

## Casticin Mitigates Renal Damage Injured by Ischemia Reperfusion: A Biochemical Study

Omer Topdagi<sup>1</sup>, Ayhan Tanyeli<sup>2</sup>, Fazile Nur Ekinci Akdemir<sup>3</sup>,  
Derya Güzel Erdogan<sup>4</sup>, Ersen Eraslan<sup>5</sup>, Mustafa Can Guler<sup>6</sup>, Elif Polat<sup>7</sup>

<sup>1</sup> Department of Internal Medicine, Faculty of Medicine, Atatürk University, Erzurum, Turkey.

<sup>2,6</sup> Department of Physiology, Faculty of Medicine, Atatürk University, Erzurum, Turkey.

<sup>3</sup> Department of Nutrition and Dietetics, High School of Health, Ağrı İbrahim Çeçen University, Ağrı, Turkey.

<sup>4</sup> Department of Physiology, Faculty of Medicine, Sakarya University, Sakarya, Turkey

<sup>5</sup> Department of Physiology, Faculty of Medicine, Bozok University, Yozgat, Turkey.

<sup>7</sup> Department of Nutrition and Dietetics, Faculty of Health Sciences, Erzurum Technical University, Erzurum, Turkey

ORCID; 0000-0002-9690-4447, 0000-0002-0095-0917, 0000-0001-9585-3169, 0000-0002-7618-5043, 0000-0003-2424-2269, 0000-0001-8588-1035, 0000-0003-0042-4084

**Abstract:** Renal ischemia-reperfusion is a severe health condition that may be dangerous and even cause death. This research aims to search the protective effects of casticin on renal injury induced by ischemia-reperfusion. Study groups included sham, ischemia-reperfusion, 5 mg/kg casticin+ischemia reperfusion, and 10 mg/kg casticin+ischemia reperfusion groups. Some antioxidant, inflammatory, and oxidant parameters were examined in renal tissues obtained following the experiment. We found that the inflammatory and oxidant biomarkers elevated and antioxidants declined in the ischemia-reperfusion group. However, the antioxidant values raised, and inflammatory values declined in treatment groups. These results have shown us that casticin may be a potent molecule against oxidative renal damage following ischemia-reperfusion.

### INTRODUCTION

Ischemia-reperfusion (I/R) is a pathological status in the tissues in which blood circulation temporarily interrupts<sup>1</sup>. Bypass, renal transplantation, kidney infarctions, sepsis, and especially acute renal failure (ARF) often lead to renal I/R that may result in death<sup>2,3</sup>. Approximately 7% of hospitalized patients are diagnosed with AKI (Acute kidney injury)<sup>4</sup> which contributes to 40–60% of the overall mortality rate<sup>5</sup>. Inflammation and oxidation are the main reasons for tissue damage in I/R-induced renal injury<sup>6-8</sup>. An imbalance between oxidant and antioxidant systems, called as oxidative stress, emerges in AKI following renal I/R injury<sup>9</sup>. Various factors contribute to I/R injury via vascular hyperpermeability, inflammatory reactions, and circulating cytokines<sup>10</sup>. The neutrophils and macrophages are the main actors which play a role in the inflammatory response<sup>11,12</sup>. Researchers have spent too much energy to make progress on illuminating the hidden cellular and molecular pathways in renal I/R injury for beneficial therapies. Still, the literature seems too weak to figure out<sup>13</sup>.

An active compound casticin (3', 5-dihydroxy-3, 4', 6, 7-tetramethoxyflavone), which has been isolated from *V. trifolia* or *V. rotundifolia*, is a member of flavonoids<sup>14</sup>. Experimental studies suggest that casticin has anticancer and anti-inflammatory effects in pulmonary tissue<sup>15-19</sup>. Casticin statistically reduced reactive oxygen species (ROS) release through inhibition of tumor necrosis factor-alpha (TNF- $\alpha$ )<sup>20</sup>. Our research investigated casticin to alleviate the I/R-induced renal injury.

### MATERIALS and METHODS

#### *Experimental Animals and Ethical Approval*

Atatürk University Experimental Animal Ethics Committee confirmed the current study. Atatürk University Experimental Animals Research and Application Center supplied Wistar-type male rats and allowed the experimental procedures to be performed. The animals were housed in cages with appropriate laboratory standards including 55±5% humidity, 12 light/12 darkness and 22±2 °C temperature. Rats had free access to both food and water. 12 hours before the experiment, all animals were debarred from food but were allowed to drink water.

