Prophylactic Effect of *Potentilla fulgens* on Renal Ischemia-Reperfusion Injury in Rats

**Efecto Profiláctico de *Potentilla fulgens* sobre la Lesión por Reperfusión por Isquemia Renal en Rata**

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**SUMMARY:** We aimed to investigate the possible protective effects of *Potentilla fulgens* on kidney tissue with ischemia-reperfusion using immunohistochemical methods. Wistar rats were grouped as sham, ischemia, ischemia-reperfusion (I/R) and I/R treated with *Potentilla fulgens*. Renal vessels of the left rat kidney were clamped for 60 minutes for ischemia, IR group had 6 h of reperfusion. 400 mg/kg *Potentilla fulgens* were given intraperitoneally 5 days before ischemia+reperfusion procedure. Biochemical analysis (MDA, GSH and MPO) of samples were performed. Kidney tissues were fixed with 10 % neutral formalin and routine paraffin tissue follow-up protocol was applied, stained with routine Hematoxylin and Eosin. ADAMTS-5 and Caspase-3 immunostaining was applied for immunohistochemistry and examined under a light microscope. In the ischemia group, inflammation and congestion in the vessels and increased ADAMTS-5 expression in glomerular cells and tubule cells were observed. In reperfusion, an increase in degenerative glomerular cells, tubule cells and intertubular connective tissue and inflammatory cells ADAMTS-5 expression was observed. In the P. fulgens group, degeneration and inflammation decreased and positive ADAMTS-5 expression was observed. In the ischemia and ischemia reperfusion group, increased apoptotic appearance and Caspase-3 positive expression in glomerular and tubular cells, and negative expression in most cells in the P. fulgens group. *Potentilla fulgens* are thought to stop apoptotic cell development at a certain stage, which affects the cytokine mechanism and plays an important role in the reduction of inflammatory cells and angiogenic regulation.

**KEY WORDS:** *Potentilla fulgens*; Renal ischemia, rat; ADAMTS-5; Caspase-3.

**INTRODUCTION**

Ischemia-reperfusion injury is an important model for testing therapeutic interventions in pathophysiological events leading to acute renal failure (Wang et al., 2012). Damage due to nutritional and oxygen restriction (ischemia) and recovery (reperfusion) is the main cause of acute kidney injury in developed countries with open heart surgery, and sepsis are important triggers (Bellomo et al., 2012). In the mechanism of occurrence of kidney, I/R damage, inflammatory mediators, adhesion molecules and various cytokines have been reported to cause chronic kidney damage if they do not recover within a short time (Fan et al., 2012).

*Potentilla fulgens* is an alpine plant of Western Himalayas which is consumed in all parts of the world for its promising medicinal properties. Pharmacologically, the aerial and root portions of the plant are reported to have antioxidant (in vitro models), antitumor, hypoglycemic and antihyperglycemic activities (Syiem et al., 2009; Kaul et al., 2011). The general signs and symptoms of toxicity, food and water intake and mortality rates of animals were observed within 72 h post-treatment. From these observations, LD50 was calculated using SPSS software (Chen et al., 2005). In general, the expression of Adamts5 at many of these locations or in cell types appears to be a constitutive feature of their phenotype. Many of these sites are known to express versican, an aggregating proteoglycan that was recently identified as a substrate of ADAMTS5 (Longpré et al., 2009). Caspase-dependent apoptosis was previously believed to be the only form of programmed cell death; however, emerging evidence suggests that necrosis could also be regulated (Ashkenazi & Salvesen, 2014). Apoptosis and necrosis, tissue damage caused by kidney I/R play a role in inducing inflammation (Heinzelmann et al.,

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procedure was applied for 6 hours. 400 mg/kg was intraperitoneally given 5 days before ischemia + reperfusion procedure. The level of MDA in the IR group rats was significantly higher than the control group (p <.001). The level of MDA has decreased significantly after treatment with Potentilla fulgens compared to the IR group. (p <.001).

The level of GSH in the IR group rats decreased significantly (p <.001) compared to the control group, and the MPO level was found higher than the control group. After treatment with Potentilla fulgens, GSH and MPO values were significantly close to control (Table I). The total score of kidney histomorphological damage was significantly lower in the control group compared to ischemia and ischemia reperfusion group (p <0.01). Histomorphological damage total scores in the ischemia-reperfusion + Potentilla fulgens (PF) group were slightly above the control group (Table I).
Table I. Statistical analysis of MDA, GSH, MPO, degeneration in tubular cells, vascular dilatation and congestion, inflammation values of the groups.

<table>
<thead>
<tr>
<th></th>
<th>MDA</th>
<th>GSH</th>
<th>MPO</th>
<th>Degeneration in tubular cells</th>
<th>Vascular dilatation and congestion</th>
<th>Inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>33.54±2.83</td>
<td>1.19±0.12</td>
<td>3.81±0.49</td>
<td>0.44±0.53</td>
<td>0.44±0.53</td>
<td>0.38±0.52</td>
</tr>
<tr>
<td>Ischemia</td>
<td>51.96±1.96*</td>
<td>0.70±0.10*</td>
<td>7.85±0.83*</td>
<td>3.67±0.50*</td>
<td>3.56±0.53*</td>
<td>3.67±0.50*</td>
</tr>
<tr>
<td>I/R</td>
<td>51.71±2.31*</td>
<td>0.72±0.12*</td>
<td>8.14±0.70*</td>
<td>3.78±0.44*</td>
<td>3.56±0.53*</td>
<td>3.78±0.44*</td>
</tr>
<tr>
<td>I/R+PF</td>
<td>35.52±3.48</td>
<td>1.15±0.10</td>
<td>3.78±0.41</td>
<td>1.11±0.60</td>
<td>0.78±0.44</td>
<td>0.89±0.60</td>
</tr>
</tbody>
</table>

Values with different superscript show statistical significance between groups, p * <0.01.

