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INVESTIGATION OF PROTECTIVE ROLE OF THYMOQUINONE AND CERATONIA SILIQUA IN ASTHMATIC RATS AND THEIR EMBRYOS

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Ameerah Fadhil Ahmed AHMED

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IN
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ETHICAL STATEMENT

I hereby declare that in this thesis study I prepared in accordance with thesis writing rules of Gazi University Graduate School of Natural and Applied Sciences;

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INVESTIGATION OF PROTECTIVE ROLE OF THYMOQUINONE AND *CERATONIA SILIQUA* IN ASTHMATIC RATS AND THEIR EMBRYOS

(Ph. D. Thesis)

Ameerah Fadhil Ahmed AHMED

GAZİ UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

December 2019

ABSTRACT

The objective of this study is to investigate co- administration of Thymoquinone (TQ) and *Ceratonia silique L.* (CS) effect on lung tissue oxidative events, some cytokines and growth factor levels of serum in asthmatic pregnant rats as well as studying changes of both the histologically and the immunohistochemically of lung tissue using light microscopy in asthmatic pregnant rats and their embryos. For this purpose, 18 female wistar rats were divided randomly to 3 groups: asthmatic pregnant group (I), asthmatic pregnant with TQ and CS group (II) and asthmatic pregnant with dexamethasone group (III). The co-administiration of TQ and CS decreased the MDA levels in lung tissues while the production of NO levels increased. Also, increased GSH and AA levels of lung tissue were found. There is no statistically signifcant alterations between all groups in terms of TNF-α levels. However, there are statistically significant alterations between all groups in terms of IL-1β and IL-13 levels. Various histological changes in lung tissues of group I were revealed. However, treatment of TQ and CS was prevented these changes in group II. Immunohistochemically, vascular endothelial growth factor (VEGF) staining levels increase was observed in group I, while there was a significant decrease in group II than it is in group III. A significant observed in the co- administration of TQ and CS group and dexamethasone group in lung tissue of embryos when compared the histological changes. However, VEGF staining levels of lung tissues of embryos significant decreased in dexamethasone group than in co-administration of TQ and CS group. In this study, the co-administeration of TQ and CS has a protective effect on lung inflammation in asthmatic pregnant rats. In addition, this two herbal mixture has a possitive effect both by reducing lipid peroxidation of lung tissue and by showing a remarkable effect on some cytokine levels which have an important role in inflamation. TQ and CS was thought to be a promising treatment agent for asthma in the future due to this mitigating features.

Science Code : 20317

Key Words : Asthma, Oxidative strees, Cytokines, TNF- α, VEGF, Thymoqinone, *Ceratonia siliqua L.*

Page Number : 99

Supervisor : Prof. Dr. Şule COŞKUN CEVHER

ASTIMLI GEBE RATLARDA VE EMBRİYOLARINDA THYMQUINONE'NİN VE *CERATONIA SILIQUA*'NIN KORUYUCU ROLÜNÜN ARAŞTIRILMASI

(Doktora Tezi)

Ameerah Fadhil Ahmed AHMED

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ÖZET

Bu çalışmanın amacı, astımlı gebe ratların akciğer dokusunda ve serumlarında oksidatif olayları, bazı sitokinler ve büyüme faktörü düzeylerine Thymoquinone (TQ) ve *Ceratonia siliqua L.* (CS) etkisinin araştırılması ayrıca astımlı gebe ratlar ile embriyoların akciğer dokularında ışık mikroskopu kullanılarak hem histolojik hem de immünohistokimyasal olarak TQ ve CS etkisinin incelemesidir. Bu amaçla 18 dişi wistar ratta, astım modeli oluşturuldu. Daha sonra dişi ratlar gebe bırakıldı. Astımlı olan gebeler, rastgele altışarlık üç gruba ayrıldı: Birinci gruba (I), hiçbir uygulama yapılmadı. İkinci gruba (II), gebeliklerinin son beş gününde TQ ve CS, gavaj yoluyla uygulandı. Üçüncü gruba (III) gebeliklerinin son beş gününde intraperitonal enjeksiyon ile deksametazon uygulandı. TQ ve CS'nin uygulandığı birinci grubun (II) akciğer dokusunda MDA seviyeleri azalma, NO seviyeleri artış tesbit edilmiştir. Ayrıca, akciğer dokusunda GSH ve AA seviyeleri artmıştır. TNF-α düzeyleri açısından tüm gruplar arasında istatistiksel olarak anlamlı bir farklılık bulunamamıştır. Ancak tüm gruplar arasında IL-1β ve IL-13 seviyeleri açısından istatistiksel olarak anlamlı değişiklikler gözlenmiştir. I. grupta akciğer dokularında çeşitli histolojik değişiklikler ortaya çıkmış. Bununla birlikte, II. grupta uygulanan TQ ve CS, söz konusu değişikliği önlemiştir. İmmünhistokimyasal olarak I. grupta vasküler endotel büyüme faktörü (VEGF) boyanma seviyeleri artmışken II. grupta III. gruba göre anlamlı bir azalma gözlenmiştir. Üç gruba ait embriyoların akciğer dokularındaki histolojik değişiklikler kıyaslandığında ise birinci grupta (VEGF) boyanma seviyelerinde artış gözlemlenirken TQ ve CS uygulanan ikinci grupta ve deksametazon uygulanan üçüncü grupta (VEGF) boyanma seviyelerinde azalma tesbit edilmiştir. Bu çalışmanın sonucuna göre, TQ ve CS'nin birlikte uygulanması, astımlı gebe ratlarda akciğer iltihabı üzerinde koruyucu bir etkiye sahiptir. TQ ve CS'nin, söz konusu inflamasyonda hafifletici etkisinden dolayı gelecekte astım hastaları için umut vadeden bir tedavi aracı olduğu düşünülmektedir.

Bilim kodu : 20317

Anahtar kelimeleri : Astım, Oksidatif stres, Sitokin, TNF-α, VEGF, Timokinon, *Ceratonia siliqua L.*

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LIST of SYMBOLS and ABBREVIATIONS

Symbols and abbreviations used in this study are presented below with their explanations.

Symbols	**Explanations**
α	Alfa
β	Beta

Abbreviations	**Explanations**
15-HETE	15-Hydroxy eosa tetra enoic acid
5-HPETE	5-Hydroxy peroxy ascosatrinwik acid
5-LO	5-Lipoxygenase
AA	Ascorbic acid
ABC	Avidin-biotin complex
AHR	Airway hyperresponsiveness
ASM	Airway smooth muscle
BAL	Bronchoalveolar lavage
Cox	Cyclooxygenase
CS	Ceratonia siliqua
CysLTs	Cysteinyl. Leukotriene
ELISAs	Enzyme-linked immunosorbent assays
GM-CSF	Granulocyte macrophage colony stimulating factor
GPCRs	G-Protein receptors
GSH	Glutathione
H&E	Hematoxylin and Eosin
H_2O_2	Hydrogen peroxide
i.p.	Intraperitoneal
ICAM	IC adhesion molecular
IgE	Immunoglobulin E
IL-13	Interleukin 13
IL-1β	Interleukin 1 beta
LTB4	Leukotriene B 4

Abbreviations	Explanations
LTs	Leukotrienes
MDA	Malondialdehyde
MDCs	Macrophages derived from macrophages
MHC	Major histocompaitibility complex
NADPH	Nicotin amide adenin dinucleotide phosphate
NF-κB	Nuclear factor kappa B
NK	Natural killer cells
NO	Nitric oxide
NS	Nitrosative stress
O•-	Superoxide
OFRs	Oxygen free radicals
OH•	Hydroxyle
OS	Oxidative stress
OVA	Ovalbumin
PGs	Prostoglandins
PLA2	Phospholipids
PLGF	Placenta groth factor
Se	Selenium
SOD	Superoxide dismutase
TACE	TNF-α converting enzyme
TARCs	Thymus and the regulating activation chemokines
Th2	T helper 2
TNF-α	Tomur necrosis factor alpha
TQ	Thymoqiunone
VEGF	Vascular endothelial growth factor
VPE	Vascular permeability

1. INTRODUCTION

In the last decades the prevalence of asthma has dramatically increased. Inn accordance with the last report of World Health Organization about 300 million people worldwide have attract from asthma and 255,000 deadly from asthma [1].

An increased airway responsiveness, reversible airways obstruction and airway inflammation it is a characterized of common chronic disease terms as asthma [2].The pathogenesis of airway inflammation in asthma involved from mast cells releaseing of inflammatory moderator that caus bronchoconstriction and many inflammatory cells involve: eosinophils, macrophages and neutrophils [3]. Chemical drugs such as fast relaxation and long-range control drugs which management smooth muscle stenosis and decreased inflammation were used in treatment of this disease. When these drugs are effective to relief the symptoms of disease, they have side effects too that make these drugs unwanted to use for long time. So, scientists are processing with conventional or folk drug in parallel with modern medicines. Between these drugs is an herbal source *Nigella sativa* has been used as conventional therapy of asthma as well as another inflammatory disease [4, 5]. The effective component of *N. sativa* is thymoquinone (TQ) that has been research for its antioxidant, antiinflammatory and anticancer effects in both in vitro and in vivo models [6, 7, 8]. TQ is an intense inhibitor of the inflammatory changes incorporated with asthma [9]. It is inhibiting T helper 2 (Th2) cytokines and eosinophil leakage into the airways and decreases allergic airway inflammation; this proof indicates its potency antiinflammatory role along the allergic reaction in the lung. However, few is known regarding the elements and techniques underlying these all effects [10]. For the time being, *Ceratonia siliqua L.*(carob) has been paid attention on economically, medically, food additives (Locust bean gum is also known as carob gum, carob bean gum, carobin, E410), in pharmaceutical and cosmetic productions [11, 12]. CS seeds in particula generous resource of complicated polymers of flavonoids like proanthocyanidin, ellagitannin, and gallotannin. These phytochemicals have been applied in medicament in order to its pharmacological effects and have free radical scavenging actions against numerous diseases [13].

The major role in the airway inflammation and determinants of asthma severity are the endogenous and exogenous reactive oxygen (ROS) and nitrogen species (NS) [14]. ROS

can generate from inflammatory cells (such as activated eosinophils, neutrophils, monocytes, and macrophages) and resident cells (such as epithelial and smooth muscle cells). [15-17] Resources of $O_2^{\bullet-}$ first include nicotinamide adenine dinucleotide phosphate (NADP) oxidase-dependent complex, the cytosolic xanthine oxidase system, and the mitochondrial respiratory chain. $O_2^{\bullet-}$ automatically or enzymatically dismutates to hydrogen peroxide (H_2O_2). In biological systems both $O_2^{\bullet-}$ and H_2O_2 interact with iron and other metal ions and form OH^- [18-20]. Eosinophils, neutrophils, and monocytes contain peroxidases that are a catalyst for the interaction between H_2O_2 and halides leading to the formation of hypohalides, such as HOCl. In addition, $O_2^{\bullet-}$ may also react with nitric oxide (NO) to form peroxynitrite ($ONOO^-$), a potent ROS [16-20]. ROS can rush proteins to form carbonyls and oppose with nitrogen species and tyrosine to form nitrotyrosine. In studies of murine and human, tyrosine nitration was shown to increase after allergen exposure in sensitized mice or atopic asthmatic humans [21]. Also, ROS reacts with lipieds to rescue isoprostane and ethane [22-24]. In the exhaled breath condensate in adults and children with asthma as a result, 8-isoprostane, a biomarker of lipid peroxidation, was elevated [23]. Asthma attacks and experimental antigen challenges are both associated with the immediate formation of O_2, where they are high in antigen challenge sites that persist throughout the final stage of asthma [24]. NO is the main nitrogen species produced in the lung. The oxidation of NO with oxygen results in the formation of nitrite, which is the substrate for eosinophil peroxidase (EPO) and myeloperoxidase (MPO) [25]. NO react with $O_2^{\bullet-}$ to form ONOO-, which can nitrate tyrosine residues and thus damage enzymes and structural and functional proteins [15-17, 25]. High NO levels are associated with an increased risk of asthma, asthma severity, and high response to bronchodilator factors [22, 26]. The balance of cellular functions during OS depends on the proper induction of antioxidant protection mechanisms. Cells have plenty of protective mechanisms to put a stop to malignant effectuate of ROS and NS called antioxidant defense system [17, 18]. Non-enzymatic antioxidants include ascorbic acid, glutathione, albumin, α-tocopherol, uric acid, and β-carotene [27-30]. The major enzymatic antioxidants of the lungs are SODs, catalase, and glutathione peroxidases as well as heme oxygenase 1, thioredoxins, peroxiredoxins, and glutaredoxins [27]. Asthma is characterized by the loss of antioxidant activities [31].

Many studies have indicated that a large trick of medical and racy plant, as well as berry and leaves of some berry plants, perhaps consumed as a native resource of free radical scavenging compination and their antioxidant characteristic [32, 33].

The effects of TQ on respiratory tract inflammation in an allergic asthma mouse model were examined by i.p of TQ prior to the airway challenge of OVA sensitized mice and caused a significant decrease in lung eosinophilia and high Th2 cytokines both in vitro and in vivo. Stimulation of lung cells with OVA, TQ also decreased the elevated serum levels of OVA-specific IgE and IgG1. Histological examination of lung tissue demonstrated that the compound significantly inhibited allergen-induced lung eosinophilic inflammation and mucus-producing goblet cells [10]. In another study the oral administration of TQ and its metabolite dihydrothymoquinone (25, 50, and 100mg/kg for 5 days to mice) showed superoxide anion scavenger activity in different tissues [34]. The anti-in-ammatory /immunomodulatory activity of NS has been recently reviewed in asthma. NS extracts and active components (including TQ, nigellone and alpha-hederine), anti-histaminic, anti-eosinophilic, anti-leukotrienes, anti-immunoglobulin and reduced proin-ammatory cytokines (interleukins-2, 4, 5, 6, 12 and 13) in vitro and in vivo models [35].

Because of the potential negative effects of artificial food additives on human health, people interest in natural products in the human diet in recent years. As far as it can be determined by our literature research, no works have been reported until now, the capacity of co-administration of TQ and CS on asthmatic mothers and their fetus.

The goal of this research is to find out the effect of co- administration of TQ and CS against asthma and OS caused by OVA at pregnant rats and biochemical and histological evaluations in their embryos.

2. GENERAL INFORMATION

2.1. Pathophysiology and Pathogenesis of Asthma

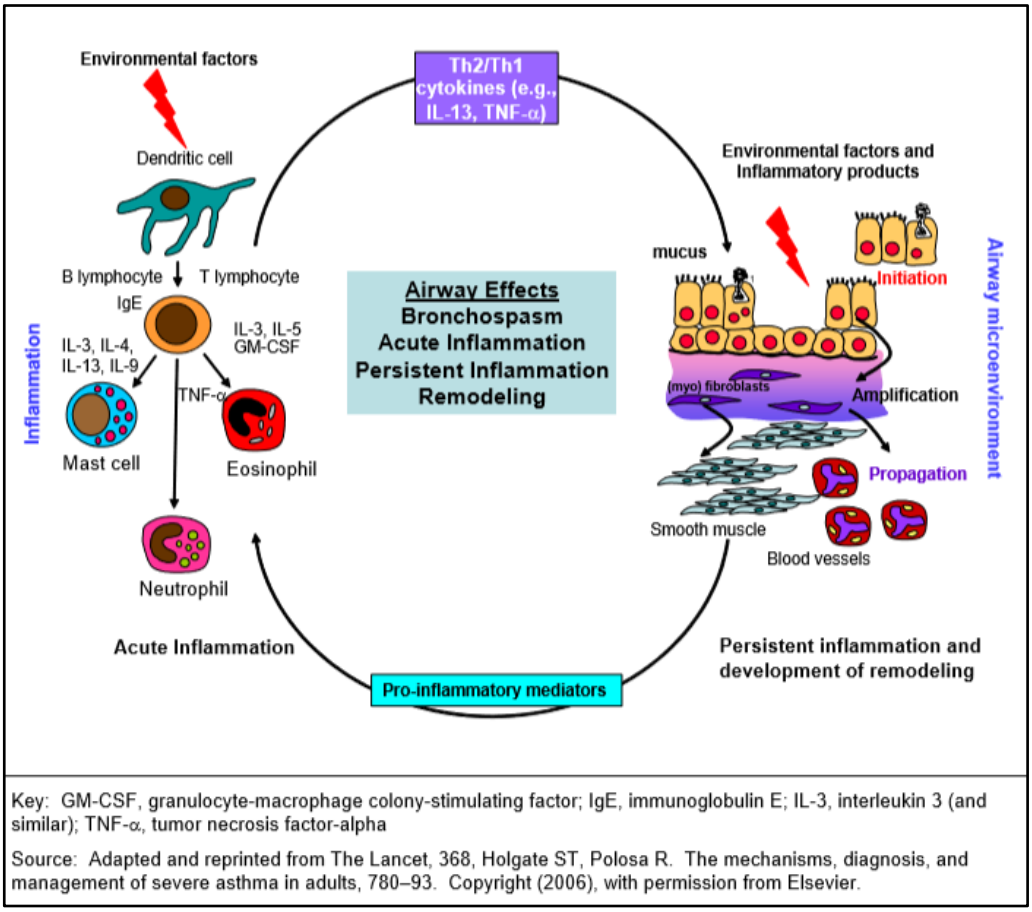
Reducing air flow in asthma is frequent and occurs due to a variation of changes in the airway, these contain:

2.1.1. Bronchoconstriction

In asthma, the predominant physiological occurrence commanding to clinical sign is contraction of the airway and following attempt with airflow. In severe asthma exacerbation, bronchial sphincter muscle contractionary (bronchial stenosis) rapidly happen to tight the airway in reaction to subjection to a variation of stimulant, including allergens or provocative. Acute bronchial stenosis caused by allergens results in the release of Immunoglobulin E(IgE)-dependent mediators from mast cells that include tetrapazate, leukotriene, histamine and prostaglandin, which by a direct route contract the smooth muscles of the airway [36]. Aspirin and non-steroidal anti-inflammatory drugs can also cause severe blockage of airflow in some patients. The evidence suggests that this non-IgE-based response also includes a mediator of the airways [37].

2.1.2. Airway edema

When the disease becomes more stable and inflammation is more advanced, other factors act to reduce airflow. These include edema, inflammation, excessive mucus secretion and the formation of insisted mucus plugs, as well as structural changes like hypertrophy and hyperplasia of the airway smooth muscle (Figure 2.1). These recent changes may not respond to the usual treatment [38].



Key: GM-CSF, granulocyte-macrophage colony-stimulating factor; IgE, immunoglobulin E; IL-3, interleukin 3 (and similar); TNF-α, tumor necrosis factor-alpha

Source: Adapted and reprinted from The Lancet, 368, Holgate ST, Polosa R. The mechanisms, diagnosis, and management of severe asthma in adults, 780–93. Copyright (2006), with permission from Elsevier.

Figure 2.1. Factors limiting airflow in acute and persistent asthma [39].

2.1.3. Airway hyperresponsiveness (AHR)

Excessive response of the airway - a bronchial strait response to an extensive range of stimuli - is considered one of the main features of asthma, but it is not necessarily unique. The degree to which the over-response of the airway can be determined through the mitigating reactions to defiance with methacholine is associated with the violence of clinical asthma. Mechanisms affecting excessive airway response include inflammation, dysfunctional nervous organization, and textural changes; inflammation come out to be a key agent in adjust the grade of excessive response to the airway. Treatment directed towards reducing inflammation can reduce excessive response to the airway and improve control of asthma [38].

2.1.4. Airway remodeling

In some people with asthma, the reduction of airflow may be only partially reversible. Permanent structural changes can occur in the airway; these are associated with gradual loss of lung function that is not completely prevented or reversed by current therapy (Figure 2.1). Remodeling of the airway involves the activation of many structural cells, with consequent changes in the airway increasing obstruction and response of airway and making the patient less responsive to treatment [39]. The remodeling necessitates thickening of the airway walls, with an increase in submucosal tissue, the adventitia and smooth muscle [40, 41]. These features vary in asthma and chronic obstructive pulmonary disease [40] in allergic and nonallergic asthma [42] and with the severity of asthma [40].

