

# The Effects of Myricetin Against Testicular and Lung Injury Induced by Testicular Ischemia Reperfusion Model

Mirisetin'in Testiküler İskemi Reperfüzyon ile İndüklenen Testis ve Akciğer Hasarına Karşı Etkileri

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## Abstract

Objective	Testicular torsion may lead to testicular atrophy and male infertility if untreated within some hours. Since ischemia reperfusion (I/R) injury is a reason for the harmful effects during testicular torsion, antioxidant agents are among the targets of science to deal with reactive oxygen species (ROS). As a flavonoid member, myricetin (3, 3', 4', 5', 7'-hexahydroxyflavone, MYR) has antioxidant, anticancer, anti-inflammatory, antiviral, and antidiabetic activities.
Materials and Methods	In this experiment 32 Wistar albino male rats were randomly divided into 4 groups (n=8). Group I was defined as the sham group. In group II, I/R group, testicular torsion detorsion was performed. In group III (MYR 25) and group IV (MYR 50), MYR was administrated intraperitoneally at 25 and 50 mg/kg doses, 30 minutes before detorsion. The testicular and lung tissue samples were examined biochemically.
Results	In both testis and lung tissues, MYR decreased TOS, MDA, and MPO levels at both high and low doses compared to the I/R group. Besides, SOD values increased in MYR treatment groups compared to the I/R group.
Conclusion	MYR performed promising effects on testicular and lung tissues following testicular I/R injury according to biochemical parameters.
Keywords	myricetin; torsion detorsion; ischemia reperfusion; testis; lung.

## Öz

Amaç	Testis torsiyonu birkaç saat içinde tedavi edilmezse erkeklerde testis atrofiğine ve kısırlığa neden olabilir. Antioksidan ajanlar testis torsiyonu ile baş etmede hedefler arasındadır. Mirisetin (myricetin, 3, 3', 4', 5', 7'-heksahidroksiflavon, MYR), antioksidan, antikanser, anti-inflamatuar, antiviral ve antidiyabetik aktivitelere sahiptir.
Gereç ve Yöntemler	32 adet Wistar albino erkek sıçan rastgele 4 gruba (n=8) ayrıldı. Grup I sham grup olarak tanımlandı. Grup II, I/R grubunda testis torsiyonu yapıldı. Grup III (MYR 25) ve grup IV'te (MYR 50), detorsiyondan 30 dakika önce 25 ve 50 mg/kg dozlarında MYR intraperitoneal olarak uygulandı. Testis ve akciğer doku örnekleri biyokimyasal olarak incelendi.
Bulgular	Testis ve akciğer dokularında MYR, I/R grubuna kıyasla hem yüksek hem de düşük dozlarda TOS, MPO ve MDA seviyelerini azaltmıştır. Ayrıca MYR uygulanan gruplarda I/R grubuna kıyasla SOD değerleri artmıştır.
Sonuç	Biyokimyasal parametreler değerlendirildiğinde, MYR testis I/R hasarını takiben testis ve akciğer dokuları üzerinde olumlu etkiler gösterdi.
Anahtar Kelimeler	mirisetin; torsiyon detorsiyon; iskemik reperfüzyon; testis; akciğer.

## INTRODUCTION

Testicular torsion is an emergency urological condition that often affects newborns, children, and adolescents, leading to infertility.<sup>1,2</sup> Testicular rotation decreases blood flow to the testicle and venous drainage is interrupted. This situation leads to ischemia and necrosis. The consequences of this injury depend on the duration and extent of the torsion.<sup>3</sup> Reperfusion therapy is required to repair ischemic tissue. However, reactive oxygen species (ROS) overproduction-related ischemia reperfusion (I/R) injury may occur during reperfusion, contributing to infertility.<sup>4</sup> I/R damage occurs due to the stimulation of an intracellular cascade involving the activation of neutrophils, inflammatory cytokines, and free oxygen radicals.<sup>5</sup> Moreover, during testicular I/R injury, newly arrived oxygen becomes toxic to testicular tissue due to ROS accumulation, testicular oxidative damage, changes in seminiferous tubule structure and function, germ cell apoptosis, and spermatogenesis damage. One of the main causes of excessive ROS accumulation in testicular tissue is the inequality in oxidation/antioxidant balance. Testicles contain powerful and complex antioxidant enzyme systems and ROS scavengers to ensure that the functions of spermatogenesis and steroidogenesis are not hampered by oxidative stress.<sup>6</sup> Clinicians widely recommend medications such as dexmedetomidine, morphine agonists, dimethyl sulfoxide, and antioxidants such as zinc, vitamin E, melatonin, and plant antioxidant extracts for I/R injury therapy.<sup>7</sup> The testicular I/R injury model is widely used in experimental animals to test various agents in testicular torsion/detorsion (TD) researches.<sup>1,3,8,9</sup>

