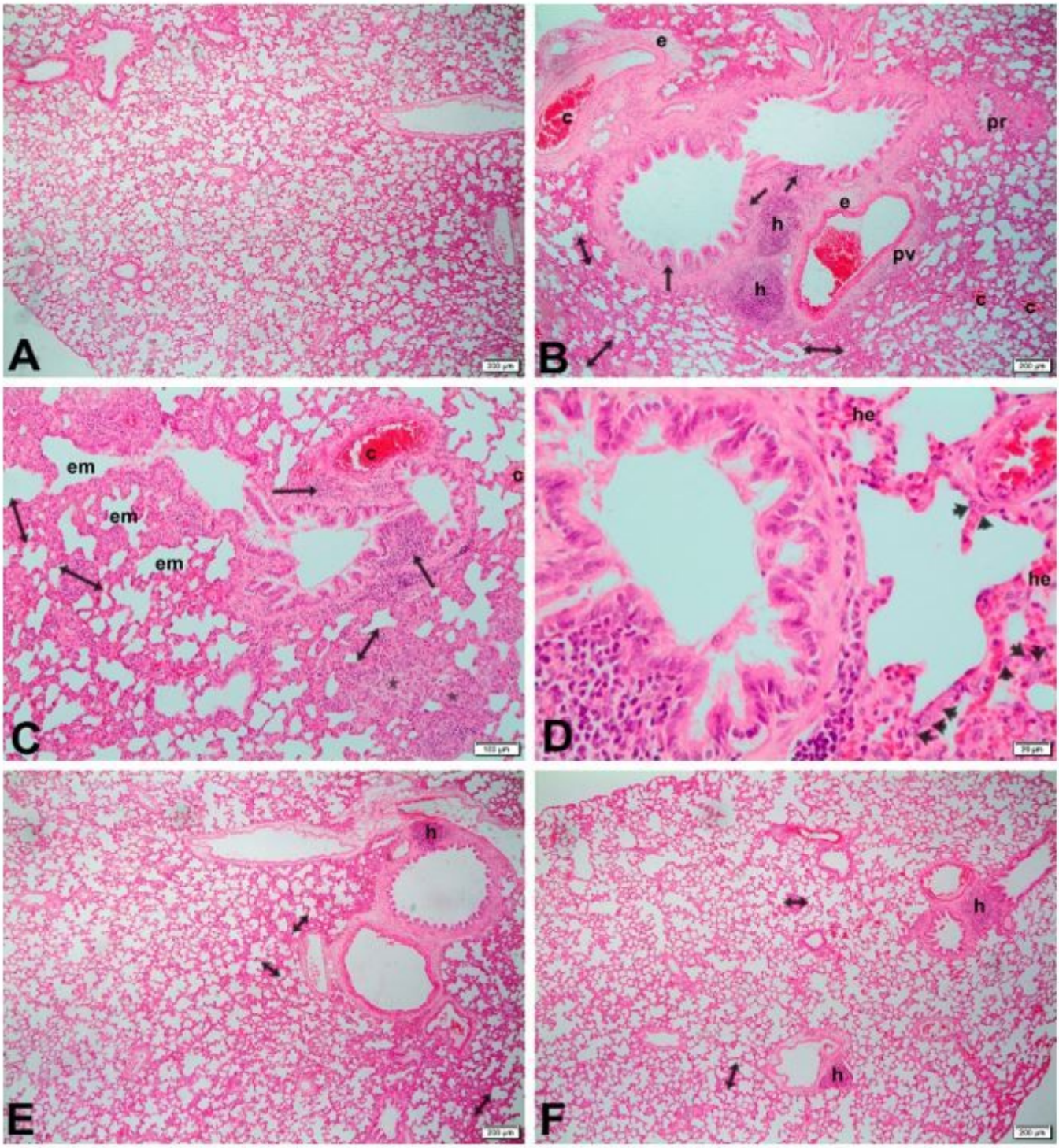

Figure 1

Representative histological changes of lung obtained from rats of different groups. (H&E). A) Control: Normal histological appearance of the lung tissues of rats. B, C, D) OVA: The appearance of moderate to severe histopathological lesions in the lung tissues of rats. E) OVA+Tyrosol: The appearance of minimal lesions in the lung tissues of rats. F) OVA+Dexa: The appearance of minimal lesions in the lung tissues of rats. [Interalveolar septum enlargement (bidirectional arrow), oedema (e), congestion (c), perivasculitis (pv), peribronchiolitis (pr), inflammatory cell infiltration in bronchiolar propria mucosa (arrow), follicular

lymphoid cell hyperplasia (h), emphysema in the alveoli (em), epithelialized areas (asterisk), proliferation (epithelization) in type-II epithelial cells (arrowhead), haemorrhage (he)].

