

Histological and Physiological Study of the Effect of Silver Nanoparticles and Omega-3 on Asthma of Male Mice Induced by Ovalbumin

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ABSTRACT

In the current study ninety one of male mice weighting (25-30 g) aged (15-17) weeks at the animal house faculty of science / university of Kufa during the period from January 2017 to September 2017. This study included some physiological and histological criteria to evaluate the protective role of omega-3(2 and 3 mg/kg) and silver nanoparticles (5 and 10 mg/kg) against asthma that induced by ovalbumin. The animals experimental are divided into 16 groups (n= 6 mice per each group) for duration of one and two months. The results showed significant increase ($p<0.05$) in the leukocyte count (eosinophil, neutrophil, lymphocyte, monocyte) in asthma group as compared with control group. Also, the results showed significant decrease ($p<0.05$) in the leukocyte count in the treated group of omega-3 and silver nanoparticles for both concentration as compared with asthma group.

The result showed significant increase ($p<0.05$) in the serum level of periostin and Galectin -3 and Interleukin -33 in asthma group as compared with control group. The histological study of lung tissue revealed that induced the tissue with ovalbumin caused necrosis, degeneration and increase of mucous in bronchiole as well as acute inflammation around bronchioles while the effect of ovalbumin in trachea tissue were sloughing, necrosis and degeneration around the epithelium of bronchioles.

Keywords: Asthma disease, Mice, Ovalbumin, Omega-3, Silver nanoparticles.

INTRODUCTION

Asthma is a chronic inflammatory disorder of the respiratory airway characterized by a multicellular process including eosinophil, neutrophil, mast cell, T-lymphocyte and CD+4 also the most important features represented by infiltration of eosinophil¹. Fatty acids Omega-3 are synthesized in the body and the body can be obtained from the diet such as naturally diet enriched in fatty fish like Salmon and tuna and fish oil supplements². There are many roles of the major components of omega-3 fish fatty acids docohexanoic acid (DHA) and eicosapentanoic acid (EPA) have been reported to have a protective function in coronary artery disease, dyslipidemia and hypertension³. Nanoparticles (NPS) structures have a range of dimension from 1-100 nm. Silver is the main products among all nanoparticles and used widely in many applications because of its broad spectrum properties, in addition to inflammatory activity the anti-microbial activity used against bacteria, viruses and fungi⁴. periostin is a useful biomarkers for bronchial asthma and it has been shown to down regulate of immune responses such as (IL-4) and (IL-13) and it can predict the hypo responsiveness to corticosteroid inhalation in asthma patients⁵. Galectin-3 are one of the

most biomarkers which have functions in variety of biological processes such as allergic pathology and inflammation also, the Galectin-3 belong to the family of B-galactose side binding animal lectins⁶. IL-33 is a member of cytokines which mostly drive the Th-1 immune responses and have many names including (IL-1F11,NF-HEV) also nuclear factor from endothelial venues . and recently described as a member of IL-1 family⁷.

MATERIALS AND METHODS

Animals

Ninety one male albino mice weighting (25-30 g) and aged between (15-17 week) were obtained from the faculty of science Thi/Qar university. The study started from January 2017 to September. 2017. Animals were housed in the animal house of faculty of Science /University of Kufa under control condition, light 12 and 12 dark hours and a temperature range from (20-24 C°).

Induction of Asthma in mice

Mice were sensitized by three intraperitoneal injection of 0.5 mg/ml of ovalbumin and 20 mg/ml of alum on days 0,7 and 14 at the days 18,19 and 20 the mice will receive ovalbumin and alum by intranasal instillation by

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micropipette and drop in its nose, mice were challenged at day 21.

Blood samples

The blood was drawn through heart puncture by using disposable syringe (5 mL), then left at room temperature for clotting, and then centrifuged at (3000 rpm) for (15) minutes, serum was isolated and stored at deep freeze in Al- Sadar medical city in Al-Najaf Al-Ashraf province until using for measurements biomarkers.

Biomarkers

The assessment of periostin, Galectin -3 and Interlukine-33 mice Elisa Kits provided by (elabscience – china)

Sandwich immunoassay technique, (enzyme-linked immuneosorbent assay – automated microtiter plate), Elisa reader (Biokit ELX 800 reader, ELX50 washer/USA).

Light microscopically study

Histological sections were prepared according to the procedure described by⁸.

