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Synergistic Effects of Thymoquinone and Carob Powder versus Dexamethasone in the Model of Asthma in Pregnant Rats: New Insights into their Therapeutic Effects

Gebe Sıçanlarda Astım Modelinde Timokinon ve Harnup Tozunun Deksametazonla Karşılaştırıldığında Sinerjik Etkileri: Terapötik Etkilerine İlişkin Yeni Görüşler

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ABSTRACT

Objective: When asthma and pregnancy coexist, the mother may be severely affected. One of the main reasons for this situation is that it is positively correlated with especially increased oxidative stress due to the co-occurrence of asthma and pregnancy. Even pregnancy alone increases oxidative stress in the body, and with the addition of asthma, an increased chain of events is added. In this sense, diet and nutrition styles can be considered as factors that regulate both events in a controlled way. Increasing anti-oxidant intake in the diet can play a leading role in eliminating the increased Reactive oxygen species; thus, this negative effect in asthma and pregnancy can be alleviated. Studies on the protective role of antioxidants in asthma are limited, but not few. The aim of this study was to reveal the strength of the effects of thymoquinone (TQ) and carob powder administration, which are known to have two different strong antioxidant effects during pregnancy, on the oxidative event in the mother with the diet management strategies.

Methods: In this study, it was tried to improve the asthmatic pregnant rat and to determine the direction of oxidant-antioxidant balance with a holistic antioxidant complex Female rats were divided into 3 groups: asthmatic pregnant group (1) sensitized via an intraperitoneal injection of ovalbumin (OVA) with alum on days 0 and 14 and exposed to aerosolized OVA 3 days over the subsequent 1 week then coupled with male rats to get pregnant; asthmatic pregnant with TQ and carob (CS) group (2) sensitized as above then administered each of TQ and CS on the last 5 days of pregnancy; and asthmatic pregnant with dexamethasone group (3) sensitized as above then received dexamethasone by intraperitoneal injection on last 5 days of pregnancy.

ÖZ

Amaç: Gebelikte astım varlığı, artan oksidatif stres nedeniyle hem anne hem de fetüs için risk oluşturabilir. Bu durumun en önemli nedenlerinden biri, özellikle astım ve gebeliğin birlikte görülmesi nedeniyle artan oksidatif stresle pozitif ilişkili olmasıdır. Tek başına gebelik bile vücutta oksidatif stresi artırır, buna ek olarak astımın eklenmesiyle olaylar zinciri daha da artar. Bu anlamda beslenme tarzları her iki olayı da kontrollü bir şekilde düzenleyen bir faktör olarak düşünülebilir. Diyette antioksidan alımının artırılması, artan reaktif oksijen türlerinin ortadan kaldırılmasında öncü rol oynayabilir; böylece astım ve gebelikteki bu olumsuz etki hafifletilebilir. Antioksidanların astımda koruyucu rolüne ilişkin çalışmalar sınırlıdır. Bu çalışmanın amacı, gebelik döneminde iki farklı güçlü antioksidan etkisi olduğu bilinen timokinon (TQ) ve harnup tozu uygulamasının diyet yönetimi stratejisi ile annedeki oksidatif olay üzerindeki etkilerinin gücünü ortaya koymaktır.

Yöntemler: Bu çalışmada, astımlı gebe sıçanlarda bütünsel bir antioksidan kompleks ile oksidan-antioksidan dengenin yönü belirlenmeye ve iyileştirilmeye çalışılmıştır. Dişi sıçanlar üç gruba ayrılmıştır: Astımlı gebe grup (1), 0. ve 14. günlerde alüminyum içeren ovalbümin (OVA) ile intraperitoneal enjeksiyon yoluyla duyarlılaştırıldı ve sonraki 1 hafta boyunca 3 gün boyunca aerosolize edilmiş OVA'ya maruz bırakıldı ve ardından gebe kalmak için erkek sıçanlarla birleştirildi; TQ ve harnup (CS) grubu (2) ile gebe kalan astımlı hastalara yukarıda belirtilen şekilde duyarlılık kazandırıldı ve daha sonra gebeliğin son 5 gününde TQ ve CS'nin her biri uygulandı; ve yukarıda belirtilen şekilde duyarlılaştırılan deksametazon grubu (3) ile astımlı gebelere gebeliğin son 5 gününde intraperitoneal enjeksiyon yoluyla deksametazon verildi.