Received :27.05.2021  
Received in revised form :02.07.2021  
Accepted :09.07.2021  
Available online :15.09.2021

Keywords:

Renal Ischemia-reperfusion  
Casticin  
Oxidative stress  
Inflammation  
Rat

Corresponding Author:  
Ayhan Tanyeli  
E-mail; ayhan.tanyeli@atauni.edu.tr  
http://dx.doi.org/10.29228/jamp.51534

Int J Acad Med Pharm,  
2021; 3 (3); 245-248



### Groups, Drugs, and I/R Model

In the current search, 32 Wistar male rats were weighed (240-260 g) and randomized into 4 groups. All rats were fixed in face-down position. The animals' back regions were shaved and cleaned with povidone-iodine. 60 mg/kg intraperitoneal (i.p.) ketamine (Ketalar®, Pfizer, Istanbul) and 10 mg/kg i.p. xylazine hydrochloride (Rompun®, Bayer, Istanbul) were administered to the rats during the experimental procedures. Casticin was procured from Sigma Aldrich Co.

In group I (sham), an incision was applied to the back region and closed with 3/0 silk suture. Any I/R model or any medication was not performed. In group II (I/R), following the incision, all renal veins and arteria were fixed with a microvascular clamp for 1 hour. And then, the blood flow was allowed for 24 hours by releasing the clamps during the reperfusion stage<sup>21, 22</sup>. In group III (5 mg/kg casticin+I/R) and group IV (10 mg/kg casticin+I/R), casticin was administered to the rats i.p. 30 minutes before the reperfusion. Later, the I/R model was carried out. When the experiment ended, high dose anesthesia was used for the rat sacrifice. The renal tissues were moved away. The tissue samples were washed and maintained frozen until the biochemical examination.

### Biochemical assessments

Total antioxidant status (TAS) and total oxidant status (TOS) were detected with the available kits (Rel Assay Diagnostics). TOS to TAS ratio, the oxidative stress index (OSI), was preferred as another oxidative stress indicant. SOD evaluation was predicated on the production of superoxide radicals created via xanthine and the xanthine oxidase system, which performs a reaction with nitro blue tetrazolium to form formazan dye<sup>23</sup>. Lipid peroxidation amounts in ovarian tissue were measured by assessing malondialdehyde (MDA) using the thiobarbituric acid test<sup>24</sup>. The activities of myeloperoxidase (MPO) in the renal tissues were forecasted according to methods defined by Bradley et al.<sup>25</sup>.

TNF- $\alpha$  level was measured at 450 nm using rat TNF- $\alpha$  platinum ELISA commercial kit (eBioscience, catalog no: BMS622). Interleukin-1 $\beta$  (IL-1 $\beta$ ) level was measured on an ELISA reader with an enzyme linked immunosorbent assay kit (Elabscience, catalog no: E-EL-R0012) at 450 nm wavelength.

### Statistical analysis

All the results were shown as Mean $\pm$ Standard deviation (SD). Results were determined using One-way ANOVA and then Tukey test for pairwise comparisons of groups. The differences were accepted as significant when  $P < 0.05$ .

## RESULTS

There was no loss in rats due to experimental applications. Table 1 describes OSI, TOS and TAS, indicating the balance between them. In the comparison of I/R group with the sham group, TOS and OSI values increased statistically while TAS value was decreased. In the comparison of the treatment groups with the I/R group, changes in all parameters were statistically significant ( $P < 0.05$ ). When the treatment groups compared each other, the difference in TAS and OSI values was statistically significant ( $P < 0.05$ ).

**Table 1.** Comparisons of TAS, TOS, and OSI levels among the experimental groups

| Experimental Groups (n=8) | TAS (mmol Trolox Eq/L)           | TOS ( $\mu\text{mol H}_2\text{O}_2$ Eq/L) | OSI (arbitrary unit)             |
|---------------------------|----------------------------------|---|----------------------------------|
| Sham                      | 2.77 $\pm$ 0.22                  | 6.71 $\pm$ 0.29                           | 0.24 $\pm$ 0.02                  |
| I/R                       | 1.72 $\pm$ 0.14*                 | 8.91 $\pm$ 0.69*                          | 0.52 $\pm$ 0.06*                 |
| Casticin 5 mg/kg+I/R      | 2.26 $\pm$ 0.23* <sup>#</sup>    | 7.54 $\pm$ 0.59* <sup>#</sup>             | 0.33 $\pm$ 0.04* <sup>#</sup>    |
| Casticin 10 mg/kg+I/R     | 2.54 $\pm$ 0.13 <sup>†,‡,w</sup> | 7.20 $\pm$ 0.76* <sup>#</sup>             | 0.28 $\pm$ 0.03 <sup>†,‡,w</sup> |

