Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2023;48(1):268-277
DOI: 10.17826/cumj.1174607

RESEARCH

The effects of quercetin on erythropoietin, hypoxia-inducible factors-1α, 2α, and pyroptosis related genes expression levels in inflammation induced by TNF-α and LPS in HepG2 cells

Quercetin'in HepG2 hücrelerinde, TNF-Alfa ve LPS ile indüklenen enflamasyonda, eritropoietin, HIF-1α, HIF-2α ve piroptoz ile ilgili genlerin ekspresyon seviyeleri üzerine etkileri

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Abstract

Purpose: TNF-α, which increases in the circulation as a result of systemic inflammation, suppresses the production of the erythropoiesis regulating hormone, erythropoietin (EPO) and causes inflammation anemia. The anti-inflammatory activity of quercetin has been reported in different studies. However, no study has been found showing the effect of this situation on EPO gene expression. For this purpose, we evaluated the effects of quercetin on inflammation and, more importantly, its effects on EPO, suppressed in the inflammatory environment and Hypoxia-inducible factors (HIF), which are stimulators of EPO synthesis. In addition, we aimed to evaluate the effect of quercetin on pyroptosis, which is defined as pro-inflammatory programmed cell death.

Materials and Methods: We created inflammation models with TNF-α or LPS using HepG2 cells in vitro. In these inflammation models, we evaluated the effects of quercetin on proinflammatory mediators TNF-α, IL-1α, NF-κB gene expressions, EPO, HIF-1α, HIF-2α gene expressions, as well as pyroptosis-related caspase 1 and IL-18 gene expressions.

Results: Quercetin showed inflammatory effects by increasing TNF-α, IL-1α, and NF-κB mRNA levels. Consistent with this inflammatory effect, EPO mRNA expression was suppressed. HIF-1α and HIF-2α mRNA levels were increased.

Öz

Amaç: Sistemik inflamasyon sonucu dolaşımda artan TNF-α, eritropoezi düzenleyici hormon eritropoietin (EPO) üretimini baskılayarak inflamasyon anemisine neden olur. Quercetin'in antiinflamatuar aktivitesi farklı çalışmalarda rapor edilmiş ancak bu durumun EPO gen ekspresyonu üzerine etkisini gösteren bir çalışmaya rastlanmamıştır. Bu amaçla quercetin'in inflamasyon ve daha da önemlisi inflamatuar ortamda baskılanan EPO ve EPO sentezini uyaran Hipoksi ile indüklenebilir faktörler (HIF) üzerindeki etkilerini değerlendirdik. Ayrıca proinflamatuvar programlı hücre ölümü olarak tanımlanan piroptozis üzerine quercetin'in etkisini değerlendirmeyi amaçladık.

Gereç ve Yöntem: İn-vitro HepG2 hücrelerini kullanarak TNF-α veya LPS ile inflamasyon modelleri oluşturduk. Bu inflamasyon modellerinde, quercetin'in proinflamatuar mediatörler TNF-α, IL-1α, NF-κB gen ekspresyonları, EPO, HIF-1α, HIF-2α gen ekspresyonları ve ayrıca piroptozla ilişkili kaspaz-1 ve IL-18 gen ifadesi üzerindeki etkilerini değerlendirdik..

Bulgular: Quercetin, TNF-α, IL-1α, NF-κB mRNA düzeylerini yükselterek inflamatuar etkiler göstermiştir. Bu enflamatuar etkiyle uyumlu olarak, EPO mRNA ekspresyonu baskılanmıştır. HIF-1α ve HIF-2α mRNA seviyeleri yükselmiştir.

Sonuç: HIF'lerdeki artışın, inflamatuar sitokinlerin ve NF-κB'nin EPO üretimi üzerindeki baskılayıcı etkisini

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Received: 13.09.2022 Accepted: 15.03.2023

Conclusion: These results suggested that the increase in HIFs could not prevent the suppressive effect of inflammatory cytokines and NF-κB on EPO production. However, it has been observed that it tends to suppress proinflammatory cell death by decreasing caspase 1 and IL-18 mRNA levels. These results show that quercetin may show conflicting effects, and further studies are needed to test its safety.
Keywords: Anemia of inflammation, erythropoietin, hypoxia-inducible factors, TNF-α, quercetin

engelleyemediğini düşündürmektedir. Ancak kaspaz-1 ve IL-18 mRNA düzeylerini düşürerek proinflamatuar hücre ölümünü baskılama eğiliminde olduğu gözlemlenmiştir. Bu sonuçlar, quercetin'in çelişkili etkiler gösterebileceğini ve güvenliğini test etmek için daha ileri çalışmalara ihtiyaç olduğunu göstermektedir.