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ABSTRACT

Results: The co-administration of TQ and CS decreased malondialdehyde levels, increased nitric oxide, ascorbic acid, and glutathione levels in lung tissue. In addition, the levels of some proinflammatory cytokines were decreased in the serum. The cumulative protective effects of TQ and CS were successful in reducing inflammation in the lungs.

Conclusion: The administration of TQ and CS may be considered as a candidate for a new herbal blend or adjuvant for asthmatic pregnant, given the side effects of cortisol.

Keywords: Asthmatic pregnant, carob, thymoquinone, oxidative stress, inflammation

ÖZ

Bulgular: TQ ve CS'nin birlikte uygulanması akciğer dokusunda malondialdehit seviyelerini azalttı ve nitrik oksit, askorbik asit ve glutatyon seviyelerini artırdı. Ayrıca, serumda bazı proenflamatuvar sitokinlerin seviyesi azalmıştır. TQ ve CS'nin kümülatif koruyucu etkisi akciğerdeki enflamasyonu azaltmada başarılı olmuştur.

Sonuç: Kortizolün yan etkileri göz önüne alındığında, TQ ve CS'nin uygulanması astımlı gebelerde yeni bir bitkisel karışım veya adjuvan adayı olarak düşünülebilir.

Anahtar Sözcükler: Astımlı gebe, harnup, timokinon, oksidatif stres, enflamasyon

INTRODUCTION

Asthma is a common disease that affects children and adults, particularly pregnant women. Maternal asthma brings not only the mother herself but also the morbidity and mortality associated with the fetus (1). Therefore, being both asthmatic and pregnant at the same time puts a huge burden on both the mother and the newborn (2).

Asthma is a fairly a disease with a high prevalence in the community and involves a complex interaction of various factors, such as inflammation, bronchial hyperresponsiveness, and airflow obstruction. The predominant character leading to clinical symptoms is contraction and inflammation of the smooth muscle, resulting in narrowing and airway obstruction. Numerous triggers, such as irritants and drugs, may cause bronchoconstriction in some patients. Airway inflammation may lead to blood vessel proliferation, mucus hypersecretion, subepithelial fibrosis, inflammatory cell infiltration, and smooth muscle hyperplasia (3,4). In asthma, many inflammatory cells (mast cells, neutrophils, macrophages, etc.) are related in the airway inflammation pathogenesis (5). Reactive oxygen species (ROS) are released as a result of cell metabolism. ROS stimulate histamine release and mucus secretion by contracting airway smooth muscles (6). All these reactions lead to increased inflammation. Both increased oxidants due to the open airways to the outside environment and the presence of airway inflammatory cell-derived ROS cause high levels of oxidative stress in the lung (7). An article on asthma susceptibility in mice found elevated levels of certain cytokines both in the lungs and systemically, particularly differences in T helper 2 (TH2) and TH17 cytokine levels (8).

Strong anti-inflammatory drugs, such as inhaled corticosteroids and anti-leukotrienes, are the basis for the treatment of asthma symptoms in the long term. Although these drugs are beneficial for the treatment of asthma, they have several side effects. Recently, traditional and herbal approaches have attracted attention in every field and constitute an important part of health systems worldwide (9). One of them, Nigella sativa has been known as an alternative treatment for asthma and other inflammatory diseases. Nigella sativa, which is traditionally used as a food and in the treatment of asthma, bronchitis, eczema, and inflammation, has various important biological effects (anti-inflammatory, anti-tumor, antioxidant) (10). Most of these biological properties have been associated with TQ, the primarily metabolite of Nigella sativa essential oil. TQ inhibits cycloxyoxygenase and lipoxygenase inflammation, decreases the ratio of TH2 cytokines and eosinophils. TQ is an inhibitor of asthma and inhibits eosinophil infiltration and decreases allergic airway inflammation. However, the mechanism of action of TQ during asthma treatment has not been fully elucidated (11-13).

