*Research Article / Araştırma Makalesi*

# Assessment of the Effects of Quercetin on Lung Injury After Hind Limb Ischemia Reperfusion in Rats

## Quercetin'in Sıçanlarda Alt Ekstremite İskemi Reperfüzyonu Sonrası Akciğer Hasarı Üzerindeki Etkilerinin Değerlendirilmesi

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**Abstract**

**Background:** Quercetin has antioxidant and anti-inflammatory effects. Although there are previous animal experiments investigating Quercetin's effect on ischemia reperfusion (IR) injury in the literature, studies involving effect of lower extremity IR on remote organ are rare.
**Materials and Methods:** 18 male Wistar Albino rats were randomly divided into 3 groups, 6 in each group as; Control (C), Ischemia-reperfusion (IR), IR-Quercetin, (IR-Q). Their weights were between 200-250 g. 30 minutes before the procedure20 mg/kgQuercetin was administered via intraperitoneal route. In the IR groups, infrarenal abdominal aorta was clamped by an atraumatic microvascular clamp. After 120 minutes of ischemia and reperfusion was achieved for another 120 minutes. When reperfusion period ended, tissue samples were taken from the lungs. Malondialdehyde (MDA) level, superoxide dismutase (SOD) and catalase (CAT) enzyme activity and histopathological parameters were compared.
**Results:** We found the MDA level in the IR group higher than the control group ($p<0.0001$). Lower MDA level was found in the IR-Q group compared tothe IR group ($p=0.012$). SOD and CAT enzyme activity in the IR group was notably lower in the control group ($p<0.0001$, $p<0.001$, respectively). Higher SOD and CAT enzyme activities were found in the IR-Q group compared to the IR group ($p=0.012$, $p<0.001$, respectively). Neutrophil infiltration/aggregation, alveolar wall thickness and total lung injury score were notably higher in IR group than in C group ($p=0.001$, $p=0.002$, $p<0.0001$, respectively). In addition, a statistically significant decrease was observed in the Quercetin treated group in neutrophil infiltration/aggregation, alveolar wall thickness and total lung injury score compared to the IR group ($p=0.023$, $p=0.022$, $p=0.002$, respectively).
**Conclusions:** We determined that intraperitoneally administered Quercetin at a dose of 20 mg/kg30 minutes before ischemia in rats reduces lipid peroxidation, oxidative stress and reduces the damage caused by IR in lung histopathology.Study findings suggest that Quercetin has a lung protective effect when administered before IR.

**Key Words:** Quercetin, Reperfusion Injury, Lung, Malondialdehyde, Superoxide Dismutase, Catalase

**Öz.**

**Amaç**: Quercetin antioksidan ve antiinflamatuar etkilere sahiptir. Literatürde daha önce Quercetin'in iskemi reperfüzyon hasarı üzerindeki etkisini araştıran hayvan deneyleri olmasına rağmen, alt ekstremite iskemi-reperfüzyonun uzak organ üzerindeki etkisini içeren çalışmalar nadirdir.
**Materyal ve Metod:** 18 adet erkek Wistar Albino sıçan, her grupta 6 adet olmak üzere rastgele olarak Kontrol (C), İskemi-reperfüzyon (IR), IR-Quercetin, (IR-Q) 3 gruba ayrıldı. Ağırlıkları 200-250 gr arasındaydı. İşlemden 30 dakika önce intraperitoneal olarak 20 mg/kg Quercetin verildi. IR ve (IR-Q) gruplarında infrarenal abdominal aorta atravmatik mikrovasküler klemp ile klemplendi. 120 dakika iskemiyi takiben 120 dakika süreyle reperfüzyon sağlandı. Reperfüzyon süresi sona erdiğinde akciğerlerden doku örnekleri alındı. Malondialdehit (MDA) düzeyi, süperoksit dismutaz (SOD) ve katalaz (CAT) enzim aktivitesi ve histopatolojik parametreler karşılaştırıldı.
**Bulgular:** IR grubunda MDA düzeyini kontrol grubuna göre daha yüksek bulundu ($p<0,0001$). IR-Q grubunda IR grubuna göre daha düşük MDA düzeyi bulundu ($p=0.012$). IR grubundaki SOD ve CAT enzim aktivitesi kontrol grubunda belirgin şekilde daha düşüktü (sırasıyla $p<0.0001$, $p<0.001$). IR-Q grubunda IR grubuna göre daha yüksek SOD ve CAT enzim aktiviteleri bulundu (sırasıyla $p=0.012$, $p<0.001$). Nötrofil infiltrasyonu/agregasyonu, alveolar duvar kalınlığı ve toplam akciğer hasarı skoru, IR grubunda C grubuna göre belirgin olarak daha yüksekti (sırasıyla $p=0,001$, $p=0,002$, $p<0,0001$). Ayrıca, Quercetin ile tedavi edilen grupta nötrofil infiltrasyonu/agregasyonu, alveolar duvar kalınlığı ve toplam akciğer hasarı skoru IR grubuna göre istatistiksel olarak anlamlı düşük bulundu (sırasıyla $p=0.023$, $p=0.022$, $p=0.002$).
**Sonuç:** Sıçanlarda iskemiden 30 dakika önce 20 mg/kg intraperitoneal olarak uygulanan Quercetin'in lipid peroksidasyonunu, oksidatif stresi azalttığını ve akciğer histopatolojisinde IR'nin neden olduğu hasarı azalttığını belirledik. Çalışma bulgularıQuercetin'in IR öncesi uygulandığında akciğer koruyucu etkisi olduğunu düşündürüyor.