Statistical Analysis

The data of present study were articulated as (Mean± Standard Error), the statistical analysis were calculated by using megastat and Graphpad prism, when P value <0.05

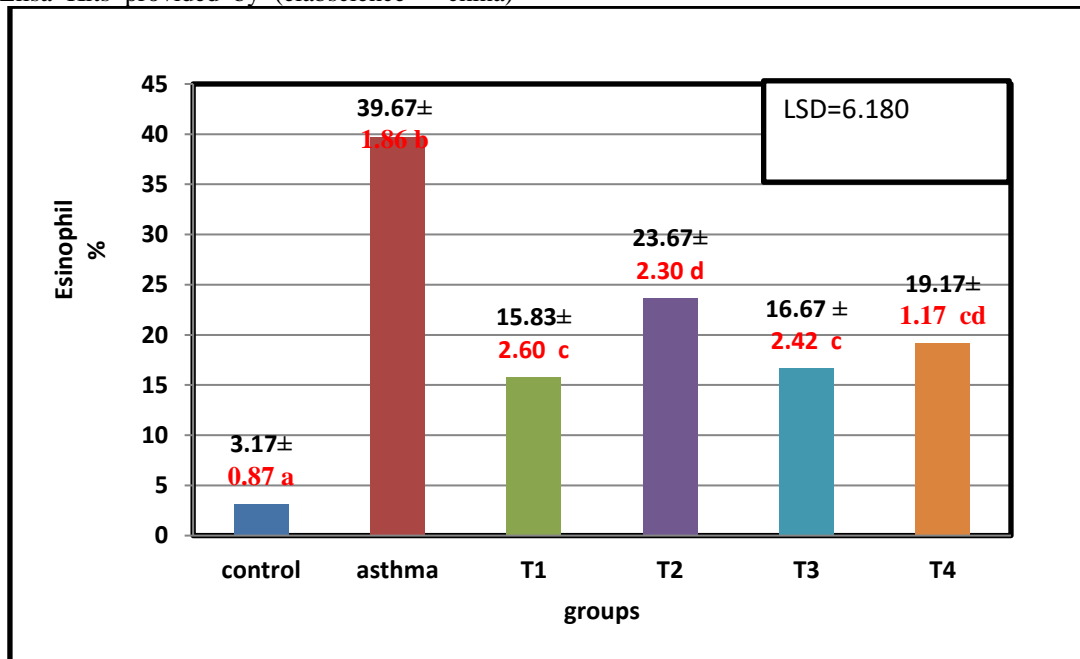


Figure 1: Effect of two concentration of omega -3 and silver nanoparticles on eosinophil count in blood in male mice after induced with ovalbumin for 21.

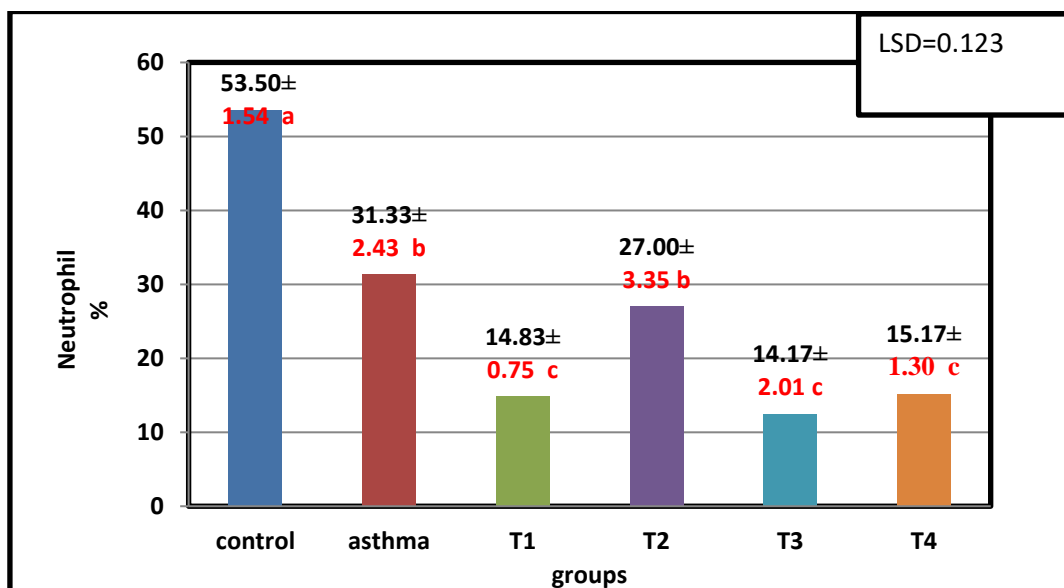


Figure 2: Effect of two concentration of omega -3 and silver nanoparticles on neutrophil count in blood in male mice after induced with ovalbumin for 21 days.