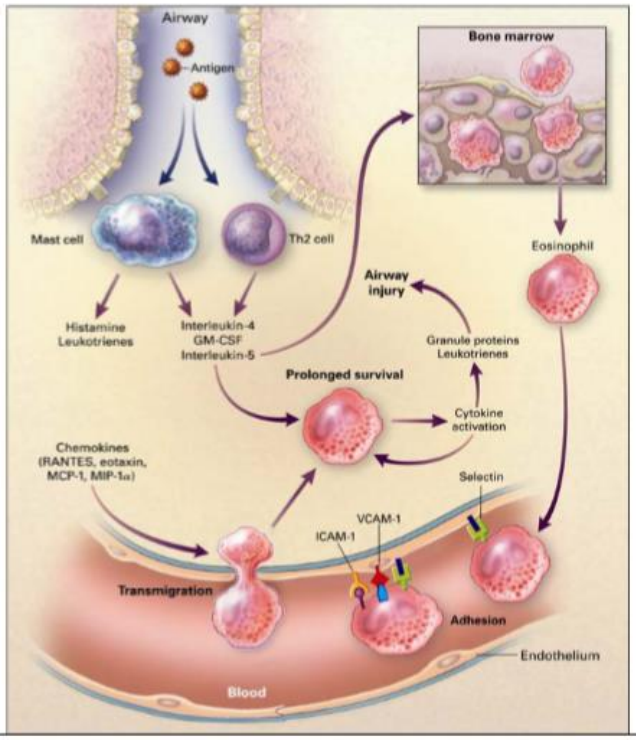
2.2. Pathophysiologic Mechanisms in the Development of Airway Inflammation

Inflammation has a major role in the pathophysiology of asthma. As reputed in the explanation of asthma, inflammation of the airway includes the coaction of a lot of cell kinds and various moderators with the bronchial tubes leading finally to the pathological typical features of the disorder:

2.2.1. Inflammatory cell

Lymphocytes

The development and regulation of bronchitis in asthma was further enhanced by the discovery and characterization of flock of lymphocytes, Th1 and Th2, with significant intermediate inflammatory properties and effects on airway activity (Figure 2.2).



Inhaled antigen activates mast cells and Th2 cells in the airway. They in turn induce the production of mediators of inflammation (such as histamine and leukotrienes) and cytokines including interleukin-4 and interleukin-5. Interleukin-5 travels to the bone marrow and causes terminal differentiation of eosinophils. Circulating eosinophils enter the area of allergic inflammation and begin migrating to the lung by rolling, through interactions with selectins, and eventually adhering to endothelium through the binding of integrins to members of the immunoglobulin superfamily of adhesion proteins: vascular-cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1). As the eosinophils enter the matrix of the airway through the influence of various chemokines and cytokines, their survival is prolonged by interleukin-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF). On activation, the eosinophil releases inflammatory mediators, such as leukotrienes and granule proteins, to injure airway tissues. In addition, eosinophils can generate GM-CSF to prolong and potentiate their survival and contribution to persistent airway inflammation. MCP-1, monocyte chemotactic protein; and MIP-1α, macrophage inflammatory protein.

Figure 2.2. Airway inflammation [36]

Following the discovery of these distinct flock of lymphocytes in animal models of allergic inflammation, evidence emerged that in human asthma, the shift or inclination towards the character Th2-cytokine led to the eosinophilic inflammation that characteristic of asthma [43]. In addition, the production of Th2 cytokines (eg, interleukin-4 (IL-4), IL-5, and IL-13) can also be explained by excessive production of IgE, presence of eosinophils and the development of excessive response to the airway. There is also be a decrease in a subset of lymphocytes, regulative T cells, which typically inhibit Th2 cells, in addition to increasing natural killer cells (NK) that slip great numbers of Th1 and Th2 cytokines [44, 45]. T lymphocytes, over with another airway occupant cells, can so adjust the evolution and rate of airway remodel.Though it is an simplism of a complicated procedure to define asthma as a type Th2 disease, identify the emphasis of *n* families of cytokines and chemicals has advanced our mentality of the development of airway inflammation [46, 47].

Mast cells

Actiuation of mucous mast cells triggers bronchodilators (cystenyl leukotriene, prostaglandin D2, histamine) [48, 49, 50]. Although sensitizing allergens occur through highly convergent IgE receptors, which is probably the best suitable interaction, sensitive mast cells also can be stimulated by prophylactic stimulant to explain bronchospasm motivate by exercise. Increased amount of mast cells in the smooth muscle of the airway possibly associated with an excessive response in the airway [51].

Eosinophils

There are an increasing number of bronchioles in most people with asthma, not all [52, 53]. These cells carry inflammatory enzymes, create leukocytes, and show a large range of pro-inflammatory cytokines. Rise in eosinophils are frequently associated with an increase violence of asthma. Moreover, many works suggest that asthma treatment with corticosteroids decrease circulat and airway eosinophils in collateral to clinical healing. However, the role and additive of eosinophils in asthma is subject to re-evaluation based on studies conducted on antiretroviral therapy IL-5 meaningfully reduced eosinophils but did not influence control of asthma [54].

Neutrophils

Neutropenia is increased in the airways and sputum for people with violent asthma, pending acute exacerbation, and in the asset of smok. Its pathological physiology residue unclear; mayhap a determining of non-response to corticosteroid therapy [55]. In pulmonary function, regulation of the recruitment, reactivation and modification of neutrophils is again understudy, but leukotriene B4 may lend to these processes [56, 57].

Dendritic cells

These cells act as primary cells to introduce antigens that interface with allergens from the exterior of the airway and then transmigrate to local lymph nodes to interface with regulating cells and eventually alert the production of Th2 cells from pure cells [58].

Macrophages

The most numerous bronchial cells are macrophages can be activated by allergens throu low-IgE receptors to slip inflammatory mediators and cytokines that increase inflammatory response [59].

Resident cells of the airway

The target of an asthma response are not only smooth muscles in the airway (by succumbing to the narrowing of airflow blockage) but they contribute to it too (by producing their own family of prophylactic mediators. As a result of inflammation in the airway and the descent of growth factors, smooth muscle cells in the airway can afford activation, constriction and hypertrophy-events that can affect asthmatic dysfunction in the airway [38].

Epithelial cells

The airway epithelium is a lining cell of another airway that is critically involved in asthma [60]. The generation of inflammatory mediators, the recruitment and activation of inflammatory cells, and the infection of respiratory tract infections can lead to the emergence of epithelial cells to infect the epithelium itself or to procreate extra inflammatory mediators. The reparation processing, after harm epithelium, may be unusual in asthma, which promotes the disincentive injury that consist in asthma [38].

2.2.2. Inflammatory mediators

Chemokines

Chemokines are important in the recruitment of inflammatory cells in the airways and are mainly expressed in epithelial cells [46]. Eotaxin is relatively selective for eosinophilic, while the thymus and the regulating activation chemokines (TARCs) and macrophages derived from macrophages (MDCs) induct Th2 cells [61]. There is growing appreciation for the role of this family of mediators in managing many aspects of injury, repair, and asthma [38].

Cytokines

In asthma cytokines direct modify the inflammatory response and may adjust its violence. The cytokines reproduce from Th2 cover IL-5, which is essential for flunk and involution of eosinophils, and IL-4, which is important for the differentiation of Th2 cells and with IL-13 prominant for the formation of IgE. The main cytokines include tumor necrosis factor (TNF-α), which amplifies the inflammatory response, granulocyte-macrophage colony-stimulating factor (GM-CSF), which prolongs permanence of eosinophil in airways and IL-1β [62].

Cysteinyl-leukotrienes

Cysteinyl-leukotrienes reproduce mostly from mast cells are strong bronchoconstrictors. They are the exclusively mediator whose specific inhibition associated with asthma symptoms and improved lung function [63]. Last works have indicated that leukotriene B4 by recruiting neutrophils can subscribe to the inflammatory process [64].

Immunoglobulin E

Immunoglobulin E (IgE) is the antibody responsible for stimulating allergic reactions and is important in causing allergic diseases and the development and continuation of inflammation. IgE attaches to cell surfaces via a specific high-convergence receiver. The mast cell contains large numbers of IgE receptors; these, when activated by interaction with the antigen, release a wide range of intermediaries to initiate acute bronchospasm as well as to release pro-inflammatory cytokines to sustain primary airway inflammation [50, 65]. Other cells, basal cells, stem cells, and lymphocytes also contain highaffinity IgE receptors. The evolution of monoclonal antibodies towerds IgE showed that decreasing IgE is efficient in the treatment of asthma [66, 67].

2.3. The Role of Leukotrienes in Allergic Diseases

Lipids are the key component of cell membranes in addition to its primary role as a food source. Lipids derivatives such as leukotrienes (LTs) and prostaglandins (PGs) play essential roles in inflammatory and immune responses and act as signaling molecules. LTs are divided into two categories, the cysteinyl LTs (CysLTs: LTC4, LTD 4 and LTE4) and

the chemoattractant leukotriene B4 (LTB4), which carries only the hydroxyl markers [68, 69]. LTs of arachidonic acid are generated via the 5-lipoxygenase pathway (5-LO), which are representative intermediates of lipid or biologically active lipids. LTs by binding to G protein receptors exert their biological effects (GPCRs) [70]. Sub species of different LT receptors exert unique functions [71].

2.3.1 Overview of the 5- Lipoxygenase pathway

In response to various biological stimuli, arachidonic acid is released from the sn-2 posture phospholipid membrane by A2 phospholipase [72, 73]. Then to generate PGs and LTs, respectively they are metabolized by cyclooxygenase (COX) and lipoxygenase (LO) routes [74]. There are named according to the position of carbon in which one oxygen molecule is combined which are at least six different types of mammary lipoxygenase. Between them, 5-LO is the most widely studied research, which is chiefly expressed in granulocytes, macrophages and mast cells [75]. First the arachidonic acid is oxidized through the 5-LO enzymatic activity of the 5-hydroxy peroxyacosatriteinwick acid (5HpETE) at C-5 followed by an unstable mediator, A4 leukotriene (LTA4); 5 HpETE acts in coordination with 5-LO activated protein (FLAP) in a manner interdependent on Ca^{2+} [76]. LTA4 is transform to LTB4 by LTA4 Hydrolase [77] or by leukotriene C4 synthase (LTC4S) conjugated to reduced glutathione to produce CysLT (LTC4) [78]. Then by extracellular peptides, LTC4 is release from the cell and transform to LTD4 and LTE4, the more stable CysLTs [79] (Figure 2.3). In a studies of mouse models of ovalbumin-induced inflammation of airway show that mice deficient in 5-LO are unable to synthesize perceptible levels of LTs and thus decrease inflammation levels [80]. Zileuton, A 5-LO inhibitor, has been adopted to treat asthma, the only agent that can inhibit LT production [81].

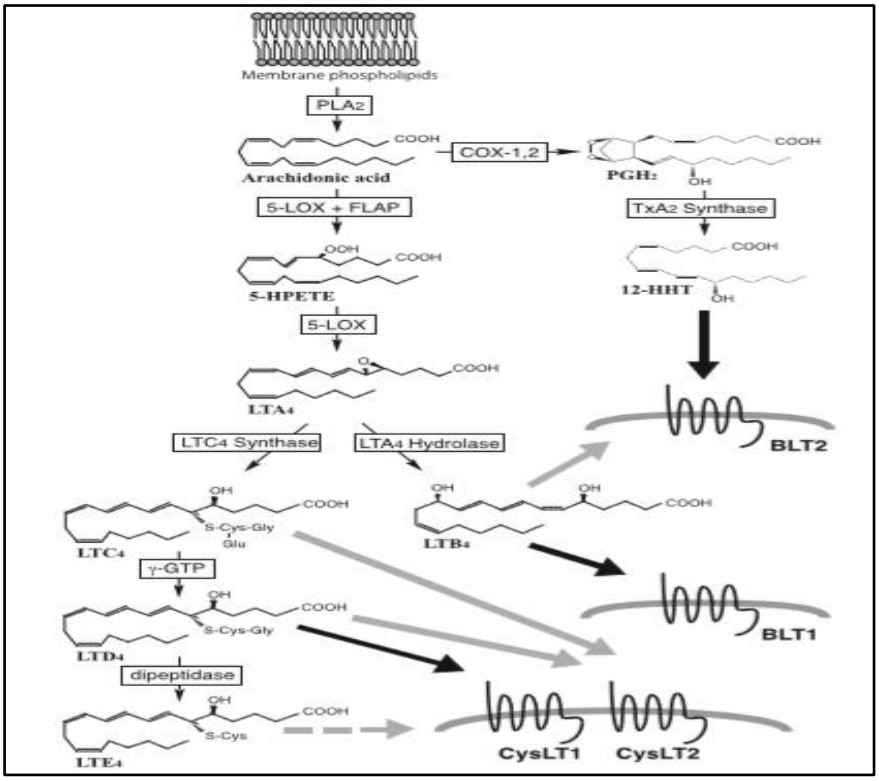


Figure 2.3. The arachidonic acid progressive generates leukotrienes from membrane phospholipids. COX, cyclooxygenase; PLA2, phospholipase A2; 5-LOX, 5-lipoxygenase; 5-HPETE, 5-hydroperoxyeicosatetraenoic acid; FLAP, 5lipoxygenase activating protein; [82].

2.3.2. Leukotriene B4 and asthma

In asthma the real role of LTB4 till now uncertain. In contrast to the mediators of bronchoconstriction (CysLTs), LTB4 is believed to be a pro-inflammatory mediator responsible for activation, duration and mobilization of leukocytes, including neutrophils and eosinophils [83]. BLT 1, plays a role in asthma too, the high-affinity receptor for LTB4. BLT1-deficient mice are reluctant to OVA-induced allergic AHR and show reduced bulk of eosinophils, lymphocytes and dendritic cells in the lungs [84].

2.3.3. Interleukin-13 (IL-13) and asthma

IL-13 is a pleiotropic Th2 cytokine produce from an assortment of inflammatory cells and structural cells, including the airway epithelium. IL-13 is produced by CD4+ T cells, NK T cells, mast cells, basophils, eosinophils, and nuocytes [85]. It involved as a central regulator of IgE synthesis, excessive mucus secretion, AHR and fibrosis [86]. Animal models show that IL-13 stimulates goblet cells and excessive mucus secretion [87]. In

airway epithelial cells, IL-13 is known for load 15-Lipoxygenase-1 (15-LO1), an important enzyme in the arachidonic acid pathway, which forms a stable 15-hydroxyeosatetraenoic acid (15-HETE) of arachidonic acid metabolism. Human epithelium is linked to 15-LO1 expression with asthma severity [88]. IL-13 is expressed excessively in sputum, bronchial mucosa, peripheral blood, and mast cells in the smooth muscle bundle of the airway in asthma, supporting its role in causing AHR [89, 90].

Interleukin-13 function

IL-13 is a pleiotropic cytokine that extend its impact on a large range of cell types. It has a variety of functions that are critical to balance and immune repair, but when uncontrolled contributes to the phenotype of asthma [91]. As a Th2 cytokine, IL-13 is involved in many of the IL-4 interleaved properties, where both cytokines enhance B cell proliferation, alter and synthesize the IgE class in B cells, and promote surface expression of antigens such as CD23 and major histocompatibility complex (MHC) class II [92]. IL-13 is involved in mediating the effective Th2 function [93]. In monocytes and macrophages, IL-13 can inhibit the product of many pro-inflammatory mediators like IL-1, prostaglandins, TNF-α and ROS [94]. IL-13 has been reported to have direct effects on eosinophils, including enhanced eosinophilic survival, activation, and recruitment [95]. Furthermore, IL-13 has been shown to promote multiplication and cholinergic-induced contractions of smooth muscle cells in vitro [96]. It also induces epithelial cell proliferation [97].

2.3.4. Interleukin 1-beta (IL-1β)

IL-1β is a pivotal cytokine that is centrally involved in both local and systemic immune responses. In asthma patient, IL-1β has been involved in the early phase of both the inflammatory response and adapts airway responsiveness, and the high levels of IL-1β protein have been shown to be existing in the airways of asthma patients [98]. Moreover, there is an evidence demonstrates that IL-1β modify airway constriction and repose responses by direct action on the airway smooth muscle (ASM) itself [99]. Taken together, this evidence highlights the important role of IL-1β in the pathophysiology of asthma. There are a two distinguished receptor: IL-1 type-I (IL-1RI) and type-2 (IL-1RII) that relate to both IL-1a and IL-1b [100]. The receptor that responsible for producing the biological effects that are attributed to IL-1 signaling is IL-1RI [101]. In contrast, the IL-

1RII receptors do not have peptide signaling of the functional cytoplasm, therefore acting as trap receptors associated with IL-1 and mitigating IL-1 signals [102]. In addition, IL-1RII receptors can be cut into a soluble form (sIL-1RII), which is associated with high-density IL-1b, which reduces the amount of IL-1β available [103].

2.4. Tumor Necrosis Factor- α (TNF-α) and Asthma

An important cytokine in the innate immune response is TNF-α that before activation of the adaptive immune system provides immediate defense against invasive organisms [104]. Mainly it is produced by macrophages in response to the activation of membrane-related identification molecules, which assign the face crop of common bacterial cells such as polysaccharide, carbohydrates and lipopolysaccharides. It is initially produced as a biologically active 26kD membrane stable portent protein [105]. Fundamentally, this is cleaved posteriorly by TNF- α converting enzyme (TACE) to release the 17kD free protein [106]. Which these proteins take Figure into active biologically homotrimers [107]. Then that work on the ubiquitously expressed TNF- α receptors 1 and 2 (p55 and p75 or TNFRi and TNFRii) [108]. The interaction of ligand receptors in signals within cells without normalization of the compound causes the phosphorylation in nuclear factor κB (NF- κB) to operate the p50-p65 subunit, which cooperate with the DNA chromatin structure to increase transcription of inflammatory genes, like IL-8, IL-6 and TNF-α itself (Figure 2.4). The probability that TNF-α conduce to the irregular response of inflammatory appearing in the asthma tract is upturned by the results of TNF-α mRNA increaseing [109] and protein in the airway of asthmatic patients [110]. Furthermore, the development of AHR and an airway neutrophilia caused by inhaled recombinant TNF- α to normal subjects [111]. In asthmatic patients the TNF-α administration leads to an increase in AHR as measured in decreases PC20 methacholine [112]. The mechanism underlying these observations is not fully clarified: it may have a direct effect of TNF-α on ASM [113] or is mediated through the release of cysteinyl-leukotrienes LTC4 and LTD4 [114]. TNF-α stimulates the release of histamine directly from human mast cells [115] and share in a positive autocrine loop that activate human mast cell cytokine secretion [116]. TNF-α can therefore be involved in smooth muscle / mast cell interaction and particularly this is important in the development of AHR (figure 4). It is increases expression epithelial of adhesion molecules like ICAM-1 and V-CAM-1, which plays an important role, is the conduction of T-Cells to the lung and in the subsequent development of AHR [117]. In addition to its association with asthma in

general, TNF-α contains several characteristics that may be related to asthma, including recruitment of neutrophils, induction of glucocorticoid resistance, myocyte proliferation and stimulation of fibroblast growth and maturation to myofibroblasts by elevate TGF-α expression [118].

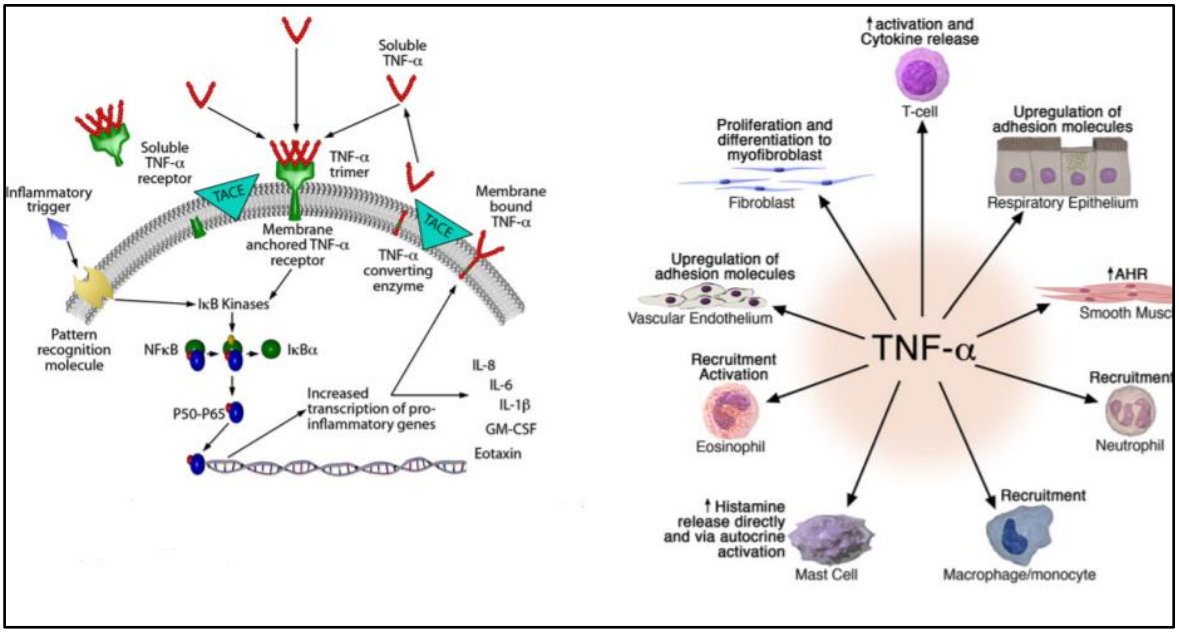


Figure 2.4. Summary of TNF-biology and signaling and the role of it in the pathogenesis of asthma. TNF-a plays a central role in many of the features of the asthma paradigm by exerting important effects on both inflammatory and structural cells [119].