**Anahtar kelimeler:** Quercetin, Reperfüzyon Hasarı, Akciğer, Malondialdehit, Süperoksit Dismutaz, Katalaz

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## Introduction

Ischemia reperfusion (IR) damage to skeletal muscle is inevitable in some clinical situations. Ischemia reperfusion injury causes a serious number of morbidity and mortality and its physiopathology has been investigated by many researchers so far. Inadequate or no blood supply for muscle tissue cause ischemia and when the blood supply is re-established reperfusion may unexpectedly cause increased ratio of mortality and morbidity due to extended inflammation and necrotic and apoptotic events caused by reactive oxygen species (ROS) (1). IR injury may not be limited to local muscle tissue and may spread to distant organs. Although thrombus or embolism is generally the main factor (2), there may be many reasons such as free-flap reconstruction, various orthopedic surgery and trauma (3,4).

Toxic ROSplay a main role while tissue destruction and organ dysfunction develop after ischemia-reperfusion injury. Free radicals provide this effect by disrupting the structure of molecules in the cell like DNA, protein, lipid, and carbonhydrate (5). During ischemic period, because of the decreasing phosphates rich in energy [adenosine triphosphate (ATP)], their degradation products' concentration level of tissue (hypoxanthine) elevates. However, hypoxanthine produces superoxide anions in the presence of oxygen and thus the peroxynitrite ($OONO^-$) and hydroxyl radical ($^{\cdot}OH$) are produced(6). Inflammation and various enzymatic reactions produce reactive oxygen species (ROS, from a variety of enzymatic sources) that leads to organ dysfunction and damage. This organ dysfunction and ischemia reperfusion damage is not only limited to the ischemic area, but also spread to distant organs. As following IR damage to the extremitiesmorbidity and mortality are generally caused by lung injury in the clinic, lungs are the main topic of this study. The interval between the ischemia and lung injury makes it even more interesting to find a cure to reduce this distal organ injury as this interval gives us an opportunity at the same time (7). Pulmonary edema develops due to increased vascular permeability following lung damage. Previous studies have shown that inflammatory mediators, neutrophils and partially free oxygen radicals have a role in remote organ damage caused by ischemia reperfusion (8). Various pharmacological agents have been tried in treatment to limit or prevent this damage to the remote organ (9). However, as far as we can tell**,** no studies have examined the effects of this compound on the lungs following IR damage to the muscles.

Antioxidants have scavenging and suppressing effect on free radicals. In this way, they inhibit or deactivate the effect of oxidants in various ways (10,11). ROS formation isdecreased by antioxidant treatment. Thus, this prevents IR-induced tissue damage and organ dysfunction. Nowadays, a rising interest has occured for natural antioxidants defending the human body against ROS and free radicals (12). Nearly all plant materials including plant based food products have these natural antioxidants havephenolic compounds (13). Flavonoids are the main group of the antioxidants. They also include flavanols,flavanones and flavanonols (14).

Antioxidant rich diets are also supported in the society nowadays. By scavanging free radicals these diets help to reduce the risk of many diseases including cancers, cardiac diseases. Many leaves, fruits, vegetables and grains contain Quercetin, which is the most putative flavanol (15). Flavonoids have an important role as an antidiabetic, antioxidative, anti-inflammatory, and antitumor agent (16,17). Quercetin has proven effects in inhibiting damage of oxidation to various tissues such as brain, pancreas, urinary bladder, heart,kidney, liverand cavernosal tissues (18).Besides being a strong antioxidant, Quercetin is a radical scavenger (19).

Our aim was to examine the preventive effect of Quercetin on remote organ after IR in experimental muscle injury. For this purpose biochemical [Malondialdehyde (MDA),Superoxide Dismutase(SOD), Catalase (CAT)enzyme activity level] and histopathological parameters were analyzed.

## Materials and Methods

### *Animals and Experimental Protocol*

The procedures in the experiment were carried out according to the permission of the Gazi University Institutional Local Animal Care and Use Committee (Ethical number: G.Ü.E.T-16-066). Wistar Albino rats (n= 18) between the age of 10 and 12 weeks and weighingbetween 200-250 g were used. The rats were housed in a temperature 20-21 $^{o}$C and maintained on a 12/12 reversed light cycle anduntil 2 hours before the anesthesia procedure all had access to food freely.

Rats were anesthetized by intramuscular xylazine hydrochloride 10 mg/kg (Alfazyne, 2%; Ege Vet, Ltd., Izmir, Türkiye) and ketamine (Ketalar; Parke-Davis; Pfizer, Inc., New York, NY, USA) at 75 mg/kg. Midline laparotomy was done under general anesthesia.

Rats were casually divided into three groups (n=6) as; Control (C), Ischemia-reperfusion (IR), IR-Quercetin group.

***Control group:*** AfterMidline laparotomy no additional surgical intervention was performed. After 4 hours lung tissue was collected and animals were sacrificed.