TOS; Total Oxidant Status; TAS; Total Antioxidant Status; OSI; Oxidative Stress Index. Data are presented as mean $\pm$ SD.  $\dagger p < 0.05$  and  $* p < 0.001$ , compared to the sham group.  $\# p < 0.001$ , compared to the I/R group.  $w p < 0.05$ , Casticin 5 mg/kg group compared to Casticin 10 mg/kg group.

Table 2 and Table 3 summarize the statistical comparisons of SOD, MDA, MPO, TNF- $\alpha$  and IL-1 $\beta$  concentrations in four experimental groups. When the I/R group compared the sham group, MDA, MPO, TNF- $\alpha$  and IL-1 $\beta$  levels increased while SOD value decreased. When the low dose casticin group was compared to the sham group, a statistically significant difference was determined among SOD, MDA, MPO, and IL-1 $\beta$  levels ( $P < 0.05$ ). No difference occurred between the high dose casticin and sham groups in terms of these biochemical markers. When the I/R group compared to the treatment groups, it has been found statistically significant for these parameters ( $P < 0.05$ ). When treatment groups compared to each other, SOD, MPO, and MDA concentrations were statistically different ( $P < 0.05$ ).

**Table 2.** Comparisons of SOD, MPO and MDA levels among the experimental groups

| Experimental Groups (n=8) | SOD (U/mg protein)                | MPO (U/g protein)                   | MDA ( $\mu\text{mol/g}$ protein) |
|---------------------------|-----------------------------------|-------------------------------------|----------------------------------|
| Sham                      | 585.26 $\pm$ 81.27                | 42317.04 $\pm$ 4502.82              | 78.29 $\pm$ 6.15                 |
| I/R                       | 312.22 $\pm$ 14.56*               | 81137.75 $\pm$ 11247.01*            | 121.46 $\pm$ 6.78*               |
| Casticin 5 mg/kg+I/R      | 482.00 $\pm$ 31.37 <sup>†,‡</sup> | 48754.24 $\pm$ 3073.69 <sup>#</sup> | 86.76 $\pm$ 6.96 <sup>†,‡</sup>  |
| Casticin 10 mg/kg+I/R     | 541.37 $\pm$ 32.37 <sup>#</sup>   | 41100.26 $\pm$ 2444.79 <sup>#</sup> | 79.93 $\pm$ 5.28 <sup>#</sup>    |

SOD; Superoxide Dismutase; MPO; Myeloperoxidase; MDA; Malondialdehyde. Data are presented as mean $\pm$ SD.  $\dagger p < 0.05$  and  $* p < 0.001$ , compared to the sham group.  $\# p < 0.001$ , compared to the I/R group.

**Table 3.** Comparisons of TNF- $\alpha$  and IL-1 $\beta$  levels among the experimental groups

| Experimental Groups (n=8) | TNF- $\alpha$ (pg/mg protein)       | IL-1 $\beta$ (pg/mg protein)          |
|---------------------------|-------------------------------------|---------------------------------------|
| Sham                      | 24806.70 $\pm$ 4005.91              | 27437.80 $\pm$ 2247.17                |
| I/R                       | 38941.60 $\pm$ 5115.58*             | 57318.61 $\pm$ 4081.52*               |
| Casticin 5                | 24189.92 $\pm$ 2580.67 <sup>#</sup> | 32975.10 $\pm$ 6497.85 <sup>†,‡</sup> |
| Casticin 10               | 25632.11 $\pm$ 1967.94 <sup>#</sup> | 28368.37 $\pm$ 2359.93 <sup>#</sup>   |

IL-1 $\beta$ ; Interleukin-1 $\beta$ ; TNF- $\alpha$ ; Tumor necrosis factor- $\alpha$ ; Data are presented as mean $\pm$ SD  $\dagger p < 0.05$  and  $* p < 0.001$ , compared to the sham group.  $\# p < 0.001$ , compared to the I/R group.