Anahtar kelimeler: İnflamasyon Anemisi, eritropoietin, hipoksi ile indüklenebilir faktörler, TNF-α, quersetin

INTRODUCTION

Anemia of inflammation (IA) is the second most common type of anemia after iron deficiency anemia worldwide and is the most common anemia in hospitalized or chronically ill patients[1]. It is common in patients with diseases that cause prolonged immune stimulation, including infection, autoimmune diseases, and cancer.

Systemic inflammation causes immune cell activation and synthesis of many proinflammatory cytokines. IA results from pathophysiological pathways that act through a stimulated immune system. The first way is that cytokines increased in inflammation induce hepcidin, the main regulator of iron homeostasis. The second pathophysiological pathway, which we will also focus on, is that cytokines, Interleukin-1 (IL-1) and Tumor Necrosis Factor- alpha (TNF-α), which increase during inflammation, prevent the formation of erythropoietin (EPO), which is the regulatory hormone of erythropoiesis. Observational studies in IA patients have shown lower-than-expected levels of the hormone EPO for most degrees of anemia[2].

EPO is a glycoprotein hormone responsible for erythropoiesis. Human erythropoietin is mainly produced by peritubular fibroblasts in the kidney. Small amounts of EPO also occur in the liver, bone marrow, spleen, lung, and brain[3]. Endogenous EPO production is mainly regulated by hypoxia, and this regulation takes place at the transcriptional level. When the oxygen supply of the human body decreases, transcription factors defined as hypoxia-inducible factors (HIF) are activated and induce the expression of many genes, including the EPO gene. Although HIF-1α was discovered during EPO studies, HIF-2α has been identified as the primary transcription factor that stimulates EPO expression[4]. However, the EPO promoter contains a binding site for transcriptional factors such as GATA-binding factor 2 (GATA-2) and Nuclear factor kappa B (NF-κB), as well as the hypoxic response element. Activation of these transcriptional factors and inflammatory cytokines suppresses EPO and its inducibility by hypoxia. NF-κB is a common mediator of proinflammatory cytokines and can modulate the expression of many genes involved in inflammation. It is known that IL-1 and TNF-α suppress EPO gene expression by inducing NF-κB activation[5].

In recent years, interest in the use of natural products such as quercetin and flavonoids has increased to treat different diseases. Quercetin is found in fruits and vegetables as a plant metabolite, often in low amounts as a conjugated quercetin molecule with sugar residues. In contrast, in its Aglycone-free form, quercetin is often marketed as a dietary supplement in high doses. Many studies have shown that quercetin acts as an anti-inflammatory, anti-apoptotic, antioxidant and anti-cancer agent[6]. The anti-inflammatory activity of flavonoids and the underlying mechanisms have been widely studied. Several studies in recent years have shown that quercetin can increase EPO expression and HIF-1α levels in a concentration-dependent manner[7,8].

This study was designed to evaluate the effects of quercetin on inflammation, and its effects on HIFs, which regulates EPO synthesis, which is suppressed in the inflammatory environment. In addition, we aimed to evaluate the effect of quercetin on pyroptosis, which has been defined as proinflammatory programmed cell death in recent years. According to current literature, studies reporting the anti-inflammatory activity of quercetin conclude that it suppresses TNF-α synthesis. However, the effect of it on EPO gene expression has not been demonstrated yet. For this purpose, HepG2 cells, a liver carcinoma cell, frequently used in similar studies due to intense EPO synthesis, are used in our study. As an experimental model, an inflammation model was created under two different conditions by applying TNF-α directly to cells in vitro

conditions and by applying lipopolysaccharide (LPS), which is isolated from the cell wall of bacteria and shown in many studies to increase TNF-α.