**IR group:**Infrarenal segment of the aorta was clamped with a vascular clamp.Then, 2 hours later the clamp was removed and reperfusionwas allowed for 2 more hours. In the end, lung tissue was collected and rats were sacrificed.

***IR group with Quercetine:*** 20 $mg.kg^{-1}$Quercetine(Quercetın Anhydrous, Sigma-Aldrich, Q4951-10G) was given intraperitoneally for 30 min before the ischemia period.After-Midline laparotomy infrarenal segment of the aorta was clamped with a vascular clamp. Then, 2 hours later the clamp was removed and reperfusion was allowed for 2 more hours. After collecting lung tissuesamplesat the end of the reperfusion period, which lasted 2 hours, rats were sacrificed.

Intracardiac blood samples (up to 10 ml) were obtained.

Histopathological and biochemical parameters were analyzed after the reperfusion period (20).

***Oxidative and antioxidant Parameters***

Lung tissue was washed withdeionized, cold (4 °C) water to discard blood contamination and then homogenized with Heidolph Instruments homogenizer (GMBH & CO KGDiax 900 GermanyR) at 1000 U for about 3 min. Initial preparation of the tissues is required for measurements on cell contest. After 60 min. ofcentrifugation at 10,000 g the upper clear layer was taken. After reduction of NBT to $NBTH_2$measurement ofabsorbance increase at 560 nm was the main method used for SOD activity measurement (21). The amount of enzyme protein resulted in fifty per cent inhibition in $NBTH_2$ reduction rate was defined asone unit of SOD activity and U/mg protein was used to express.

To measure MDA levels Van Ye et al method was used, thiobarbituric acid reactive substances (TBARS) assay (22). MDA level, as MDA or similar substances react with TBA and this reaction results in production of pink pigment with an absorption maximum of 532 nm. Sampleis mixed with 20 % (w/v) trichloroacetic acidin room temperature and the precipitate is then centrifuged at 3000 rpmfor 10 min. An aliquot of the supernatant is then placed into an equal volume of 0.6% (w/v) TBA in a boiling water bath for 30 min. Sample and blank absorbance were read at 532 nm (UV/VIS -1601 Shimadzu Spectrophotometer, Japan) after coolingand the results expressed as nmol/mg protein, based on a standart graph where 1,1,3,3-tetramethoxypropane has been used as the MDA standard. Lowry O method is used to determine the samples' protein levels and bovine serum albumin was used as standard protein (23).

Measurement of absorbance decrease due to $H_2O_2$ consumption at 240 nm by Aebi H method was used for CAT activity (24)**.**

***Histopathological Analysis***

10% neutral formalin solution was used to fix in after lung tissue samples were removed. After that,samehistologist, who was blinded to the study examined the lungs with light microscopy. A 200-400 times magnified microscopywas used to evaluate a total of 10 randomhematoxylin and eosine (H&E) stained sections. Light microscope was used to examine stained slides. Lung injury degree was measured by using infiltration of neutrophils and thicknes of alveolar walls. A score was given to each parameter and 0 point for any, 1 point for quite little, 2 points for middle, 3 points for severe.Total lung injury score was calculated by adding the two scores (25).

***Statistical Analysis***

*Statistical analysis was performed with SPSS 20.0 statistical software (SPSS, Chicago, IL, USA). p<0.05 was considered to indicate a statistically significant difference. The data were expressed as Mean ± Standard Deviation (Mean ± SD) and histopathological paramaters median (25%-75%). Shapiro-Wilk test was used to analyze each categorical variable. Bonferroni Correction test, Kruskal-Wallis, Mann-Whitney U testswere used to test histopathological and biochemical parameters.*

## *Results*

***Histopathological evaluation***

Normal lung tissue morphology is shown by H&E staining in Figure 1. Severe acute inflammatory processes,degenerative cells, neutrophils, macrophages and hemorrhage were detected in the IR group (Fig. 2). The IR-Q group showed hemorrhage, inflammation, vascular congestion and edema, too (Fig. 3). But the key point is the acute inflammatory process was mild. Quercetin significantly prevented degenerative changes of the lung. Fig. 3 shows an improvement of the inflamed lung tissue morphology in the quercetin treated group (Group IR-Q). Histopathological changes in Group IR-Q were significantly less than IR group. In group IR total lung injury score, neutrophil infiltration/aggregation and alveolar wall thickness were notably higher compared to the group C (p=0.001, p=0.002, p<0.0001, respectively). In addition, there was statistically significant decrease in alveolar wall thickness, neutrophil infiltration/aggregation, and total lung injury score in the IR-Q group (p=0.023, p=0.022, p=0.002, respectively) (Table 1).