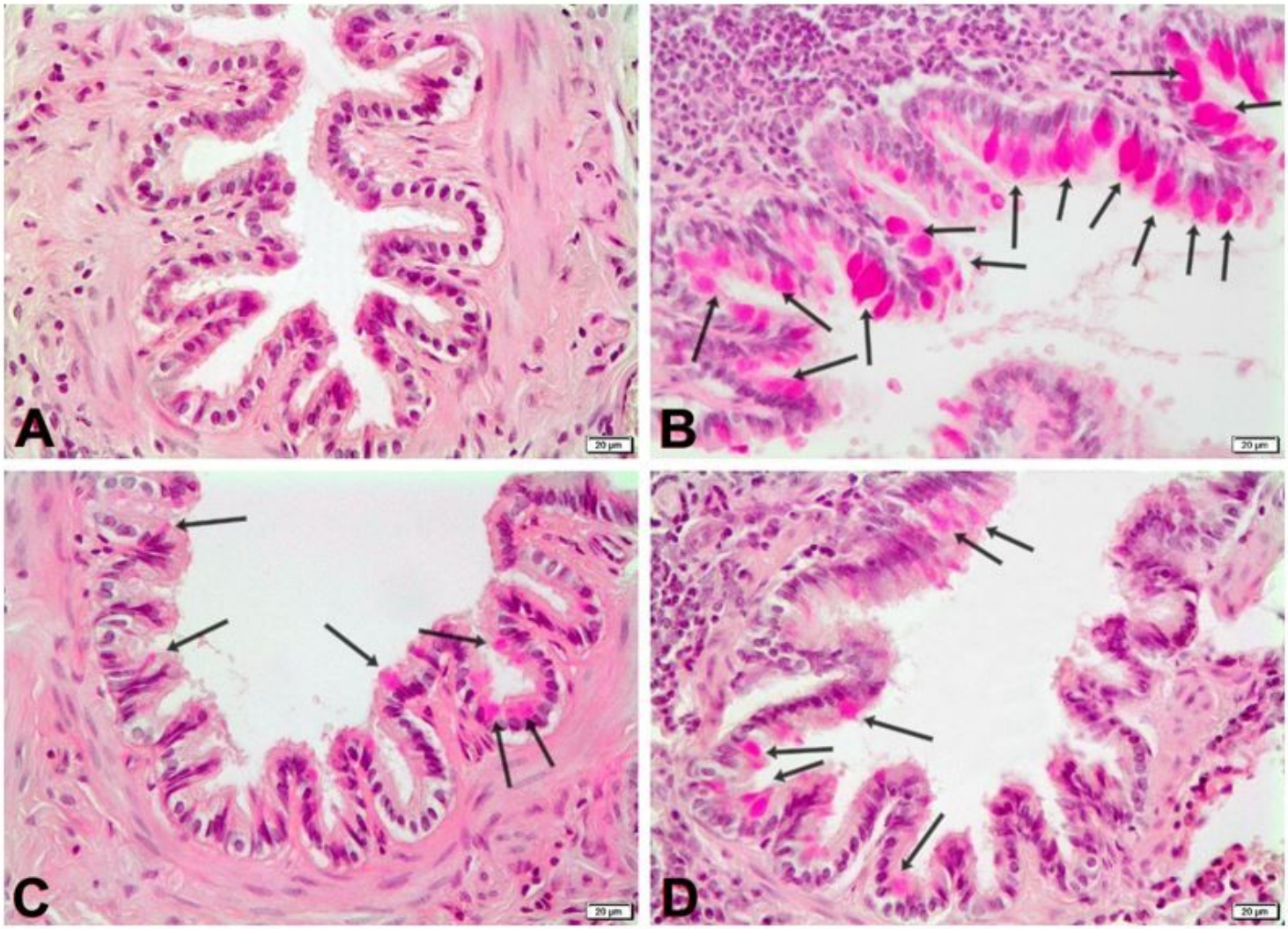

Figure 2

Representative of mucus secretions (arrows) in the PAS-stained sections of lung obtained from rats of different groups. A) Control, B) OVA, C) OVA+Tyrosol, D) OVA+Dexa group.

