



## Protective Effects of Hydroxytyrosol on Renal Ischemia-Reperfusion Injury in Mice

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### Abstract

**Background:** Renal ischemia reperfusion (IR), one of the most important causative mechanisms of acute kidney injury, is a major clinical problem that occurs in some hospitalized patients. The aim of this study was to investigate the effects of hydroxytyrosol (HT) on renal ischemia-reperfusion injury in mice.

**Methods:** Male mice were randomly assigned to three groups (N = 9): (1) sham operated, (2) renal IR (45 min ischemia followed by 24 h reperfusion), or (3) renal IR+HT (5 mg/kg orally for 7 days before renal IR and one hour before IR). Animals were sacrificed and the blood and kidney tissue samples were collected for glutathione (GSH) and malondialdehyde (MDA) evaluation.

**Results:** Renal IR injury led to decreases in renal tissue GSH and increases in MDA levels compared to sham operated. Hydroxytyrosol partially inhibited the IR-induced decrease in GSH activity. In addition, in the renal IR+HT group, MDA was increased compared to the renal IR group.

**Conclusions:** These findings suggest that HT diminished oxidative stress in renal tissue after renal IR.

**Keywords:** Ischemia reperfusion, Acute kidney injury, Hydroxytyrosol, Oxidative stress.

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antithrombotic profile.<sup>4,5</sup> Virgin olive oil components are bioavailable in humans and have shown antioxidant properties. One of the most important antioxidant compounds of virgin olive oil are dietary phenols. Phenols are compounds with an aromatic ring structure with one or more hydroxyl groups. Bioavailability studies in humans show that the absorption of olive oil phenols is probably larger than 55–66 mol%. Hydroxytyrosol (HT, also known as dihydroxyphenylethanol) is one of the most potent phenolic antioxidants in extra virgin olive oil, which can be attributed to the electron donating ability of hydroxyl groups in the ortho position and subsequent formation of stable intramolecular hydrogen bonds with the phenoxyl radical.<sup>6-8</sup>

Studies in recent years have shown that HT diminished oxidative stress in hepatoma cells and spleen. HT exerted its antioxidant effect by restoring the values of antioxidant enzyme such as SOD and catalase activities near control values.<sup>9, 10</sup>

This study was designed to evaluate the protective effects of HT as an antioxidant on renal damage after renal IR and also highlight the possible useful effects of the usage of olive oil as complementary therapies in AKI patients in order to improve the side-effects of procedures such as vascular surgery and elective urological operations.

### Materials and Methods

Male mice weighing 25–35 g were housed under controlled environmental conditions (24±2°C and 12 h light–dark cycle) and had free access to standard rat chow and tap water. Animal care was in compliance with the guidelines of the Animal and Human Ethical Committee of Shahroud University of Medical Sciences. Animals were randomly assigned to three groups (N = 9): 1) sham operated, 2) renal IR (45 min ischemia followed by 24 h reperfusion), or 3) renal IR+HT (2 mg/kg orally for 7 days before renal IR and one hour before IR).<sup>5</sup>

An established model of renal IR injury in mice was used.<sup>3</sup> Briefly, mice were anesthetized with intraperitoneal pentobarbital sodium (60 mg/kg). Then a midline incision was made and the renal pedicles were bluntly dissected and occluded with non-traumatic vascular clips for 45 min. After ischemia, clamps were removed gently and the kidneys were observed for an additional 5 min to ensure reflow process. Next, the incision was closed with a 4-0 silk suture. The animals were then returned to their cages and allowed to recover. During the period of renal ischemia, animals were kept well hydrated with warm sterile saline and were maintained at a constant body

## Introduction

Acute kidney injury (AKI) is a major clinical problem that occurs in some hospitalized patients, especially in intensive care units. Renal ischemia reperfusion (IR) is one of the most important causative mechanisms of AKI and is associated with various clinical settings including shock, sepsis, kidney transplantation, vascular surgery, and elective urological operations.<sup>1,2</sup> Although there are advances in renal replacement therapy, AKI still continues to be associated with a high mortality rate.<sup>2</sup>

Although the exact mechanism of renal IR injury is not completely understood, oxidative stress have been identified to play a key role in this process. The abundance of polyunsaturated fatty acids makes the kidney an organ particularly vulnerable to reactive oxygen species (ROS) attacks.<sup>3</sup>

The Mediterranean diet, rich in virgin olive oil, improves oxidative stress, endothelial function, inflammation, and risk factors for cardiovascular disease such as the lipoprotein profile, blood pressure, glucose metabolism, and

temperature on a heating pad. Sham-operated mice underwent a surgical procedure identical to those of IR mice except that clamps were not applied. Animals were sacrificed after reperfusion of 24 h, and the blood and kidney tissue samples were collected for subsequent experiments.

Plasma concentrations of blood urea nitrogen (BUN) and plasma creatinine (Cr) were evaluated by colorimetric methods using commercially available kits.<sup>3</sup>

Malondialdehyde (MDA) level was evaluated in renal tissue by its reaction with thiobarbituric acid according to the Esterbauer and Cheeseman method.<sup>11</sup> Renal glutathione (GSH) was assayed according to the Griffith method 5, 50-Dithiobis 2-nitrobenzoic acid was used as a chromogen and the absorbance of the reduced chromogen was measured at 412 nm.<sup>12</sup>

Results are expressed as means±S.E.M. The statistical significance was determined by using one-way analysis of variance followed by Tukey's post-hoc test. Results with a  $p < 0.05$  were considered significant.