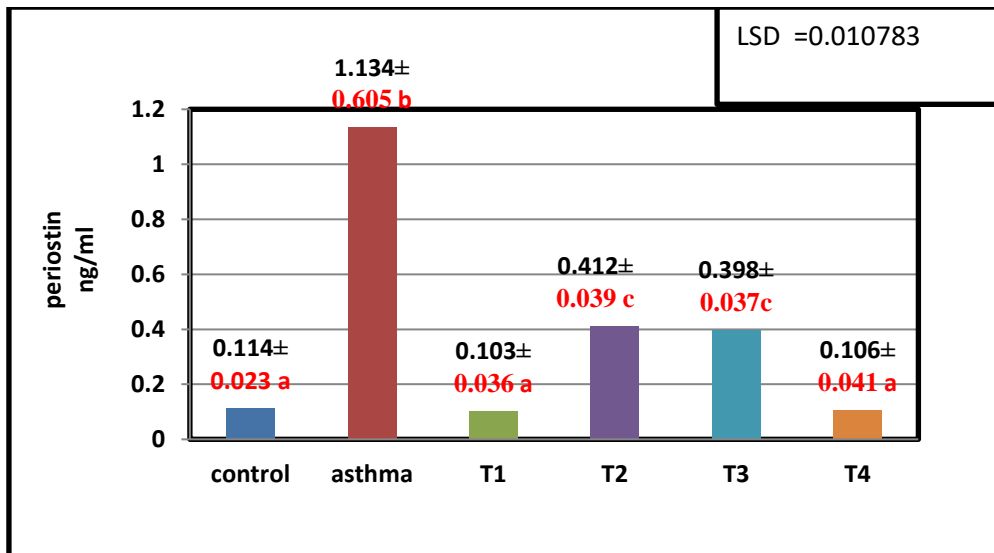


Figure 3: Effect of two concentration of omega-3 and silver nanoparticles on periositin serum level in male rats treated with ovalbumin for 21 days .

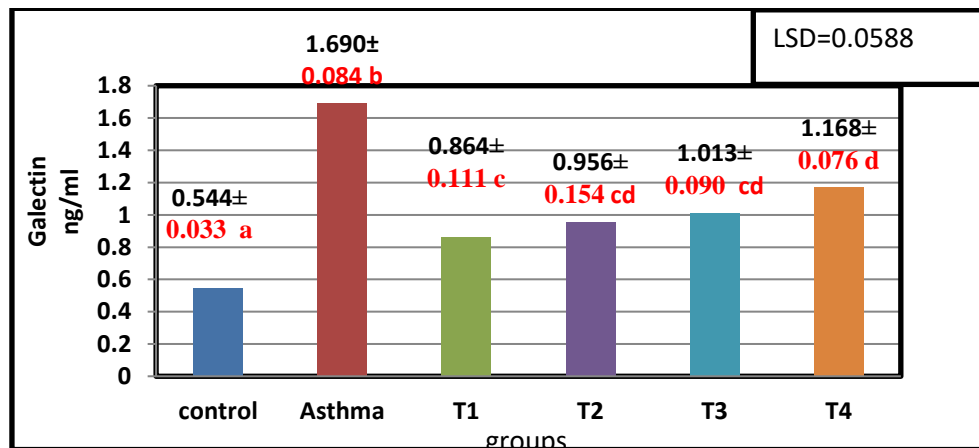


Figure 4: Effect of two concentration of omega-3 and silver nanoparticles on Galectin serum level in male rats treated with ovalbumin (protective groups for 21 days).

