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The Therapeutic Responses of Resveratrol against Ovalbumin-Induced Asthma Influenced Via Upregulation of Forkhead Box Transcription Factor in Rats

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Abstract : Asthma fist attack incidents can happen at any age, with the majority of occurrences happening in people under the age of 10, with boys more commonly affected than girls. However, in adults, women are more often affected than men. Asthma in adults is sometimes associated with exposure to particular items during work, including pesticides, and wood dust. The purpose of this study was to determine if Resveratrol (RES) could reduce the allergic asthma that ovalbumin (OVA) induces in rats. 60 adults, 10-week-old rats were divided into the following groups at random: The first group (G1) was regarded as naive, the second group (G2) consisted of rats getting resveratrol, the third group (G3) consisted of asthma-induced rats using ovalbumin and the fourth group of rats (G4) received both resveratrol therapy and asthma induction. Blood samples were taken at the end of the experiment to assess total white blood cells and red blood cells, and Broncho Alveolar Lavage Fluid (BALF) to assess redox system activity. Furthermore, lung and trachea tissue were collected for histopathology. Moreover, the Foxp3 gene was detected in the spleen. Data analysis showed that the number of total white blood cells decreased significantly $p<0.05$ after resveratrol treatment. While the number of red blood cells was increased statistically by $p<0.05$ with resveratrol therapy. Resveratrol increased Glutathione (GSH) concentration significantly $p<0.05$ and decreased malondialdehyde concentration significantly in BALF. Resveratrol was able to alleviate the epithelial mucosa thickness, mucosal obstruction, and light mononuclear leukocyte infiltration in trachea tissue and attenuate severe interstitial pneumonia with infiltration of mononuclear leukocytes, congestion of interstitial tissue, and alveolar dilation in lung tissue. Overall, the study concluded that resveratrol possesses the biological actions that are necessary to boost the pulmonary system's defences against the harmful effects of ovalbumin-induced asthma.

Keywords: Asthma, Resveratrol, Rat, FOXP3 , BALF.

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Introduction

One of the disorders in which T-regulatory function is compromised in asthma, and asthmatics' reaction to oral or systemic steroids is largely owing to the propensity of these drugs to cause immunosuppression by activating T-regulatory cells (1) Treatment of these diseases is limited by the unavailability of specific drugs that the miRNAs' modulation of the Th2-dependent processes' subsequent routes (2) Excessive reliance on long-acting corticosteroids with possible side effects also complicates the situation (3,4) As demonstrated by prevent study

resveratrol significantly reduces various models of autoimmune illness exhibit inflammation (5,6). In addition, Resveratrol is just as effective in treating asthma as drugs, according to a study. Resveratrol was first reported and isolated from the root of the white hellebore plant by a Japanese researcher and in 1963 used in traditional Japanese and Chinese medicine (7,8) Resveratrol was discovered in grapes, and in the early 1990s became well known and was also identified in wine (9). Originally, resveratrol was thought to play a role in avoiding the heart problems that red wine consumption is related to. When Jang and colleagues showed that resveratrol was active against three key phases of carcinogenesis, the anticancer impact of this molecule on cancer was recognized. (i.e., initiation of carcinogenesis). initiate, motivate, and progress (10). Resveratrol's beginnings can be traced beginning in the early 1990s, when epidemiological research showed that, despite the prevalence of cardiovascular risk factors, the incidence of myocardial infarction in France was only one-third of that in the United States., illustrated by high body fat. Drinking Alcohol, lack of exercise, and smoking too much. This phenomenon, often referred to as the "French paradox"**,** no bold ethically this sentence should be removed attributed to the fact that the French regularly drink red wine with meals (one L. of red wine contains 3-5mg of resveratrol, and further implies that red wine consumption may reduce mortality and morbidity associated with coronary heart disease (CHD) and atherosclerosis(11).

Research problem

Asthma is a common lung condition that causes occasional breathing difficulties. It affects people of all ages and often starts in childhood, although it can also develop for the first time in adults.