Understanding the regulatory effects of quercetin on inflammatory activation and EPO will provide new insights into the development of flavonoid preparations to prevent inflammatory diseases and treat anemia in inflammatory diseases.

MATERIALS AND METHODS

All the experiments were carried out in the molecular physiology laboratories of the Physiology Department of Medical Faculty/ Osmangazi University.

Cell culture

HepG2 cells (the American Type Culture Collection (ATTC), Rockville, MD), were cultured asdescribed previously [9].

MTT assay

24 hours later, after seeding in 96-well plates, HepG2 cells were treated with LPS (from Escherichia coli; Sigma-Aldrcih, Darmstadt, Germany) (1 ng/ml, 10 ng/ml, 100 ng/ml, 1 μg/ml, 10 μg/ml, 100 μg/ml) or Quercetin (Sigma-Aldrcih, Darmstadt, Germany) (1 μM, 10 μM, 25 μM, 50μM, 100 μM) or without LPS and Quercetin as a control for 24 hrs (n= 8). MTT colorimetric assay was used to evaluate cell proliferation [9]. The number of viable cells was determined in terms of absorbance at 550 nm using a microplate reader (AMR-100; ALLSHENG, Hangzhou, China). According to MTT results, the doses of 100 ng/ml for LPS and 10 μM/ml for quercetin were chosen. The dose of TNF-α mentioned in a previous study in our laboratory was used for inducing inflammation in HepG2 cells.

Experimental Groups

1. Control; had only complete medium.
2. Quercetin; 10 μM/ml quercetin added in complete medium.
3. TNF-α; 20 ng/ml TNF-α added complete medium.
4. LPS; 100 ng/ml LPS added complete medium.
5. TNF-α+Quercetin; 20 ng/ml TNF-α and 10 μM/ml quercetin added in complete medium.
6. LPS+Quercetin; 100 ng/ml LPS + 10 μM/ml quercetin added complete medium.

qRT-PCR

After detecting the doses as 10 μM for quercetin, 20 ng/ml for TNF-α and 100 ng/ml for LPS, we incubated and treated the cells in 24-well plates (we decided n number as 6 according to similar studies [7]) . Total RNA was isolated from the cells using the GeneJET RNA Purification Kit (Thermo Scientific, USA). The concentration and purity of the RNA were spectrometrically measured using NanoDrop 1000 (Thermo Scientific, USA). Isolated RNA samples were converted to complementary DNAs (cDNAs) using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA) at 42°C for 60 min and 70°C for 5 min. cDNA samples were stored at −80°C until further analysis. Expressions were measured using the Syber-Green qRT-PCR Kit (Thermo Scientific, USA). cDNA synthesis was verified by the detection of the β-actin transcript, which was used as an internal control. Relative differences in expression were determined using the comparative threshold cycle ($2^{-\Delta\Delta Ct}$) method with the data derived from minimum of three independent experiments.

Statistical analysis

We used GraphPad Prism6 software (San Diego, USA) for statistical analysis. Kolmogorov–Smirnov test was used to assess the normality of the distribution of the investigated parameters. Differences were tested by one-way ANOVA with Tukey's test as post hoc (for MTT analysis and, HIF-1α, NF-κB, IL-1α, HIF-2α), and Kruskal–Wallis with Dunn's test as post hoc (for EPO, TNF-α, Caspase-1, IL-18) for normally distributed or not, respectively. Data were expressed as mean-± standard deviation. $P < 0.05$ were considered statistically significant.

RESULTS

MTT

According to our data obtained from the MTT assay, we chose the 10 μM dose for quercetin as the most viable dose. Considering all the doses of LPS didn't show any different effect, we decided to go on the experiments with 100 ng/ml dose for LPS according to a similar study (Figure 1)[10].

For the dose of TNF-α to be used in the experiments, the doses applied in a previous study using the same cell line in our laboratory were taken as reference[9].