CS (carob), which is grown extensively in the Mediterranean region, has a vital role and attracts attention in the food and medicine industries for economic and environmental reasons (Battle and Tous). Carob seed is a rich source of flavonoids such as gallotannin, ellagitannin, and proanthocyanidin. These phytochemicals have free radical-scavenging activities and are used against many diseases because of their pharmacological properties (14). Carob powder is a rich source of Niacin, B6, vitamins C, D, E, folic acid, and other valuable fatty acids (15). Therefore, it can be suggested that the protective effects against lipid peroxidation caused by ROS in tissues and the prevention of the depletion of the antioxidant enzyme; SOD, CAT, and glutathione (GSH) are attributed mostly to its chemical content. Plant flavonoids have also been reported to alleviate gingival inflammation via the suppression of nuclear NF-κβ translocation and myeloperoxidase activity, which may explain the reported inhibition of the pro-inflammatory mediators (15,16).

During pregnancy, there is a superimposed increased oxidative stress burden brought on by both pregnancy and asthma. The protective effects of exogenous antioxidants against oxidative stress are important in pregnancy with asthma. In this sense, herbal approaches attract attention today. Despite the increased oxidative stress resulting from asthma complications and asthma exacerbations during pregnancy, it has been reported that an increase in antioxidant supplements alleviates these effects (16).

To date, there is only one histologic and immuno-histological study which was done our laboratory related to the cumulative effect of TQ and CS in pregnant asthmatic rats (2). In this new study, our hypothesis is whether TQ and CS will have a positive effect on oxidative events and some indicator cytokines in pregnant rats with asthma and will show a potential synergistic effect.

MATERIALS AND METHODS

Experimental Design

The experimental design was carried out as described in our previous study (2). The experimental procedures were confirmed by Ethics Committee of Gazi University for Animal Experiments (approval number: G.Ü.ET-16.035, date: 21.03.2016). The experimental groups were designed as shown in Table 1.

Sensitization and inhalational exposure: All animals were sensitized according to the methods of Moura et al. (17) and Yang et al. (18) and the experimental timeline is shown in Figure 1.

Sample collection: Ketamine and xylazine were intramuscularly injected (IM) to anesthetize the rats. Lung samples from all rats were dissected after sacrificed by high dose of anesthetics, washed thoroughly with saline and kept in liquid nitrogen until they were held in the laboratory and then kept at -30 °C. Blood samples were collected in clot activator tubes then they centrifuged in 3000 rpm for 5 min. and the serums were kept at -30 °C.

Biochemical Analyses

Determination of MDA, NOx, AA, and GSH levels: Malondialdehyde (MDA) levels in both serum and tissue were measured by the method of Casini et al. (19). In this method, MDA reacts with thiobarbituric acid. The NOx levels in tissue were measured spectrophotometrically by the Griess reaction at 540 nm (20). Roe and Kuther's method modified by Berger et al. (21) was used for measuring ascorbic acid (AA) level in both tissue and serum. A modified Ellman method for GSH spectrophotometrically determination in tissue was used (22). Plasma RSH was determined using the spectrophotometric method (23).

Measurement of some cytokine levels: Some serum levels were measured with commercial kit [serum interleukin-1 beta (IL-1β) & tumor necrosis factor-alpha (TNF-α) DIAsource, BE and serum IL-13 Sunredbio, CHN].

Table 1. Experimental group design

Group 1 (n=6)	Asthmatic pregnant
Group 2 (n=6)	Asthmatic pregnant with TQ and CS treated
Group 3 (n=6)	Asthmatic pregnant with dexamethasone treated

TQ: Thymoquinone, CS: Carob.

Statistical Analysis

Data were given as means ± standard deviations, and the differences between groups were made using ANOVA post-hoc Tukey using the SPSS software package (IBM, USA) $p<0.05$ was considered statistically significant.