There's currently no cure, but there are simple treatments that can help keep the symptoms under control so it does not have a big impact on your life.

Aim of the study

1- Exploring the role of resveratrol in tissue integrity of lung and liver in ovalbumin-induced asthma in rats.
2- Evaluating the effect of resveratrol as an anti-inflammatory agent in the lung.
3- Detecting ability of resveratrol supplement as an antioxidant.

Materials and methods

Ethical approval

The College of Veterinary Medicine at the University of Baghdad received approval to conduct this scientific investigation in book No. 1438/P.G.

Animals of the study and treatment

60 adult male albino Wister rats, 10 weeks old, with an average weight of 195-200 g were used in this study. The rats were adopted in the Baghdad University College of Veterinary Medicine animal facility in Baghdad, Iraq. They were kept in well-ventilated plastic cages, given a typical pellet diet throughout the experiment, and given unfettered access to water. Between 20 - 25 °C.(12) The rats were subjected to alternating periods of light and darkness over the course of the 12-hour trial. The bed is changed twice a week. Randomly, four equal groups of rats were created. The initial group was considered nave (G1), rat in the second group (G2) received 100 mg of resveratrol orally daily for 14 days based on (Authors) (12), and the third group (G3) was sensitized by intravitreal injection). On day 0, add 250 g ovalbumin eggs (Sigma-Aldrich, USA) and aluminum hydroxide, 4 mg/ml Sensitive mice receive 50 g

OVA suspension in 50 µl sterile phosphate-buffered saline, injected intravenously on day 7, as described in Alharris, E; et al.(13) rats of the fourth group (G4) combined treatment as in G2 and induced asthma.

BALF collection and infiltrated mononuclear cell count

Bronchoalveolar lavage fluid (BALF), Was taken from the lungs and used to measure the redox system. In order to collect BALF, the trachea was sutured before the lung and trachea were surgically removed (14). In order to aspirate the fluid, sterile PBS was administered via the trachea. To obtain the antioxidant-containing supernatants, the collected fluid was centrifuged. To identify the antioxidants, present in BALF, ELISA was carried out using MyBiosource and in accordance with the manufacturer's procedure.

Blood collection

Blood samples were obtained from heart puncture while the subjects were under general anesthesia with an overdose of a Ketamine 60 mg/kg and Xylazine 12 mg/kg cocktail (15,16). Total white blood cell counts and total red blood cell counts were measured.

Histopathology

Harvested lung samples were embedded in paraffin, cut into 5 µm slices, fixed in 10% formalin, stained with hematoxylin and eosin, and examined under a microscope.

Genes expression of FOXP-3 and GAPDA gene

According to https://www.scopus.com/authid/detail.uri?authorId=58000932700 Ahmed and Mohammed(2022), Q-PCR was used to ascertain the expression of FOXP-3 in the spleen (Table 1). To this end, from each group, total RNA was extracted and utilized to create cDNAs using the Real MODTM Green W2 2x qPCR mix(17).

Table (1): Primer sequences of expression of genes.

Primers	Reverse	Forward
FOXP-3	5TTTATGCATCAGCTCTCCACTGTAG3	5′GAGAAGACTCCAGTGGCAGCAGTA3
GAPDA	5′ GCAGCGATATCGTCATCCAT 3′	5′ CCGCGAGTACAACCTTCTTG 3,

Statistical analysis

Use GraphPad Prism v8 software (San Diego, CA, USA) for all statistical analyses. Five mice in each group were used in each experiment. Multiple comparisons were performed using one-way ANOVA and then Tukey's post-analysis was performed. The statistics are considered significant at $p< 0.05$ (17).

Results and discussion

Antioxidant status (Malondialdehyde and Glutathione) in the BALF of the rats

Figure (1-A) displays., that malondialdehyde expression in rats BALF increases statistically by $p < 0.05$ in the G3 group in comparison to G1, G2, and G4 groups Additionally, MDA shows no significant changes in the G2 group compared with the G1, and G4 at $p < 0.05$. As can be seen in Figure 1 –B, the GSH value in rats' downregulation in the G3 group signification compared to the G4, and G2 groups at $p < 0.05$, In addition, GSH expression statistically elevated in the G2 group with respect to the G1, and G3 groups at $p < 0.05$, as well as group G4, increases dramatically compared to G3, and G1 $p<0.05$.