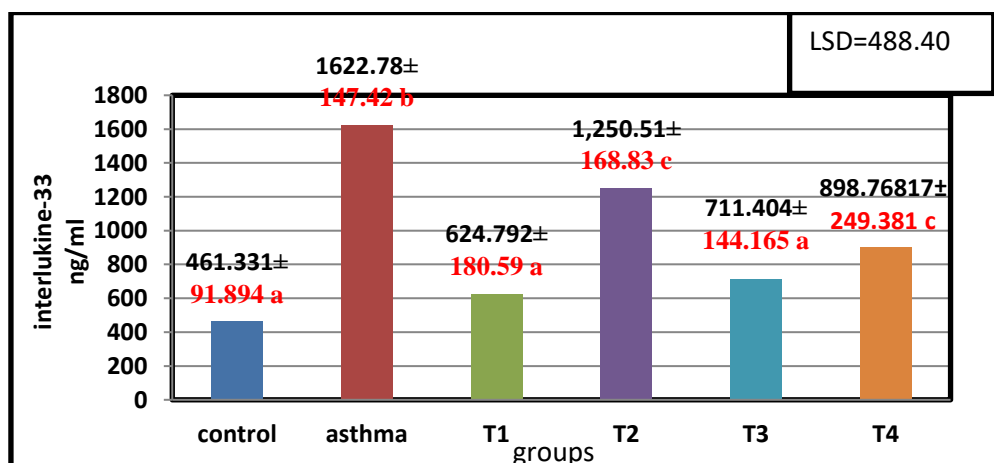


Figure 5: Effect of two concentration of omega-3 and silver nanoparticles on interleukine 33 serum level in male rats treated with ovalbumin for 21 days.

was statistically significant⁹.

Effect of two concentration of omega-3 and silver nanoparticles on eosinophil count

RESULTS

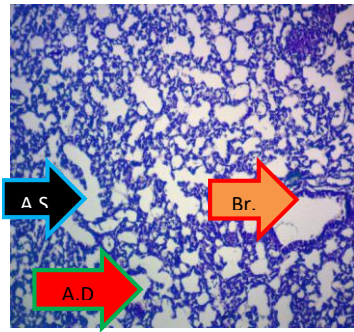


Image 1: Histological section of male mice lung (control) showed normal structure (A.S) alveolar sac, (A.D) alveolar duct and (Br.) bronchiole. H&E 200X.

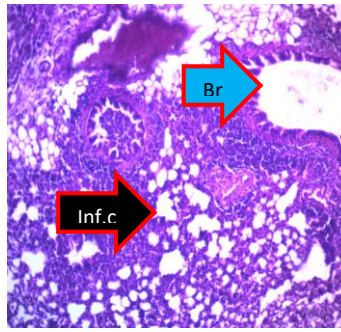


Image 2: Histological section of male mice lung (asthma) showed neutrophilic inflammation (inf.c) and bronchiole (Br.). H&E 400X.

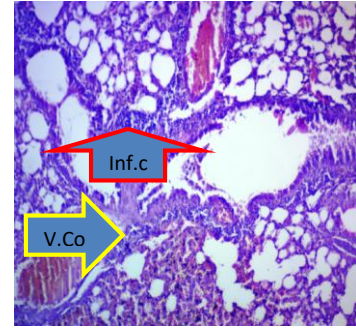


Image 3: Histological section of male mice lung treated with ovalbumin and silver nanoparticles for 21 days showed little inflammatory cell (inf.c) around bronchioles and congestion in blood vessel (V.Co). H&E 400.

Figure(1) indicated significant increase in eosinophil count ($p < 0.05$) in asthma groups for 21 days (39.67 ± 1.86) compared with control groups (3.17 ± 0.87), also, significant decrease ($p < 0.05$) in the treated groups (T1,T3) (15.83 ± 2.60), (16.67 ± 2.42), respectively compared with asthma group. the figure also, found significant decrease ($p < 0.05$) in the treated group (T2,T4) (23.67 ± 2.30), (19.17 ± 1.17) compared with asthma group. this figure indicated significant increase in eosinophil count in treated groups (T1,T2,T3,T4) (15.83 ± 2.60), (23.67 ± 2.30), (16.67 ± 2.42), (19.17 ± 1.17) compared with control (3.17 ± 0.87). The T1 and T3 groups were significant ($p < 0.05$) than T2. no significant differences ($p > 0.05$) between T1,T3, and T4.

The result of Figure(2) indicated significant decrease ($p < 0.05$) in the neutrophil in the asthma group compared with control group (53.50 ± 1.54) for 21 days. this figure also, showed significant decrease ($p < 0.05$) in the treated groups (T1,T3,T4) (14.83 ± 0.75), (14.17 ± 2.01), (15.17 ± 1.30) compared with asthma (31.33 ± 2.43), while indicated no significant differences ($p > 0.05$) in neutrophil count in the treated groups (T2) (27.00 ± 3.35) compared with asthma group also, indicated a significant decrease ($p < 0.05$) in all treated groups (T1,T2,T3,T4) compared with control (53.50 ± 1.54). the treated group (T2) were showed significant increase ($p < 0.05$) in neutrophil count compared with T1,T3 and T4. no significant differences ($p > 0.05$) between (T1),(T2) and (T3).