RESULTS

In this study, the in the dexamethasone treatment group, the MDA levels increased the most. MDA levels decreased in treated group with TQ and CS (group 2) ($p<0.05$) (Table 2). On the other hand, serum MDA levels decreased in TQ + CS treatment group (Table 4). Application of TQ + CS were decreased serum MDA levels. The lung tissue increased in combination of TQ and CS (Table 2, $p<0.05$).

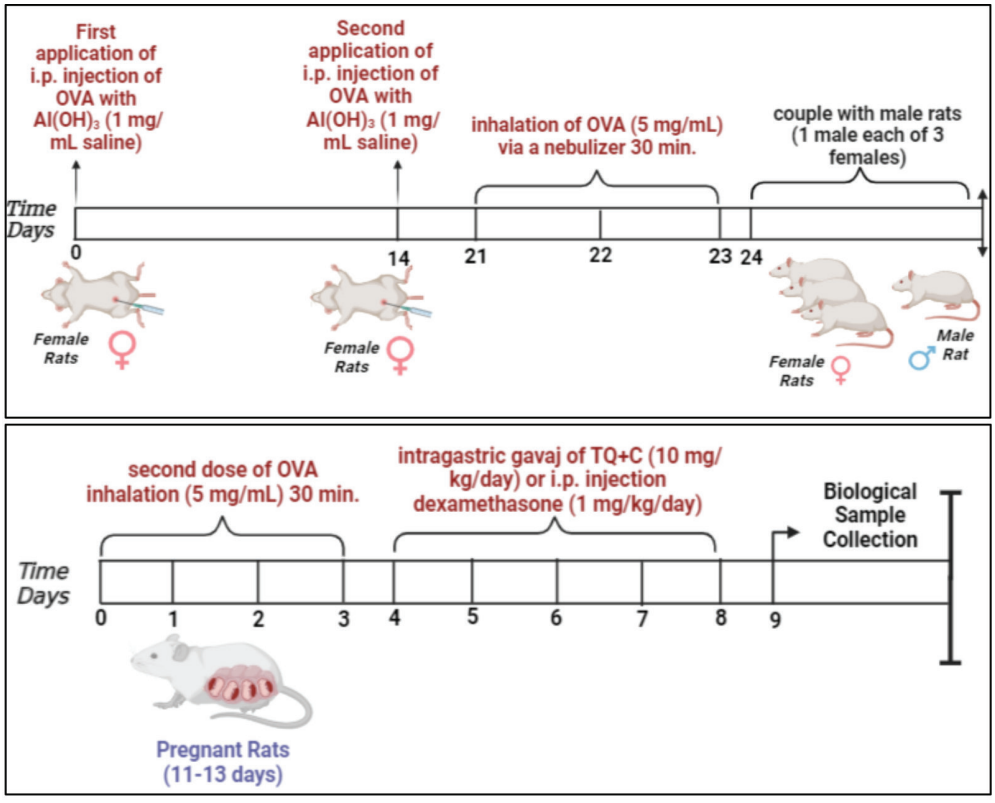

Figure 1. Experimental timeline. To develop a model of asthma, female rats were first treated OVA for 14 days (i.p. injection). At the end of 14 days, a second OVA treatment was performed (i.p. injection). On days 21, 22, and 23, OVA was administered by inhalation. Female rats in an asthma model were created with OVA, were mated with male rats. Pregnant rats aged 1-13 days were given OVA by inhalation for 3 days, and the asthma model was repeated. TQ + CS treatment was administered pregnant rats for 5 days. Tissue serum and plasma of the rats sacrificed at the end of the treatment were separated and subjected to biochemical analysis.

TQ: Thymoquinone, CS: Carob, OVA: Ovalbumin.

In our study, application of dexamethasone increased lung tissue GSH levels (p<0.05) (Table 2). The tissue levels of AA increased after the dual administration of TQ + CS (with parallel lung tissue NO levels) (p<0.05) (Table 2).

TQ and CS administration did not show any change in serum TNF-α levels (Table 3). TNF-α levels in dexamethasone group was in increasing trend when compared to both (p>0.05) (Table 3). Our findings showed that dual TQ + CS administration significantly decreased both IL-1β and IL-13 levels in asthmatic rat serum. The WBC differential content in the serum was shown in Table 5. The eosinophil percentage was significantly decreased in the treatment group compared with the other groups (p<0.05).