## Results

Forty-five minutes of ischemia followed by 24 h of reperfusion significantly increased plasma BUN and creatinine (Cr) levels in IR group compared with sham group. Hydroxytyrosol administration diminished the IR-induced increase in plasma BUN and Cr (Table 1).

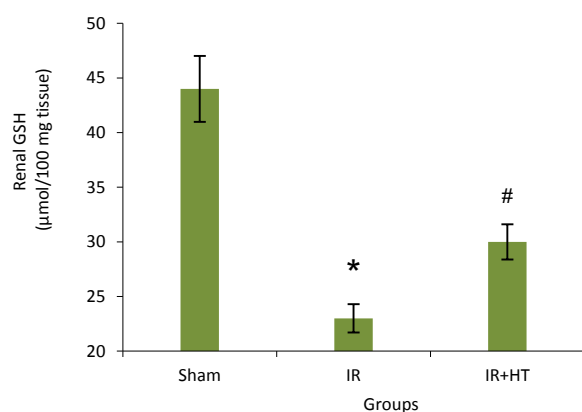
**Table 1. Plasma BUN and Cr concentrations**

Groups	BUN	Cr
Sham	25.45±2.19	0.21±0.02
IR	77.01±6.61*	0.53±0.08*
IR + HT	54.93±4.22**	0.39±0.07**

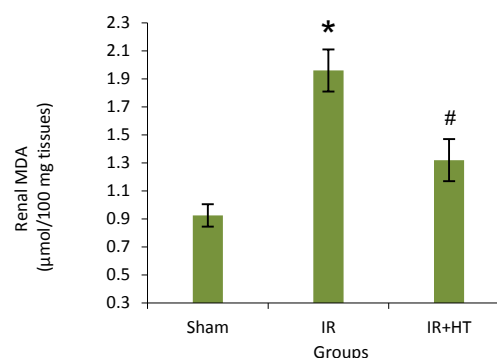
\*Denotes  $P < 0.05$  compared with sham

\*\*Denotes  $P < 0.05$  compared with IR,  $N = 9$ .

Renal IR injury led to decreases in renal tissue GSH and increases in MDA levels compared to sham operated group. Hydroxytyrosol partly inhibited the IR-induced decrease in GSH activity. In addition, in the renal IR+hydroxytyrosol group, MDA was increased compared to renal IR group. (Figures 1 and 2).



**Figure 1. Renal GSH (mean ± SEM):** Note: \*Denotes  $p < 0.05$  versus sham group and # Denotes  $p < 0.05$  versus IR group.



**Figure 2. Renal MDA (mean ± SEM):** Note: \*Denotes  $p < 0.05$  versus sham group and # Denotes  $p < 0.05$  versus IR group.

## Discussion

Renal IR damage is one of the most common causes of AKI. Ischemia-reperfusion is a serious problem that affects the outcome of various surgical operations such as organ transplantation and surgical revascularization.<sup>1,13</sup>

Olive products are rich in natural antioxidants that may inhibit oxidative stress, which often occurs during the development of many diseases. Compared to other phenolic compounds in olive oil, hydroxytyrosol displays much more effective antioxidant characteristics, such as the scavenging of free radicals, breaking peroxidative chain reactions, preventing lipid peroxidation, inhibiting hypochlorous acid derived radicals, and others.<sup>8</sup>

This study represents a renal IR model which induces renal oxidative stress. Renal IR caused BUN and Cr elevation in blood compared with sham group. After 45 min of ischemia followed by 24 h of reperfusion, significant decreases in GSH and increases in MDA were seen. These findings confirmed that renal IR ischemia induced renal oxidative stress.

In 2016, Lisha Zhao et al. demonstrated that renal IR induces oxidative stress in renal tissues. They reported that renal levels of superoxide dismutase (SOD), glutathione (GSH), and MDA markedly decreased after renal IR (45 min Ischemia followed by reperfusion for 24 h).<sup>14</sup>

Bircan et al. reported that renal IR (45 min ischemia followed by 3 h reperfusion) increased MDA, SOD, and catalase in renal tissue.<sup>15</sup>

In our study, oral hydroxytyrosol administration diminished oxidative stress in renal tissue after renal IR. GSH significantly increased and MDA significantly decreased in renal IR+Hydroxytyrosol group compared with renal IR group.

Kedechi et al. showed in 2016 that the supplementation of HT in incubating media significantly improved sperm viability and decreased sperm DNA oxidation via a decrease in ROS level.<sup>16</sup>

Merra et al. (add year) showed that HT improved the antioxidant indices in spleen in induced oxidative stress with cadmium-intoxicated rats. They evaluated SOD, catalase, and thiobarbituric acid reactive substances (TBARS) as antioxidant indices.<sup>9</sup>

In conclusion, renal ischemia and reperfusion induced oxidative stress resulted in increased levels of MDA and decreased levels of GSH in renal tissues. However, oral hydroxytyrosol consumption significantly increased the glutathione level and decreased the malondialdehyde level. In addition, hydroxytyrosol has antioxidant effects in renal tissues after IR. Therefore, consumption of HT in these clinical situations can improve oxidative stress and diminish injury of kidney.

### Conflict to Interest

The authors declared that they have no conflict of interest.

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