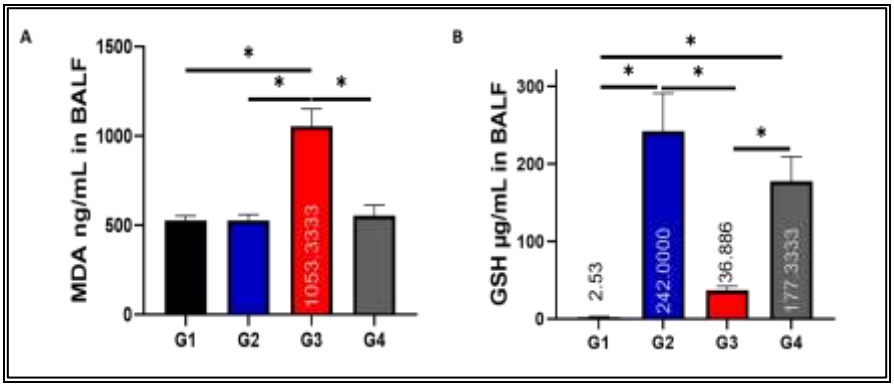

Figure (1-A) the effect of Resveratrol, asthma, and their combination in malondialdehyde concentration in BALF (ng/ml) *p<0.05. (2-B) The effect of Resveratrol, asthma, and their combination in total serum Glutathione concentration in BALF *p<0.05.

Figure (2-A) displays, that the total numbers of white blood cells are elevated significantly p<0.05 in the G3 group compared to the G1, G2, and G4 groups. Additionally, there is an increase dramatically in the G4 group with respect to the G1 group. As can be seen in Figure **2 –B**, the total number of red blood cells in rats' downregulation the statistic of the G3 group as opposed to G1, G2, and G4 groups at $p < 0.05$, In addition, the total numbers of red blood cells no statistical differences in the G1group with respect to the G2, and G4 groups at $p < 0.05$.

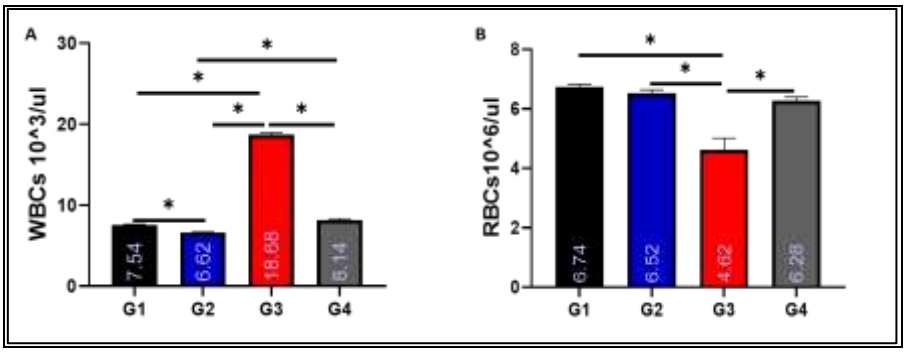

Figure (2-A) The effect of Resveratrol, asthma, and their combination on white blood cells(10^3/ul) in adult rat.(2-B) The effect of Resveratrol, asthma, and their combination on Red blood cells (10^6/ul) in adult rats.

