



Effect of Quercetin and Resveratrol on Raftlin, 8-iso Prostaglandin F2alpha Levels in Renal Ischemia-reperfusion Injury

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ABSTRACT

Background: Renal ischemia reperfusion injury (IRI) can be frequently observed in mandatory clinical situations such as organ transplantation, as well as in undesirable clinical scenarios such as embolic, thrombotic causes and hypotensive shock. This damage is an important pathological process that can occur in a wide clinical spectrum, such as acute renal failure and permanent renal failure. The effects of Quercetin and Resveratrol, substances with high antioxidant capacity that may have a positive effect on this pathological process, on renal IRI were examined by looking at Raftlin and 8-iso Prostaglandin F2alpha (8-iso-PGF2 a) levels.

Methods: Forty-two male Wistar-albino rats were divided into six groups (Control group; n=7, I/R group; n=7, Sham group; n=7, Quercetin group; n=7, Resveratrol group; n=7, Quercetin and resveratrol group; n=7). Quercetin (100 mg/kg) and resveratrol (100 mg/kg) was administered intragastrically in the treatment group. Only sham group was administered with 0.9% serum physiologic (NaCl). Raftlin and 8-iso-PGF2 a values were measured by ELISA method. In the histopathological examination, the groups were evaluated in terms of hemorrhage, myocardial edema, myocytolysis and polymorphonuclear leukocyte infiltration.

Result: It was observed that both quercetin and resveratrol molecules decreased Raftlin and 8-iso-PGF2a values. The group in which the two molecules were given together had the lowest values and it was determined that this value approached the control group value. Quercetin and resveratrol are thought to have a protective effect on renal IRI.

Key words: 8-iso Prostaglandin F2alpha, Quercetin, Raftlin, Renal ischemia-reperfusion injury, Resveratrol.

INTRODUCTION

Ischemia is the deoxygenation of the tissue due to the reduction of blood reaching the tissues, while reperfusion is the reoxygenation (O₂) of the tissue by restoring the blood flow (Yapca *et al.*, 2013). Renal ischemia reperfusion injury (IRI) can be frequently observed in mandatory clinical situations such as organ transplantation, as well as in undesirable clinical scenarios such as embolic, thrombotic causes and hypotensive shock. This damage is an important pathological process that can occur in a wide clinical spectrum, such as acute renal failure (ARF) and permanent renal failure (PRF). Renal tissue is extremely sensitive to ischemia reperfusion (IR). It has been shown that in case of insufficient oxygen supply of tubular epithelial cells, it can progress to permanent tissue damage and even cause tissue death (Bonventre *et al.*, 2011) IR can result in many metabolic processes in the cell, such as endoplasmic reticulum stress and the formation of reactive oxygen species (ROS) (Devarajan, 2006; Kalogeris *et al.*, 2016). In recent years, quercetin and resveratrol, which have high antioxidant capacity against ROS production, have been widely used. The inevitable injuries may occur after infarction, sepsis and organ transplantation and this phenomena exacerbate tissue damage by initiating an inflammatory cascade including ROS, cytokines, chemokines and leukocytes activation (Jang and Rabb, 2009; Sharfuddin and Molitoris, 2011). In the kidney, IRI contributes to pathological conditions called ARF that is a

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clinical syndrome with rapid renal dysfunction and high mortality rates (Kellum *et al.*, 2011; Hoste *et al.*, 2006). The pathophysiology of IRI in renal is very complex but some pathological pathways such as activation of neutrophils, release of reactive oxygen species and other inflammatory mediators including adhesion molecules and a variety of cytokines are involved.

Quercetin is a flavonoid found in various vegetables and fruits and has antioxidant, antiapoptotic and anti-inflammatory effects (Harwood *et al.*, 2007; Al Rasheed *et al.*, 2017). It has been determined that quercetin has a protective effect on IRI in many different tissues (Gholampour and Sadidi, 2018).

Resveratrol is a stilbene group compound that is synthesized in high amounts in the leaves and bark of the grapevine plant and has phytoalexin properties. According to the results of various studies, it has been revealed that resveratrol has various biological properties such as antioxidant and anti-inflammatory (Hamza and El-Shenawy, 2017; Meng *et al.*, 2021). Resveratrol reduces IRI in tissues due to its radical scavenging properties (Fu *et al.*, 2018).