DISCUSSION

Asthma and pregnancy remain a worrying issue today, as both the mother and the fetus struggle to cope with the heavy burden of these conditions. Both being pregnant and having asthma are known as conditions in which the oxidative stress load is higher than normal in women.

Antioxidants are one of the best solutions that come to mind in terms of supporting against increased oxidative stress in pregnancy. Asthma is a serious condition manifested by asthma exacerbations in pregnant women. Asthma exacerbation rates are known to increase and trigger with pregnancy, and this rate is not negligible (24). In the treatment of asthma, corticosteroids are used cautiously because they do not pose a risk to both the mother and the fetus, but they are still hesitant by the patients even at the minimum dose. Natural methods are also often preferred to prevent the onset of asthma symptoms and exacerbations, ensure optimal lung function, and manage risk factors for poor asthma outcomes and comorbidities. Taking advantage of plants is one of them.

Asthma occurs when chronic inflammation in the airway remodeling (25). The respiratory response in IgE-mediated asthma involves mast cell degranulation and histamine and leukotriene mediators. Sensitization with ovalbumin (OVA) is known to stimulate and

Table 2. 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 (µg/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*	1.01±0.23	20.59±0.91*
Asthmatic pregnant with dexamethasone (III)	17.56±0.30	18.40±1.65	3.99±0.77a	14.64±0.85

Each value is the mean ± standard deviation of 6 animals per group. *p<0.05 when compared to group 1 and 3, ap<0.05 when compared to group 1 and 2. TQ: Thymoquinone, CS: Carob, GSH: Glutathione, AA: Ascorbic acid.

Table 3. TNF-α, IL-1β and IL-13 levels in the 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

Each value is the mean ± standard deviation of 6 animals per group. *p<0.05 when compared to groups 1 and 3. TQ: Thymoquinone, CS: Carob, GSH: Glutathione, AA: Ascorbic acid.

Table 4. 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 ± standard deviation of 6 animals per group. *p<0.05 when compared to group 1. TQ: Thymoquinone, CS: Carob, GSH: Glutathione, AA: Ascorbic acid, MDA: Malondialdehyde.

Table 5. Differential WBC count in the serum of pregnant rats

Groups	Monocytes percentage, (%)	Lymphocytes percentage, (%)	Neutrophils percentage, (%)	Eosinophils percentage, (%)
Asthmatic pregnant (1)	4.83±1.77	71±6.32	18.5±6.10	5.50±3.20
Asthmatic pregnant with TQ + CS (2)	3.50±1.89	68±11.87	25.83±10.67	2±1*
Asthmatic pregnant with dexamethasone (3)	4.67±2.42	42.33±19.73a	45.67±11.91a	5±1.91

*p<0.05 when compared to 1 and 3 groups. ap<0.05 when compared to 1 and 2 groups.
TQ: Thymoquinone, CS: Carob, WBC: White blood cell

increase inflammatory cell infiltration and airway responsiveness (26). Tissue lung MDA level increased in control group. Our findings were consistent with those of a previous study (27). MDA is a common indicator of lipid peroxidation. You et al. (28) showed that superoxide anion and hydrogen peroxide released in large amounts from activated inflammatory cells in the pulmonary alveoli lead to an increase in MDA and maintain inflammation. In consistent with above studies in our study, we found increased lipid peroxidation accompanied by OVA exposure in lung tissue.

Biochemical results content of the lung tissue and serum: Carob pod treatment (aqueous extract) might mitigate lipid peroxidation in brain and heart. It is also thought to play a role in protecting against cardiovascular and neuronal diseases (29,30). Otherwise, the anti-inflammatory activities of thymoquinone (TQ) support the effect of it on lung inflammation (31). The effects of Nigella sativa on inflammation, asthma, and the immune system have been reported (10). Although it is thought that TQ shows its effects through its antioxidant properties in Th1 and Th2 cells, its mechanism of action is not fully understood (32). It shows that TQ and CS, which have a strong antioxidant potential, reduced the harmful effects of ROS. In our study, the in dexamethasone treatment group, the MDA levels increased the most. MDA levels decreased in treated group with TQ and CS (group 2) ($p<0.05$) (Table 2). On the other hand, serum MDA levels decreased in TQ + CS treatment group (Table 4). Application of TQ + CS were decreased serum MDA levels. This decrease may be due to the antioxidative properties of TQ and CS. It is thought that TQ and CS treatment, which are used as potential antioxidants during asthma attacks, reduces oxidative biomarkers and thus may improve asthma.