Antioxidant mechanisms act by enzymatic or non-enzymatic pathways. Among the non-enzymatic parts of those mechanisms, flavonoids directly neutralize ROS through the donation of hydrogen (H), inducing antioxidant enzymes or affecting cell signaling.<sup>10</sup> As a member of flavonoids, myricetin (MYR) (3, 3, 4, 5, 5, 7-hexahydroxyflavone), has antioxidant<sup>11</sup>, anticancer<sup>12</sup>, anti-inflammatory,<sup>13</sup> and antiviral<sup>14</sup> activities.

MYR, present in some vegetables, fruits, and plants,<sup>15,16</sup> is mainly in the form of glycosides.<sup>17</sup> It is absorbed by the gastrointestinal tract and mostly metabolized by the liver. The metabolite of MYR is excreted into urine as 3,5-dihydroxyphenylacetic acid.<sup>11</sup>

Here, it was planned to determine the possible beneficial effects of MYR against testicular I/R-induced testicular and lung injuries.

## MATERIALS AND METHODS

### Animals and Ethics

All procedures performed in this study were approved by Atatürk University Animal Experiments Local Ethics Committee (protocol no: 28.06.2018/146). The study was performed at the Experimental Animals Research and Application Center, Atatürk University (August, 2019). All animal experiments were carried out in accordance with the guidelines on human's animal use and care for laboratory animals for biomedical research published by the National Institutes of Health (8th edition, 2011), and the Helsinki Declaration was followed. 32 male Wistar albino rats were randomly divided into 4 groups (n=8). Rats were kept at a 12-hour dark-light cycle at 22°C. Before the experimental process, animals were debarred from food and allowed free access to water. All surgical interventions to animals were performed under anesthesia as 10 mg/kg, intraperitoneal (i.p.) xylazine hydrochloride (Rompun, Bayer, Istanbul), and 60 mg/kg, i.p. ketamine (Ketas, Pfizer, Istanbul). The scrotum regions of the animals were shaved and disinfected with 10% povidone-iodine.

**Group I (sham group):** The testicles were dissected through a longitudinal scrotal incision, and then the incision was sutured without any intervention.

**Group II (I/R group):** Scrotal incision was performed, and testicles were rotated clockwise 720 degrees to form bilateral testicular torsion. After 2 hours of torsion, 2 hours of detorsion was performed.<sup>9,18,19</sup>

**Group III (MYR 25) and Group IV (MYR 50):** Same procedures were carried out with the I/R group. MYR was administered i.p. 30 minutes before detorsion at 25 mg/kg and 50 mg/kg doses as described in a previous I/R study.

The testicular tissue samples were removed following the experimental process and stored at 80°C until analysis.

### Evaluation of Biochemical Parameters in Testicular Tissues

Each tissue sample was weighed as 100 mg and homogenized in 2 ml phosphate buffer. Following the homogenization, they were centrifuged at 5000 rpm for 20 minutes at +4°C and transferred to the tubes to be stored at -80°C. Measurement of malondialdehyde (MDA), the final product of lipid peroxidation, was performed using the method of Ohkawa et al.<sup>20</sup> Myeloperoxidase (MPO) activity was evaluated using the technique used by Bradley et al.<sup>21</sup> Superoxide dismutase (SOD) level was determined using the method of Sun et al.<sup>22</sup> MDA, MPO, and SOD levels were measured using a spectrophotometer. The measurements of total antioxidant status (TAS) and total oxidant status (TOS) were determined with the commercial kit (Rel Assay Diagnostics). TOS to TAS ratio was admitted as the oxidative stress index (OSI).

### Statistical analysis

SPSS 20 (SPSS Corporation, Chicago, IL, USA) statistics program was preferred for data analysis. Results were presented as Mean±Standard Error (SE), and p<0.05 was considered statistically significant. Statistical analysis was

performed via One-way analysis of variance, and the difference between groups was determined by Tukey post hoc test.