It is thought that quercetin and resveratrol which have both antioxidant and anti-inflammatory effects, may have a healing effect on renal IRI. Raftlin is one of the raft-associated proteins that may exert renal IRI healing effects (Saeki *et al.*, 2003). Raftlin, which is affected by oxidative stress in the body in vascular inflammation, causes a decrease in tumor necrosis factor-alpha (TNF- α), interleukin-6 (IL-6), interleukin-8 (IL-8) and C-reactive protein (CRP) due to the reduction of inflammation and free oxygen radicals in the body (Devouassoux *et al.*, 2007). 8-iso-PGF2 α , produced non-enzymatically from arachidonic acid as a result of lipid peroxidation, has been used as a reliable indicator of oxidative damage in recent years.

There are many studies in the literature about renal IRI. However, no study has been conducted examining the effects of Quercetin and Resveratrol molecules on Raftlin and 8-iso Prostaglandin F2alpha (8-iso-PGF2 α) levels and renal IRI. Our study is the first to examine Raftlin and 8-iso-PGF2 α levels in renal IRI. It was aimed to examine the possible effects of quercetin and resveratrol against IR-related renal damage using histological and biochemical parameters.

MATERIALS AND METHODS

Animals

This research was carried out in the medicinal biochemistry laboratory of Kahramanmaraş Sutcu Imam University between February and August 2022..

In this study, 42 female albino Wistar rats (*Rattus norvegicus*) weighing between 250-300 grams were used. Rats were kept in a 45-55% humidity environment with a 12-hour day/night light period. After the surgery, necessary wound care and supervision were performed under the same conditions. Rats were fed ad libitum as pellets without using a special diet. The subjects were obtained from Kahramanmaraş Sutcu Imam University Laboratory Animal Breeding and Experimental Research Center and their care and surgery procedures were performed in the same institution.

Study groups

Rats were randomly divided into 6 groups, each containing seven animals. The numbers were calculated using the pilot study as an example.

In all ischemia groups and sham group: After laparotomy, the intestines were lateralized to the right side to reach the retroperitoneal area, the left kidney was identified and the renal hilum was clamped with a bulldog

clamp for 60 minutes. Reperfusion was achieved after 45 minutes (Tercan *et al.*, 2021).

Control group (n=7)

Nothing implemented.

IR group (n=7)

Only IR was performed.

Sham group (n=7)

1 mL serum physiologic (0.9% NaCl) was given intragastrically for one week. Afterwards, IR was performed.

Que-IR group (n=7)

100 mg/kg/day quercetin (sigma-aldrich Q4951) was given intragastrically in 1 mL 0.9% NaCl for one week. Afterwards, only IR was performed (Yang *et al.*, 2016).

Res-IR group (n=7)

100 mg/kg/day resveratrol (sigma-aldrich R5010) was given intragastrically in 1 mL 0.9% NaCl for one week. Afterwards, only IR was performed (Shaito *et al.*, 2020).

Que+Res-IR group (n=7)

100 mg/kg/day quercetin and 100 mg/kg/day resveratrol were given intragastrically in 1 mL 0.9% NaCl for one week. Afterwards, only IR was performed.

Surgical method

The subjects were subjected to general anesthesia with 50 mg/kg ketamine and 20 mg/kg xylasin intraperitoneal injection before the procedure. Depths of anesthesia of the subjects; It was constantly controlled by finger withdrawal and motor movements. Surgical intervention was performed by a single surgeon. After cleaning the abdominal hair in the supine position, skin antisepsis with povidone-iodine was applied to the abdominal skin. Median laparotomy was performed. In all groups, the abdomen was entered and the intraperitoneal structures were lateralized to the right side. Meanwhile, 3 mL 0.9% NaCl was applied to the intra-abdominal cavity to prevent insensible losses.

The left kidney was found and its hilum was isolated in all groups. 60 minutes of ischemia was performed to all groups except the control group. During ischemia, the abdominal organs were moistened with warmed sterile 0.9% isotonic and the abdomen was temporarily closed. After 45 minutes, bulldog clamps were opened and renal reperfusion was achieved (Tercan *et al.*, 2021). From all subjects, the left kidney was identified and removed from surrounding adhesions for histopathological evaluation and biochemical analysis. The extracted kidney tissue was divided into two equal parts.

Biochemical analysis

Tissue homogenate preparation

1 g 9 volumes to tissues (vol/wt) PBS (pH 7.4) were added and It was homogenized. It was centrifuged at 2000 xrpm

for 20 minutes and the upper supernatants were removed. It was kept at -80°C until the working day.