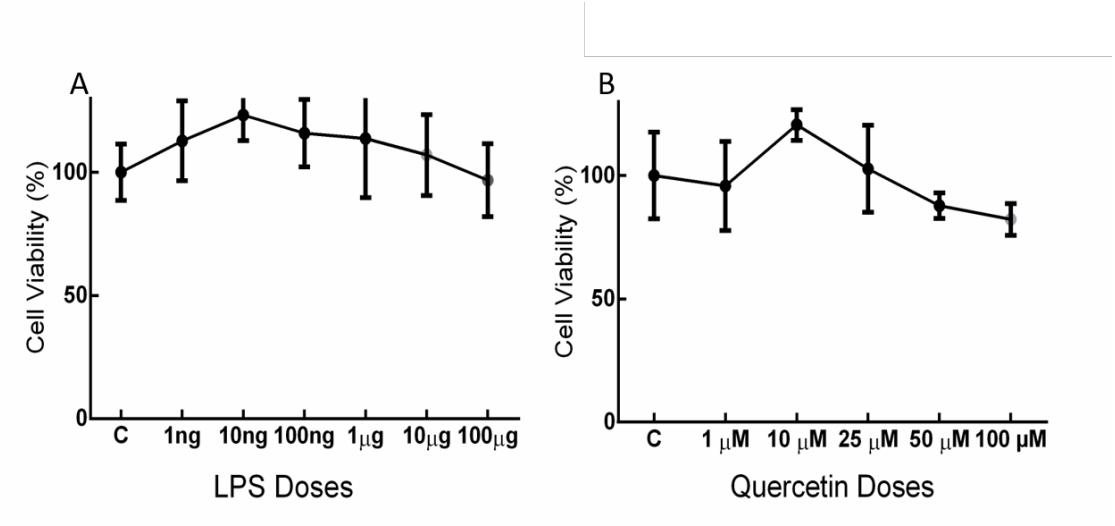

Figure 1. Effect of LPS and Quercetin treatment on the viability of HepG2 cell lines. (A) The cells were treated with different concentrations of LPS (1 ng/ml, 10 ng/ml, 100 ng/ml, 1 μg/ml, 10 μg/ml, 100 μg/ml) and (B) Quercetin (1 μM, 10 μM, 25 μM, 50 μM, 100 μM) for 24 hours. Percentages of viable cells after treatment were determined by MTT assay. LPS: lipopolysaccharide.

qRT-PCR results

In all the groups, Epo mRNA levels decreased but not significantly according to the control (Figure 2). HIF-1α mRNA levels increased significantly in the groups given quercetin with TNF-α and quercetin with LPS (Figure 3). HIF-2α mRNA levels increased significantly in the groups given quercetin and quercetin with TNF-α (Figure 4). In all the groups treated with quercetin, NF-KB and IL-1α mRNA levels increased significantly (Figure 5-6). TNF-α mRNA levels increased significantly in the groups given quercetin alone and quercetin with LPS (Figure 7). Caspase-1 mRNA levels also decreased in all the quercetin-given groups significantly (Figure 8). A significant increase is only detected in the TNF-α given group for IL-18 mRNA (Figure 9).

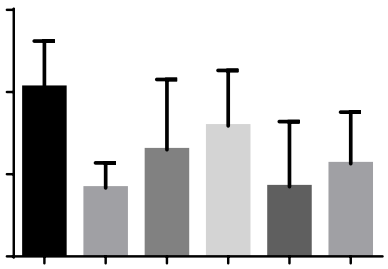

Figure 2. Expression of EPO gene in HepG2 cell lines treated with quercetin (10 μM), TNF-α (20 ng/ml), LPS (100 ng/ml), quercetin (10 μM) + TNF-α (20 ng/ml), quercetin (10 μM) + LPS (100 ng/ml) compared to control group. Data is presented as mean ± SD. LPS: lipopolysaccharide, TNF-α: Tumor Necrosis Factor alpha.

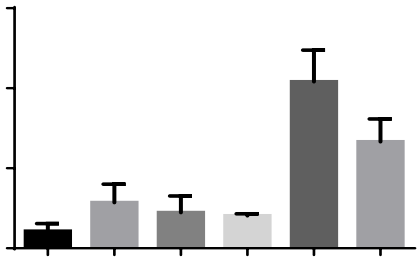

Figure 3. Expression of HIF-1 α gene in HepG2 cell lines treated with quercetin (10 μM), TNF-α (20 ng/ml), LPS (100 ng/ml), quercetin (10 μM) + TNF-α (20 ng/ml), quercetin (10 μM) + LPS (100 ng/ml) compared to control group. Data is presented as mean ± SD. ***P<0.001. LPS: lipopolysaccharide, TNF-α: Tumor Necrosis Factor alpha.