Histopathological changes

Figure (3-A) shows a section of the lung (G1) showing: normal alveolus pneumocytes type-II (Black arrow), inter alveolar septum (Red arrow) normal alveolar macrophages (Blue arrow) H&E stain. 400x.(3-B) shows a section of the lung (G2) shows: normal alveolus pneumocytes type-II (Black arrow), interalveolar septum (Red arrow) normal alveolar macrophages (Blue arrows).H&E stain.400x. (3-C): section of the lung (G3) shows severe interstitial pneumonia with infiltration of mononuclear leukocytes (Red arrow), congestion of interstitial tissue (Black arrow), and alveolar dilation (Black arrows).H&E stain 400x. (3-D) shows a section of the lung (OVA+R2) showing: normal alveolus pneumocytes type-II (Black arrow), interalveolar septum (Red arrow) normal alveolar macrophages (Red arrow) H&E stain400x.

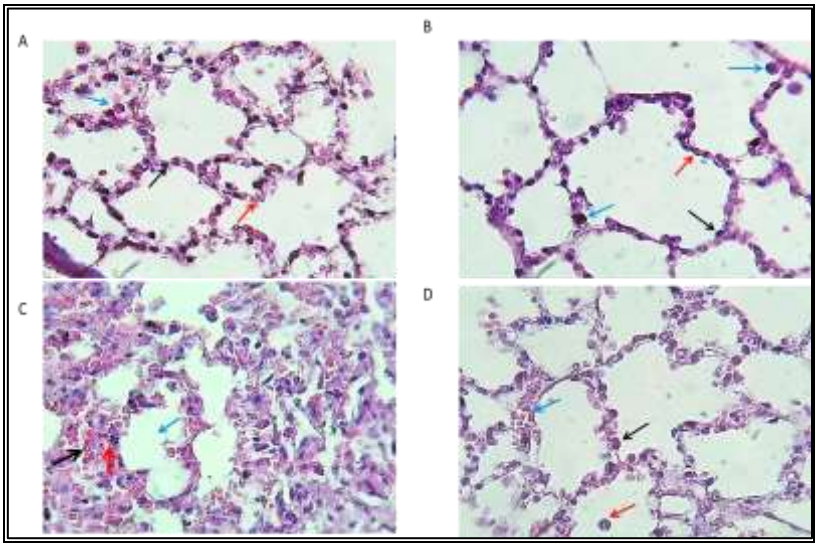

Figure (3 A-D) shows the histopathological changes in resveratrol, Asthma, and their combination on lung tissue.

Figure (4-A) section of trachea (G1) shows: epithelialized mucosa (Blue arrow), sib mucosal leukocytes (black arrow) and fibrocytes (Red arrow), and cartilage (Asterisk).H&E stain 400x(4-B) section of trachea (G2) shows: Norma mucosal thickening with normal epithelium (Blue arrow), normal subepithelial connective tissue (Red arrows) and normal content of mononuclear leukocytes (Black arrow)H&E stain 400x.(4-C) section of the trachea (G3) shows: thickening of epithelial mucosa (blue arrow), mucosal congestion (Black arrow), and mild infiltration of mononuclear leukocytes (Red arrow).H&E stain 400x(4-D) section of the trachea (G4) shows: Normal epithelial cells (Blue arrow), mild thickening of subepithelial connective tissue associated with cellulitis (Asterisk), moderate infiltration mononuclear leukocytes mainly macrophages (Black arrow) & lymphocytes (Ed arrow)H&E stain 400x.