Measurement of biomarkers

Clotting is expected to occur in the collected tissue samples. Later It was centrifuged at 1500 rpm for 10 minutes. Prefixes were kept at -80°C until the day of analysis. Raftlin and 8-iso-PGF2 α levels in tissue samples were determined using enzyme-linked immunosorbent assay (ELISA) kits (My Bio Source, catalog number: MBS1603767, USA; My BioSource, catalog number: MBS1600179, USA) as per manufacturer's guidelines.

Histopathological evaluation

Renal tissues were fixed in 10% formalin solution at room temperature for three days and underwent routine histological procedures. Sections of 4-5 μm thickness taken from the paraffin blocks obtained were stained with Hematoxylin-eosin and Masson-trichrome dyes and viewed under a Zeiss LSM 900 microscope and evaluated.

Statistical analysis

The SPSS (Statistical Package for Social Sciences) 25.0 program was used for statistical analysis. The results were given as mean $\pm$ standard deviation. In the evaluation of biochemical data, the non-parametric Kruskal-Wallis test was used to determine the differences between the groups and the Mann-Whitney U test was used to evaluate the difference between the two groups at $p < 0.05$.

RESULTS AND DISCUSSION

Our study showed that quercetin and resveratrol molecules create differences in raftlin and 8-iso-PGF2 α levels on renal IRI (Fig 1, 2). Compared to the control group, the Raftlin value of all renal IRI groups and the sham group, except the group in which quercetin and resveratrol were given together, was found to be statistically significantly higher ($p < 0.05$). There was no statistically significant difference between the renal IRI group in which quercetin and resveratrol were given together and the control group ($p = 0.994$). It was observed that the raftlin value increased with IR damage. It was determined that the raftlin value decreased statistically in the groups given quercetin and resveratrol ($p < 0.05$). In fact, a significant decrease in the raftlin value was observed in the group where the two molecules were administered together and it was found to approach the control group value. When the effectiveness of quercetin and resveratrol is compared, it has been observed that quercetin reduces the raftlin value much more than resveratrol. However, no statistically significant difference was detected ($p = 0.285$) (Fig 1).

The changes in Raftlin and 8-iso-PGF2 α levels between groups were found to support each other (Fig 1, 2). It was observed that the level of 8-iso-PGF2 α increased in the IR groups and the sham group. Based on the raftlin level, it was found that the quercetin molecule, which has a high effect on renal IRI, also had the same effect on the 8-iso-PGF2 α level. Compared to the control group, there was

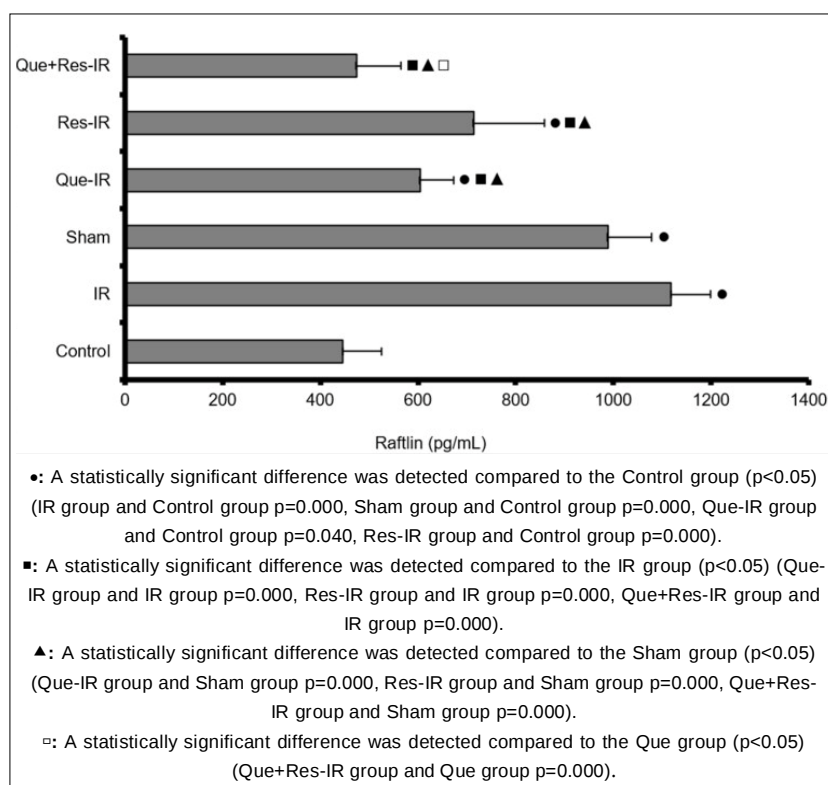


Fig 1: Raftlin values of groups.