**Table 1.** Histopathological datas of lung tissue [Median (25%-75%)]

| | Group C (n=6) | Group I/R (n=6) | Group IR-Q (n=6) | P** |
|---|---|---|---|---|
| **Neutrophil infiltration/ aggregation** | 0 (0-1) | 2.5 (1-3)* | 1 (0-1)& | 0.003 |
| **Alveolar wall thickness** | 0 (0-1) | 2 (1-3)* | 0.5 (0-1)& | 0.001 |
| **Total score** | 1 (0-1) | 4 (2.75-6)* | 1.5 (0-2.25)& | <0.0001 |

*C: Control, I/R: Ischemia/Reperfusion, IR-Q: Ischemia Reperfusion- Quercetin*

*p **Significance level with Kruskal Wallis test p <0.05*

** p <0.05: compared to Group C*

*& p <0.05: Compared to Group I/R*

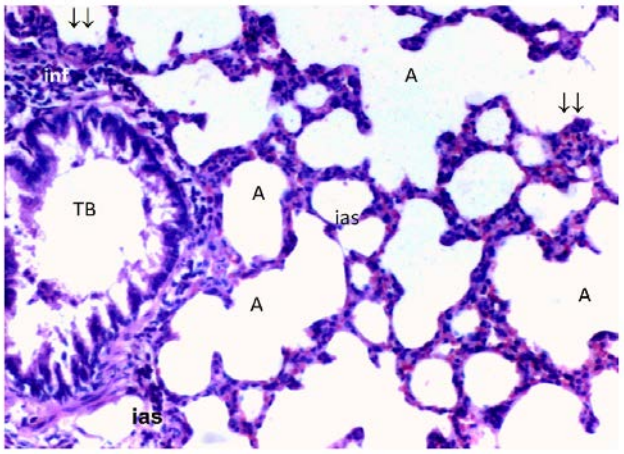


**Figure 1. Control group:** Normal appearance of lung tissue. (H&E: he matoxylin and eosin X100) (A: Alveoli, TB: Terminal bronchiole, ias: interalveolar septum, inf: inflammation, ↓↓: (septum) thickening).

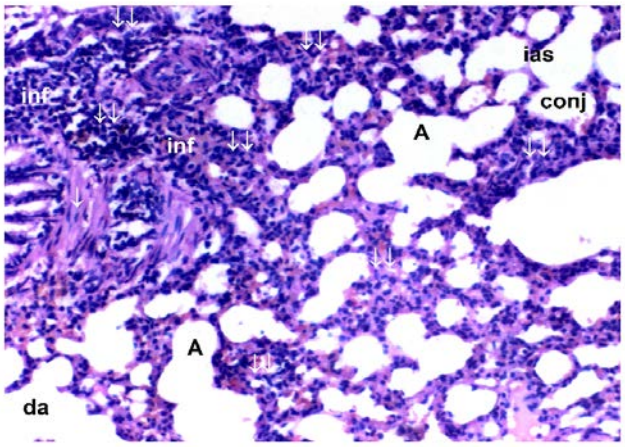


**Figure 2. I/R group:** Light microscopic view of lung tissue; (H&E: hematoxylin and eosin X100) Degenerative cells, severe acute inflammatory processes, neutrophils, macrophages and hemorrhage were seen in the IR group. (A: Alveoli, da: ductus alveolaris, ias: interalveolar septum, inf: inflammation, ↓↓: (septum) thickening, ↓: pulmonary vessel thickening, conj: capillary congestion).

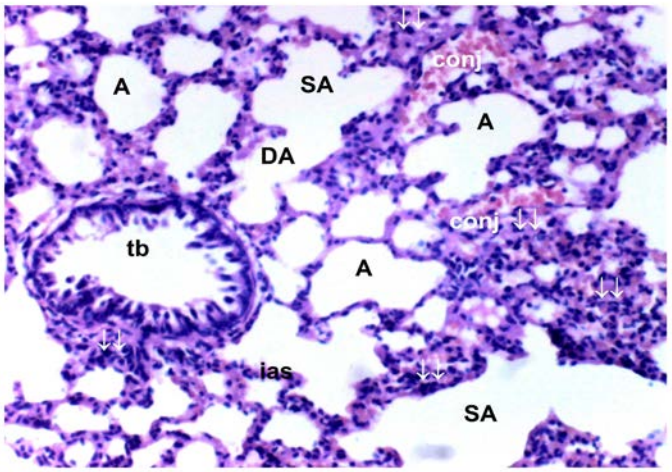


**Figure 3. IR-QUERCETIN group:** Light microscopic view of lung tissue; (H&E: hematoxylin and eosin X100) Acute inflammatory process was mild. Administration of Quercetin in rats significantly prevented degenerative changes in the lung. (A: Alveoli, tb: terminal bronchiole, da: ductus alveolaris, SA: saccus alveolaris, ias: interalveolar septum, inf: inflammation, ↓↓: (septum) thickening, conj: capillary congestion).

***Biochemical Evaluation***

Lung tissue MDA levels and CAT ,SODenzyme activities in groups were shown infigure 4,5,6. Our findings showed that MDA level in the IR group was significantly higher compared to the control group (p<0.0001).MDA level in the IR-Q group was significantly lower than the IR group (p=0.012) (Figure 4). Lower SOD and CAT enzyme activity weredetected in IR group compared to the control group (p<0.0001, p<0.001, respectively).When compared to IR group, SOD and CAT enzyme activities were fairly higher in the IR-Q group ( p<0.001, p=0.012, respectively) (Figure 5,6).