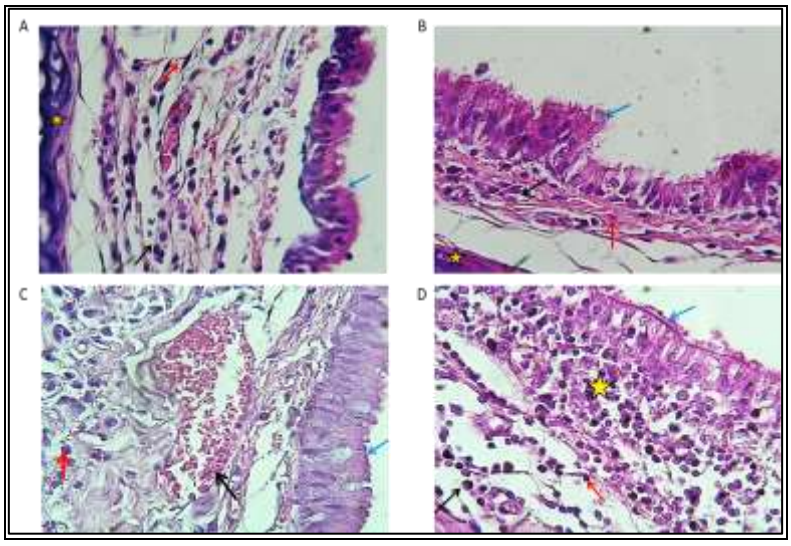

Figure (4 A-D) shows the histopathological changes in resveratrol. Asthma, and their combination on trachea tissue.

FOXP3 gene validation in the spleen

Figure (5A) displays., FOXP3 expression in rats decreased by p 0.05 for the significance of the G3 group in comparison to the G4, and G2 groups., additionally, FOXP3 signification increased G2 group compared with the G1, G3, and G4 at $p < 0.05$. as well as there is an increase in G4 group expression compared to G1 group at $p < 0.05$.

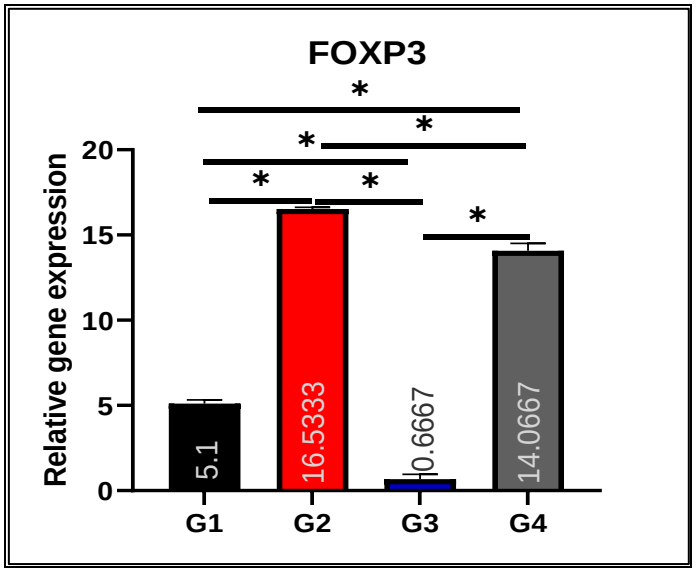

Figure (5): The effect of RES, Ova- Ova-induced asthma, and their combination on *FOXP3* in adult male rats.

*** P< 0.05 is the statistical threshold for group differences**

Several studies of children with allergies have shown a dramatic elevation of MDA in the BALF (18,19). The only study that took into account population information of the naive group showed no dramatic difference in MDA concentration in the BALF of asthmatic and non-asthmatic people (20). In addition, inhalation therapy in adults with asthma appears to have positive outcomes on the pulmonary oxygenation status of the patients (21). The main restriction is due to the intrusiveness and intricacy of the implementation of BALF, which makes it difficult to use it in therapeutic settings to monitor the level of inflammation and oxidative stress in patients with allergic respiratory illnesses (22). Glutathione may be directly impacted by asthma, The metabolic reduction of glutathione due to genetics in the Fluid lining of the lungs has been shown to increase levels of extracellular glutathione and interfere with the turning on of the EGF receptor (Epidermal Growth Factor), the generation of the mucus gene of airway epithelial cells and airway hyperresponsiveness in a cytokine-driven asthma model (22). Glutathione was recognized to improve the bioavailability of a significant smooth muscle relaxant for the airways, in addition to antagonizing histamine released by mast cells (23,24). Glutathione depletion may promote contractility of the airways by inhibiting the impact of NO (25**).** It is possible that glutathione depletion of local inflammatory cells may lead to increased glutathione concentration in