Nitric oxide (NO) is a messenger molecule produced by several NO synthases, and it plays an important role as a neurotransmitter, vasodilator, and bronchodilator in the lungs. It can promote the inflammatory response in asthma (33). The lung tissue increased in combination of TQ and CS (Table 2, $p<0.05$). However, previous studies have shown that TQ decreases NO levels (34,35). We suggest that vitamin C-rich CS leads to an increase in NO levels. d'Uscio et al. (36) pointed out that long-term treatment with vitamin C increases NO synthesis activities and vascular tetrahydrobiopterin levels, which supports our result.

GSH is a vital for antioxidant defense system and airway cells activities (37). In our study, application of dexamethasone increased lung tissue GSH levels ($p<0.05$) (Table 2). Dexamethasone is used in the treatment of asthma and is a member of the glucocorticoid drug class. Glucocorticoids are very effective in treating asthma, and they have various effects on the inflammatory response, such as reducing antigen-induced infiltration of eosinophils and cytokine production (38). Long-term use leads to side effects such as oxidative injury, nuclear DNA damage, and mitochondrial dysfunction (39). Thus, alternative treatment methods are required. Our finding showed similarities to Ismael and Shaffie's results (40). They found that fish oil and fish oil + dexamethasone alleviated OVA-induced modifies in lung function tests. GSH levels in group 2 also increased however insignificant ($p>0.05$). Abd El Aziz et al. (41) reported that TQ improved LPS-induced GSH reduction. This is a remarkable study of the dual effects of TQ and CS in an experimental asthmatic model, and there is no literature finding about the effectiveness of CS in the lung tissue of asthmatic rats. It is believed that the antioxidant properties of carob are due to its polyphenolic and other metabolites.

AA (vitamin C) alleviates the harmful effects of ROS (42). In this study, the tissue levels of AA increased after the dual administration of TQ + CS (with parallel lung tissue NO levels) ($p<0.05$) (Table 2). The enormous vitamin C content in carob powder probably mediated the increased AA levels in lung tissue in group 2 (15). Similarly, oral TQ administration has been shown to increase the levels of antioxidants such as vitamin C (43). When all these findings are evaluated together, it is thought that the increased AA levels detected in the lung tissue of group 2 are due to exogenously administered TQ + CS. On the other hand, whether the AA level in the serum was not statistically different between the TQ and CS applied group and the control group, it was in an increasing trend in the TQ + CS group of AA level. Some mammals, such as humans and rats, cannot manufacture AA in their bodies, so they must take food containing AA. Hence, it can be thought that there is an interaction between AA and GSH. Our results showed that when dexamethasone administration was compared with TQ and CS, application, there was an inverse relationship between AA and GSH.