2.5. Vascular Endothelial Growth Factor (VEGF)

A central role in the creation of new blood vessels (vasculogenesis) during the development of embryo is VEGF cytokine [120]. It was mentioned earlier as vascular permeability factor (VPF) [121]. Many VEGFs within the cytokine family, have been described: VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E and the placenta growth factor (PlGF). VEGF code genes are found on chromosome 6p21 [122]. Primarily VEGF works on the endothelial cells of blood and lymphatic vessels, stimulating their migration and division (Figure2.5). Inflammatory cells, like mast cells, eosinophils and macrophages, enounce receptors of VEGF (VEGF Receptor-1, VEGFR-1) that VEGFR-1, in order, induc chemotactic migration of these cells [123]. It is 50,000 times most strong than histamine in doing so. A test of sputum in patients with bronchial asthma detected significant concentrations of VEGF in addition to Cyst-LTs [124]. In a studies of VEGF roles in the

pathogenesis of bronchial asthma reported that this factor can stimulate allergic inflammation in the bronchial tree and participate formation of remodeling. In other studies, about positive relationship between VEGF concentrations in sputum, lung tissue and severity of bronchial asthma was showed [125]. According to Lopez Guiso et al, the remodeling of the airway in patients with bronchial asthma starts by destroying the bronchial endothelial and releasing VEGF from the endothelial cells [126], it was reported that Th17 lymphocytes infiltrating bronchial epithelium stimulate production of VEGF. Comparison in vitro analysis in children of bronchial epithelium diagnosed with asthma and health controls showed an excess capacity of bronchial epithelial cells for the production and release of VEGF in asthma patients [127].

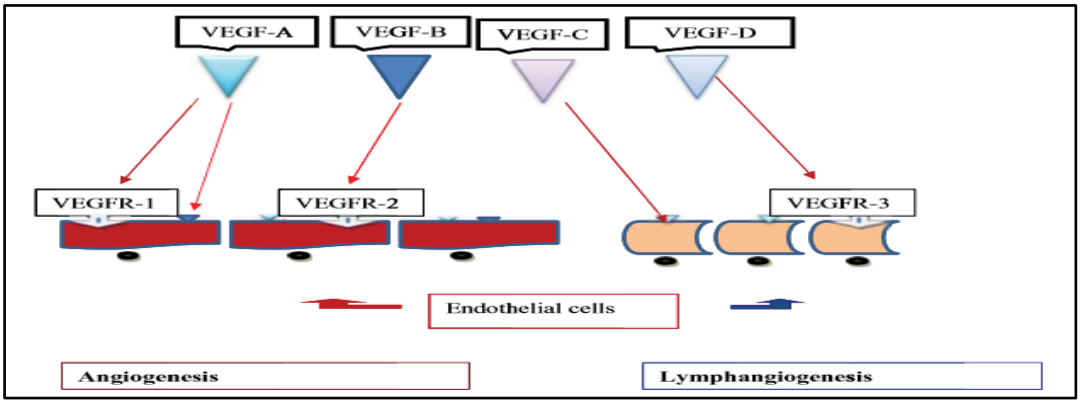


Figure 2.5. VEGF-α acts by receptors: VEGF-C, VEGF-D activate VEGFR-3 and stimulate lyphangiogenesis. VEGFR-1, VEGFR-2, but activates mainly VEGFR-2 and induce angiogenesis [122].

2.5.1. Angiogenesis and vascular endothelial growth factor

Many of clinical research studies and animal models demonstrated that VEGF is the major mediator involved in lung neovascularisation and a strong pro-angiogenic growth factor that acts a key role in vascular remodelling both in normal and pathological events [128, 129, 130]. VEGF-A isoform 165 (VEGF165) is thought to be the most effective pro angiogenic member of the VEGF family. VEGF stimulate endothelial cell proliferation and migration and is expressed in various vascularised organs including the lung [131]. Many of cell populations reproduction VEGF including CD34 cells, macrophages, eosinophils, mast cells, fibroblasts and ASM cells [132, 133]. Curiously, VEGF manufacture by airway structural cells are induced by TH17 cells and compress by TH1 cells [134, 127]. VEGF is

mainly synthesized by ASM in the vitro, while this production can be altered in response to a number of stimuli, such as TNF-α, TGF-β1, IL-1β, angiotensin II, endothelin-1 and nitric oxide [135, 136].

2.6. Oxidative Stress

Oxygen free radicals (OFRs) are molecules or atoms that includ oxygen and present in their outer orbit an unpaired electron. These radicals can interact with other molecules by interfere opposed to them, take off their electrons and revise their molecular textures [137]. In the organism the major metabolic tract of the oxygen is linked to the total decrease of oxygen to the water, with the integration of 4 electrons in the peripheral part of the respiratory chain. If oxygen reduction involves fewer electrons during the respiratory chain, intermediary OFRs will be generated. Singlet oxygen, hydroxyl ($OH^{\bullet}$), superoxide ($O_2^{\bullet}$-) and hydrogen peroxide (H_2O_2) are the most common OFRs.

2.6.1. Oxygen free radicals Production

As mentioned in advance, the total decrease of molecular oxygen in water is achieved by receiving 4 electrons at the end of the respiratory chain. However, if oxygen decrease confuses few electrons, other OFRs can be create, as display down:

$O_2 + e^- \rightarrow O_2\bullet^-$ (superoxide radical)

$O_2 + H_2O \rightarrow HO^{\bullet}_2 + OH^-$ (per hydroxyl radical)

$HO^{\bullet}_2 + e^- + H^+ \rightarrow H_2O_2$ (hydrogen peroxide)

$H_2O_2 + e^- \rightarrow OH^{\bullet} + OH^-$ (hydroxyl radical)

Hydroxyl radical OH•

OH• can be created radically in biologically relevant systems via multiple reactions. One is the Fenton chemistry. The resulting UV-induced hemolytic splitting of the O-O link in H_2O_2 makes OH•, this can be thought to occur for H2O2 generated in skin exposed to sunlight.

$H\text{-}O\text{-}O\text{-}H \longrightarrow 2OH^{\bullet}$

OH• radical can be generated from ozone. In addition, $OH^{\bullet}$ has been suggested to emerge during ethanol metabolism and may be during decomposition of peroxynitrous acid. Other sources of OH• includes:

1. Ionizing radiation [138]. OH• radicals are responsible for a great side of the damage done to cellular DNA, proteins and lipids by ionizing radiation. DNA damage, especially double-strand breaks, it is thought to be an important damaging episode; mostly as double-strand breaks cannot be fixed by the cell easily.

2. From hypochlorous acid reacting with $O2^{\bullet-}$ [139]:

$$HOCl + O_2\bullet- \longrightarrow O_2 + Cl^- + OH\bullet$$

3. Ultrasound, lithotripsy and freezed-drying [140].

The superoxide radical

The O2•- radical is lesser interactive than the OH• radical and is created thru the decrease of oxygen by one electron. Underneath physiological terms, it is mainly created in microsomes, mitochondria and peroxisomes [141]. Its half-life period is lengthy than that of the OH• radical, as well as it can interact with molecules for a long time. In neutral or alkaline conditions O_2•- dismutation is catalyzed by the O_2•- dismutase enzyme (SOD) [142].

$$O_2\bullet- + O_2\bullet- + 2H^+ \longrightarrow H_2O_2 + O_2$$

Hydrogen peroxide

Fundamentally the H_2O_2 radical is created by the protonated O_2•- radical in a low pH perimeter. Though H_2O_2 is not a actual OFR, it can act with active oxidation minerals like copper and iron, resulting in new OFRs.

$$H_2O_2 \longrightarrow 2\ OH\bullet \text{ (hemolytic fission)}$$

H_2O_2 can degrade certain hem proteins (including myoglobin, hemoglobin and cytochrome *c*) to release irons [143]. Also, it has a longlife span and lofty dispersion capability thru fermented cellular membranes (like their ability to propagate with water capacity), which increases the toxic effect of re-oxygenation [144].

2.6.2. Biologic resources of free radicals

The master biologic tract of the OFR creation is the transference of the electron linked to the mitochondrial membranes. It is believed that Ubiquinone - cytochrome b is the most likely O_2•- formation sites [145]. A large portion of the O_2•- radicals formed in the mitochondria are converted to H_2O_2 by mitochondria O_2•- dimetazase, and H_2O_2 molecules may migrate to cytosol. Microsomes and nuclear membranes may also be involved in e-transport system through cytochrome P450 and B5, which can produce OFRs. The shift in the location of the isoenzyme cytochrome P450 may also influence the possibility of forming OFR through a process that is still unclear. However, this phenomenon is thought to be associated with the fluctuation of the cytochrome P450 through total lofty and falling-power turn status. Switching to a lofty-power status may increase the manufacture of O_2•- and H_2O_2 required to increased decrease of the P450 cytochrome [146]. Though OFRs are frequently create in recombinant circle oncoming from the mitochondrial chain, they possibly produced by cytoplasmic sources, like xanthine oxidase. Xanthan oxide causes the hypoxanthine reagent with oxygen, creating O_2•- and uric acid [147]. Also, cells procreate OFR use another resource, contain oxidase enzymes (flavin dehydrogenase, aldehyde oxidase, cyclooxygenase, cytochrome P450 oxidase system and NADPH oxidase), the carrier system for microsomal electrons and nuclear membranes and autoxidation of small molecules (catecholamine, Flavin, and hydroquinone) [137].

2.6.3. Free radicals' sources in the lung

Several cells of the lung parenchyma, like type-2 alveolar cells, endothelial cells, clara cells, alveolar macrophages and airway ciliated cells can create OFRs [148]. The systems that generate OFRs in the lungs are like those seen in other tissues.

2.7. Oxidative Stress in Asthma

Inflammatory cells in asthma that are enlisted to the airways includ neutrophils, eosinophils and macrophages, are a great resource of ROS and NO that have a major role in the asthma pathogenesis [149] (Figure2.6).

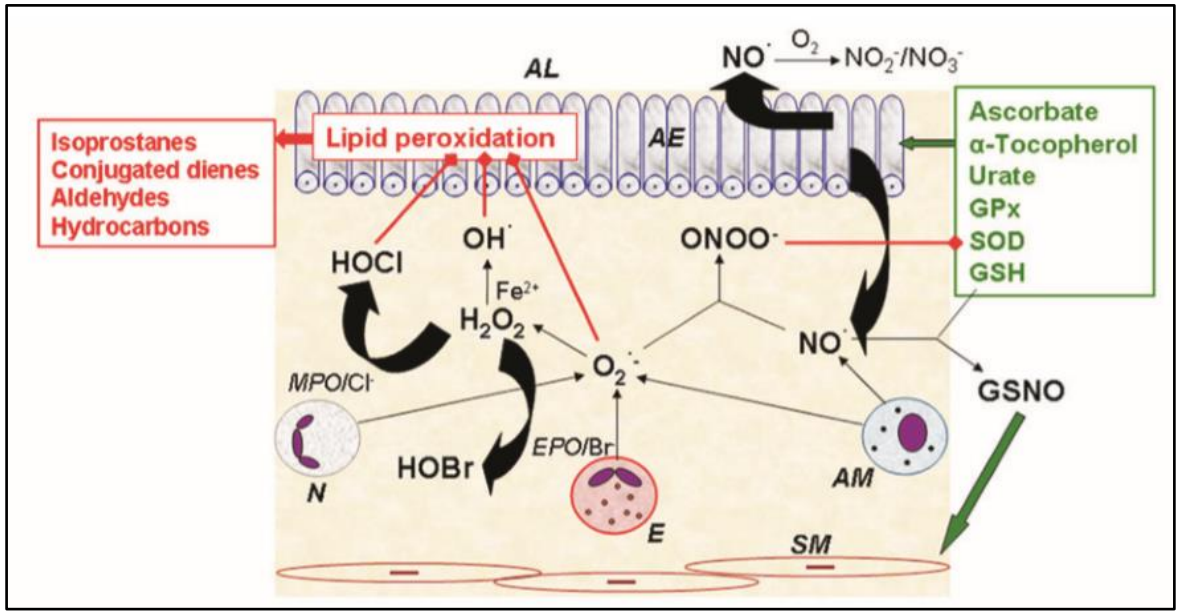


Figure 2.6. Diagrammatic Representation of Sources of Oxidative Stress in Asthms: Schematic acting of resources of oxidative stress in asthma and the intracellular and extracellular antioxidants and enzymes that opposed oxidative stress [149]

The NADPH oxidase of eosinophils (E), neutrophils (N) and alveolar macrophages (AM) in the airways of asthmatic patients procreate superoxide anion which spend dismutation to H_2O_2. Neutrophil myeloperoxidase (MPO) catalyses the effect of H_2O_2 with Cl^- to form HOCl, when eosinophil peroxidase (EPO) catalyses the creation of HOBr. In the existence of Fe^{2+}, H_2O_2 so spend a fenton effect to create hydroxyl radicals. These reactive oxygen species start peroxidation of poly-unsaturated fatty acids in cell membranes ensure in the creation of isoprostanes and other products of lipid peroxidation. Superoxide so act with NO• made by airway epithelial (AE) cells and AM to form peroxynitrite, which might inactivate proteins like SOD by nitrate tyrosine residuals. NO• insert the airway lumen (AL) and is assing in the exhaled breath of patients with asthma. NO• so acts with glutathione (GSH) to create S-nitrosoglutathione (GSNO) which has a bronchodilating action on smooth muscle (SM) [149].

Describes oxidative stress damage that occurs when ROS overwhelms the host's antioxidant defenses. Oxidative stress might trick amajor role in the pathophysiology of asthm [150, 151] it might be a prevalent pathway cause to tissue loss. Figure 2.7 is a clarify illustration of how to be exposed to a diversity of distinct matter like allergens, gaseous pollutants, chemicals, drugs, bacteria, viruses [152] command to activation and mobilization of cells in the airway in asthma, contain neutrophils, mast cells, eosinophils, macrophages, lymphocytes, and platelets. As shown in Figure 7, allergy response including the obtain immune system are characterized by the creation of IL-5, subsequent recruitment and activation of eosinophils. In contrast, stimulants acting through the innate immune system produce IL-8, subsequent recruitment and neutrophil activation. However, both of these pathways lead to ROS production, primarily due to the respiratory explosion of active cells. Activated cells in inflammatory cells respond to a "respiratory explosion," which involves oxygen uptake and next slip of ROS in the ambient cells. Pending the respiratory boom, a decreased nicotinamide adenine dinucleotide phosphate-dependent superoxide generating system is actuate and slips O2•- into the cell. The decomposition effect, which is stimulated by superoxide dismutase (SOD), ends in the creation of H_2O_2, which, in the asset of halide ions (eg, Cl, I-, and Br), will act to the formation of hypohalose acid (eg HOBR/ HOCl). In eosinophils, this action is stimulated by eosinophil peroxidase (EPO). In neutrophils, this action is stimulated by myelobroxidase. HOCl / HOBr might then act with O_2•- or Fe^{2+} to create other powerful oxidant, possibly radical hydroxyl (OH). So, pending this "respiratory explosion", the cells inside the body launched loud concentrations of O_2•-, H_2O_2 and OH, HOCl / HOBr which might seep in the ambient cells leading to increased free radicals in the tissues of the airway. In addition, inflammatory cells in asthmatics have an increased ability to create free radicals equate to controls, contributing more to lofty concentrations of ROS [153-155]. Excessive nitrogen species can also be produced by people with asthma. The cytokines might induce increased nitrosol creation (NO•) [156] which acts with O_2•- For the formation of peroxinitrite, a toxic type of cells that has much adverse effects, contain lipid oxidation [157]. No• also it can be transformed to nitrite, which can oxidize proteins. This might be throu nitrous tyrosine, in an EPO-stimulated action [158]. High levels of nitrotherosine were observed in asthma patients [159]. So, the surplus amounts of ROS and RNS created by asthma patients might get over antioxidant defenses of host and lead to oxidative stress.

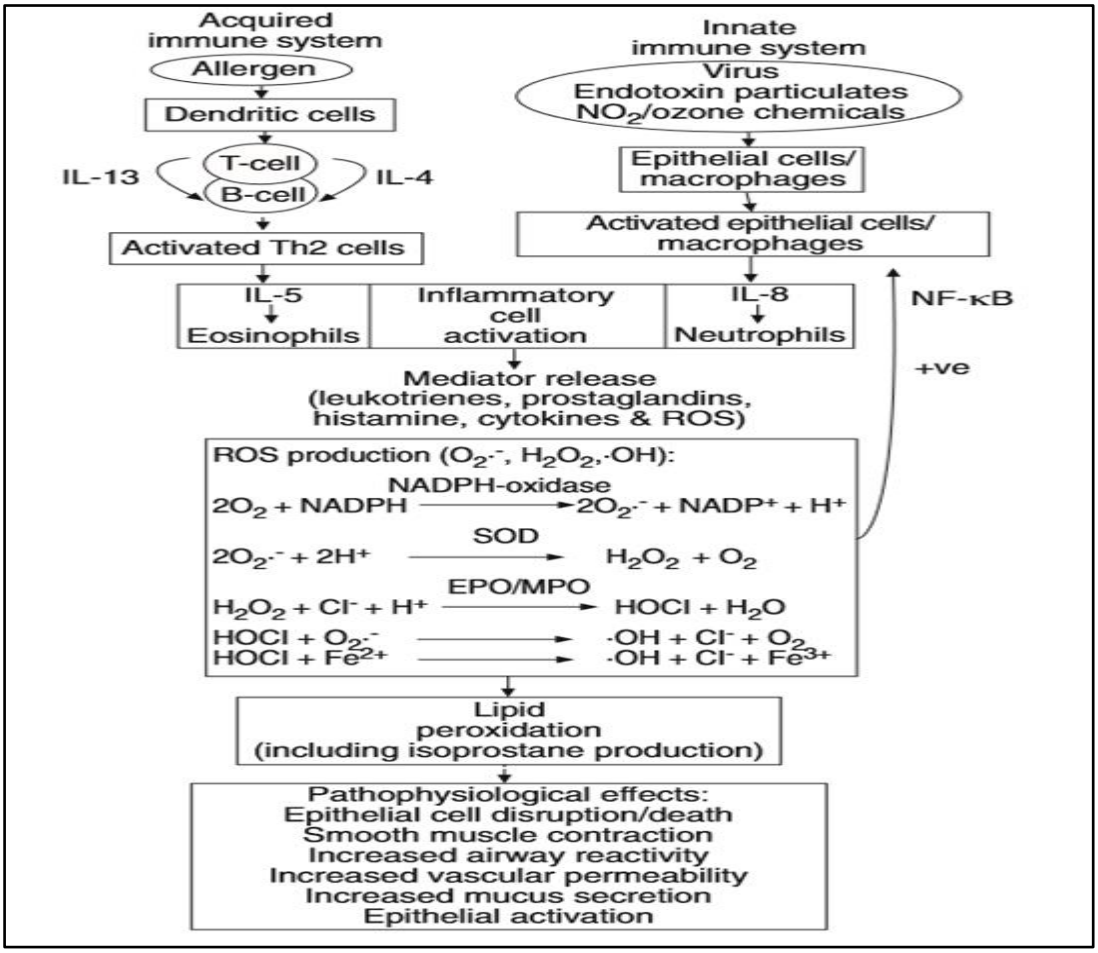


Figure 2.7. Mechanisms Leding to Lipid Peroxidation in Asthma: In asthma mechanisms leading to lipid peroxidation. Th2: T-helper type-2 cells; NO_2: nitrogen dioxide; IL: interleukin; ROS: reactive oxygen species; H_2O_2: hydrogen peroxide; O_2•-: superoxide; •OH: hydroxyl radical; SOD: superoxide dismutase; NADPH: reduced nicotinamide-adenine dinucleotide phosphate; NADP: nicotinamide-adenine dinucleotide phosphate; EPO: eosinophil peroxidase; MPO: myeloperoxidase; NF: nuclear transcription factor [152].

2.8. Defense Systems Against Oxidative Aggression

The antioxidant defense system consists of a set of items that countract the dangerous effects of OFRs. The classification proposed from Davies to classify the antioxidant defense system was made by distinct it into prime and second defense. The Prime defense contain antioxidant complexes like vitamins (A, E and C), uric acid, glutathione and antioxidant scavenger enzymes (like SOD, catalase and the peroxidases). Second defense contain proteolytic enzymes, lipolytic enzymes, phospholipases, endonucleases, DNA repair enzymes, exonucleases and ligases [160]. In all mammalian cell systems, the low form of tripeptide GSH is the most abundant molecular weight thiol. The chemical

diversity of decreased GSH, specially its coaction with different oxidizing constituents like O_2, H_2O_2 and OH, makes it effective. Decreased GSH is found in lofty concentricity in bronchoalveolar lavage (BAL) fluid, grant security opposed to oxidative damage to the lungs. The dominance of decreased GSH was proven in studies in which its consuming was regarding to a big hazaed of pulmonary disease [161].