asthmatic and COPD patients (26,27). Resveratrol plays an important role as an antioxidant., According to Caruso et al. resveratrol's three hydroxyl groups' electrophilic hydrogen atoms are the cause of its antioxidant activity(29) Compared to the Asthma group, resveratrol therapy dramatically boosted RBC count and lowered WBC count. This might be a result of Resveratrol's capacity to activate poly-ADP-ribose polymerase 1 (1 PARP 1), which in turn activates genes that work together to protect cells from oxidative harm (30, 31). This result is in line with studies by Nurgül et al. (32) that examined the preventive effects of resveratrol against sodium fluoride-induced hematological and biochemical changes in rats. They found that the group that got both fluoride and resveratrol experienced less hematological alteration. By inhibiting the SEB-activated T cells' metabolic may be altered via the mTOR pathway and RES., preventing them from proliferating and causing them to enter a more quiescent state (33). An ongoing inflammatory condition affecting the airways called is asthma. It is distinguished by airflow restriction and episodic narrowing of the airways, representing an overreaction of the airways to various stimuli. Airway studies during exacerbations of asthma show an inflammatory infiltrate of neutrophils, eosinophils, and mast cells (34). In addition, cytogenetic analysis of resveratrol in healthy human blood lymphocytes showed that it significantly reduced mitotic-induced mutagenicity (35). Our results demonstrate that by blocking the production of inflammatory mediators, resveratrol reduces airway remodeling and asthma-induced inflammation. There is little scientific evidence to support the claim that resveratrol directly reduces tissue permeability. To assess the impact of inflammation on mucosal barrier integrity, type II alveolar epithelial cells were co-cultured with splenocytes previously treated with resveratrol (36). Resveratrol markedly attenuated the effect of oxidative stress on the male reproductive system, renal damage, and liver hepatotoxicity (37,38,39). These results provide credence to the idea that asthma involves both local and systemic inflammation (40) severe symptoms are characteristics of asthma. Leptin and VEGF levels were up, but sRAGE levels were down (41**)** Asthma patients are anaemic. The risk of developing anemia within a period of time is higher than in healthy adults (42).

Resveratrol has been shown to inhibit IL-17 production by blocking Th17 cell differentiation and reducing their activity. By reducing IL-17 levels, resveratrol may help reduce the inflammatory response that accompanied our findings (43) Resveratrol's ability to shield erythrocytes against the shape-changing effects of HClO (44). By activating the Nrf2 pathway, resveratrol prevents normal human bronchial epithelial cells from experiencing paraquat-related mitochondrial damage, oxidative stress, inflammation, and fibrotic responses (45). Resveratrol combats asthma in a variety of ways by lessening symptoms, lowering inflammation, and enhancing biochemical markers. Pharmacotherapy reduces the amount of mucus in the respiratory system, reduces alveolar edema, relaxes the smooth muscle of the respiratory tract, and reduces bronchial hyperresponsiveness (46) peroxisome proliferator-activated receptor PPAR-mediated L1-RTP Retotranspoition in somatic cells is directly modulated by resveratrol, and sirtuins 6 (SIRT6) and Mitogen-activated protein kinase (MAPK) signaling have strong interactions that may help prevent

illness (46). A transcription factor called FOXP3 is crucial for the growth and operation of regulatory T cells (Tregs). Tregs, a subgroup of T cells with immunosuppressive characteristics, aid in regulating and controlling the immune response to prevent uncontrollable inflammation and autoimmune disease. It has been demonstrated that resveratrol increases FOXP3 expression and activity, which increases Treg activity and can help maintain immunological homeostasis and decrease inflammation (47). In the FOXO3 gene, the polymorphism ofrs 13217795 CTSNP was linked to the emergence of asthma disease. Patients with asthma were more likely to carry the T allele than the C allele (48) For the Iraqi population, Al-Qadhi and Al-Saadi found the SNP (1805011AC) of the IL-4R gene in relation to allergic asthma (49, 50).

Conclusion

Due to its well-known anti-inflammatory and antioxidant capabilities, resveratrol may be utilized as a supplement to provide protection for asthma patients exposed to ovalbumin.

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Conflict of interest

There are no competing interests.

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