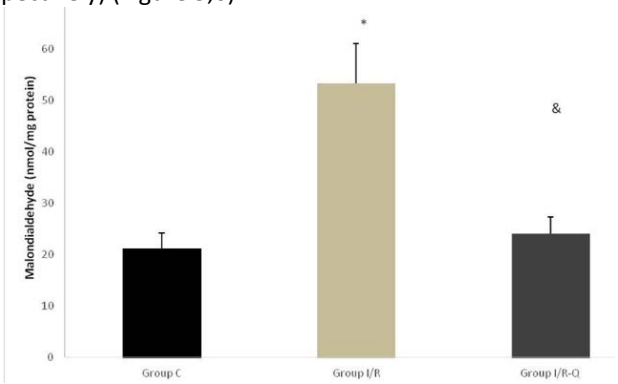


**Figure 4.** Malondialdehyde level of lung tissue
C:Control, I/R:Ischemia/Reperfusion, IR-Q: Ischemia Reperfusion-Quercetin
** p <0.05: compared to Group C, & p <0.05: Compared to Group I/R*

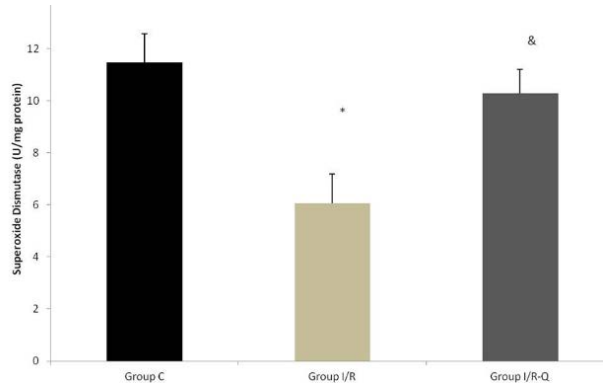


**Figure 5.** Superoxide Dismutase enzyme activity of lung tissue
C:Control, I/R:Ischemia/Reperfusion, IR-Q: Ischemia Reperfusion-Quercetin
** p <0.05: compared to Group C, & p <0.05: Compared to Group I/R*

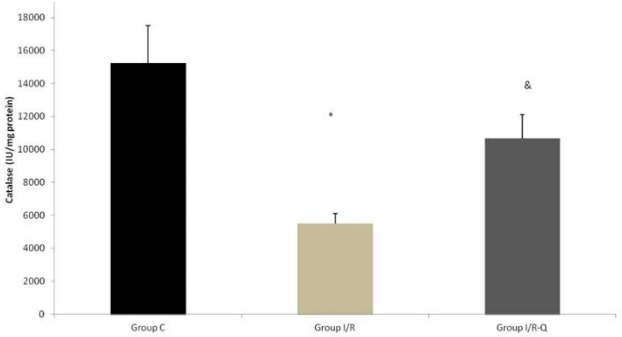


**Figure 6.** Catalase enzyme activity of lung tissue
C:Control, I/R:Ischemia/Reperfusion, IR-Q: Ischemia Reperfusion-Quercetin
** p <0.05: compared to Group C, & p <0.05: Compared to Group I/R*

## Discussion

In the present study, as lung related problems caused by extremity IR injury are the major causes of morbidity and mortality in our clinical experience, we demonstrated that Quercetin treatment prevents remote organ (lung) damage caused by IR injury, helps to maintain lung tissue morphology and attenuates IR induced lipid peroxidation. IR injury initiates a sequence of events that results in cellular damage and organ dysfunction (26). As a prominent component of the multiple organ dysfunction syndrome acute lung injuryis the main cause of the lethal events associated with IR injury(27). Neutrophil activation has an important role in IR damage. By inflammatory cytokine production and release of oxygen free radicals endothelial cell damage occurs and causes major pathological damage in lung injury (28). Generation of inflammatory cytokines like IL-6 is prevented by Quercetin and reperfusion injury is decreased (29).

Free oxygen radicals occur during IR injury have an important role in the tissue damage. So antioxidant agents are beneficial for the physiopathological changes appear during IR. We investigated Quercetin as a protective agent for remote organ injury after skeletal muscle IR injury.Free oxygen radicals cause lipid peroxidationdue to peroxidation of polyunsaturated fatty acids in the cell membrane (30). Formation of lipid peroxides such as the MDA is caused by oxidation of polyunsaturated fatty acids. This also causes enzymatic or chemical deterioration by $O_2^-$ or OH (31). Lipid peroxidation triggered by oxygen free radicals is one of the important causes of lung injury (32). In our study, after muscle IRwe measured MDA levels in the lung as lipid peroxidation marker and lung damage was found to be associated with high MDA levels (33). In our study, Quercetin as an inhibitor of lipid peroxidation significantly decreases the MDA levels. Lipid peroxidation leads to structural and functional damage to the cells and that results in cellular injury (34). SOD and CAT are antioxidants produced endogenously and take role in protecting the cell against ROS by catalysing their conversion to less reactive species (30,31,34). SOD converts superoxide radicals ($O_2^-$) to hydrogen peroxide ($H_2O_2$) and oxygen ($O_2$). SOD activity of lungtissuewas significantly reduced in the IR group compared with C group and by treatment of Quercetin increased tissue SOD activity was observed compared to IR group. CAT is primary enzymatic defence against $H_2O_2$ generation. Statistically significant difference in the CAT enzyme activity was found between IR and C groups. Tissue CAT enzyme activity was notably elevated in IR-Q group compared to IR group.

In the present study, in the IR group disruption of alveolar architecture was indicated clearly by light microscopy. Also neutrophil infiltration in histopathological sections in the IR group was observed. However, Quercetin decreased this histological damage in sections from the IR-Q group. It appears that Quercetin can significantly attenuate leukocyte recruitment in the lung tissues.