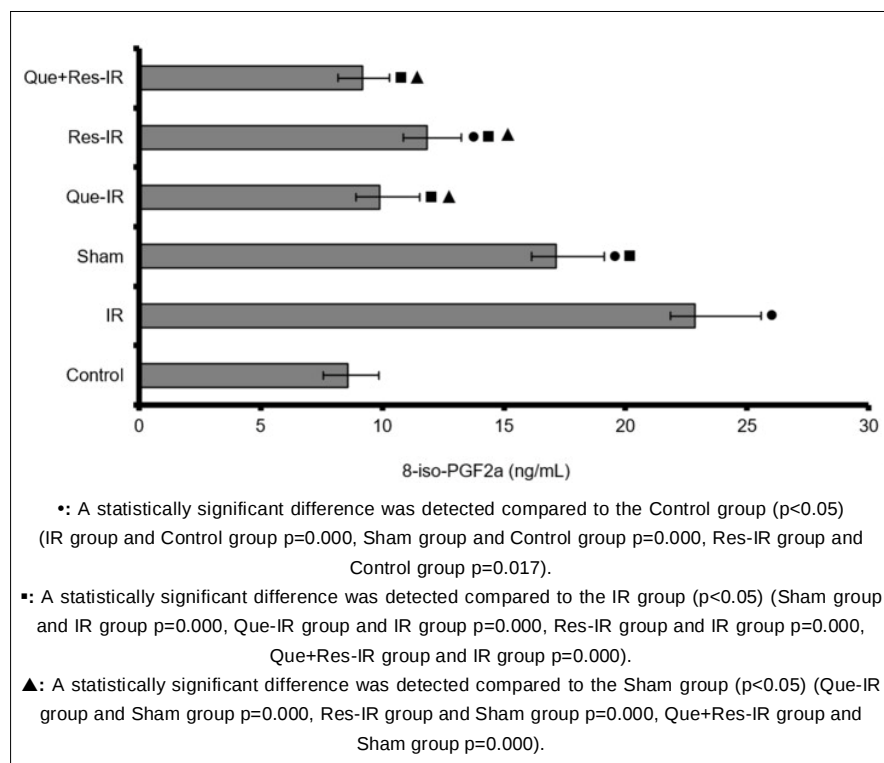


Fig 2: 8-iso-PGF2α values of groups.

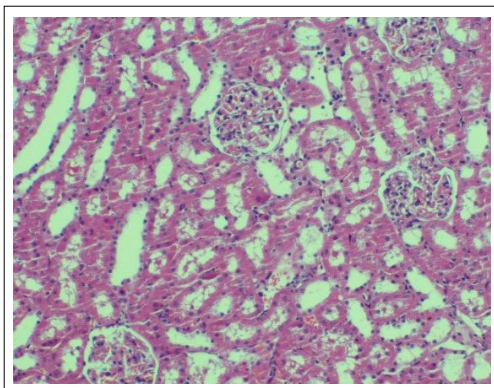


Fig 3: Normal histological tubular in control group.

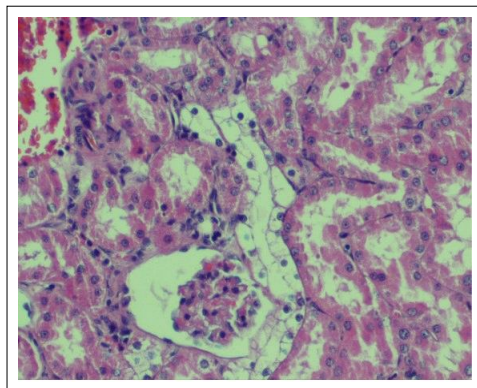


Fig 5: Slight hydrophobic changes in Que+Res-IR group.

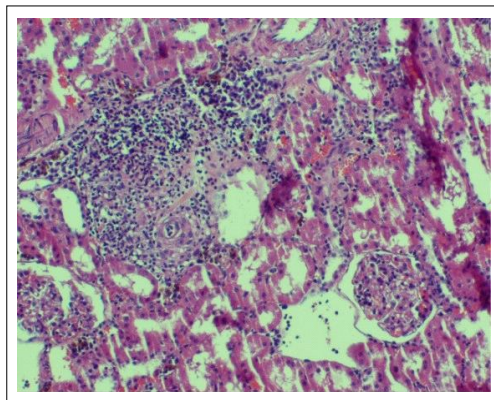


Fig 4: Significant inflammation and tubular dilatation in the tubules in the IR and Sham groups.