Biomarkers

Effect of two concentration of omega-3 and silver nanoparticles on periostin level in serum of male mice treated with ovalbumin for 21 days

Figure(3) indicated significant increase ($p < 0.05$) in the periostin level in the asthma group (1.134 ± 0.605) compared with control (0.114 ± 0.023), also, the figure showed no significant differences ($p < 0.05$) in periostin level in the treated group (T1) (0.103 ± 0.036) of 2mg/kg of omega-3 and (T4) of 10 mg/kg of silver nanoparticles (0.106 ± 0.041) compared with control group (0.114 ± 0.023), while found significant

increase ($p < 0.05$) in the group (T2) for 3 mg/kg (0.412 ± 0.039) of omega-3 and (T3) 5mg/ kg silver nanoparticles (0.398 ± 0.037) compared with control group (0.114 ± 0.023). also, this figure showed significant decrease ($p < 0.05$) in the periostin level in the group (T3,T4) (0.398 ± 0.037), (0.106 ± 0.041) for silver nanoparticles compared with asthma. this figure also indicated no significant differences ($p > 0.05$) in the periostin level in the protective group (T1,T4) of omega-3 (0.103 ± 0.036), (0.106 ± 0.041) compared with asthma group. while indicated significant increase ($p < 0.05$) in periostin level in the treated group (T2,T3) (0.412 ± 0.039), (0.398 ± 0.037) compared with control.

Galectin-3

the figure(4) showed significant increase ($p < 0.05$) of galectin-3 level in the asthma group for 21 days (1.690 ± 0.084) compared with control (0.544 ± 0.033), also showed significant decrease ($p < 0.05$) in galectin level in all protective group (T1,T2,T3,T4) for both duration (0.864 ± 0.11), (0.956 ± 0.154), (1.013 ± 0.090), (1.168 ± 0.067) one and two month to omega -3 and silver nanoparticles respectively compared with asthma. this figure showed significant increase ($p < 0.05$) in galectin-3 level in all treated group (T1,T2,T3,T4) (0.864 ± 0.111), (0.956 ± 0.154), (1.013 ± 0.090), (1.168 ± 0.067), compared with control (0.544 ± 0.033). T4 group of silver nanoparticles was significant increase ($p < 0.05$) than other protective group T2,T2,T3.

Interleukin -33

This figure(4-52) showed significant increase ($p < 0.05$) in the interleukin-33 level in the asthma group 21 days (1622.78 ± 147.42) compared with control (461.331 ± 91.894) also, this figure showed significant decrease ($p < 0.05$) in interleukin- 33 level in the treated group (T1,T3) (624.792 ± 180.59), (711.404 ± 144.165) compared with asthma and T2, T4 group. also, showed significant decrease ($p < 0.05$) in the interleukin 33 in the treated group (T2,T4) ($1,250.51 \pm 168.83$), (898.768 ± 249.381) compared with asthma the figure showed no significant differences ($p < 0.05$) in the interleukin level

in the treated group (T1,T3) compared with control ,while occurs significant increase ($p<0.05$) in the interleukin level in other treated group compared with control .

Histological results

Histological section of male mice lung(control) showed normal histological structure (image 1) and can identify gas exchange area :Alveolar sac (Al.S) , Alveolar duct(Al.D) and bronchioles(Br.) which are adjacent to blood vessel and surrounded by alveoli and showed ciliated simple columnar epithelium of bronchus. Histological section of male mice lung treated with ovalbumin for 21 days(asthma) showed congestion around bronchioles (con.) and sever neutrophilic inflammation (S.N.inf) around another bronchioles (image 2) histological section of male mice lung treated with ovalbumin and silver nanoparticles (5mg/kg) for 21 days appearance congestion in blood vessel (con.) and inflammatory cell around bronchioles (image 3) histological section of male mice lung treated with ovalbumin and silver nanoparticles (10 mg/kg) for 21 days showed congestion in blood vessel (con.) and inflammation between alveoli(inf.) (image 4).

DISCUSSION

The present study indicated a significant increase ($p<0.05$) in the eosinophil count in asthma group. The result of current study agreement with study of 9 that suggested the effector functions of eosinophil comes from the release of lipid mediator and proteins such as cytokines and granule protein so that, the degranulation of eosinophil in asthma lead to production of several cytotoxic –cationic granule protein. The increase in eosinophil lead to more cytotoxic effect and harmful for foreign invaders within the body¹⁰. The increase number of eosinophil is an important characteristics features of severe asthma and associated with severity of disease.