2.9. Antioxidant Deficiencies in Asthma

2.9.1. Antioxidant enzymes

Oxidative stress might so consist in the lungs of asthma patients due to the changing statment and actions of antioxidant enzymes that play a staminal role in protective lung tissue from the toxical effects of ROS. Initially there was a lack of glutathione peroxidase (GPx), which alert the reducing of H_2O_2 and lipid hydroperoxides by GSH, in platelets of patients with aspirin sensitivity and chronic asthma [162] and this finding has been confirmed in leukocytes and erythrocytes of both atopic and nonatopic patients with asthma [163, 164]. The low GPx activity might be united with nominal grade of selenium in these patients [165]. However, in a study in patients with acute or non-severe asthma compared with controlled individuals, GPx serum efficincy and GP_X levels of extracellular protein were not different [166]. GSH is a major antioxidant in the lung and it set in highest concentrations in the lung epithelial liner than in blood [167]. In a study of BAL fluid for patients with mild asthma, total GSH concentration was reported to be higher than normal persons [168] and a latter study reported that this might due to high concentrations of oxidized GSH in bronchial lavage and BAL means for patients with mild asthma [169]. However, pulmonary epithelial cells can increase their production of reduced GSH as an adapable reaction to oxidative stress [170]. Elevated GSH in the blood and erythrocyte oxidized GSH were also reported in cases of asthma compared to those under proper control [163, 164]. Taken together, these studies suggested that increasing of oxidative stress in asthma might contribute to promoting oxidation and synthesis of GSH in the lungs. Also, GSH has been implicated in the mechanism by which repeated consumption of paracetamol can be linked to an increased risk of asthma [171]. For patients with asthma, the activity of SOD has been consistently reported in airway epithelial cells [172]. And this appears to have been the result of airway inflammation, because the use of inhaled cortical steroids normalizes the activity of cellular cytosolic CuZn-SOD in

epithelial cells of bronchial [173]. Later work has shown that while the SOD activity is present, erythrocyte serves to increase [163, 164]. CuZn-SOD serum activity, but not protein, reduced in asthmatics patients and this was associated with the extent of blockage of airflow [167]. OS and NS may disrupt SOD activity in asthma patients with an rise in apoptosis, remodeling and shedding the epithelial cells [167].

2.9.2. Vitamins

Vitamins E, C and A all are antioxidants, but the potential relationship between asthma and vitamin C (ascorbic acid) has received the very caution. Several epidemiological studies and population reserch from the United Kingdom, the United States, the Netherlands and China suggested that nominal dietary inlet of vitamin C is associated with reduced lung function [174, 175, 176, 177]. Also, lower dietary intake of vitamin C was associated with increased bronchial reaction [178]. In line with these records of reduced intake of vitamin C among asthma patients, the investigation indicate that vitamin C concentrations in plasma are low in asthmatic patients, specially those with severe asthma, and that the liner of the lung and concentrations of sputum induced for vitamin C, also fallen in moderate patients with asthma [179, 180]. In the common population some works though have shown no sovereign effect of vitamin E (α-tocopherol) on function of lung [176] when Vitamin E concentrations in plasma and lung decreased in patients with asthma [165, 169].

2.9.3. Minerals

As an essential component of GPx, selenium (Se) plays an important role in the antioxidant defense system. In a study of asthmatic patients from New Zealand, the UK and Australia have fixed lowering in Se blood levels [156, 165, 181]. However, the rate of asthma is not increasing in North Korea and China where Se lack is particular [174]. Also, there are Cu, Mn, and Zn ingredient of the enzyme of antioxidant, SOD, but aside from one paper that bronchial reactions and etesian allergic tick are in reverse intrested to dietary intakes of Mn and Zn, respectively [178].

2.9.4. Other dietary and non-dietary antioxidants

Flavonoids are a subset of a great range of multivitamin polyphenols antioxidant compositions that are synthetized by plants. A large group study of about 10,000 Finnish adults set up a moderate reverse relationship between asthma and flavonoids [182] and in a study from the UK observed the reverse association of red wine and apple consumption with asthma, a potential protective antioxidant effect of flavonoids [183]. In asthmatic patients increased consuming of the soya isoflavone, genistein, was related to the best function of the lung [184] and a high consuming of soya isoflavones, associated with a decreased expansion of allergic rhinitis in Japanese pregnant women [185]. In a study showed that the low concentration of bilirubin in plasma was an important indicator of acute asthma, concentrations of plasma albumin were also lower in patients with acute asthma. Bilirubin works with vitamin E to prevent lipid peroxidation in low-density lipoproteins [186], while albumin, the most protein of plasma, has an antioxidant role as a result of its combinations of sulfhydryl and is consideration to coordinate glutathione levels in the epithelial cells of the lung [187]. It has been recommended that nominal concentrations of albumin plasma in asthma patients might due to disease reactivity or corticosteroid therapy [188].

2.10. Current Treatments for Asthma

In the past decade, asthma treatments have confirmed long-term deactivation of airway inflammation and relief of symptoms with quick-acting bronchodilators (primarily aerosolized beta-agonists) [189]. The most effective agents available for controlling asthma symptoms and improvement in pulmonary function is inhaled corticosteroids [190] but the potential side effects when used in increased doses led to the use of adjuvant therapies [191]. Treatment with long - acting beta stimuli, theophylline and leukotriene antagonists, all have proven to help control asthma while minimizing inhaled corticosteroids [192, 193, 194]. It was found that long-term glucocorticoids lead to dysfunction in mitochondria in addition to oxidative injury to the mitochondrial and nuclear DNA [195]. Immunotherapy for conventional allergies can be effective in many but not all patients [196]. DNA vaccines and other molecular methods of down-regulating antigen-specific Th2-mediated responses are being studied [197]. Complementary and

alternative medicines (CAMs) are applied in furthermore than 80% of the world's citizens and have become a major part of health care grid worldwide [198].

2.11. Asthma and Pregnancy

Asthma can greatly affect pregnancy results if not controlled well. The National Asthma Education and Prevention Program (NAEPP) affirm that during pregnancy preserving optimal control of asthma is lead for the well-being and health of both mother and her baby [199]. Pregnant women may be ambivalent about taking medications during pregnancy because of concerns about harmful effects on the fetus; however, epidemiological evidence is very strong in favor of using drugs to control asthma. In fact, control of asthma is particularly important during the first three months when organic formation occurs. Congenital malformations were significantly more common in women with asthma who experienced asthma during the first three months [200]. In general, paternal asthma rising the hazard of pre-eclampsia, congenital malformations, low birth weight, perinatal mortality and premature birth. About 4.1% of all pregnant women had an asthma attack in the previous year (pre-pregnancy) [201]. One study showed statistically significant increases in gestational diabetes, small for gestational age newborns and cesarean delivery for women with moderate–severe asthma, even with optimal control, when compared to controls without asthma [202]. The need for oral steroids was independently predictive of prenatal birth to and weeks and low birth weight [203].

2.12. Embryo Development

Fertilized egg is a begins of developmental sequence, moves to maturity, then repeatition itself throu breeding and is known as the life cycle. Biologists of developmental divide the life cycle of animals into three major periods: fetal improving, postnatal improving, and puberty. Embryonic development is a semester of fetal improving, which can be divided into phases of fertilization, cleavage, gastrulation, organogenesis, and histogenesis [204]. Fertilization is the uniting of egg and sperm. The egg is a very large cell, loaded with nutrients to support early embryo development. Sperm is a highly specialized cell in its function in determining and fertilizing an egg. After fertilization by sperm, the fertilized egg is named a zygote. The early embryo improves in the egg duct before migration to the uterus for implantation. Embryos spend a complicated row of morphogenetic move during

gastrulation, in which embryonic cells reposition and migrate. At the end of gastrulation, the embryo occurs of three germ layers that will spend anymore morphogenetic move and interplay to create the alphabet of primal organs. During organic generation, the growing fetus show off the fundamental body plan and get functionally jurisdiction. These developmental handles include a range of well-balanced propagation and differentiation handles through spatial and temporary chacks of molecular and cellular programing [205].

During embryo development oxygen is essential [206]. After fertilization in the egg channel, the earlyer embryo challenges the reduced oxygen gradient when mobile to the reproductive system. The yolk sac acts a majore role in early fetal growth because it gives oxygen and foods to the fetus pending the post-implantation semester. Once the fetal and cycle systems are founded, the fetus becomes lesser responsive to the maternal oxidative state [207]. Though oxygen is necessary for the improving of embryos, the use of oxygen as an energy substrate so exposure a potency risk through the creation of OS and NS as native products of oxygen metabolism, in particul the $O\bullet^-$ and $OH\bullet$ [208].

Oxidative stress is related with impaired early growth and fragmented embryos [209]. It can alert apoptosis of the egg and early embryo [210]. In study in mouse showed that ROS has a specified task during hatching the blastocyst from zona pellucida [211]. Embryos tolerate a specific detonation of ROS generation at hatching. Also, the generation of ROS might be a major regulation system for programed cell death in the blastocyst [212]. The clef to the instrumentation of ordinances pending embryo improving are the equation of reductive and oxidative (redox) homeostasis [213]. Exact chack of cellular redox is necessary for organized cell duty, which is particularly important for complicated developmental handle pending embryonic development [214]. The imbalance in the redox leads to growth retardation, organ malformations, and the generation of the mutant and even fatal fetus [215]. Antioxidant enzymes act a major role in protective the developing embryos from oxidation for the adult organism. Unlucily, the enzymatic action of many antioxidant enzymes in embryos is very under than that of adults. So, the embryos are in particula responsive to oxidative loss [216].

Minor molecule antioxidants conten uric acid, ascorbic acid, GSH and tocopherol. The major cellular non-protein sulfhydryl compound is GSH (γ-glutamyl cysteinyl glycine). It is infected in the defence opposite oxidative loss in both the male and female gametes

[217]. GSH concentrations in mature sperm bit by bit decrease pending sperm formation. Also, GSH has been infect in protect the meiotic spindle morphology of the oocyte. It acts a major role in the arrangement of a mature oocyte to receive a sperm and in oocyte growing [217]. Pending pre-implantation improving, GSH levels of fetal are constantly decreasing [218]. After the pre-implantation phase, the ability of the embryos to GSH synthesis is low, the concentrations of the GSH decrease with the continued early division and with the delayed post-transplant blastocyst formation [218].

2.13. Thymoquinone and Asthma

TQ is the bioactive component of the extract of the *Nigella sativa*. Recent years have seen hundreds of research reports on its therapeutic biological effects as an anti-inflammatory, anti-diabetic, antihistamine, anti-cancer agent [219]. *N. sativa* is among the promising medicinal plants used nowadays to alleviate the symptoms of allergy and asthma, in which many of the reported pharmacological effects are due to TQ; the basic major active of the volatile oil [220]. TQ (2-isopropyl-5-methyl-1, 4-benzoquinone) is the initial component of the volatile oil of N. sativa seeds. A rich fraction of TQ (TQRF) and TQ in doses ranging from 0.5 to 1.5 g / kg and 20 to 100 mg / kg body weight respectively for 8 weeks showed a significant inhibitory activity against OH• development compared with untreated mice [221]. It inhibits cyclooxygenase and 5-lipooxygenase pathways of arachidonic acid metabolism [222]. Its effect as a bronchodilator was demonstrated by inhibition of the constituent dose of thromboxane B2 and leukotriene B4 [223]. TQ may have an anti-inflammatory effect during lung response by inhibiting prostaglandin D2 synthesis and TH2-induced immune response [224]. A few studies have also shown that TQ may have protective effects against lipid peroxidation process [208]. It has been reported that TQ stimulate relaxation in the guinea pig isolated trachea by prevent the effect of histamine and serotonin on smooth muscles [225]. The study suggested that the effect of TQ relaxation may be associated with the prevention of lipoxygenase products by blocking the non-selective receptors of serotonin and histamine, the findings of the study confirmed the traditional use of black seeds for asthma treatment. TQ also prevented Ca^{++} signals and narrowing of airway caused by acetylcholine chloride ACh in smooth muscle cells in the airway. The results also confirmed the use of TQ-containing plants to treat asthma [226]. Treatment of TQ decreased inflammatory and vascular response by inhibiting VEGF expression. The study suggested that TQ may be useful for the treatment of asthma by

modifying the inflammation [227]. Some anti-inflammatory properties of TQ may be related to adenosine receptors that are affected by TQ [228]. A study indicated that TQ suppressed IL-2 by more than 50%, reduced IL-6 by more than 50%, and incite a more than 50% decreasing in PGE2 in T-lymphocytes and monocytes [229]. The high capability and low systemic toxicity of TQ make it a promising alternative to ordinary therapeutic drugs [230].

2.14. *Ceratonia siliqua L.*

CS is an evergreen tree belongs to Leguminoseae (Fabaceae) family and Caesalpinaceae sub-family. It has brutal and sown species. The CS tree has been grown since ancient times in very countries of the Mediterranean basin and has a major economic and environmental rate [231]. CS bean is a generous resource of valued compounds like phenolic compounds, dietary fiber, minerals and d-pinitol. The chemical combination of the grain changes with genetic, ambient, harvesting factors and climatic [232]. CS fruit contains high amounts and types of food like dietary fiber, sugar, minerals and phenolic. Have 23-27% dietary fiber, 62-67% of total sugar, 4-6% protein [233]. CS contains several types of minerals like potassium (843-1215 mg / 100 g), magnesium (63-326 mg / 100 g), calcium (251-361 mg / 100 g), phosphorus (85-681 mg / 100 g) and has 3944.7 mg / kg of total phenol. It has been discovered that CS fruit contains 24 different phenolic compounds as well as the very common gallic acid [234].

2.14.1. CS bean products

CS powder (flour)

CS flour can be used as immunization spy for creates like pasta, tarhana and some diet products [235, 236]. CS flour is used as cacao substituent. CS flour does not contain theobromine and caffeine [237]. Rich in protein and low in calories, sensory properties are nice and suitable for people with gastrointestinal disease [238]. CS powder is an important source of vitamins E, D, C, Niacin, B6 and folic acid; vitamins A, B2 and B12 are provided at lower levels. CS powder oil consists of 17 fatty acids, mostly oleic acid, linoleic, nectaric and steric, 40.45%, 23.19%, 11.01% and 3.08%, respectively [239].

CS syrup

In Turkey CS syrup is called as “pekmez”. The syrup is rich in vitamins, polyphenols and minerals. Also, it provides high energy for people.

Locust bean gum

Locust bean gum is widely used as an addition in the food production. The major characterist of this mastic is the performance of a lofty thickness gel construction in a wide row of pH. It used in several types of foods as thickener and stabilizer [240].

Dietary fiber

CS fiber is the major secondary product to create CS syrup. It is mostly consisting of unsolvable fibers. The digestion of these fibers is passing slowly [241]. CS fibers have great potential to produce dietary supplements and functional foods [242].

D- pinitol

D-Pinitol can imitative the ability of insulin for lowering and balancing the blood sugar in diabetes type 2 patients [243]. Diabetes and the relationship of d-pinitol are an important topic for the world and there are different studies on this subject [244].

Many studies have shown that CS and its products can promote human health and help prevent specific chronic diseases. In particular, they show antiproliferative and apoptotic activity against cancer cells, they are suggested to treat diarrhea symptoms, and they present antihyperlipidemia and antidiabetic effects due to high antioxidants, polyphenols and high content in fibers of carob and its products [245].

3. MATERIALS AND METHODS

3.1. Chemicals

Thiobarbituric acid (TBA), butylated hydroxytoluene (BHT), trichloroacetic acid (TCA), ethylenediaminetetraacetic acid (EDTA), met phosphoric acid, 5,5'dithiobis-(2nitrobenzoic acid) (DTNB), trihydroxymethyl aminomethane (Tris), Sodium hydrogen phosphate(NA_2HPO_4), Sodium phosphate($NAHPO_4$), Sodium citrate ($NA_3C_6H_5O_7$), potassium dihydrogen phosphate (KH_2PO_4), Potassium phosphate dibasic (K2HPO4), N-(1-Naphthyl)ethylene diamine dihydrochloride (NEDD), Sulfanilamide (SULF), Phosphoric acid (H_3PO_4), Hydrochloric acid (HCL), Vanadium(III)chloride (VCL_3), Zinc sulfate ($ZNSO_4$), Nitrite(NO_2-),Sodium dodecyl sulfate (SDS), Perchloric acid ($HCLO_4$), Ascorbic acid, Cupric sulfate ($CUSO_4$), Thiourea(CH_4N_2S), 2,4-Dinitrophenylhydrazine (NDPH), 1,1,3,3- Tetramethoxypropane (TEP), ethanol, potassium chloride (KCL), sulfuric acid(H_2SO_4), TQ (2-isopropyl-5-methyl-1, 4-benzoquinone), OVA and aluminum hydroxide of technical grade used in this study were supplied by Sigma Chemical Co. (St. Louis, MO, USA). Kits for the analysis of cytokines (IL-13, IL-1β) were provided by Sunredbio Ref DEE201120099 and Diasource ImmunoAssays Ref KAP1211 S. A. 2. Rue du Bosquet B-1348 Lovain-La-Neuve Belgium. Growth factors (TNF-α and VEGF) were provided by DIAsource ImmunoAssays Ref KAP1751 S. A. 2. Rue du Bosquet B-1348 Lovain-La-Neuve Belgium. CS powder from herbalist and sodium chloride (NaCl) used in the present stud were obtained from a pharmacy in Ankara.

3.2. Animals

Female rats (Wistar albino) in the weight range of 200-250 g were provided from the Experimental Animal Research Center, Gazi University (GÜDAM, Ankara, Turkey), and were housed in three groups, each group containing six rats. The animals were housed at 20 ± 2°C with 12:12 h reverse light/dark cycle and given free access to standard laboratory chow for rodent with water in stainless cages and received humane care according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals.

3.3. Ethical Approval

All procedures in this study were in accordance with the Guide for the Care and Use of Laboratory Animals. This study was approved by Ethics Committee of the Gazi University of science faculty (Code No: G. U. ET-16. 035).

3.4. Experimental Design

The rats were at random disunited into three groups every having six rats. Group I (asthmatic pregnant): they were induced to asthma by OVA. Group II (asthmatic pregnant with TQ and CS treated): the asthmatic rats received 10mg\kg\day each of TQ and CS once a day during last 5 days of pregnancy. Group III (Asthmatic pregnant with Dexamethasone treated): the rats received 1mg\kg\day Dexamethasone [246] once a day during last 5 days of pregnancy.

TQ was dissolved in normal saline (PF %0, 9 isotonic) 10mg\kg [247] using water bath kept at 60°C and the solution was prepared fresh just before gavage administration. CS was dissolved in normal saline (PF %0, 9 isotonic) at room temperature.

3.5. Sensitization and Inhalational Exposure

Groups of OVA were sensitized to ovalbumin (grade V, ref. A5503-1G, Sigma Aldrich), in accordance with the procedures of Moura et al. [248] and Yang et al. [249]. They were effective sensitized by intraperitoneally injections (i.p.) of OVA (1mg/ml normal saline) with alum (1mg/ml normal saline) (Reagent grade, 239186-25G, Sigma Aldrich) that an adjuvant on days 0 and 14. Rats were challenged for 30 min with inhalation of OVA by a nebulizer (Handyneb, SN. NGM 769576) coupled to a plastic box on the days 21, 22 and 23. After the last inhalation of OVA rats were coupled with male rats (1 male each of 3 females). After proving rat's pregnancy by the veterinarian (between 11-13 pregnant days), they were attacked of asthma by a second doses of OVA (5mg/mL) inhalation for 3 days. Then in last 5 days of pregnancy rats in group II were received mix of TQ (274666-5G, Sigma Aldrich) and SC solutions by intragastric gavage and rats in group III were received dexamethasone doses by i.p injection. All pregnant rats from each group were sacrificed 24h after the final doses of treatments (in the last 1-2 days to birth) then the blood samples

of pregnant rats were collected, and lungs of pregnant rats and heir embryos were collected.

3.6. Preparation of Blood Samples and Lung Homogenates:

All rats were anaesthetized by an injection of ketamine (40 mg/kg) and xylazine (2 mg/kg) intramuscularly. Blood samples were obtained from posterior vena puncture in an anesthetized rat.

3.6.1. Blood samples

Blood samples (3 ml) were put into disposable glass tubes with clot activator. Samples were left to clot at room temperature then centrifuged at 3000 rpm for 5 min for serum separation. Serum samples were stored at -30ºC for analysis of MDA, RSH, AA, IL-1β, IL-13, and TNF-α level. For differential WBC count 2 ml of blood sample was collected into the test tube containing anticoagulant EDTA for counting differential WBC. Differential cell counts were done on a thin slide prepared with a smearing blood sample, using Leishman's stain. According to staining and morphological criteria, differential cell analysis was carried out under a light microscope by counting 100 cells, and the percentage of each cell type was calculated.