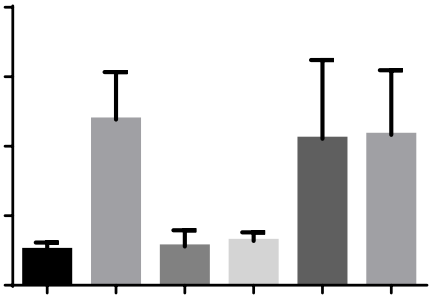

Figure 5. Expression of NF-κB gene in HepG2 cell lines treated with quercetin (10 μM), TNF-α (20 ng/ml), LPS (100 ng/ml), quercetin (10 μM) + TNF-α (20 ng/ml), quercetin (10 μM) + LPS (100 ng/ml) compared to control group. Data is presented as mean ± SD. *P<0.05. LPS: lipopolysaccharide, TNF-α: Tumor Necrosis Factor alpha.

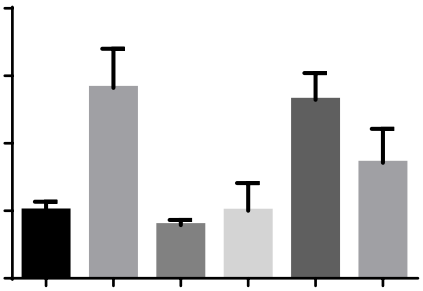

Figure 4. Expression of HIF-2α gene in HepG2 cell lines treated with quercetin (10 μM), TNF-α (20 ng/ml), LPS (100 ng/ml), quercetin (10 μM) + TNF-α (20 ng/ml), quercetin (10 μM) + LPS (100 ng/ml) compared to control group. Data is presented as mean ± SD. ***P<0.001. LPS: lipopolysaccharide, TNF-α: Tumor Necrosis Factor alpha.

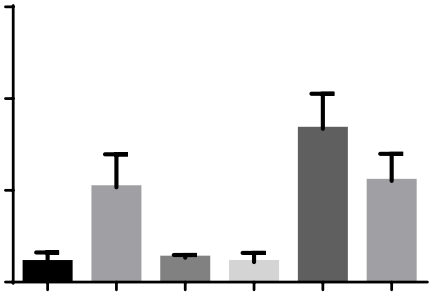

Figure 6. Expression of IL-1α gene in HepG2 cell lines treated with quercetin (10 μM), TNF-α (20 ng/ml), LPS (100 ng/ml), quercetin (10 μM) + TNF-α (20 ng/ml), quercetin (10 μM) + LPS (100 ng/ml) compared to control group. Data is presented as mean ± SD. **P<0.01, ***P<0.001. LPS: lipopolysaccharide, TNF-α: Tumor Necrosis Factor alpha.

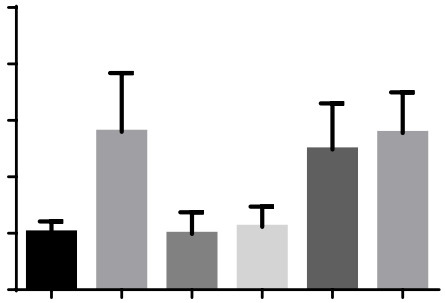

Figure 7. Expression of TNF-α gene in HepG2 cell lines treated with quercetin (10 μM), TNF-α (20 ng/ml), LPS (100 ng/ml), quercetin (10 μM) + TNF-α (20 ng/ml), quercetin (10 μM) + LPS (100 ng/ml) compared to control group. Data *is presented as* mean ± SD. *P<0.05. LPS: lipopolysaccharide, TNF-α: Tumor Necrosis Factor alpha.