Flavonoids play an important role as an anti-inflammatory, antitumor and antioxidative agent. Vegetables, seedsand fruits naturally have Quercetin as a flavonoid. It has a strong antioxidant capacity and especially protective activity against free radical and oxidative tissue damage (35). Quercetin's protective effect against IR injury in several organs like ovarium, liver and heart was shown in earlier studies (18, 36).Recently, Quercetin's neuroprotective and antioxidant effect has been demonstrated (37). Liu et al. have found that Quercetin shows protective effect by decreasing MDA levels in myocardial IR injury (36). We have found increased tissue MDA levels in IR group compared to control group. But in the quercetin treated group MDA levels were decreased. Lipid peroxidation was attenuated notably in Quercetin treated group.

SOD, CAT as antioxidant enzymes play important role in IR injury. In previous studies it has been shown that Quercetin has a protective effect against IR injury in heart, skeletal muscle, renal tissueby increasing SOD and CAT activities (36,38). Our findings for remote organ ischemia reperfusion injury treated with Quercetin were similar with previous studies for IR injury of primer organ. Lung tissue SOD and CAT activity treated with Quercetin was increased compared to the IR group. There are studies reported the protective effects of Quercetin on lung inflammation, lung cancer, and lipopolysaccharide (LPS)-induced oxidative stress in the lung(39).Huang et al.(40) also found similar results with us on their experimental study. Quercetin pretreatment reduced inflammation and oxidative stress in rats with LPS-induced acute lung injury. But we still don't know the exact mechanism of quercetin on enzyme activities.

Based on the present findings, pretreatment with Quercetin can reduce lung injuries resulting from muscle IR. Inhibition of neutrophil aggregation, as well as increase in antioxidant enzymes in the injured lung, might be the underlying mechanisms. Further research is required to confirm the clinical effectiveness of this compound.

## Conclusion

We determined 20 $mg.kg^{-1}$Quercetin administered intraperitoneally 30 minutes before ischemia reduces oxidative stres, lipid peroxidation and decreases the damage caused by IR in rats' lung histopathology. Quercetin applied before IR in rats has a protective effect. We believe that when the findings of our study are supported by other studies, the protective effects of Quercetin on IR damage will be demonstrated in detail and the indications for use will expand.

***Ethical Approval:*** Gazi University Institutional Local Animal Care and Use Committee (Ethical number: G.Ü.E.T-16-066).

***Author Contributions:***
*Concept: M.A., Y.K.*
*Literature Review: G.L.O., Y.K.*
*Design : M.K., Y.K.*
*Data acquisition: A.Ö., Y.K.*
*Analysis and interpretation: Y.K., A.K., T.M., G.K., M.K.*
*Writing manuscript: Y.K.*
*Critical revision of manuscript: . M.A., Y.K.*
***Conflict of Interest:*** *None*
***Financial Disclosure:*** *None*