3.6.2. Lung homogenates

The lung of pregnant rats was dissected then washed in 0.9% NaCl and one lung was used for histological examination that preserved in 10% neutral buffered formalin and the other lung placed on an ice-cold plate then immediately frozen in liquid nitrogen until they were homogenized. The lungs of embryos were fixed in normal saline 0.9% for histological and immunohistochemically study.

3.7. Histological Study

Lung samples were dissected immediately, well washed with saline and in 10% neutral-buffered formalin were fixed for 72 h at least. For half an hour all the samples were washed in tap water, in ascending grades of alcohol (absolute 70% - 90% - 95%) were dehydrated, cleared in xylene and then in paraffin wax were embedded. For light

microscopic examination, serial sections of 6 μm thick were cut and stained with hematoxylin and eosin (H&E). The sections were viewed and photographed by Olympus BX51 microscope (Olympus Optical Co. Ltd, Tokyo, Japan) [248].

3.8. Immunohistochemically Method

An avidin-biotin-complex (ABC) for formalin-fixed paraffin-embedded tissues immunohistochemical staining method was used [249]. From each paraffin block, successive sections of 5 μm of tissue were cut, then on glass slides prepared and desiccated. In xylene, tissues were deparaffinized then in graded alcohol solutions rehydrated. For blocking the action of endogenous peroxidase, 3% hydrogen peroxide in pure methanol was used for 20 min at room temperature. In a microwave the tissues were vaporized for 10 min using 0.01 M sodium citrate solution at pH 6.8 for retrieve the antigen. For 10 min at room temperature, Non-specific protein linking was saturated using 4% horse serum (Invitrogen, Burlington, Ontario) in phosphate buffered saline (PBS). By application the diluted primer VEGF to tissue segments, the slides incubated 1 hour in room temperature. In accordance with the manufacturer's directions (Vector Laboratories; Vecastain Elite ABC kit) reagent (ABC) was made and applied for 30 min in room temperature. Coloration improved by applying diaminobenzinetetrahydrochloride (DAB) reagent (Sigma Aldrich) for 10 min. For counterstained the tissues, hematoxylin was applied, then in graded alcohol dehydrated and affixed with cover glass. Semi quantitative method was used. 0: no staining, 1: low staining, 2: moderate staining, 3: severe staining.

3.9. Determination of IL-1β, IL-13 and TNF-α

Enzyme-linked immunosorbent assays (ELISAs) were performed according to the manufacturer's directions. IL-1β contained in serum was measured using a specific rat IL-1β ELISA kit (DIAsource ImmunoAssays S. A. 2. Rue du Bosquet B-1348 Lovain-La-Neuve Belgium). IL-13 contained in serum was measured using a specific rat IL-13 ELISA kit (Sunredbio Ref DEE201120099). TNF-α contained in serum was measured using a specific rat TNF-α ELISA kit (Diasource Ref KAP1751). The concentration of IL-1β, IL-13 and TNF- α is determined by comparison to the standard curve and is expressed as pg/ml.

3.10. Biochemical Analyses:

3.10.1. Determination of thiobarbituric acid reactive substances (TBARS) levels

The samples were prepared according to the method described by Kurtel et al. to determine the plasma MDA levels as a lipid peroxidation indicator. In brief, 0.5 ml aliquots were added to 1 ml of a solution containing 15% TCA, 0.375% TBA, and 0.25 NHCl. Protein precipitant was removed by centrifugation at 10.000 g for 5 min, and the supernatants were transferred to glass test tubes containing 0.02% BHT to prevent further peroxidation of lipids during subsequent steps. The samples were then heated for 15 min at 100ºC in a boiling water bath, cooled, and centrifuged to remove precipitant. The absorbance of each sample was determined at 532 nm [250].

3.10.2. Determination of sulfhydryl RSH levels

Plasma RSH was determined by spectrophotometric method. In brief, 0.5 ml aliquots of each samples were mixed with 1 ml of a solution containing 100 mM Tris-HCl (pH:8.2), 1% SDS, and 2 mM EDTA. The mixture was incubated for 5 min at 25ºC and centrifugated at 10.000 g for 5 min. DTNB (10 mM) was then added to each reaction volume and incubated for 15 min at 37ºC to allow for the formation of TNB. The absorbance of each sample was determined at 412 nm. GSH content was calculated assuming a molar extinction coefficient of 13.000 at 412 nm for TNB [251].

3.10.3. Determination of NOx levels

The NOx values were given by the sum of nitrite and nitrate, which are the stable end products of NO. NOx levels in tissue were determined by the Griess reaction. Briefly, tissue samples were homogenized in five volumes of phosphate buffer (pH = 7.5) and centrifuged at 3000 xg for 15 min. To supernatants (0.5 ml) 0.25 ml of 0.3 M NaOH were added. After incubation for 5 min at room temperature, 0.25ml of 10% ZnSO4 was added for deproteinization. This mixture was then centrifuged at 14.000 g for 5 min and then the supernatants were used for the Griess assay [252]. Nitrate levels in tissue homogenates were determined spectrophotometrically, based on the reduction nitrate to nitrite by $VaCl_3$.

3.10.4. Determination of ascorbic acid (AA)

Roe and Kuther's method modified by Berger was used [254]. Lung tissues were homogenized in mixture of ice-cold PCA / EDTA. Then homogenate was centrifuged at 15000 g (RCF) 4°C for 3 min. One tube was placed as a standard AA solution, another tube was blunted with PCA solution and the samples were prepared in the supernatant tubes. Each tube was incubated for 3 hours at 37 ° C by adding color reagent and vortexed. The temperature of the samples was brought to 0 ° C by adding H2SO4 to each tube and mixed and waited 30 minutes at room temperature. Samples were read at 520 nm against a blank. Tissue A.A levels were calculated as μg per ml tissue.

3.10.5. Determination of glutathione (GSH)

Modified Elman method for GSH determination in tissue was used. Tissue samples were homogenized in 0.15 M cold KCl then the mixture of 0.5 mL meta-phosphoric acid / EDTA / NaCl was added to homogenate for deproteinization. After 20 minutes of centrifugation at 4000 rpm at 4°C, 2 ml of 0.3M Na2Hpo4 and 0.2 ml solution of DTNB (0.4 mg / ml 1% sodium citrate) were added to 0.5 ml of supernatant. The optical density of all samples was read on the spectrophotometer at 412 nm against a blank. Tissue GSH levels were calculated as μmol per gr tissue [251].

3.11. Statistical Analysis:

Submits of data was performed by means (SD) and ranges (minimum-maximum) between-group comparisons were made using Tukey. A statistically significant was counted by value of $P < 0.05$ and these statistical analyses was made by using version 11 of the SPSS software package (SPSS Inc, USA).

4. RESULTS

4.1. Histological Results of Lung Tissues for Pregnant Rats

When the general histological structure of the groups was evaluated, group I increased inflammatory cell and eosinophils infiltration around the bronchi and bronchiole walls, increased subepithelial smooth muscle thickness and epithelial cell lengths was noticed. In group II decreased inflammatory cell infiltration, subepithelial smooth muscle and epithelial cell lengths were normal. The administration of dexamethasone revealed a very mild regulation in all the histological finding associated with asthma (Figure 4.1. A, B and C) (Table 4.1).

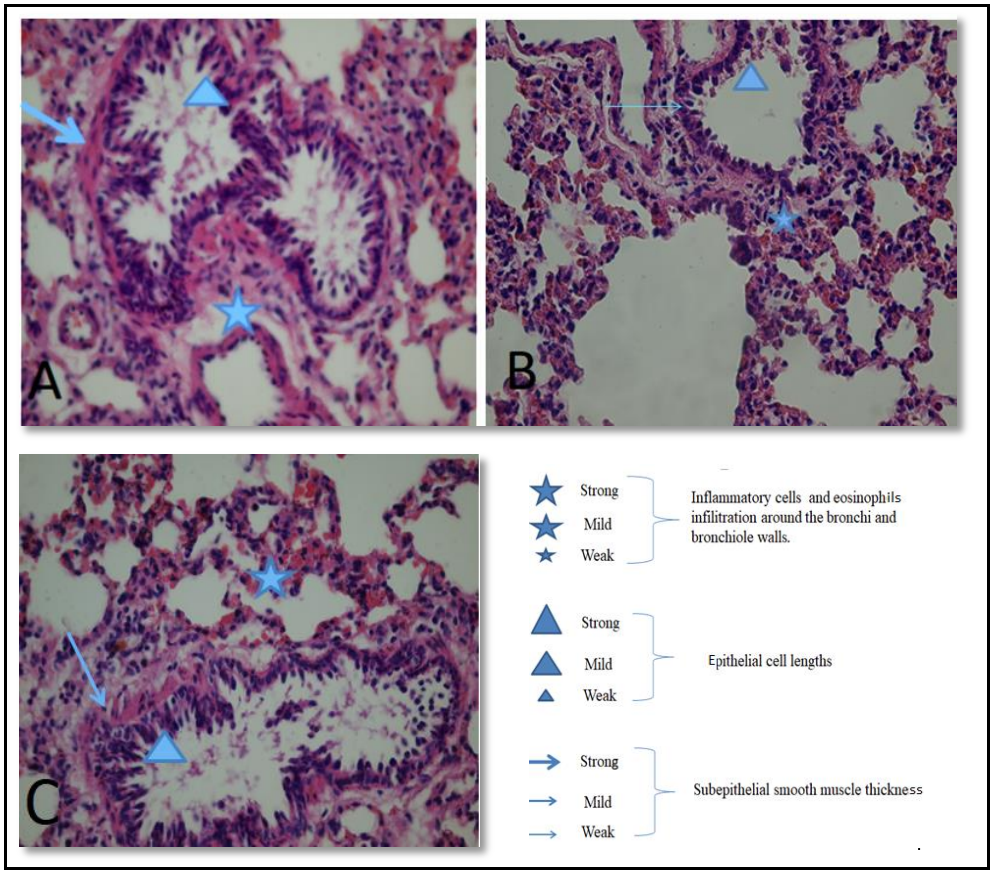


Figure 4.1. (A) H&E Lung sections of asthmatic pregnant rat. Increased inflammatory cell and eosinophils infiltration around the bronchi and bronchiole walls (★),subepithelial smooth muscle thickness (⟶) and epithelial cell lengths (▲) ×40, H&E. (B) Lung sections of asthmatic pregnant rat with TQ and CS. Inflammatory cell infiltration (★), subepithelial smooth muscle thickness (⟶) and epithelial cell lengths (▲) were normal. ×40, H&E. (C) Lung sections of asthmatıc pregnant rat with Dexamethasone. Very mild regulation in all of the histological finding associated with asthma (★, ⟶ , ▲) ×40, H&E.

Table 4.1. H & E histological semiquantitative evaluation in lung tissue.

Groups	Inflammatory cell and eosinophils infiltration around the bronchi and bronchiole walls	Subepithelial smooth muscle thickness	Epithelial cell lengths
Asthmatic pregnant (I)	+++*	+++*	+++*
Asthmatic pregnant with TQ & CS (II)	+**	+**	+**
Asthmatic pregnant with Dexamethasone (III)	++**	++**	++**

*: Significant increase, **: Significant decrease ($p < 0, 05$).

4.2. Histological Results of Lung Tissues for Embryos

When the general histological structure of the groups was evaluated, in group I increased inflammatory cell and eosinophils infiltration around the bronchi and bronchiole walls, increased subepithelial smooth muscle thickness and epithelial cell lengths was noticed. In group II decreased inflammatory cell infiltration, subepithelial smooth muscle and epithelial cell lengths were determined. The administration of dexamethasone revealed a similar finding in all of the histological finding associated with group II (Figure 4.2. A, B and C) (Table 4.2).

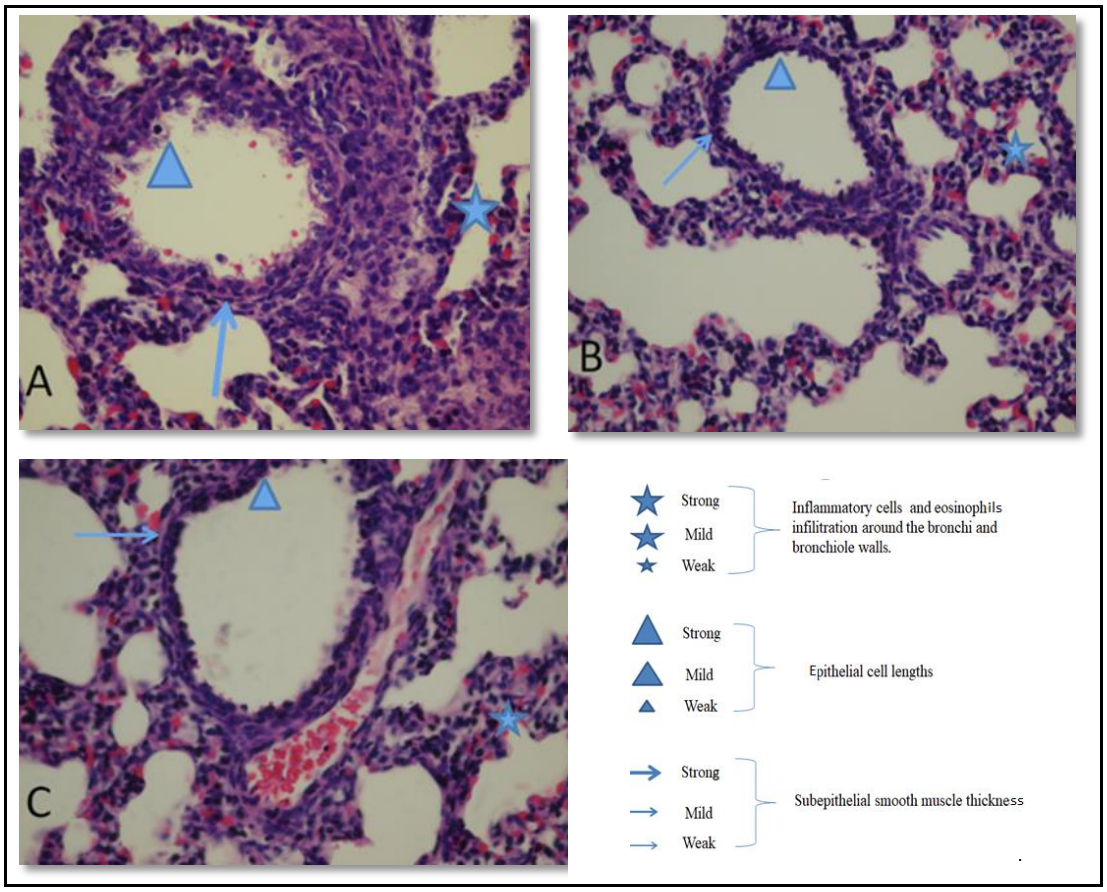


Figure 4.2. (A) H&E Lung sections of embryo from asthmatic pregnant rat. Increased inflammatory cell and eosinophils infiltration around the bronchi and bronchiole walls ( ),subepithelial smooth muscle thickness( ) and epithelial cell lengths ( ) ×40, H&E. (B) Lung sections of embryo from asthmatic pregnant rat with TQ and CS. Inflammatory cell infiltration ( ), subepithelial smooth muscle thickness ( ) and epithelial cell lengths ( ) were normal. ×40, H&E. (C) Lung sections of embryo from asthmatic pregnant rat with Dexamethasone. A similar regulation in all of the histological finding associated with asthma (, ,) ×40, H&E.

Table 4.2. H & E histological semiquantitative evaluation in lung tissue of embryos.

Groups	Inflammatory cell and eosinophils infiltration around the bronchi and bronchiole walls	Subepithelial smooth muscle thickness	Epithelial cell lengths
Embryo from asthmatic pregnant (I)	+++*	+++*	+++*
Embryo from asthmatic pregnant with TQ & CS (II)	+**	+**	+**
Embryo from asthmatic pregnant with Dexamethasone (III)	+**	+**	+**

* Significant increase, ** Significant decrease (p < 0, 05).

4.3. VEGF Immunohistochemistry for Pregnant Rats

Subendothelial VEGF staining significantly increased in group I when compared both of group II and group III (p <0, 05). In group II subendothelial VEGF staining significantly decreased when compared to I and dexamathasone group (p <0, 05). When compared to group II and Group III, decreased subendothelial VEGF staining found in the co-administration of TQ and CS group (Figure 4.3) (Table 4.3).

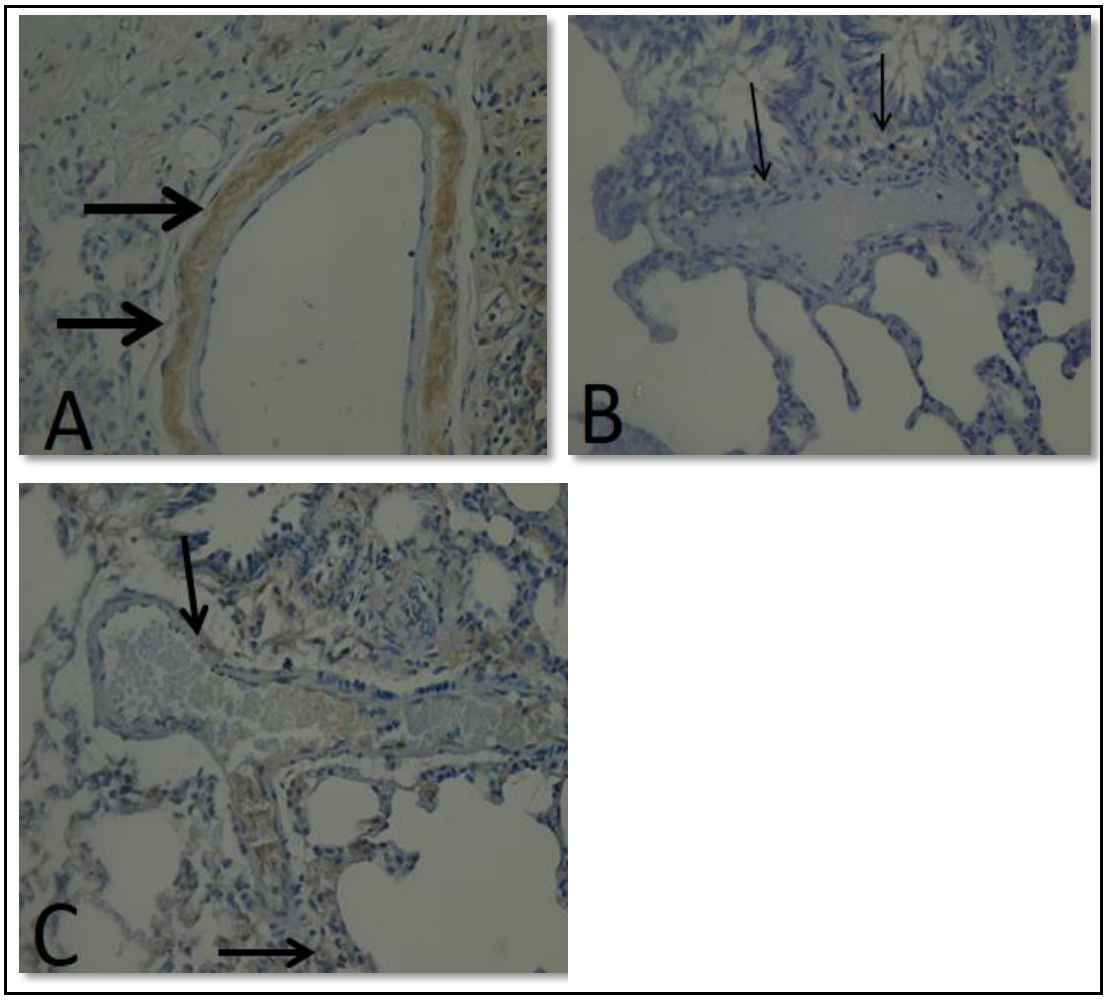


Figure 4.3. (A) VEGF Lung sections of asthmatic pregnant rat. Subendothelial VEGF levels increased (⟶) X40, VEGF Immunohistochemical staining. (B) Lung sections of asthmatic pregnant rat with TQ and CS. Subendothelial VEGF levels significantly decreased (⟶) ×40, VEGF Immunohistochemical staining. (C) Lung sections of asthmatic pregnant rat with dexamethasone. Subendothelial VEGF levels decreased (⟶) ×40, VEGF Immunohistochemical staining.

Table 4.3. Immunohistochemistry of subendothelial VEGF in lung tissue of pregnant rats.

Groups	Asthmatic pregnant (I)	Asthmatic pregnant with TQ&CS (II)	Asthmatic pregnant with Dexamethasone (III)
Subendothelial VEGF	+++*	+**	++**

* Significant increase, **Significant decrease (p < 0, 05)

4.4 VEGF Immunohistochemistry for Embryos

Subendothelial VEGF staining significantly increased in group I when compared both of group II and group III (p <0, 05). When compared group II and group III, decreased subendothelial VEGF staining found in dexmethasone group more than in the co-administration of TQ and CS group (p <0, 05) (Figure 4.4) (Table4.4).