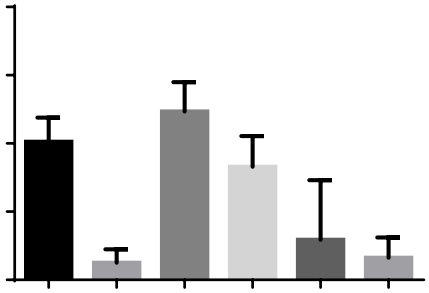

Figure 8. Expression of Caspase-1 gene in HepG2 cell lines treated with quercetin (10 μM), TNF-α (20 ng/ml), LPS (100 ng/ml), quercetin (10 μM) + TNF-α (20 ng/ml), quercetin (10 μM) + LPS (100 ng/ml) compared to control group. Data is presented as mean ± SD. **P<0.01, ***P<0.001. LPS: lipopolysaccharide, TNF-α: Tumor Necrosis Factor alpha.

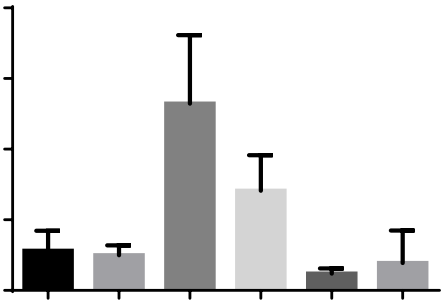

Figure 9. Expression of IL-18 gene in HepG2 cell lines treated with quercetin (10 μM), TNF-α (20 ng/ml), LPS (100 ng/ml), quercetin (10 μM) + TNF-α (20 ng/ml), quercetin (10 μM) + LPS (100 ng/ml) compared to control group. Data is presented as mean ± SD. **P<0.01. LPS: lipopolysaccharide, TNF-α: Tumor Necrosis Factor alpha.

DISCUSSION

This study was designed to evaluate the effects of quercetin on inflammation induced by LPS and TNF-α and the effect of quercetin on HIFs, which are the stimulators of EPO and EPO synthesis, in the inflammatory environment. We also evaluated the effects of quercetin on proinflammatory cell death.

In our study, an inflammation model was created using LPS, which is known to induce TNF-α synthesis in HepG2 cells. The effects of quercetin on proinflammatory mediators TNF-α, IL-1α, NF-κB gene expression, EPO, HIF-1α, HIF-2α gene expression, as well as pyroptosis-related caspase 1 and IL-18 gene expression were tested in an in vitro inflammation model. The results of our study show that quercetin can exert pro-inflammatory effects and modulate the expression of EPO and HIF, consistent with this effect. Protective effects on cell death were investigated.

There was a significant increase in TNF-α, IL-1α, and NF-κB gene expressions in the groups in which quercetin was given at a dose of 10 μM for 24 hours alone or in combination with TNF-α and LPS (Figure

5, 6, 7). It is known that TNF-α stimulates IL-1 release and causes NF-KB activation together with IL-1. A study in HepG2 cells demonstrated that treatment with TNF-α and IL-1 induces NF-κB activation [11]. NF-κB, which is the main molecular regulatory transcription factor of inflammatory responses, shows important effects on the development of many chronic diseases, including cancer. Studies reporting the anti-inflammatory properties of quercetin suggest that it inhibits TNF-α, IL-1α, and NF-κB [12]. However, consistent with our study, there are some contradictions in the literature regarding the effect of quercetin on inflammation. In a study using transgenic mice expressing an NF-κB-dependent luciferase reporter, quercetin was observed to increase LPS-induced NF-κB activation [13]. In a study in osteoblasts, it was shown that quercetin could not inhibit NF-κB translocation induced by TNF-α- [14]. In another study, it was observed that dietary quercetin did not inhibit NF-κB activation in the renal cortex in rats models of chronic glomerular disease [15]. In contrast to our findings, a study using HepG2 cells shows that quercetin reduces NF-κB activation induced by TNF-α. However, in this study, cells were pretreated with 10 μM quercetin for 4 hours and then incubated with 6 ng/ml TNF-α [16]. Quercetin administration time, TNF-α dose and administration time are clearly different from our study.

EPO gene expression was suppressed in the groups given quercetin alone or in combination with TNF-α and LPS, although it was not statistically significant compared to the other groups (Figure 2). In our study, EPO inhibition is an expected finding, consistent with the inflammatory mediator-enhancing effects of quercetin. It has been shown that proinflammatory cytokines such as TNF-α and IL-1 suppress EPO production by stimulating DNA binding with NF-κB activation. In a study using HepG2 cells, GATA-2 and NF-KB transcription factors were observed to play a role in the modulation of EPO gene expression by IL-1β and TNF-α [5]. The most important transcription factors for the induction of EPO synthesis are hypoxia-inducible factors. However, in addition to the HIFs, the EPO promoter contains a binding site for transcriptional factors such as GATA-2 and NF-KB. Activation of these transcriptional factors changes by inflammatory cytokines, binds to EPO and suppresses its hypoxic inducibility.