## References


1. Blaisdell FW. The pathophysiology of skeletal muscle ischemia and the reperfusion syndrome: a review. Cardiovasc Surg 2002;10:620–30.
2. Earnshaw JJ. Demography and etiology of acute leg ischemia. Semin Vasc Surg 2001;14:86–92.
3. Sotoudeh A, Takhtfooladi MA, Jahanshahi A,Asl AHK, Takhtfooladi HA, Khansari M. Effect of N-acetylcysteine on lung injury induced by skeletal muscle ischemia-reperfusion. Histopathlogical study in rat model. Acta Cir Bras 2012;27:168–71.
4. Takhtfooladi MA, Jahanshahi A, Jahanshahi G, Sotoudeh A, Takhtfooladi HA, Khansari M. Protective effect of N-acetylcysteine on kidney as a remote organ after skeletal muscle ischemia-reperfusion. Acta Cir Bras. 2012 Sep;27(9):611-5. doi: 10.1590/s0102-86502012000900004. PMID: 22936085.
5. Köksal E, Gülçin I, Beyza S, Sarikaya O, Bursal E. In vitro antioxidant activity of silymarin. J Enzyme Inhib Med Chem. 2009 Apr;24(2):395-405. doi: 10.1080/14756360802188081. PMID: 18830883.
6. Eltzschig HK, Collard CD. Vascular ischaemia and reperfusion injury. Br Med Bull 2004;70:71-86.
7. Pedro C, Susimeire G. Ischemia/reperfusion-induced lung injury prevention: many options, no choices. J Bras Pneumol. 2016;42(1):7-8.
8. Takhtfooladi H, Takhtfooladi M, Moayer F, Mobarakeh S. Melatonin attenuates lung injury in a hind limb ischemia-reperfusion rat model. Rev Port Pneumol (2006). 2015 Jan-Feb;21(1):30-5. doi: 10.1016/j.rppnen.2014.01.010. Epub 2015 Jan 20. PMID: 25854133.
9. Takhtfooladi MA, Takhtfooladi HA, Sedaghatfar H, Shabani S. Effect of low-level laser therapy on lung injury induced by hind-limb ischemia/reperfusion in rats. Lasers Med Sci. 2015 Aug;30(6):1757-62. doi: 10.1007/s10103-015-1786-6. Epub 2015 Jul 9. PMID: 26155904.
10. Kalin P, Gulcin I, Goren AC. Antioxidant activity and polyphenol content of cranberries (Vaccinium macrocarpon). Rec Nat Prod 2015;9:496–502.
11. Sehitoglu MH, Han H, Kalin P, Gülçin İ, Ozkan A, Aboul-Enein HY. Pistachio (Pistacia vera L.) gum: a potent inhibitor of reactive oxygen species. J Enzyme Inhib Med Chem. 2015 Apr;30(2):264-9.
doi: 10.3109/14756366.2014.915395. Epub 2014 Jun 18. PMID: 24939094.
12. Gulcin I. In vitro prooxidant effect of caffeine. J Enzym Inhib Med Chem 2008;23:149–52.
13. Cetinkaya Y, Göçer H, Menzek A, Gülçin I. Synthesis and antioxidant properties of (3,4-dihydroxyphenyl)(2,3,4-trihydroxyphenyl)methanone and its derivatives. Arch Pharm (Weinheim). 2012 Apr;345(4):323-34. doi: 10.1002/ardp.201100272. Epub 2011 Dec 7. PMID: 22147548.
14. Gülçin I, Elias R, Gepdiremen A, Chea A, Topal F. Antioxidant activity of bisbenzylisoquinoline alkaloids from Stephania rotunda: cepharanthine and fangchinoline. J Enzyme Inhib Med Chem. 2010 Feb;25(1):44-53. doi: 10.3109/14756360902932792. PMID: 20030508.
15. Gülçin İ, Tel AZ, Kirecci E. Antioxidant, antimicrobial, antifungal and antiradical activities of *Cyclotrichium niveum* (Boiss.) Manden and Scheng. Int J Food Propert 2008;11:450–71.
16. Sinha R, Srivastava S, Joshi A, Joshi UJ, Govil G. In-vitro anti-proliferative and anti-oxidant activity of galangin, fisetin and quercetin: role of localization and intermolecular interaction in model membrane. Eur J Med Chem. 2014 May 22;79:102-9. doi: 10.1016/j.ejmech.2014.04.002. Epub 2014 Apr 2. PMID: 24727463.
17. Burak C, Wolffram S, Zur B, Langguth P, Fimmers R, Alteheld B, et al. Effect of alpha-linolenic acid in combination with the flavonol quercetin on markers of cardiovascular disease risk in healthy, non-obese adults: A randomized, double-blinded placebo-controlled crossover trial. Nutrition. 2019 Feb;58:47-56. doi: 10.1016/j.nut.2018.06.012. Epub 2018 Jul 12. PMID: 30278429.
18. Chen YW, Chou HC, Lin ST, Chen YH, Chang YJ, Chen L, et al. Cardioprotective Effects of Quercetin in Cardiomyocyte under Ischemia/Reperfusion Injury. Evid Based Complement Alternat Med. 2013;2013:364519. doi: 10.1155/2013/364519. Epub 2013 Mar 14. PMID: 23573126; PMCID: PMC3612448.
19. Gülçin İ, Elmastas M, Aboul-Enein HY. Determination of antioxidant and radical scavenging activity of basil (*Ocimum basilicum*) assayed by different methodologies. Phytother Res 2007;21:354–61.
20. Kılıç Y, Özer A, Tatar T, Zor MH, Kirişçi M, Kartal H, et al. Effect of picroside II on hind limb ischemia reperfusion injury in rats. Drug Des Devel Ther. 2017 Jun 26;11:1917-1925. doi: 10.2147/DDDT.S132401. PMID: 28721011; PMCID: PMC5500556.
21. Durak I, Canbolat O, Kavutçu M, Oztürk HS, Yurtarslani Z. Activities of total, cytoplasmic, and mitochondrial superoxide dismutase enzymes in sera and pleural fluids from patients with lung cancer. J Clin Lab Anal. 1996;10(1):17-20. doi: 10.1002/(SICI)1098-2825(1996)10:1<17::AID-JCLA4>3.0.CO;2-I. PMID: 8926562.
22. Van Ye TM, Roza AM, Pieper GM, Henderson J Jr, Johnson CP, Adams MB. Inhibition of intestinal lipid peroxidation does not minimize morphologic damage. J Surg Res. 1993 Nov;55(5):553-8. doi: 10.1006/jsre.1993.1183. PMID: 8231175.
23. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951 Nov;193(1):265-75. PMID: 14907713.
24. Aebi H. Catalase. In: H.U. Bergmeyer (Ed): Methods of Enzymatic Analysis, Academic Press, New York and London, 1974; pp.673-677.
25. Tuncay A, Sivgin V, Ozdemirkan A, Sezen SC, Boyunaga H, Kucuk A, et al. The Effect of Cerium Oxide on Lung Tissue in Lower Extremity Ischemia Reperfusion Injury in Sevoflurane Administered Rats. Int J Nanomedicine. 2020 Oct 6;15:7481-7489. doi: 10.2147/IJN.S263001. PMID: 33116483; PMCID: PMC7547779.
26. Akbas H, Ozden M, Kanko M, Maral H, Bulbul S, Yavuz S, et al. Protective antioxidant effects of carvedilol in a rat model of ischaemia-reperfusion injury. J Int Med Res. 2005 Sep-Oct;33(5):528-36. doi: 10.1177/147323000503300508. PMID: 16222886.
27. Abola MTB, Golledge J, Miyata T, Rha SW, Yan BP, Dy TC, et al. Asia-Pacific Consensus Statement on the Management of Peripheral Artery Disease: A Report from the Asian Pacific Society of Atherosclerosis and Vascular Disease Asia-Pacific Peripheral Artery Disease Consensus Statement Project Committee. J Atheroscler Thromb. 2020 Aug 1;27(8):809-907. doi: 10.5551/jat.53660. Epub 2020 Jul 4. Erratum in: J Atheroscler Thromb. 2020 Dec 1;27(12):1374. Erratum in: J Atheroscler Thromb. 2021;28(3):304. PMID: 32624554; PMCID: PMC7458790.
28. Harkin DW, Barros D'sa AA, McCallion K, Hoper M, Halliday MI, Campbell FC. Circulating neutrophil priming and systemic inflammation in limb ischaemia-reperfusion injury. Int Angiol. 2001 Mar;20(1):78-89. PMID: 11455935.