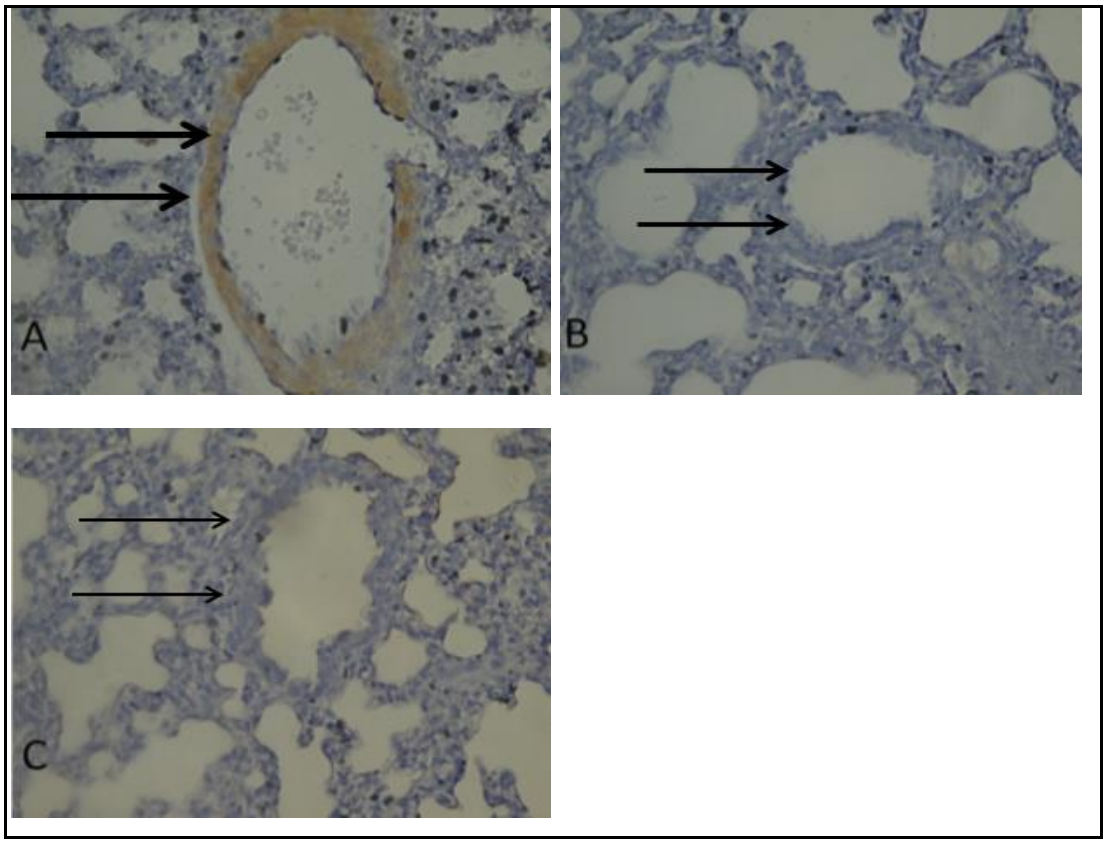


Figure 4.4. (A) VEGF Lung sections of embryos. Subendothelial VEGF levels increased (⟶) ×40, VEGF Immunohistochemical staining. (B) Lung sections of embryos with TQ and CS. Subendothelial VEGF levels decreased (⟶) ×40, VEGF Immunohistochemical staining. (C) Lung sections of embryos with dexamethasone. Subendothelial VEGF levels significantly decreased (⟶) ×40, VEGF Immunohistochemical staining.

Table 4.4. Immunohistochemistry of subendothelial VEGF in lung tissue of embryos.

Groups	Embryo from asthmatic pregnant (I)	Embryo from asthmatic pregnant with TQ&CS (II)	Embryo from asthmatic pregnant with Dexamethasone (III)
Subendothelial VEGF	+++*	++**	+**

*: Significant increase, **: Significant decrease (p < 0, 05)

4.5. Biochemical Results Content of the Lung Tissue of Pregnant Rats

The study results showed that the MDA levels decreased in group II when compared to group I and III (p < 0, 05). However, NO levels were increased in group II when compared to group I and III (p < 0, 05). With parallel lung tissue NO levels, the co-administration of TQ and CS was an increased AA level in group II when compared to group I and III (p < 0, 05). The GSH levels increased in Dexamethasone group when compared to group I and II (p < 0, 05) (Table 4.5).

Table 4.5. Biochemical results in the lung tissues of pregnant rats.

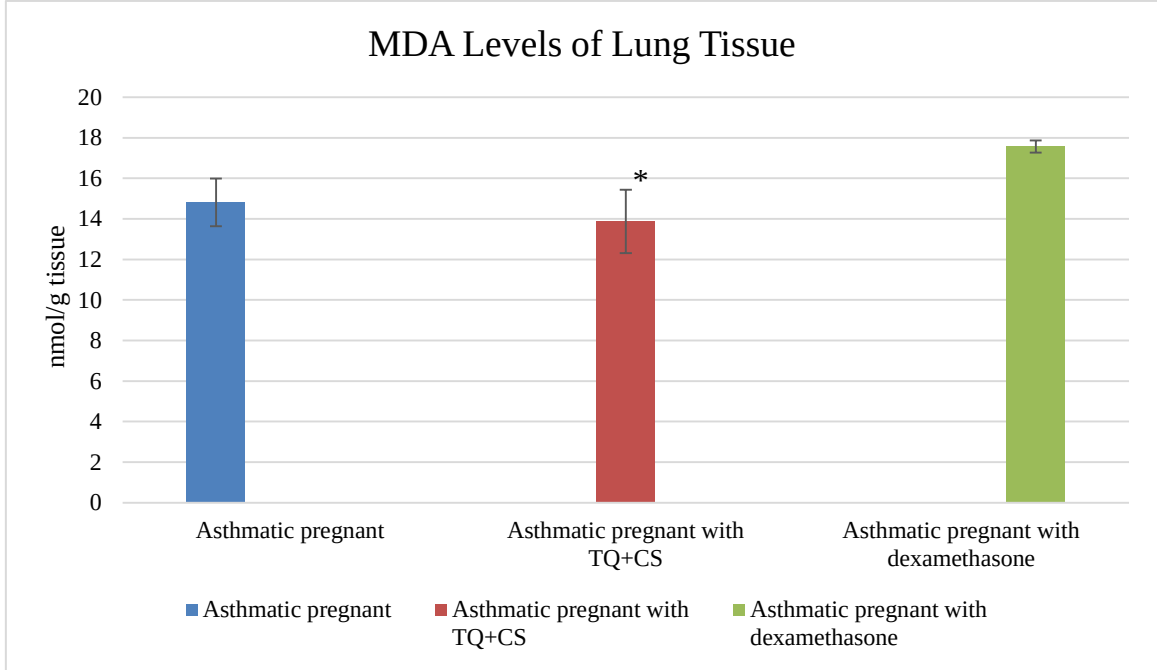
Groups	MDA levels (nmol/g tissue)	NO levels (nmol/g tissue)	GSH levels (µmol/g tissue)	AA levels (µmol/g tissue)
Asthmatic pregnant (I)	14,81 ± 1,18	19,80 ± 3,09	0,88 ± 0,18	16,71 ± 0,83
Asthmatic pregnant with TQ + CS (II)	13,87 ± 1,56*	36,36 ± 1,27[a]	1,01 ± 0,23	20,59±0,91[a]
Asthmatic pregnant with dexamethasone (III)	17,56 ± 0,30	18,40 ± 1,65	3,99 ± 0,77[b]	14,64 ± 0,85

Each value is the mean ± SD of 6 animals per group

* P < 0,05 when compared to group I and III,

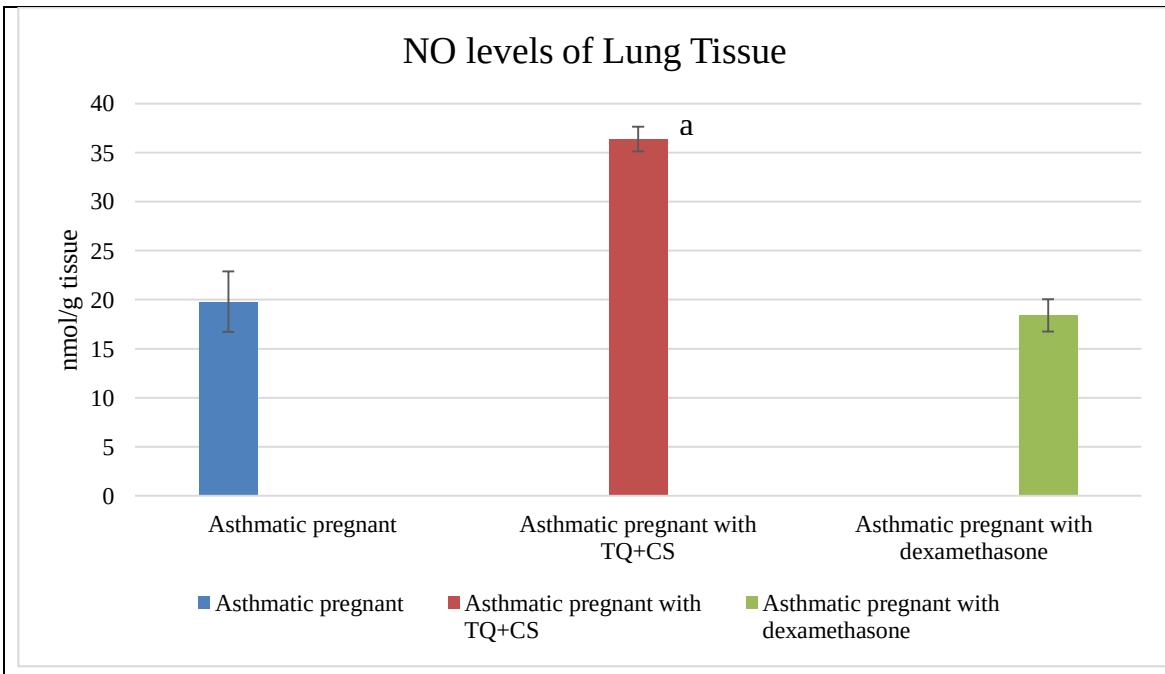
[a] P < 0,05 when compared to group I and III,

[b] P < 0,05 when compared to group I and II.



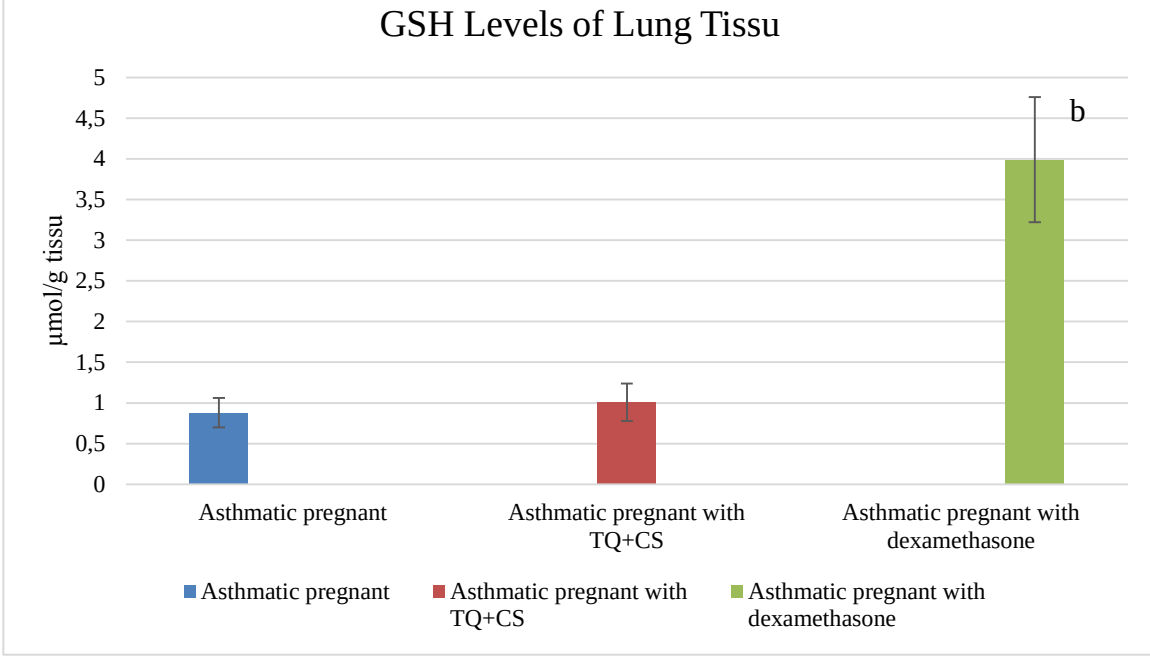
*P <0,05 when compared to group I and III

Figure 4.5. MDA levels of lung tissue



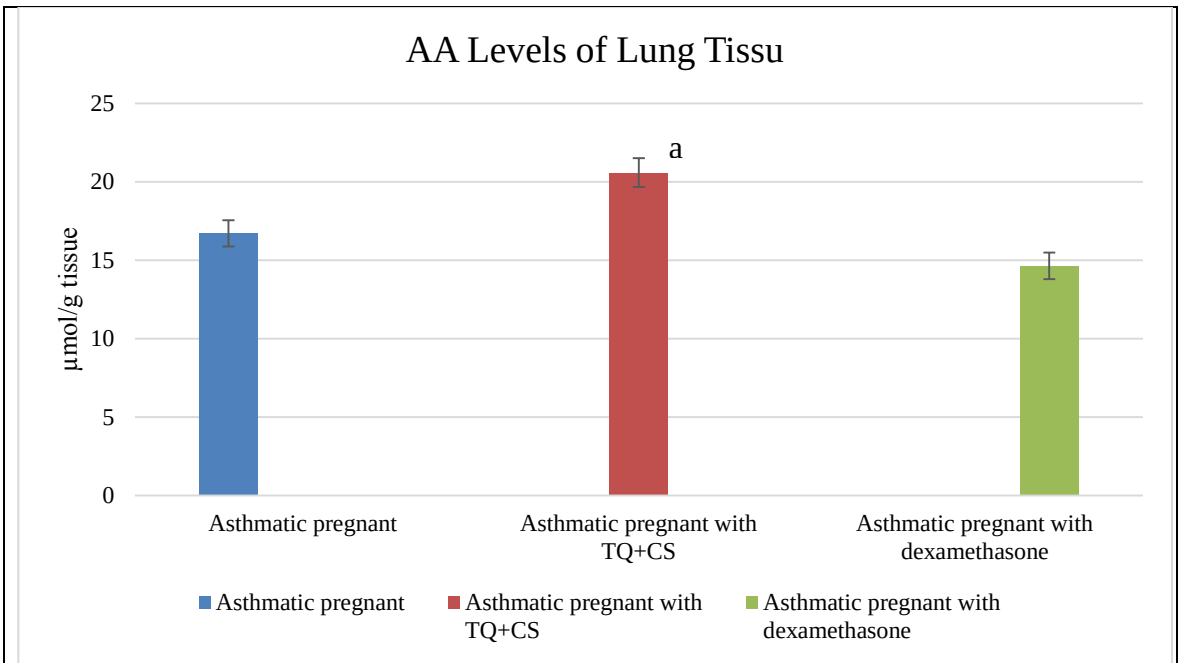
[a]P < 0,05 when compared to group I and III

Figure 4.6. NO levels of lung tissue



[b]P < 0,05 when compared to group I and II

Figure 4.7. GSH levels of lung tissue



[a]P < 0,05 when compared to group I and III

Figure 4.8. AA levels of lung tissue

4.6 TNF-α and Cytokines Results Content in Serum of Pregnant Rats

The results showed that there is no statistically signifcant alteration among groups in terms of TNF-α levels except the levels of IL-1β and IL-13. Both levels of IL-1β and IL-13 were significantly decreased in co-administration of TQ and CS group in the serum of rats when compared to I and III groups (p <0, 05) (Table4. 6).

Table 4.6. TNF- α, IL-1 β and IL-13 levels in serum of pregnant rats.

Groups	TNF-α levels (pg/ml)	IL-1β levels (pg/ml)	IL-13 levels (pg/ml)
Asthmatic pregnant (I)	1,95 ±0,66	51,99 ± 2,02	58,39 ± 0,84
Asthmatic pregnant with TQ + CS (II)	1,86 ± 1,18	34,59 ± 2,33*	46,98 ± 0,15*
Asthmatic pregnant with dexamethasone (III)	3,58 ± 1,77	53,24 ± 1,85	55,52 ± 0,08

*p < 0, 05 when compared to I and III groups

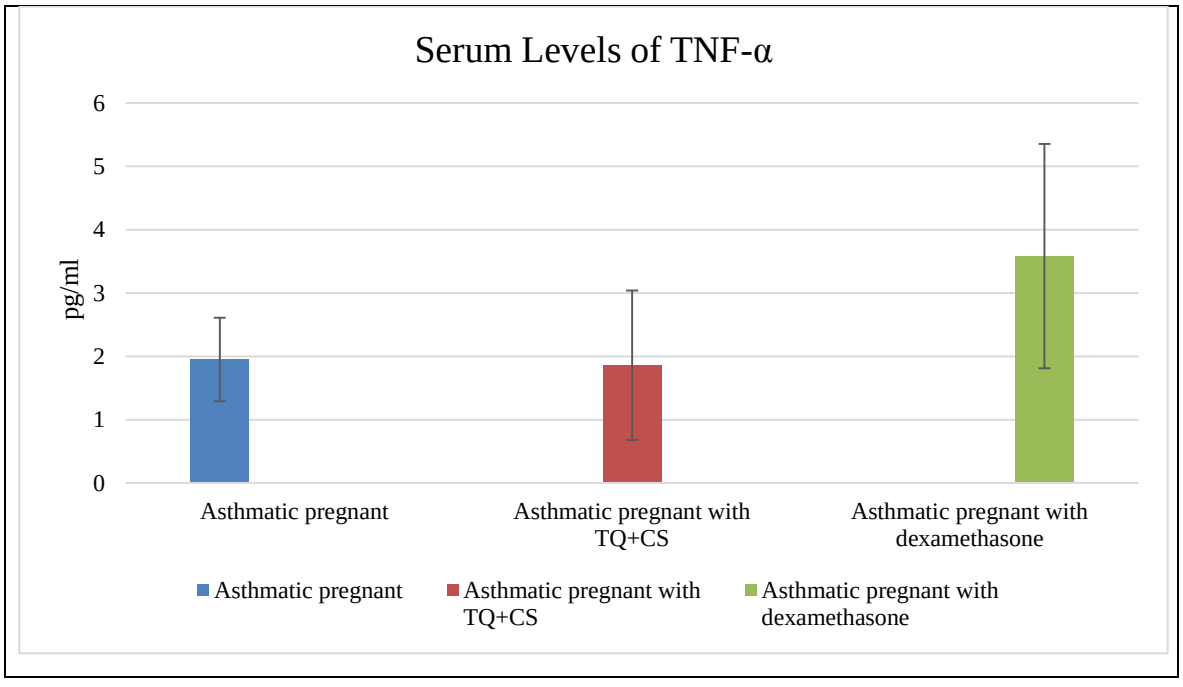


Figure 4.9. Serum levels of TNF-α.

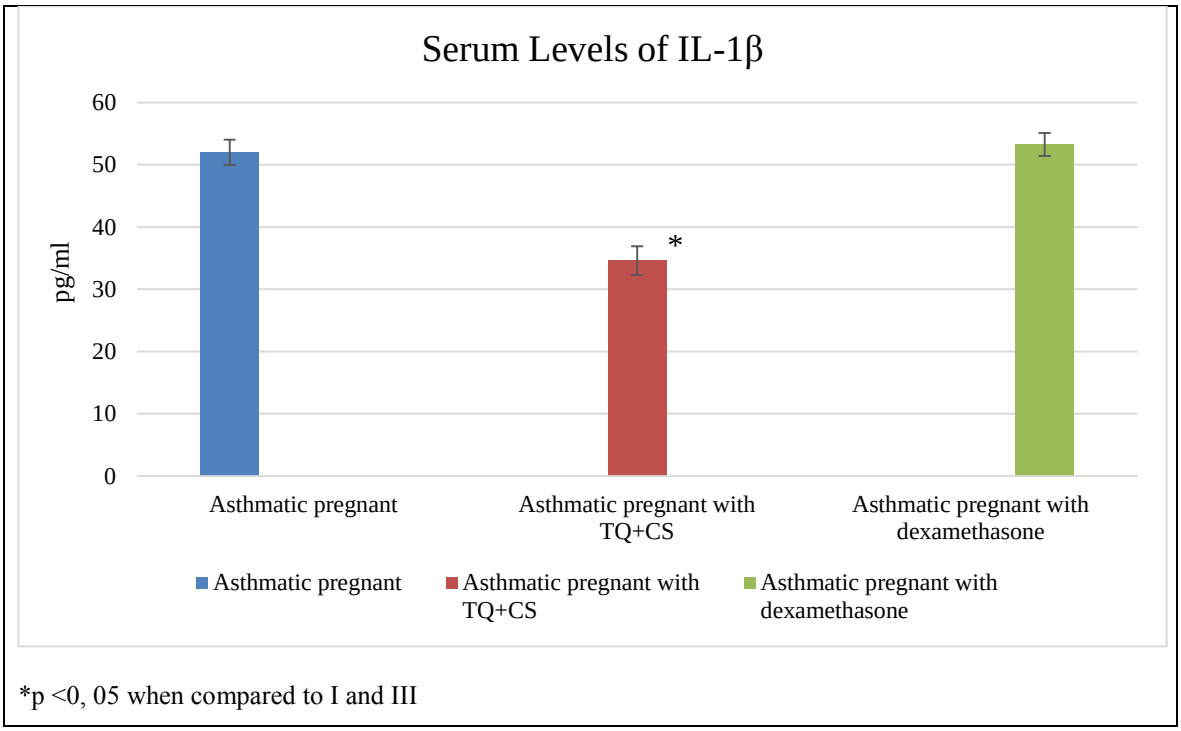


Figure 4.10. Serum levels of IL-1β.