While HIF-1α gene expression increased significantly in the groups given quercetin together with TNF-α and LPS, this increase was also observed in the group given quercetin alone, although it was not significant (Figure 3). A significant increase in HIF-2α gene expression was observed in the groups given quercetin alone and in combination with TNF, while a slight but not significant increase in HIF-2α was observed in the group given quercetin with LPS (Figure 4). Similarly, in a study using HepG2 cells, the amount of HIF-1α protein increased significantly with 10 μM quercetin [7]. However, the effect of quercetin on HIF-2α was not studied in the literature reviews. It has been reported that quercetin inhibits the enzyme prolyl hydroxylase, which uses iron as a cofactor, through Fe chelation as an antioxidant property, and thus induces the accumulation of HIFs [17].

Observation of HIF accumulation in our study may be due to this feature of quercetin. On the other hand, in another study using HepG2 cells, proinflammatory cytokines such as IL-1β and TNF-α increased HIF-1α DNA binding and HIF-1α protein amount, but EPO gene expression was suppressed despite the increase in HIFs [18]. A study in pulmonary artery smooth muscle cells showed that NF-κB binds to the HIF-1α promoter in hypoxic and normoxic conditions, increasing HIF-1α mRNA levels [19]. This revealed that the NF-κB pathway has a role in maintaining basal HIF-1α mRNA levels. Consistent with this information in our study, the increase in HIFs gene expression may be due to the inducing effect of proinflammatory cytokines and NF-κB. These results may also suggest that the cytokine-induced inhibition of EPO production was not mediated by HIFs. As evidenced by subsequent studies, inflammatory cytokines and transcription factors such as GATA-2 and NF-κB are activated and bind to the EPO gene and suppress the hypoxic inducibility of EPO. NF-κB has also been reported to compete for p300/CBP, which are cofactors required for the activation of HIFs [20,21]. It can be thought that this situation leads to the depletion of cofactors and inhibits HIF activity. Another in vitro study in HepG2 cells concluded that IL-1 inhibited the expression of HNF-4α and completely suppressed the hypoxia-inducibility of EPO by causing degradation of this factor [22]. HNF-4α is a transcription factor that increases EPO gene expression by binding to EPO together with HIF, and the binding of HNF-4 is required for hypoxic induction of the EPO gene [23]. Recent studies on HIFs show that beyond their function of maintaining

homeostasis during hypoxia, they are regulators of many genes involved in immunity and inflammation in hypoxic and normoxic conditions [24]. Consistent with all this information, our study suggested that quercetin increased HIF-1α and HIF-2α gene expression, but it could not prevent the suppressive effect of inflammatory cytokines and NF-κB on EPO.

Other parameters we evaluated in our study are the effects of quercetin on caspase 1 and IL-18, which are associated with proinflammatory cell death. Caspase 1 gene expression was significantly suppressed in groups given quercetin alone or in combination with TNF-α and LPS (Figure 8). Similarly, in the groups quercetin was given alone or in combination with TNF-α and LPS, IL-18 gene expression was significantly suppressed compared to the group given only TNF-α, but not significantly compared to the group given only LPS (Figure 9). The results show that quercetin tended to suppress pyroptosis. Studies examining the relationship between quercetin and pyroptosis have shown, consistent with our study, that quercetin reduces caspase 1 and IL-18 gene expression by suppressing the activation of NLRP3 inflammasome structures activated by TNF-α [25]. An NLRP3 inflammasome construct consists of the NLRP3 protein, the caspase uptake domain-containing protein construct (ASC), and pro-caspase 1. When stimulated by various stimuli, these inflammasome structures activate caspase 1, the driver of pyroptosis, and cause the release of IL-1β and IL-18 via caspase 1 [26]. In a study using human periodontal connective stem cells, NLRP3, procaspase-1, and caspase-1 gene expression and protein levels increased significantly after incubation with 20 ng/ml TNF-α for 24 hours, while TNF-α with 1 μM quercetin for 24 hours incubation suppressed these protein and gene levels [27]. In another study using human macrophage cells, exposure to 20 μM quercetin for 24 hours suppressed fructose-induced caspase 1, IL-1β and IL-18 gene expression and protein levels [28]. In another study using human colorectal cancer cells, quercetin suppressed E.coli-induced activation of NLRP3 inflammasome structures, significantly reducing caspase 1 activation and IL-18 production [29].