## DISCUSSION

ARF is a frequently encountered disease in clinical nephrology with a high mortality rate<sup>26</sup>. Oxygenation and oxidative stress are accepted to be a reason for AKI during renal I/R<sup>27</sup>, which damages the tubular and endothelial cells by oxidative stress, ROS generation, and acute inflammation<sup>28</sup>. Unfortunately, those events usually happen after hemodynamic occasions such as kidney transplant, heminephrectomy, hemorrhagic shock<sup>29, 30</sup>. The acute ischemia period leads to an increment in endothelium permeability and expression of different adhesion molecules attracting neutrophils into the extracellular fluid, which augment inflammation via ROS and MPO<sup>31, 32</sup>. Some researchers like Tok et al. commonly reported MPO as it significantly increases in renal tissue following I/R compared with a healthy tissue<sup>33, 34</sup>.

The inflammatory cascade is triggered by some reactions such as neutrophil infiltration, ROS production, and tubular epithelial cell activation. Among those, the neutrophil activation and infiltration through proinflammatory cytokines play the leading role in transforming the localized tissue injury into remote injury<sup>35</sup>. Literature suggests that TNF- $\alpha$  and IL-1 $\beta$  levels start to increase slightly after ischemia, gain their peak levels in few hours, and remain elevated for days<sup>36, 37</sup>. Excessive ROS release leads to cell injury by lipid peroxidation following I/R<sup>38, 39</sup>. Tissue MDA level is a valuable indicator of lipid peroxidation<sup>40</sup>. Likewise, MDA supports oxidative stress by producing ROS<sup>41, 42</sup>. The oxidative stress can be predicted by some indicative assessments like MDA, TOS, TAS, and OSI, which display the balance of redox reactions between oxidation and antioxidation<sup>43</sup>. Even with progress in diagnosis and treatment methods, ischemic renal failure is a common issue in trials<sup>44</sup>. For this reason, we aimed to improve I/R-induced renal damage by utilizing the anti-inflammatory and antioxidant features of casticin.

Literature has many studies that investigate the antioxidant and anti-inflammatory properties of casticin in experimental models. Because there is no study about casticin in the renal I/R model and our study seems original. In this study, reduction of IL-1 $\beta$  and TNF- $\alpha$  levels suggested that casticin decreases I/R-induced renal injury. Casticin, which has shown to have antitumoral, anti-inflammatory, and hepatoprotective activities<sup>19, 45-47</sup>, has been studied in mice which mitigated acute lung inflammation induced by cigarette smoke and reduced ear dermatitis and edema induced by croton oil<sup>15</sup>. Casticin has been stated to protect human chondrocytes against inflammation induced by IL-1 $\beta$ <sup>48</sup>. In human airway epithelial cells, casticin has been shown to alleviate lipopolysaccharide-induced inflammatory responses through NF- $\kappa$ B pathways<sup>49</sup>. Casticin decreases proinflammatory cytokine levels like TNF- $\alpha$ , IL-6, and IL-1 $\beta$  in lipopolysaccharide-stimulated mouse macrophages<sup>47</sup>. In a mice study, casticin has been proven to prevent ulcerative colitis induced via dextran sulfate sodium by decreasing ROS signaling and levels of proinflammatory cytokines<sup>50</sup>. Casticin has been reported to exert antioxidant effect<sup>51</sup>. Casticin was demonstrated to reduce oxidative stress against liver damage caused by methotrexate<sup>52</sup>.

Here, antioxidant and anti-inflammatory properties of casticin were demonstrated in the renal I/R model in rats. In the I/R group, TAS and SOD declined while IL-1 $\beta$ , MDA, MPO, OSI, TOS, and TNF- $\alpha$  levels elevated, and casticin treatment reversed these values.

We assessed the renal tissue for oxidative stress to investigate the potential protective effects of casticin against renal injury induced by I/R. We observed that oxidative stress and inflammation decreased with casticin.

## Conclusion

Casticin prevented I/R-induced renal injury with its antioxidant

and anti-inflammatory properties. Besides, advanced studies would be beneficial for further information about the protective mechanisms of casticin against I/R-induced renal tissue injury.

## Conflict of interest

Authors declare that they have no financial interests or personal conflicts that may affect the study in this article.

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