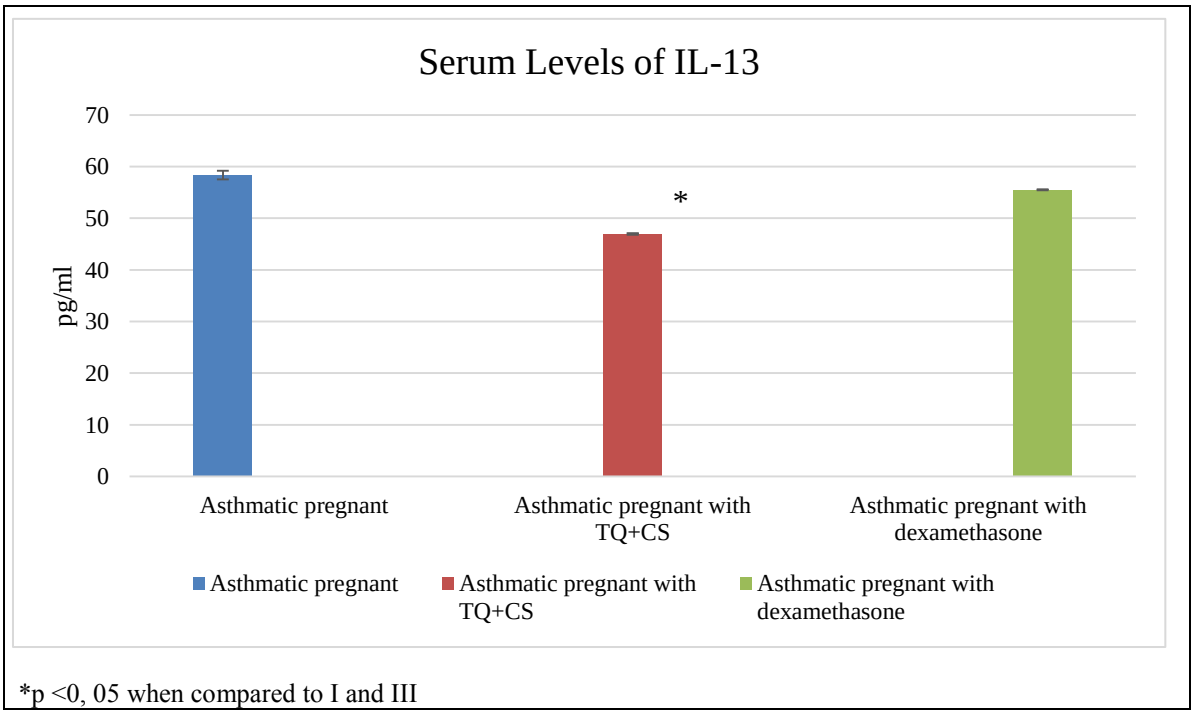


Figure 4.11. Serum levels of IL-13

4.7. Biochemical Results Content of Serum of Pregnant Rats

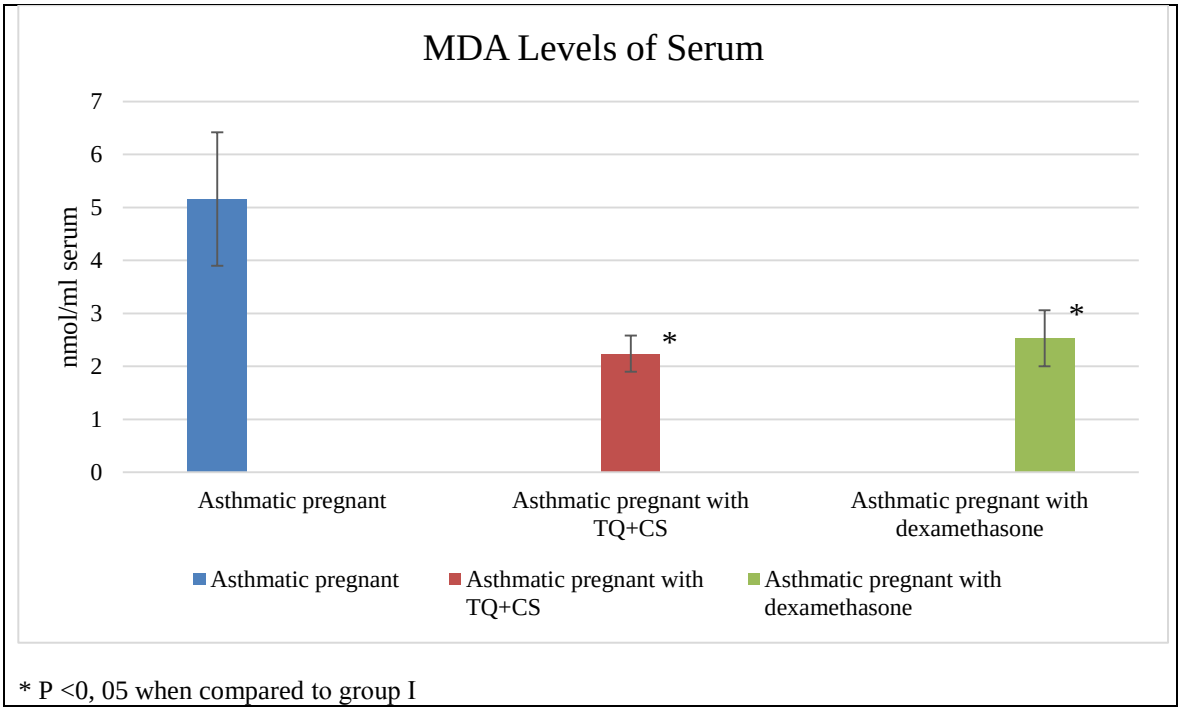
The study results showed that the MDA levels decreased in group II and III when compared to I group (p < 0, 05). There is no significant difference between groups in the terms of RSH levels (p > 0, 05), but the co-administration of TQ and CS increased the RSH levels.The AA levels in the serum of pregnant rats were not change between all groups (p > 0, 05) (Table4.7).

Table 4.7. Biochemical results in the serum of pregnant rats.

Groups	MDA levels (nmol/ml serum)	RSH levels (nmol/ml serum)	AA levels (nmol/ml serum)
Asthmatic pregnant (I)	5,16 ± 1,26	357,26 ± 97,85	2,03±0,23
Asthmatic pregnant with TQ + CS (II)	2,24 ± 0,34*	467,93 ± 82,23	1,90 ± 0,19
Asthmatic pregnant with dexamethasone (III)	2,53 ± 0,53*	407,31 ± 59,27	3,30 ± 0,21

Each value is the mean ± SD of 6 animals per group

* P < 0, 05 when compared to group I



* P <0, 05 when compared to group I

Figure 4.12. MDA levels of serum.

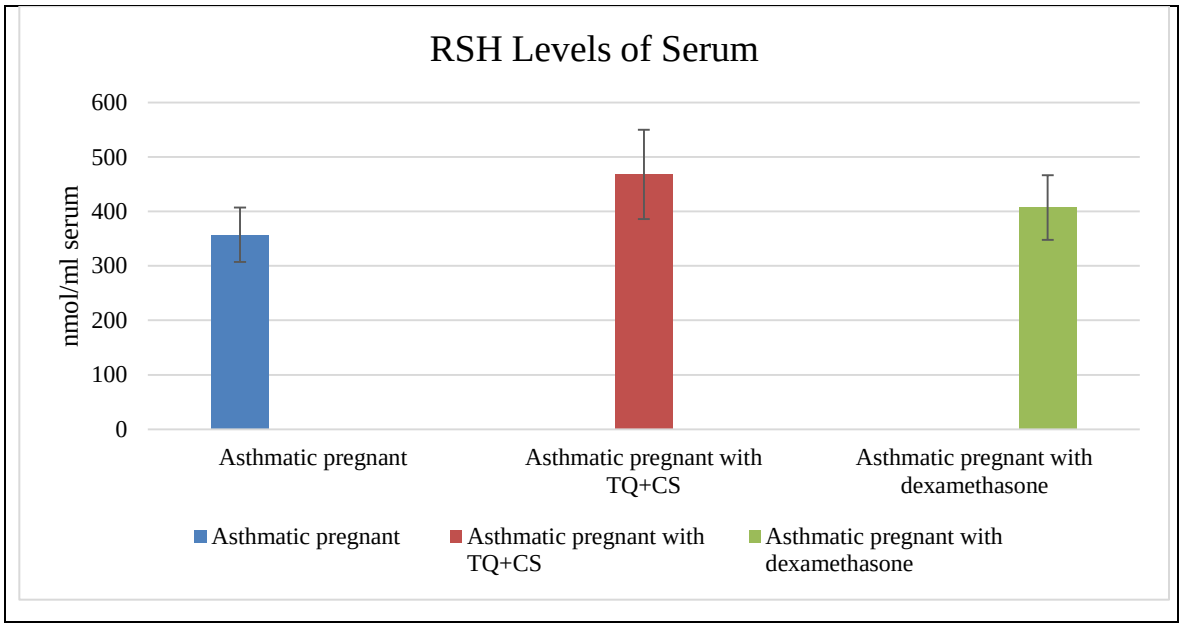


Figure 4.13. RSH levels of serum.

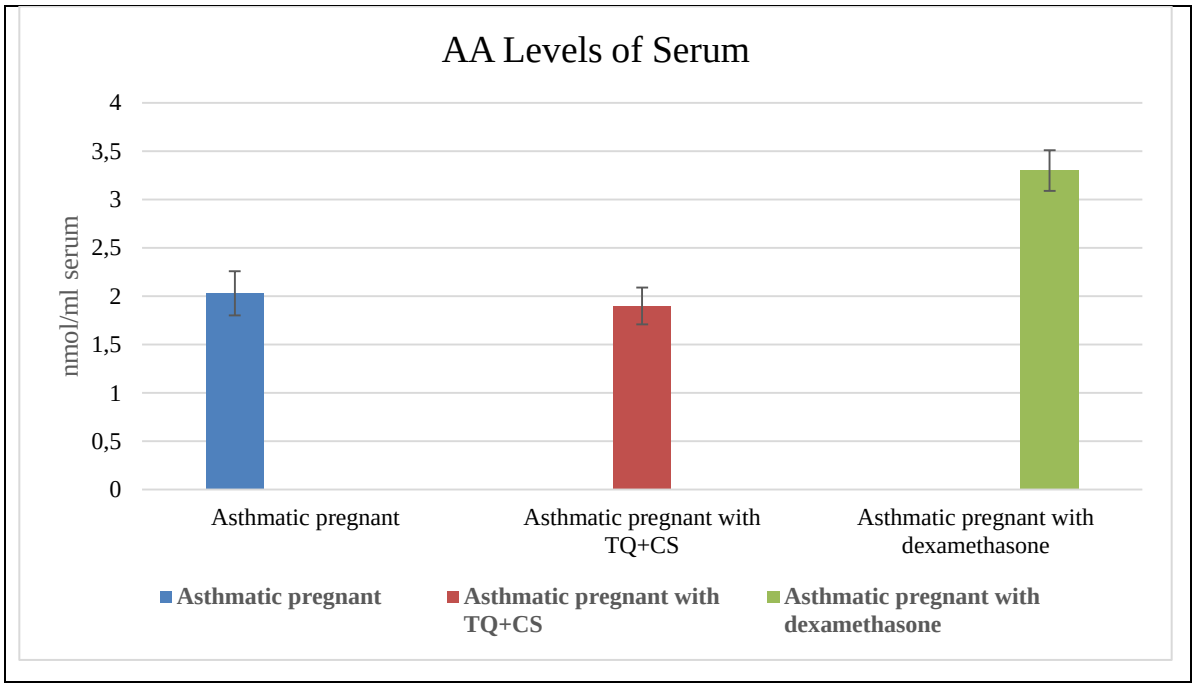


Figure 4.14. AA levels of serum

4.8. The Result of Differential WBC Count in the Serum of Pregnant Rats

The WBC differential count in the serum of pregnant rats showed that there is no significant difference between groups in differential Monocytes, while the differential of Lymphocytes was significant decreased and Neutrophils was significant increased in

dexamethasone group when compared both I and II groups (p < 0, 05). The differential Eosinophils was significant decreased in co –administration of TQ and CS group when compared to I and III group (p < 0, 05) (Table 4.8).

Table 4.8. Differential WBC count in the serum of pregnant rats

Groups	Monocytes percentage%	Lymphocytes percentage%	Neutrophils percentage%	Eosinophils percentage%
Asthmatic pregnant rat (I)	4,83 ± 1,77	71 ± 6,32	18,5 ± 6, 10	5,5 ± 3,20
Asthmatic pregnant rat with TQ and CS (II)	3,5 ± 1,89	68 ± 11,87	25,83 ± 10,67	2 ± 1*
Asthmatic pregnant rat with dexamethasone (III)	4,67 ± 2,42	42,33 ± 19,73[a]	45,67 ± 19,11[a]	5 ± 1,91

* P < 0, 05 when compared to I and III groups.

[a] p < 0, 05 when compared to I and II groups

5. DISCUSSION

Di Cosmo et al [255] showed that OVA treatment of animals significantly participated in the bronchial constriction and damage of lung tissue. Wills-Karp et al [256] were noticed that OVA sensitization of animals led to significant infiltration of inflammatory cells into the lung as observed by bronchoalveolar lavage and histological examination. In the study of Serap CM et al [257] inflammatory cell infiltration and epithelial damage were observed in the lung tissues of asthmatic mice.

Similar findings were found in our study in accordance with the above studies. The above mentioned studies support our work. In present study, OVA – induced asthma in group I, we found significantly increased the inflammatory cell and eosinophils infiltration around the bronchi and bronchiole walls, increased subepithelial smooth muscle thickness and epithelial cell lengths ($p < 0,05$). In group II the histological changes in group I were significantly decreased ($p < 0,05$). This study is quite remarkable in that it is the first study showing the effects of the combined use of TQ and CS on pregnant rats. However, only use of TQ has been shown similar results in the lungs of asthmatic mice [257, 258]; to date, there is no study showed the effective role of CS in the alleviation or treatment of asthma.

In group III the use of dexamethasone as a treatment of asthma was significant decreased the inflammatory cell and eosinophils infiltration around the bronchi and bronchiole walls, subepithelial smooth muscle thickness and epithelial cell lengths in the lung tissues of rats ($p < 0,05$) however, dexamethasone`s effect was less than it is in group II. Dex from glucocorticoids is frequently used in models of allergic asthma as standard reference [259]. Glucocorticoids extremely block T cell pro-inflammatory cytokine manufacture and that's why decrease infiltration of inflammatory granulocytes as eosinophils [260, 261].

Our results show similar findings with the results of the above-mentioned investigators, and the co-administration of TQ and CS contributed to healing by reducing the signs of inflammation associated with asthma.

This is the first study about the effect of TQ and CS application on lung tissue of rat embryos from asthmatic pregnant rats. In the literature, no studies have been found on this

subject, namely the co-administration of TQ and CS, especially in relation to histological changes. There was no difference between treatment and dexamethasone groups in terms of H&E staining of lung embryos. Both experimental groups had a positive recovery in the lungs of the embryos. When the effects of corticosteroids are considered, it is seen that the treatment application for dexamethasone is regulator and its expected effects.

Asthma develops as a result of chronic inflammation and remodeling in the respiratory tract [262]. Findings such as increased vascularity and increased number of blood vessels in submucosal patients have raised this question. The increase in the number of vessels is mainly due to factors related to endothelial cell proliferation [263]. Angiogenesis occurs due to the differentiation and proliferation of endothelial cells and vascular permeability and VEGF plays a key role in the process of asthma [264].

Serap CM et al observed that submucosal immunopositive vessels in chronic asthma group increased significantly [257]. Enhancement in vascular format, number and exterior space and ultra-statement of VEGF and VEGF receptors is great certificated in asthma airway [263, 265, 266, 267, 268]. In our study, the subendothelial VEGF level significantly increased in asthma group ($p < 0, 05$). The result of Serape CM et al [257] confirms our result of asthma group increasing angiogenesis.

Serap CM et al demonstrated that the application of TQ alone in asthmatic mice decreased submucosal VEGF and epithelial VEGF [257]. This finding was like our result. Subendothelial VEGF significantly decreased ($p < 0, 05$) in group II. In our study, the co-administration of TQ and CS together in the lungs of asthmatic pregnant rats alleviated or treated the damages caused by OVA.

Although TQ has been reported to reduced asthma symptoms and inflammatory markers, there is no literature on the effect of TQ and CS on concomitant use of asthma. Respiratory response in asthma is in the form of Ig E-mediated, mast cell degranulation and histamine and leukotriene mediators. These effects in TQ show anti-inflammatory effects by inhibiting the production of LTB4, thromboxane B2 and by inhibiting 5-LO and CO_X pathways in arachidonic acid metabolism [269]. Also, it has been shown to have inhibitory effects on histamine receptors [270]. The effect of carob alone in asthmatic lung tissues of animals or patients was not described yet. This is the first report evidence the positive

effect of carob and TQ in lung tissues of asthmatic pregnant rats. Preceding works have represented that locust bean juice is abundant in potassium, sodium, calcium, magnesium, iron, copper and manganese, in addition to zinc. In addition, a strong antioxidant element such as gallic acid is the very rich phenolic complex found in carob fruit (3.27 mg /g) and high tannic acid (10.2 mg/dl) was found [271]. Phenolic compounds and zinc´s antioxidant and free radical scavenging activity have previously been informed [272, 273]. In our study it can be said that the decreased in VEGF levels in lung tissue are probably the cumulative effect of antioxidant and free radical scavenger effect of carob and the decreasing angiogenesis effect of TQ.

In group III subendothelial VEGF significantly decreased ($p < 0, 05$) however the effect of dexamethasone was less than it is in co-administration of TQ and CS group. Serap CM et al [257] indicated the use of dexamethasone significantly decreased VEGF immunohistochemically changes but there is no significant between TQ and dexamethasone treated groups when it was investigated in lung tissues of asthmatic mice.

Currently, drugs used in the treatment of asthma have limited effects on structural changes. Anti-leukotrienes, theophylline and inhaled corticosteroids used in asthma may be effective in reducing the structural changes in the respiratory system when used for a long time [274]. Thus, a new or alternative treatment are needed especially this new treatment has little or no side effect when it will use for long time.

The co-administration of TQ and CS decreased the level of subendothelial VEGF in group II when compared to lung tissues of untreated embryos in group I. This decrease was statistically significant. However, the effect of dexamethasone administration on subendothelial VEGF level showed a decreasing effect in terms of VEGF level in both asthma group and the co-administration of TQ and CS treatment group.

Asthma develops as a result of chronic inflammation and remodeling in the respiratory tract [262]. Sensitization followed by inhalational exposure to OVA is known to increase airway responsiveness and increase inflammatory cell infiltration into the airways [275]. Mauser et al [276] suggested that provocation with allergen (among others, OVA) caused microvascular infiltration and edema, thus causing swelling of the inflamed organ. In our study, the MDA levels in lung tissue increased in group I. These findings are consistent

with Farzaneh Shakeri et al [277], Shokry DM and El-Tarahony SA [278] and Hanene Zemmour et al [279]. Significantly increased levels of MDA were observed in sensitized rats in liver and lung and the damage of cell membranes of various tissues involved as reported by Chekchaki et al [280]. MDA is a highly reactive three-carbon dialdehydes produced as a by-product of polyunsaturated fatty acid peroxidation and arachidonic acid metabolism [281]. It is a common indicator of lipid peroxidation. The changes, accompanied by OVA exposure and increased lipid peroxidation, have been reported in the literature as a result of the penetration of allergen in asthmatic patients [282]. In consistent with above studies, the release of large amounts of superoxide anion and hydrogen peroxide by activated inflammatory cells in pulmonary alveoli is the cause of increased lipid peroxidation [283].