The increase numbers of eosinophil have been pointed by airway eosinophil in animal models which induced by IL-4 ,IL-5 and IL-13. The study of¹¹ have been documented that T cells are a major source of Th2 cytokines also, T cell subtypes has been responsible for secretion of Th2 cytokines.

The current results agree with results of^{12,13} who found that administration of fish oil omega-3 during ovalbumin-challenge diminished inflammatory air way by reducing eosinophil infiltration, inflammatory cytokines production and mucous deposition also down regulation of IgG1 and IGE production also, the expression of expression factor GATA-3 and NFkB. The association between reduction of PGF2x and leukotriene E4(LTE4) and decrease eosinophil were suggested by administration of omega-3 and proved the anti-inflammatory effect¹⁴ the attenuation of four –series –systemle-leukotrienes after fish oil-omega-3 and this compound can be synthesized by eosinophil, basophil and mast cell lead to increase permeability and contract of smooth muscles¹⁵. The roles of small size nanoparticles have been studied by several researchers and showed that the (Ag,Au and Cu) have a low toxicity

and safety for human health also the low doses 5 and 10 mg/kg were also recommended for use due to ability to protect from oxidative stress and reducing oxidative stress in the body^{15,16,17}.

The some recent studies have proved a cyto protective activities in both in vivo and in vitro and anti-inflammatory also oxidant properties of AgNo3 in ovalbumin induced asthma in mouse^{18,19}. The current results accordance with many results that showed the alteration in Galectin-3 level function may cause un increase ability to remove neutrophil and reduced recruitment during infection²⁰. Another study has been demonstrated that Gal-3 may be a negative regulator of airway neutrophil²¹. The figure (3) reviled significant increase ($p<0.05$) in Periostin level in asthma group in compare with control group.

The present study agree with study of²² that suggested the basal level of periostin is sufficient to acute response and with previous study that postulated that accumulation of periostin level have a major roles in the trafficking activation of cytokines release of leukocytes²³.

Many number of studies have proved the relation between high plasma level of periostin and eosinophilic asthma²⁴. In the figure (4) showed a significant increase ($p<0.05$) in the Galectin-3 level in serum of asthma group in compare with control group.

The study accordance with various studies that evident o play role of Galectin -1,3and 9 in various inflammation also, that are expressed by eosinophil or others cells interfere with eosinophil recruitment in allergic asthma²⁵. The figure (5) showed a significant decrease ($p<0.05$) in the level of IL-33 in asthma groups in compare with control groups.

A recent study of²⁶ have postulated that IL-33 induce of allergic asthma because the Th2 inflammation was dependent on FCYRIII and IL-33 enhance a secondary response ligation FCYRIII on antigen presenting cells to develop Th2 – mediated response in the lung some other studies have documented that a signaling of IL-33 is required for eosinophil and production of IL-5 and IL-13 from innate lymphocytes (ILC25) .therefore it considered as an essential factors for severe asthma in mice²⁷. Research of several studies have reported that IL-33 modulate a mast cell through a pathway signaling /and activated mast cell and basophil to enhance of maturation, migration, survival, adhesion and production of several cytokines as pro inflammatory in air way of asthma²⁸.

In the current results of image(2) the histo pathological section of the lung treated with ovalbumin (asthma group) showed a sever inflammation in cell around the bronchioles (S.inf). Study of²⁹ have revealed during treated of rats by fish oil and dexamethasone experimentally induced by OVA, the study proved that have a therapeutic effect on the development of asthma also, concluded that protective roles as anti-inflammatory effect The current study agree with³⁰ that found the important characteristic features of asthma is the infiltration of eosinophil and neutrophil and represent

the chronic inflammation in this disease. The image (3) revealed a normal structure in the lung tissue after treated with ova and omega-3 with two concentrations for 21 days and for one and two months. The present results accordance with previous studies that have been shown the anti-inflammatory effects of both fish oil and soybean in acute inflammation and documented the protective roles of both n=3FA in fish oil and n=6 in soybean against inflammation^{31,32}. the normal structure of image (4) after treated with silver nanoparticles for one month showed a normal cells. No previous studies documented with effects of silver nanoparticles on lung tissue (histo- pathological studies).

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