## RESULTS

In table 1, several parameters were evaluated for the testis tissues. TOS value increased in the I/R group compared to the sham group. When it was compared to the I/R group, MYR prevented the increase of TOS levels at both high and low doses. In terms of SOD, I/R reduced SOD value (a). However, both doses of MYR administration elevated the SOD levels compared to the I/R group (b) even the high dose of MYR administration made SOD levels slightly higher than the sham group did. As an indicator of neutrophil recruitment, MPO increased in the I/R group compared to the sham group (a). High dose of MYR administration prevented (b) neutrophil recruitment more than the low dose MYR did (c). The increase in MDA in the I/R group compared to the sham group indicates that there is an increase in lipid peroxidation in the I/R group (a). Both doses of MYR administration prevented lipid peroxidation (b), but the high dose of MYR prevented more than the low dose of MYR did.

In table 2, various parameters were evaluated for the lung tissues. TOS values in the I/R group increased compared to the sham group. Compared to the I/R group, high dose of MYR administration prevented the increase in TOS levels (p<0.001) more than the low dose of MYR did (p<0.05). I/R reduced the SOD value. However, both doses of MYR administration elevated the SOD value compared to the

**Table 1.** Results of Testicular I/R-induced Testis Tissues

Experimental Groups (n=8)	TAS (mmol/L)	TOS (µmol/L)	OSI (arbitrary unit)	SOD (U/mg protein)	MPO (U/g protein)	MDA (µmol/g tissue)
Sham	0,02±0,10	7,31±1,13	0,71±0,10	375,58±68,28	50391,47±9741,38	267,19±43,98
I/R	0,58±0,09 <sup>a</sup>	10,51±7,05 <sup>a</sup>	1,85±0,43 <sup>a</sup>	171,09±25,18 <sup>a</sup>	89859,19±8358,07 <sup>a</sup>	456,20±88,81 <sup>a</sup>
MYR 25	0,85±0,09 <sup>b</sup>	8,35±1,22 <sup>b</sup>	0,98±0,18 <sup>b</sup>	315,07±34,56 <sup>b</sup>	60641,02±6288,59 <sup>c</sup>	316,49±38,75 <sup>b</sup>
MYR 50	0,98±0,07 <sup>b</sup>	7,63±0,86 <sup>b</sup>	0,78±0,11 <sup>b</sup>	403,83±87,61 <sup>b</sup>	54818,35±5590,98 <sup>b</sup>	274,47±29,52 <sup>b</sup>

<sup>a</sup>p<0.001 compared to the sham group. <sup>b</sup>p<0.001 and <sup>c</sup>p<0.05 compared to the I/R group  
 TAS= Total antioxidant status, TOS= Total oxidant status, OSI= Oxidative stress index, SOD= Superoxide dismutase, MPO= Myeloperoxidase, MDA= Malondialdehyde

**Table 2.** Results of Testicular I/R-induced Lung Tissues

Experimental Groups (n=8)	TAS (mmol/L)	TOS (µmol/L)	OSI (arbitrary unit)	SOD (U/mg protein)	MPO (U/g protein)	MDA (µmol/g tissue)
Sham	0.75±0.05	11.13±1.14	1.46±0.07	299.24±59.37	408782.88±82459.90	129.07±22.52
I/R	0.49±0.03 <sup>a</sup>	15.52±1.68 <sup>a</sup>	3.15±0.50 <sup>a</sup>	144.18±23.91 <sup>a</sup>	671658.51±80920.84 <sup>a</sup>	231.53±7.73 <sup>a</sup>
MYR 25	0.73±0.04 <sup>b</sup>	13.27±1.44 <sup>c</sup>	1.81±0.10 <sup>b</sup>	261.87±31.31 <sup>b</sup>	448455.66±59076.16 <sup>b</sup>	149.09±13.28 <sup>c</sup>
MYR 50	0.77±0.09 <sup>b</sup>	11.74±1.16 <sup>b</sup>	1.52±0.13 <sup>b</sup>	284.74±34.39 <sup>b</sup>	406572.89±28178.81 <sup>b</sup>	137.35±7.57 <sup>b</sup>

<sup>a</sup>p<0.001 compared to the sham group. <sup>b</sup>p<0.001 and <sup>c</sup>p<0.05 compared to the I/R group  
 TAS= Total antioxidant status, TOS= Total oxidant status, OSI= Oxidative stress index, SOD= Superoxide dismutase, MPO= Myeloperoxidase, MDA= Malondialdehyde

I/R group (p<0.001). MPO increased in the I/R group compared to the sham group. Both doses of MYR administration prevented neutrophil recruitment compared to the I/R group (p<0.001). An increase in MDA levels was observed in the I/R group compared to the sham group (p<0.001). High dose of MYR administration prevented lipid peroxidation (p<0.001) more than the low dose of MYR did (p<0.05).