The treatment of TQ and CS in group II was decreased the MDA levels compared to OVA-sensitized group and dexamethasone group ($p < 0, 05$).

Sebai et al showed that subacute (7 days) treatment with aqueous extract carob pod can alleviate lipid peroxidation in brain and heart. Consequently, it is involved in protection against several diseases such as cardiovascular, neuronal and others [284]. The anti-inflammatory activities of both systemic and local applications of TQ support the effect of it on lung inflammation [285]. The therapeutic effect of *N. sativa* oil on patients with allergic diseases (allergic rhinitis, bronchial asthma and atopic eczema) has also been shown [286]. Ali and Blunden summarized the different pharmacological effects of *N. sativa*, including its effects on asthma, inflammation and the immune system, and indicated its different constituents [287]. However, the exact mechanism(s) of action of TQ is not fully understood. One proposed mechanism of action is its regulation of the Th1 and Th2 balance [288] and the other mechanism of the effect of TQ is its antioxidant effect [289]. These facts about TQ and CS as an antioxidant prove the activities of them to decreasing the harmful effects of ROS. In our study, in group III the MDA levels increased more than that of both I and II groups. MDA level significantly decreased in treated group with TQ and CS. This may be due to the protective effects of antioxidant features of TQ and CS. Therefore, the use of TQ and CS as treatment may improve asthma during acute asthma attack with reduced oxidative biomarkers.

NO is produced by the oxidation of L-arginine by the nitric oxide synthase enzyme. In the lung, NO is a vasodilator, bronchodilator and non-adrenergic non-cholinergic (NANC) neurotransmitter and is an important mediator that can enhance the inflammatory response in asthma [290]. NO is a by-product of airway inflammation and tissue damage and is a possible oxidative stress index in airway diseases [291]. The NO levels significantly increased in group II when compared to group I and III. Our finding was contrary to the results of Mehmet Kantar's study, which showed the role of TQ in decreased the levels of NO in the lung tissues after chronic toluene exposure in rats [292]. A. El-Mahmoudy et al demonstrated that production of free radical NO by the inducible nitric oxide synthase (iNOS) enzyme was dose- and time-dependently inhibited by TQ in the supernatants of LPS-stimulated macrophages [293]. In our study, this increasing in NO levels may be due to the rich source of C vitamin from CS (carob). D'Uscio LV et al indicated that the long-term vitamin C treatment increases vascular tetrahydrobiopterin levels and NO synthases activities [294] that it was support our result.

GSH is a tripeptide thiol that is essential for defense against oxidative damage and function of airway cells [295]. In our study, the GSH levels significantly increased in group III when compared to group I and II. Dexamethasone already has been used as asthma treatment from glucocorticoid group's drug. Glucocorticoid therapy is one of the most effective anti-inflammatory therapies available for asthma. This is due to multiple effects on the inflammatory response, including reduction of cytokine production and reduction of antigen-induced infiltration of eosinophils [296]. Long-term administration of glucocorticoids has been shown to result in mitochondrial dysfunction as well as oxidative damage of mitochondrial and nuclear DNAs [195]. Therefore, the needs of new or alternative medicine are required. Our finding showed similarities to Abeer A.A. et al result [260]. GSH level in group II also increased but was not significant. Amira E. Abd El-Aziz et al summarized that the TQ ameliorated the lipopolysaccharide LPS-induced depletion of GSH content by 86%, as compared to the LPS treated mice [297]. The effect of CS in lung tissue of rats with asthma is not reported before. It is the first study that demonstrated the effect of TQ and CS together in asthmatic pregnant rats. Therefore, the antioxidant properties of CS may be due to the polyphenolic compounds, other compound and minerals. It is also thought that the presence of TQ as a strong anti-inflammatory and antioxidant agent may be make the treatment with this mixture more effective for asthma patients and asthmatic pregnant.

AA reduces the damaging effects of ROS, such as the superoxide radical (O2-), hydrogen peroxide (H_2O_2), OH• and singlet oxygen [298, 299]. AA also reduces the injurious effects of oxidants, because it reduces the ROS and nitrogen species to more stable molecules. The levels of AA significantly increased in co-administration of TQ and CS when compared to I and III groups. Increased AA level in lung tissue at group II is probably due to high Vitamin C content in carob powder [287]. Likewise, it is known that oral administration of TQ increased antioxidants such as vitamins C [300]. When all these findings are considered together, increased AA levels in group II detected in lung tissue are possible. In other words, increased AA levels in group II are caused by externally given TQ and CS contents. Because of rats have to take Vitamin C from food and cannot syntheses themselves. By the way, AA has antioxidant action both by itself and via interaction with reduced GSH or vitamin E. For this reason, it has been suggested that there is a relationship between the AA and GSH levels. In our present study, when the GSH levels decreased in the lung tissues, the AA levels found to have increased in asthmatic pregnant with TQ and CS group. This finding shows that there is a relationship between these nonenzymatic antioxidants. Namely, while OVA caused inflammation in constituents' level as a result of oxidative stress condition in the lung of rats, TQ and CS supplementation restored the OVA-induced asthma constituents.

A central role in the pathophysiology of asthma is inflammation. It involves an interaction of many cell types and multiple mediators with the airways that at last results in the distinctive features of the disease [301]. It has been suggested that the release of mediators from mast cells localized to the airway smooth muscle is important in the pathogenesis of airway hypersensitivity and bronchoconstriction in asthma [302]. From these mediators is TNF-α. TNF-α initiate's histamine release directly from human mast cells [303] and human mast cell participate in a positive autocrine cycle that increases cytokine secretion [116]. The probability of TNF-α contributing to the inflammatory response in asthma is supported by observing increased levels of TNF-α mRNA and protein in the airways of asthmatic patients [304, 305]. The role of TNF-α in the development of various features of the asthma paradigm is summarized in the figure 4. There is evidence of increased TNF-α expression in asthmatics airways [306], TNF-α infusion results in increased airway sensitivity in Brown-Norway rats [307]. In the study, El Gazzar et al have recently proved that TQ attenuated pulmonary inflammation in a mouse model of allergic asthma by decreasing Th-2 cytokines and inflammatory cell infiltration in the lung, TQ was shown to

have beneficial effect in airway inflammation through the modulation of cytokines and growth mediators. TQ was able to suppress the inflammatory response in activated mast cells by blocking transcription and production of TNF-α. TQ enforced its effects by targeting the nuclear transactivation of pro-inflammatory transcription factor NF-κB [10]. Tekeoglu et al reported that TQ exerted an inhibitory effect on TNF-α production in rheumatoid arthritis, a well-established chronic inflammation model in rats [308]. In our study, TQ and CS administration did not produce any change in serum TNF- levels when compared to other groups, contrary to expectation, these findings suggest that TQ and CS supplementation seem to be insufficient to reduce TNF-α levels. However, the application of dexamethasone, which is widely used in clinic, could not reverse this situation. This unexpected situation with TNF-α suggests that other cytokines, such as IL-1β and IL-13, are involved in the course of inflammation rather than TNF-α and may be due to doses both of TQ and CS and to short period of treatment application.

In this study the levels of TNF-α in group III was increased when compared to both of I and II groups without any significantly changes. These increasing may be due to low dosage of dexamethasone used as asthma treatment, which caused suppressing the anti-inflammatory activity of it. The cytokine levels in this study indicated that the co-administration of TQ and CS in group II was significantly decreased the syntheses of both IL-1β and IL-13 levels in the serum of rat with asthma when compared to I and III groups. An imbalance between oxidants and antioxidants has been proposed in the pathogenesis of chronic obstructive pulmonary disease [309]. Amira E. Abd El Aziz et al showed that while LPS-induced lung injury by stimulate IL-1β production; TQ (8mg\gr) decreased it on airway-induced hypersensitivity mice [297]. TQ was shown to attenuate allergic airway inflammation in a mouse model by reducing Th1 cytokines such as IL-4, IL-5 and IL-13, as well as inhibiting eosinophil infiltration in the airways [10]. In rats exposed to water pipe smoke the pre- and post-treatment with carob aqueous extract protected and ameliorated the variations in the levels of different investigated parameters [310]. Gulay et al [311] showed that the traditional use of carob extract was non-toxic and had no significant adverse effects on the male rabbits. The antioxidant and free radical scavenging activity of phenolic compounds and zinc found in carob have already been reported [312]. Zinc can work against oxidation by binding sulfhydryl groups in proteins. Copper may retain binding sites for iron in DNA, protein and lipids [313]. Zinc also acts as an antioxidant by protecting proteins and enzymes against free radical attacks or oxidation

and by preventing the formation of free radicals. Zinc loss from biological membranes increases their susceptibility to oxidative damage and disrupts their function [314, 312]. These previous studies mentioned above support the view that TQ and CS can be used to inhibit inflammatory cytokines like IL-1-β and IL-13 that is indicated in our study.

Our findings suggest that the mixture of TQ and CS has a vital role reducing lipid peroxidation and inflammation in pregnant asthmatic rats. Therefore, we conclude that the use of these two remarkable TQ and CS in clinical practice as an adjuvant agent would be appropriate for patient`s attacks, especially in asthmatic pregnant women.

OS can have many detrimental effects on airway function, including airway smooth muscle contraction, induction of airway hyperresponsiveness, mucus hypersecretion, epithelial shedding and vascular exudation [315]. A positive correlation was reported between ROS and MDA level [143]. OH• is the most potent reactive oxygen species in the biological system that is responsible to the oxidation of polyunsaturated fatty acid of cell membrane phospholipids causing lipid peroxidation and cell damage [316]. Lipid peroxidation is of particular significance in asthma and its products have been measured in asthma. Elevated MDA levels have been observed in plasma of asthmatic patients [317-318]. In a study of asthma model in rats, the serum levels of MDA significantly increased in asthmatic group compared to control group [263]. Another study showed that oxidative stress is associated with an increase MDA and protein carbonyls in both BAL fluid and peripheral blood in asthma [319]. In patients with different airway diseases including asthma, increased MDA levels in biological fluids was observed [320].

As a result of our study, the above-mentioned studies supported our results. We found increased serum MDA levels in experimentaly asthmatic pregnant rats.

Our study indicated that the serum MDA level significantly reduced in treated groups with co-administration of TQ and CS ($p < 0,05$) more than in dexamethasone group. Therefore, treatment with this two herbal mixture can improve asthma during an acute asthma attack by reduction of oxidative biomarkers. The effects of co- administration of this tow mixture on reducing the serum MDA levels have been not studied before, therefore there is no previus studies to discussion our results. But the effect of TQ alone on the decreasing the serum MDA levels were indicated [271, 272, 321, 322]. And the antioxidant properties of

CS demonstrated in healthy rats by decreasing the lipid peroxidation was reported [323, 324].

Inhaled corticosteroid treatment causes significantly lower expression of CYBB mRNA in the NADPH oxidase system [325]. The prophylactic effects of N. sativa boiled extract was also confirmed in a study involving 29 asthmatic patients. Among the N. sativa group, the use of oral inhalers and beta-agonists, oral corticosteroids, oral theophylline, and even corticosteroid inhalers decreased at the end of the study while no apparent change in the use of drugs in the control group [326]. In our study we found that reduced serum MDA levels in the dexamethasone group ($p < 0, 05$). This effect appers to have been caused by the suppretion of inflammation, which is the natural effect of corticosteroide.

Thiols are potent antioxidants which protect cells against the oxidative stress. The most thiol antioxidant in the epithelial lining fluid is the tripeptide GSH, which is a multifunctional intracellular antioxidant and is noticed to be the main thiol-disulphide redox buffer of the cell [327]. The antioxidant capacity of thiol compounds is related to the sulphur atom, which can easily accommodate the loss of a single electron [328].

Shakeri et al. showed that thiol concentration decreased in the serum of asthmatic rats which also confirm the induction of animal model of asthma [263]. Also we indicated that the serum RSH levels decreased in asthmatic pregnant rats. We found that the treatment with TQ and CS increased serum thiol level but without any significant changes ($p > 0, 05$).

Majed et al pointed that 15 mg/kg TQ was administred i.p on 11 and 14 days of gestation to non asthmatic pregnant rats. It was observed that the dose of TQ applaed did not cause any toxicity on the development of embryos [329]. Al-Ali et al. [318] reported that the LD50 of i.p. injection for TQ in nonpregnant rats was 57,5 mg/kg. The investigators also described the toxic effect of this dose on the rat internal organs and reported that the vital visceral organs showed only congestion with no damage or necrosis. In CS as a natural extracts, A positive correlation between phenolic compounds and antioxidant capacity was observed [323, 324].

These above studies indicated that both anti-inflammatory and antioxidant properties of this tow compounds have a potent effect on thiol groups.

AA is water-soluble and is well absorbed from the gastrointestinal tract. It is involved in many physiological functions in living organisms. In a variety of other functions, the role of AA in cellular metabolism can be accounted for by its reducing properties to protect cellular components from oxidative damage. It acts as a scavenger for oxidizing free radicals and harmful oxygen-derived species, such as the hydroxyl radical, hydrogen peroxide, and singlet oxygen [330-332]. L-ascorbic acid is a dibasic acid with an enediol group built into a five membered heterocyclic lactone ring. The chemical and physical properties of ascorbic acid are related to its structure [333, 330, 334]. Ascorbic acid is metabolized in the liver, and to some extent in the kidney, in a series of reactions. The principal pathway of ascorbic acid metabolism involves the loss of two electrons [330-331]. Some studies have shown that the increase in plasma vitamin C was accompanied by an increase in the intracellular levels of the vitamin [335].

Frei et al. [336-338] have shown that vitamin C is a powerful antioxidant preventing lipid peroxidation in plasma exposed to various types of oxidative stress. It is well known that in the presence of redox-active iron, AA can act as a pro-oxidant in vitro and contribute to the formation of hydroxyl radicals, which in turn may cause lipid, DNA, or protein oxidation [339].

Serum AA levels did not show any statistical significance in all groups (p> 0, 05), but the effect on lung tissue was quite different. This effect may be explaned by the fact that it is high at the tissue level in order to eliminate the negative effects of asthma in the lung, which is probably a primary tissue than serum.

Steroids, as an anti-inflammation, not only decrease the edema in the airways and thus prevent pulmonary fibrosis but also improve pulmonary function (gas exchange) through improvement in surfactant production, stabilization of capillary walls and prevention of capillary leakage, and finally improvement in β-adrenergic system.[340].

The pathophysiology of asthma involves a variety of changes at the cellular level, due to the activity of eosinophils, mast cells, neutrophils, and T lymphocytes [341]. The eosinophil is an end-stage granulocyte derived from primordial stem cells in the bone marrow and is known to circulate through the peripheral blood stream and tissues. While normally appearing in smaller numbers in healthy people, eosinophils play a central role in

inflammation and allergic processes [342, 343, 340]. Elevated levels of eosinophils may correlate with the protective mechanism of the body against asthma, allergies, atopic dermatitis, parasitic infections, gastrointestinal disorders, and other rarer diseases [342, 343, 344]. Eosinophil recruitment and production is due to Th2 lymphocyte stimulation, with the help of cytokines including IL-3, IL-4, IL-5, IL-13, and GM-CSF [340]. Acutely, allergen binds specifically to IgE, which initiates a cascade of inflammation and activation of inciting biomarkers. Eosinophils, mast cells, and Th2 lymphocytes migrate to the area in response to specific cytokines including IL-3, IL-4, IL-5, IL-9, and IL-13 [345]. Mast cells contribute to the release of acute phase mediators and cytokines, which promote deleterious effects to healthy tissue. Eosinophils release cytokines, growth factors, and leukotrienes which cause further inflammation and produce the characteristic and recurrent symptoms of the disorder, including hyperactivity in response to various provocative factors [340]. In contrast to normal tissue seen in healthy individuals, the airway mucosa of patients with asthma has a higher amount of CD4+ T (helper) lymphocytes, eosinophils, and mast cells [346, 347]. In patients with more severe disease or during an acute exacerbation of asthma, there is a greater prevalence of neutrophilic inflammation [348, 349]. In a study by Qiu et al., comparing tissue histological sections of adult patients between the ages of 20–64 years with intermittent versus severe asthma, neutrophils and eosinophils were present in the bronchial mucosa in both patient groups. There was a significantly higher prevalence of epithelial and subepithelial neutrophils in the severe asthma population as compared to those patients with intermittent asthma. Patients with intermittent asthma had six times more eosinophils in the subepithelium biopsies than neutrophils [350]. Alternatively, patients with severe asthma had a larger accumulation of neutrophils and eosinophils in the airway mucosa when compared to the intermittent asthma and control groups, with both cell types being present in an equal ratio [346]. This finding suggests that neutrophils and eosinophils are both relevant inflammatory mediator cells in more severe asthma [351, 350]. There is evidence that eosinophils may enhance immunity via an antiviral effect, as seen in experimental mice models in association with various respiratory viruses [352]. Eosinophil, lymphocyte and macrophage rich inflammation and AHR were seen in wild-type mice sensitized and challenged with OVA [353]. In a study of guinea pig model of asthma showed increasing in eosinophils and a decrease in neutrophils, lymphocytes and monocytes in lung Lavage fluid of animals that given drinking water alone compared to the control group, but when these animals treated with both low and high concentrations of TQ, eosinophils and lymphocytes were

significantly greater than those of inhaled fluticasone propionate group [354]. The study of Talatt Abbas et al. [355] in murine model of allergic asthma showed that treatment with *N. sativa* leads to significant reductions of peripheral blood eosinophil count, which was equivalent to the effects of dexamethasone when compared to sensitized group with OVA.

The WBC differential count in the serum of pregnant rats showed that there is no significant difference between groups in differential monocytes, while the differential of lymphocytes was significant decreased and neutrophils was significant increased in dexamethasone group when compared both I and II groups ($p < 0, 05$). The differential eosinophils were significant decreased in co –administration of TQ and CS group when compared to I and III group ($p < 0, 05$)

The above studyes are agreed with our results in terms of the higher count of eosinophils in the serum of pregnant asthma group ($p < 0, 05$).

Eosinophil recruitment into and subsequent activation in the airways is regulated mainly by IL-5 and IL-13 [356]. In addition, IL-13 induces expression of VCAM-1 and eotaxin (eotaxin is a chemokine which can mediate chemotaxis of peripheral blood eosinophils) in the airway epithelium, thereby facilitating the recruitment of eosinophils into the airways [357]. The above studies explain that the high IL-13 levels in asthmatic pregnant group that we found leaded to the increase in eosinophils in group I. There is a relation both IL-13 and eosinophils in asthmatic pregnant rats through expression of VCAM-1 and eotaxin.

Our results showed significantly reducing in the number of eosinophils ($p< 0, 05$) in co-administration of TQ and CS group, and there is parallelism with reduced IL-13 levels and eosinophils count. Therefore, this application may be having a protective effect against asthma by reducing inflammatory cells. The co-administration of TQ and CS influences reducing the eosinophil status, which is an important inflammatory criterion in asthma.

The goal of the treatment of asthma is reducing airway inflammation with anti-inflammatory drugs. However, the available anti-inflammatory drugs for asthma do not lead to complete cure of airway inflammation. In fact, Global Initiative for Asthma (GINA) guideline [368] recommended the use of complementary and alternative therapies, including herbal medicine. In this respect, instead of the side effects of asthma medications

containing dexamethasone, natural, non-side effects and safe herbal products will be used as ancillary and supportive products in the treatment of asthma.The aim of our study was to achieve the same goal for the new treatment of asthma, especially during pregnancy.

In contrast to our findings, Talat Abaas et al. [355] demonstrated that dexamethasone administration had a reducing effect on neutrophil, eosinophil and lymphocyte levels in murine model of asthma. Thes probably due to the doses that we used, the duration of administration and the different subjects used.

6. CONCLUSIONS AND RECOMMENDATIONS

Our findings suggest that the mixture of TQ and CS has a vital role reducing lipid peroxidation and inflammation in pregnant asthmatic rats. Therefore, we conclude that the use of these two remarkable TQ and CS in clinical practice as an adjuvant agent would be appropriate for patient`s attacks, especially in asthmatic pregnant women. The combined use of TQ and CS may be a total/cumulative effect of TQ`s reducing the angiogenesis and the antioxidant effect of CS together. This type of application will contribute to the treatment of asthma and the preventing of attacks especially during pregnancy. At the same time, the use of TQ and CS in people with normal asthma may be used as a new promising supplement to eliminate the negative effects of asthma. Current studies will open wide prospects for the positive role of TQ and CS in alleviating and treating the symptoms of asthma.

We recommend continuing the investigation with this mixture using different doses and increasing the duration of treatment. Although previous research has shown that TQ and CS were safe to use, we recommend caution when using the ingredients, especially during pregnancy, with internationally recommended safe doses.

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