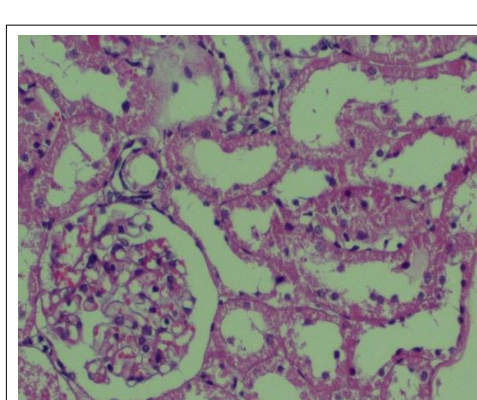


Fig 6: Mild tubular dilation in Que-IR group.

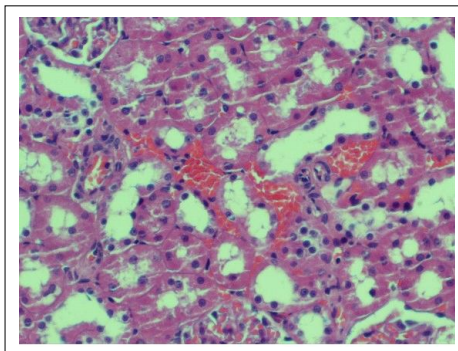


Fig 7: Vascular congestion and mild tubular dissection in the interstitium in res-IR group.

no statistically significant difference in the 8-iso-PGF2 α level between the group given quercetin alone and the groups given quercetin and resveratrol together ($p=0.724$, $p=0.986$). A statistically significant decrease in 8-iso-PGF2 α levels was detected in all groups given quercetin and resveratrol compared to the IR group ($p<0.05$) (Fig 2).

Normal histological tubular appearance in the control group (Fig 3). Significant inflammation and tubular dilatation were observed in the tubules in the IR and sham groups (Fig 4). The most positive effect was seen in the group where quercetin and resveratrol were given together (Fig 5). There was less damage in the quercetin group compared to the resveratrol group, with only mild tubular dilatation observed in this group (Fig 6). In the resveratrol group, vascular congestion and mild tubular dissection were observed in the interstitium (Fig 7).

With the resumption of blood flow in the ischemic tissue, ROS released especially by Polymorphonuclear Leukocytes (PMNL) that settle in the tissue, has an increasing effect on the destruction in the tissue. IR injury is a complex sequence involving many pathophysiological processes. Various studies have revealed that ROS play an important role in the formation of damage by initiating lipid peroxidation in IR damage (Granger *et al.*, 2015). Additionally, ROS increases tissue damage by reacting with biomolecules in living structures (Tang *et al.*, 2019). In studies conducted in previous years The effectiveness of substances that eliminate the effects of ROS through enzymatic and non-enzymatic mechanisms has been demonstrated experimentally and clinically. After ischemia, hypoxic and anoxic cell injuries occur in the kidney tissue, leading to strong local synthesis of inflammatory cytokines. These cytokines can initiate defensive physiological activities to insulate and prevent tissue damage. Besides all these, by inducing ROS to produce and collect inflammatory cells; may further exacerbate organ damage and dysfunction (Hashiguchi, *et al.*, 2005). The effects of quercetin and resveratrol on renal IRI have been investigated and their positive effects have been stated (Bagheri *et al.*, 2023). Therefore, it is very important to protect against oxidative stress and inflammation in the early stage of kidney IRI.

CONCLUSION

When searching the literature, there are no studies on raftlin and 8-iso-PGF2 α values and renal IRI. The effect of quercetin and resveratrol on kidney IRI is seen more clearly when looking at the change in raftlin and 8-iso-PGF2 α values. As a result, the findings we obtained in our study are; ROS plays an important role in renal IRI, it is thought that quercetin and resveratrol supplements may be useful against this damage.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors.

Informed consent

The study was conducted with the approval of the Experimental Animals Ethics Committee of Kahramanmaraş Sutcu Imam University.

Conflict of interest

All authors declared that there is no conflict of interest.

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