### DISCUSSION

The main pathophysiological mechanism of testicular T/D is ischemia, and subsequent reperfusion damages the testicle by twisting the spermatic cord.<sup>23</sup> These injuries are caused by the ROS produced during I/R injury. ROS causes DNA damage and apoptosis in testicular germ cells.<sup>6,18,24-26</sup> Excessive ROS production can also cause distant organ failure. ROS can cause acute respiratory failure by causing capillary edema and blood-air barrier problems in the lungs.<sup>27,28</sup> Antioxidant defense mechanisms develop in tissues to reduce this damage<sup>29,30</sup>, but tissue damage can occur if these antioxidant defense mechanisms fail. Antioxidant therapy has been suggested to prevent I/R damage.<sup>31</sup> Various drugs, enzymes, and chemical agents have been suggested for therapeutic purposes as they inhibit oxidative stress by increasing the effectiveness of antioxidant enzymes. MYR is more potent in terms of antioxidant capacity than other flavonoid types due to having more phenolic hydroxyl groups, including quercetin, kaempferol, catechin, and rutin.<sup>32</sup> It has been stated that flavonoids can play protective roles in many pathological mechanisms due to their anti-inflammatory and antiox-

idant properties.<sup>33-35</sup> This study also proved antioxidant effects of MYR on both testes (Table 1) and lungs (Table 2) as a remote organ. MYR plays a protective role on cells through inhibiting ROS generation and activating several antioxidant enzymes.<sup>36</sup> However, a study implied the antioxidant activity of MYR as dose-dependent. While higher concentrations of MYR have prooxidant property, lower concentrations perform antioxidant activity.<sup>37</sup>

MDA is a stable end product of lipid peroxidative degradation produced by ROS.<sup>38</sup> Malondialdehyde (MDA) is an indicator of lipid peroxidation in I/R injury studies because the MDA level elevates parallel to both non-enzymatic lipid peroxidation and after testicular injury as well.<sup>39</sup> Similarly, MPO, which increases in the I/R process, is an enzyme with strong prooxidative and proinflammatory properties, released by activated neutrophils. MPO is basically an indicator of neutrophil infiltration into cells.<sup>40,41</sup> In preclinical studies, it has been reported that post-ischemia testicular reperfusion leads to lipid peroxidation and an increase in tissue MDA and MPO levels.<sup>18,19,31</sup> Different doses of MYR have been reported to reduce MDA and MPO levels in many different studies.<sup>39,42,43</sup>

Superoxide dismutase (SOD) exists in different parts of the cell, such as mitochondria, cytosol, or extracellular membranes, to reduce the superoxide radicals to hydrogen peroxide.<sup>44</sup> SOD decreases in case of I/R injury.<sup>45</sup> SOD, an essential active substrate in the cell growth and differentiation process, protects the cell from injury.<sup>46</sup> Chen et al. report that MYR can lower the ROS level and increase

SOD and GPX values.<sup>47</sup> Besides, MYR has been reported to have strong antioxidative stress properties by regulating the Nrf2/HO-1 signaling pathway.<sup>47,48</sup> Our study demonstrated the effects of MYR on some antioxidant enzymes in testicular and lung damage created with the testis I/R model. The balance between I/R and oxidant/antioxidant systems in testicular and lung tissues, which was disrupted, approached the baseline levels in the 25 mg/kg and 50 mg/kg MYR groups.

As a result, we found that 25 and 50 mg/kg MYR administration 30 minutes before detorsion protected the testicle and lung against I/R damage caused by oxidative stress in rats. MYR's protective effect appears to be due to its antioxidant and anti-inflammatory properties. MYR may reduce testicular I/R damage in humans, but it is early to say this with the available data. Therefore, different studies are needed to obtain more and more detailed data.

#### **Ethical Statement**

**All procedures performed in this study were approved by Atatürk University Animal Experiments Local Ethics Committee (protocol no: 28.06.2018/146).**

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