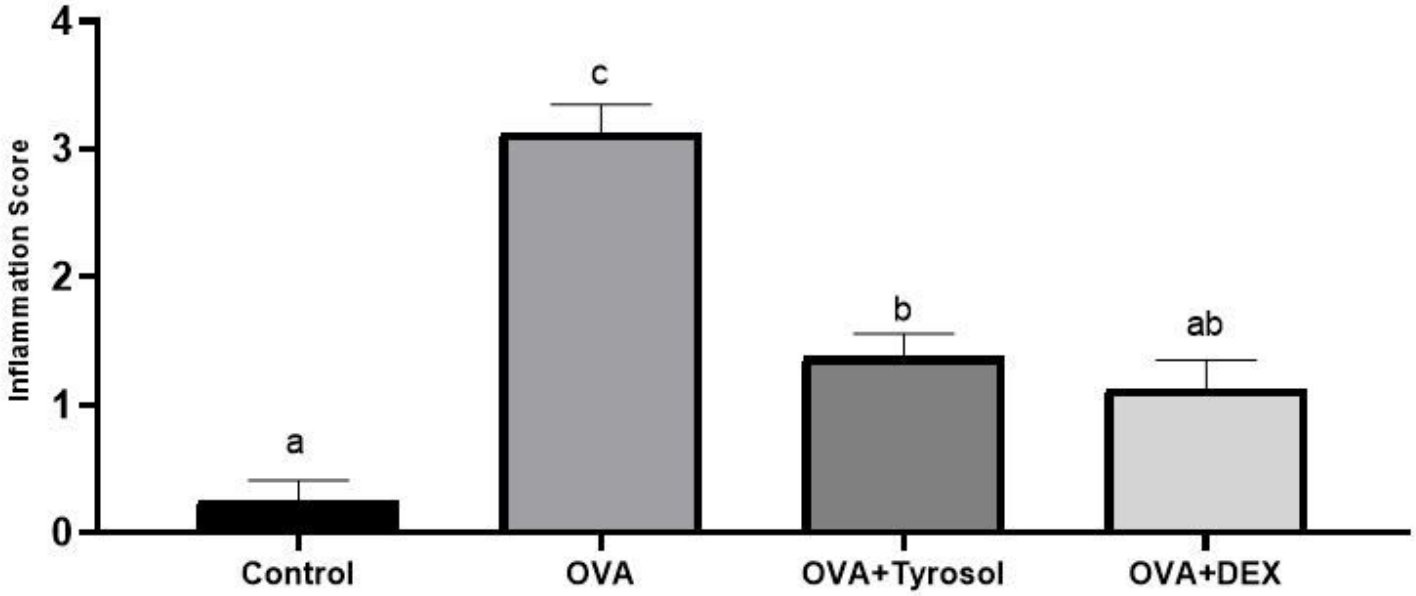

Figure 3

Effects of tyrosol on the inflammation score of pulmonary tissues. P:0.000 Data are expressed as mean ± SEM. Different superscript letters (a, b, c) within the same column show statistically significant differences between the groups. (DEX: Dexamethasone)

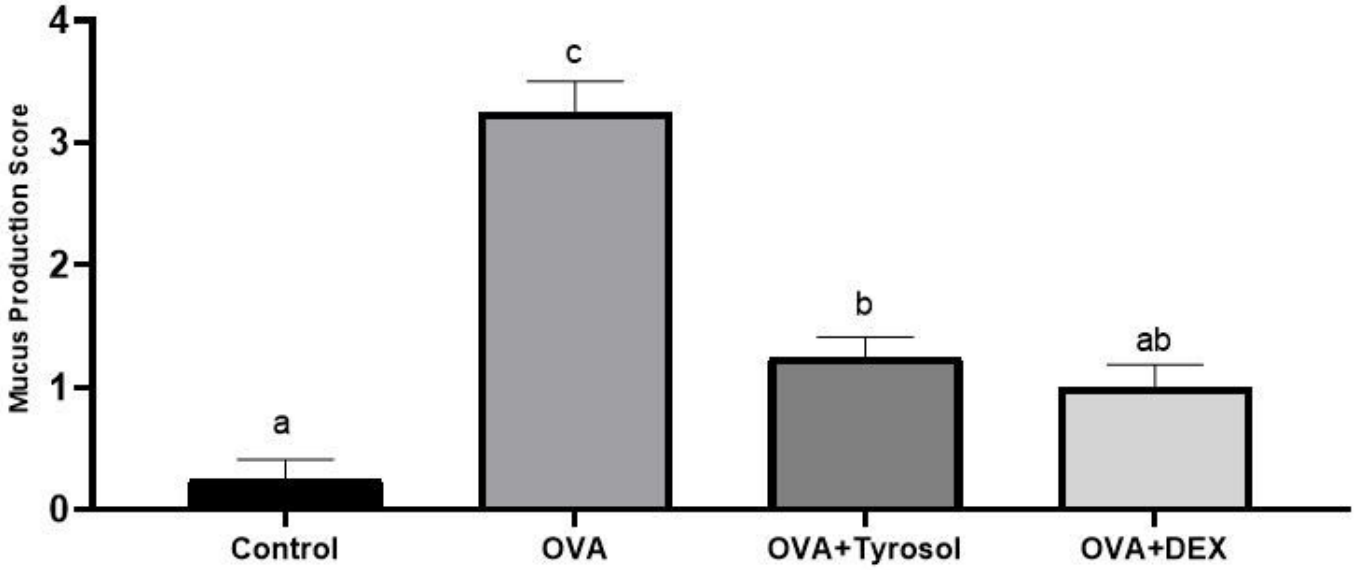

Figure 4

Effects of tyrosol on the Mucus Production Scores (MPS) of pulmonary tissues. P:0.000 Data are expressed as mean ± SEM. Different superscript letters (a, b, c) within the same column show statistically significant differences between the groups.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- NFKBElisa.xlsx
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