To show that quercetin suppresses pyroptosis while increasing pro-inflammatory mediators is an important finding of our study. Studies reporting quercetin as an anticancer agent indicate that it induces apoptotic cell death in tumor cells [30]. It is thought-provoking that the HepG2 cell line, which we preferred in our study due to its intense EPO synthesis, is a tumor cell and quercetin tends to suppress pyroptosis in this cell line. A study in HepG2 cells reported that quercetin might exert dose-dependent cytoprotective and cytotoxic effects [7]. It has been reported that low concentrations and short-term administration of quercetin have antioxidant and anti-apoptotic effects, and at high concentrations, it disrupts the antioxidant defense system, causing oxidative damage and increasing apoptosis [31-33]. As a result, the effect of quercetin may vary according to concentration, application time, applied cell type or culture conditions, and contradictory effects may be seen at the same time.

The main limitation of our study is that our results consist of mRNA analyzes.

This study aims to examine the effects of quercetin on inflammation and HIFs, which are the stimulators of EPO and EPO synthesis in the inflammatory environment, therefore it is important since no other study has examined the effect of quercetin on EPO synthesis in the inflammatory environment and will be a pioneer for more comprehensive studies. At the same time, our results are important in terms of emphasizing that quercetin may show contradictory effects. Therefore, it shows that flavonoid preparations marketed as natural products in the treatment of diseases should be used with caution, and more comprehensive studies are needed to test their safety.

Yazar Katkıları: Çalışma konsepti/Tasarımı: MO, BA; Veri toplama: MO, BA, RO, AK; Veri analizi ve yorumlama: MO, BA, SK, NE; Yazı taslağı: MO, BA; İçeriğin eleştirel incelenmesi: MO, BA, SK, NE; Son onay ve sorumluluk: MO, BA, SK, RO, AK, NE; Teknik ve malzeme desteği: MO, BA, NE; Süpervizyon: SK, NE; Fon sağlama (mevcut ise): yok.
Etik Onay: Bu çalışma in vitro bir çalışma olduğundan, etik onay gerekli değildir.
Hakem Değerlendirmesi: Dış bağımsız.
Çıkar Çatışması: Yazarlar çıkar çatışması olmadığını beyan ederler.
Finansal Destek: Yazarlar finansal destek beyan etmemişlerdir.
Yazarın Notu: Bu çalışma Eskişehir Osmangazi Üniversitesi Bilimsel Araştırma Projeleri Koordinasyon Birimi tarafından 2018-2004 sayılı hibe kapsamında desteklenmiştir. Bu çalışmanın özgün bulguları, 14-16 Eylül tarihlerinde Londra/İngiltere'de düzenlenen Europhysiology 2018 Kongresi'nde poster olarak sunulmuştur.

Author Contributions: Concept/Design : MO, BA; Data acquisition: MO, BA, RO, AK; Data analysis and interpretation: MO, BA, SK, NE; Drafting manuscript: MO, BA; Critical revision of manuscript: MO, BA, SK, NE; Final approval and accountability: MO, BA, SK, RO, AK, NE; Technical or material support: MO, BA, NE; Supervision: SK, NE; Securing funding (if available): n/a.
Ethical Approval: As this study is an in-vitro study, no ethical approval is necessary.
Peer-review: Externally peer-reviewed.
Conflict of Interest: The authors declare that there are no conflicts of interest.
Financial Disclosure: Authors declared no financial support

Acknowledgement: This work has been supported by Eskisehir Osmangazi University Scientific Research Projects Coordination Unit under grant number #2018-2004. Initial findings of this study were presented as a poster in Europhysiology 2018 Congress, 14-16 September in London/ UK.

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