29. Zahedi M, Ghiasvand R, Feizi A, Asgari G, Darvish L. Does Quercetin Improve Cardiovascular Risk factors and Inflammatory Biomarkers in Women with Type 2 Diabetes: A Double-blind Randomized Controlled Clinical Trial. Int J Prev Med. 2013 Jul;4(7):777-85. PMID: 24049596; PMCID: PMC3775217.
30. Birdane FM, Cemek M, Birdane YO, Gülçin I, Büyükokuroğlu ME. Beneficial effects of Foeniculum vulgare on ethanol-induced acute gastric mucosal injury in rats. World J Gastroenterol. 2007 Jan 28;13(4):607-11. doi: 10.3748/wjg.v13.i4.607. PMID: 17278229; PMCID: PMC4065985..
31. Pérez JM, Arenas FA, Pradenas GA, Sandoval JM, Vásquez CC. Escherichia coli YqhD exhibits aldehyde reductase activity and protects from the harmful effect of lipid peroxidation-derived aldehydes. J Biol Chem. 2008 Mar 21;283(12):7346-53. doi: 10.1074/jbc.M708846200. Epub 2008 Jan 22. PMID: 18211903.
32. Olguner C, Koca U, Kar A, Karci A, Işlekel H, Canyilmaz M, et al. Ischemic preconditioning attenuates the lipid peroxidation and remote lung injury in the rat model of unilateral lower limb ischemia reperfusion. Acta Anaesthesiol Scand. 2006 Feb;50(2):150-5. doi: 10.1111/j.1399-6576.2006.00938.x. PMID: 16430534.
33. Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol 1990;86:421–3.
34. Henderson PW, Singh SP, Weinstein AL, Nagineni V, Rafii DC, Kadouch D, et al. Therapeutic metabolic inhibition: hydrogen sulfide significantly mitigates skeletal muscle ischemia reperfusion injury in vitro and in vivo. Plast Reconstr Surg. 2010 Dec;126(6):1890-1898. doi: 10.1097/PRS.0b013e3181f446bc. PMID: 21124129.
35. Hazafa A, Rehman KU, Jahan N, Jabeen Z. The Role of Polyphenol (Flavonoids) Compounds in the Treatment of Cancer Cells. Nutr Cancer. 2020;72(3):386-397. doi: 10.1080/01635581.2019.1637006. Epub 2019 Jul 9. PMID: 31287738.
36. Liu H, Guo X, Chu Y, Lu S. Heart protective effects and mechanism of quercetin preconditioning on anti-myocardial ischemia reperfusion (IR) injuries in rats. Gene. 2014 Jul 15;545(1):149-55. doi: 10.1016/j.gene.2014.04.043. Epub 2014 Apr 24. PMID: 24769323.
37. Dok-Go H, Lee KH, Kim HJ, Lee EH, Lee J, Song YS, et al. Neuroprotective effects of antioxidative flavonoids, quercetin, (+)-dihydroquercetin and quercetin 3-methyl ether, isolated from Opuntia ficus-indica var. saboten. Brain Res. 2003 Mar 7;965(1-2):130-6. doi: 10.1016/s0006-8993(02)04150-1. PMID: 12591129.
38. Zhang W, Wang Y, Yang Z, Qiu J, Ma J, Zhao Z, et al. Antioxidant treatment with quercetin ameliorates erectile dysfunction in streptozotocin-induced diabetic rats. J Biosci Bioeng. 2011 Sep;112(3):215-8. doi: 10.1016/j.jbiosc.2011.05.013. Epub 2011 Jun 14. PMID: 21664865.
39. Sul OJ, Ra SW. Quercetin Prevents LPS-Induced Oxidative Stress and Inflammation by Modulating NOX2/ROS/NF-kB in Lung Epithelial Cells. Molecules. 2021 Nov 17;26(22):6949. doi: 10.3390/molecules26226949. PMID: 34834040; PMCID: PMC8625571.
40. Huang R, Zhong T, Wu H. Quercetin protects against lipopolysaccharide-induced acute lung injury in rats through suppression of inflammation and oxidative stress. Arch Med Sci. 2015 Apr 25;11(2):427-32. doi: 10.5114/aoms.2015.50975. Epub 2015 Apr 23. PMID: 25995762; PMCID: PMC4424260.