Cytokine levels in serum: Scott et al. (44) examined female fetuses with moderate asthmatic mothers such as placental expression of TNF-α and some interleukins and found increased cytokine levels compared to control. When asthma and pregnancy coexist, an associated increased inflammation in maternal and placental circulation is considered (45,46). In inflammatory diseases, such as asthma, the levels of cytokines such as TGF-β, TNF-α, IL-6, IL-8, and IL-10, change and contribute negatively to the progression of the disease (47). Osei-Kumah et al. (48) suggested that some cytokine concentrations were increased but the others were not during pregnancy. In another study, they found some serum levels to be unchanged, such as interleukin, GMCSF, Interferon gamma, and TNF-α, in obese pregnant women (47). Our study differs from these studies in that it is an experimental animal study, but this is in parallel with the fact that they have similar metabolic pathways. Considering this context, increased cytokine levels were detected in pregnant rats with asthma. The co-administration of TQ and carob provides a significant decrease in serum cytokine levels, and inflammatory processes turned it into a positive direction. Inflammation plays an essential role in asthma pathophysiology. This includes the interaction of various cell types and mediators with the airway, which reveals the characteristic features of the disease. Mediators of airway smooth muscle have been suggested to be important in asthma (49). One of these mediators is TNF-α stimulates histamine (50), and human mast cells participate in a positive autocrine cycle that increases cytokine secretion (51). Increased levels of TNF-α in the airways of asthmatic patients suggest that TNF-α is involved in the inflammatory process in asthma (52,53). There is evidence that TNF-α expression is increased in the airways in asthma (53). El Gazzar et al. (54) reported that TQ reduced Th-2 and inflammatory cell infiltration in the lung, thereby alleviating pulmonary inflammation TQ blocks the production of TNF-α by targeting nuclear transactivation of nuclear factor-kappa, thus exerting a positive effect in reducing airway inflammation (54). Tekeoglu et al. (55) showed in a rheumatoid arthritis model with chronic inflammation that TQ inhibited TNF-α production. Surprisingly in our study, TQ and CS administration did not show any change in serum TNF-α levels (Table 3). According to

TNF-α results, other cytokines (IL-1β and IL-13) take a greater role in the inflammation process and may be due to the application doses of TQ + CS and short-term treatment administration. TNF-α levels in dexamethasone group was in increasing trend when compared to both ($p>0.05$) (Table 3). Low doses of dexamethasone used to treat asthma may cause these increases. Our findings showed that dual TQ + CS administration significantly decreased both IL-1β and IL-13 levels in asthmatic rat serum. The imbalance between oxidant and antioxidant metabolites is thought to be involved in the pathogenesis of chronic obstructive pulmonary disease (56). Abd El Aziz et al. (41) conducted that while LPS-induced lung injury by stimulate IL-1β production; TQ (8 mg/g) decreased it on airway-induced hypersensitivity mice. Another study revealed that, TQ decreased IL-4, IL-5 and IL-13 in airway (12). In addition, it has been reported that carob extract is non-toxic, does not have serious side effects and contains zinc and phenolic components that have antioxidant activity (57,58). Zinc can suppress oxidation by binding to sulfhydryl groups in proteins, and it can activate the binding site of copper and iron in lipid, protein, and DNA (59). Another metabolite that protects biomolecules against ROS damage or formation is zinc. Zinc deficiency in biological membranes increases oxidative damage and impairs their functions (57,60). These studies prove that TQ and CS can inhibit inflammatory pathway cytokines.

Differential WBC content: The pathophysiology of asthma occurs a variety of changes at the cellular level as a result of the activity of eosinophils, mast cells, neutrophils, and T-lymphocytes (5). The WBC differential content in the serum was shown in Table 5. The eosinophil percentage was significantly decreased in the treatment group compared with the other groups ($p<0.05$).

CONCLUSION

Our findings lead us to consider that, dual applying of TQ and CS plays a critical role in reducing lipid peroxidation and consequently inflammation in pregnant asthmatic rats. Thus, we believe that the administration of dual TQ and CS can be used as a potential adjuvant in clinical practice, especially at the time of an attack in pregnant women with asthma.

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Ethics

Ethics Committee Approval: The experimental procedures were confirmed by Ethics Committee of Gazi University for Animal Experiments (approval number: G.Ü.ET-16.035, date: 21.03.2016).

Informed Consent: Patient approval has not been obtained as it is performed on animals.

Authorship Contributions

Concept: Ş.C.C., Design: Ş.C.C., B.B., Supervision: Ş.C.C., Resources: Ş.C.C., Materials: Ş.C.C., A.F.A., Data Collection or Processing: A.F.A., M.E., E.G.G.P., Analysis or Interpretation: A.F.A., M.E., E.G.G.P., Literature Search: A.F.A., E.G.G.P., Writing: A.F.A., Ş.C.C., Critical Review: Ş.C.C., B.B., Other: Ş.C.C.,

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