DISCUSSION

Ischemia is an important blockage occurring in the circulatory system and the damage it will cause depends on ischemic time. The reperfusion mechanism takes place as a return and determines the result of the damage (Daemen et al.). The severity of ischemia / reperfusion (IR) injury depends on the ischemic duration and the adequacy of the collateral circulation. In the pathophysiology of renal ischemia reperfusion, inflammation and tubular epithelial cell apoptosis occur, causing excessive production of Reactive oxygen species (ROS), cell apoptosis and release of inflammatory cytokines, causing tissue damage (Li et al., 2014). MDA as a marker of lipid peroxidation shows the degree of peroxidation after renal ischemia injury. GSH, GSH-Px, CAT and SOD are the most important endogenous antioxidant enzymes and are found in high concentrations in kidney cells. GSH removes superoxide radicals and protects protein thiol (-SH) groups from oxidation. MPO and oxidation products are considered critical pathogenic factors in many kidney diseases and contribute to the development of different kidney damage (Day et al., 2005). GSH is an endogenous tripeptide and is found in kidney cells, especially at high concentrations. GSH removes superoxide radicals and protects protein thiol groups from oxidation (Ross, 1988). In our study, MDA values in ischemia and ischemia-reperfusion groups were high and decreased after Potentilla fulgens treatment (Fig. 1). It was observed that P. Fulgens decreased the lipid peroxidation. In addition, the level of GSH in IR group rats decreased significantly (p <0.01) compared to the control group, and MPO level was found higher than the control group. After treatment with Potentilla fulgens, significant GSH and MPO values were close to control (Fig. 2). After ischemic damage in the kidneys, tubular necrosis, fibrosis and inflammatory cells have been proliferated and accumulated in the damage area. It has been reported that apoptotic cell death directly or
Fig. 3. Hematoxylin-Eosin staining of kidney tissue of all groups. a) No changes were observed in the glomerular structures in the cortex region of the control group kidney sections and in the formations in the juxtaglomerular apparatus area. Epithelium cell sizes in proximal and distal tubules were observed in normal view. b) In the ischemia group, degenerative changes in cells in the glomerular area, deterioration in Bowman capsule structure, inflammatory cell infiltration in the juxtaglomerular region, dilatation in the blood vessels were observed. In the proximal and distal tubule lumens, irregularity, pyknosis and apoptotic appearance were observed in the cell nuclei. c) In the ischemia-reperfusion group, there was an increase in inflammation cells in a wide area around the glomerular structures along with necrosis, glomeruli and tubules. Nucleus loss was observed in the nuclei of tubular cells with apoptosis. d) In the ischemia-reperfusion + *Potentilla fulgens* group, there was a decrease in degenerative appearance in the cells in the glomerular structure, and lumens of tubular structures, while apoptotic changes were observed in some of the regular cells.

indirectly contributes to I/R-induced inflammation and the resulting tissue damage (Day *et al.*, 2006; Delbridge, 2007). In our study, an increased inflammatory cell infiltration around the glomerulus and tubule in the ischemia group (Fig. 3b), advanced necrotic structures and apoptotic cells in the reperfusion group were observed (Fig. 3c). In the group treated with *P. Fulgens*, it was observed that the inflammation decreased and the necrotic structures were low and apoptotic cells decreased (Fig. 3d).

ADAMTS-5 is also known as aggrecan-2, a major component of extracellular matrix and ADAMTS-5 gene known to be expressed in bladder, cervix, esophagus, placenta and uterus (Porter *et al.*, 2005). The role of ADAMTS-5 in renal injury has not yet been studied.

In our study, we observed that ADAMTS-5 expression increased in kidney damage that developed with ischemia (Fig. 4b) and after ischemia reperfusion (Fig. 4c).
increased inflammatory cell, and increased extracellular matrix. We found moderate ADAMTS-5 expression in some extracellular areas with a decrease in inflammatory cells in *Potentilla fulgens* application (Fig. 4d). ADAMTS-5 can play important pathogenic roles in the progression of kidney damage. Baloglu et al. (2018) has stated that treatment of *P. fulgens* in spinal cord injury may decrease the apoptotic cell count of nerve and glial cells, causing cytokine mechanism to decrease, inflammatory cells and angiogenic progression. There are no studies on *P. fulgens* treatment on kidney ischemia reperfusion. Tunc et al. (2015) have demonstrated that *Potentilla fulgens* treatment on intestinal ischemia reperfusion injury can inhibit cellular apoptosis of mucosal cells and trigger cell proliferation and reduce intestinal permeability to accelerate regeneration and repair of the small intestinal mucosa. In the study of Acar et al. (2016), *P. fulgens* treatment in ovarian torsion prevented degeneration in tuba uterina epithelium, blocking of blood vessels and inflammatory cell infiltration. They suggested using *Potentilla fulgens* as an antioxidant. Caspases are a family of genes maintaining homeostasis through regulating cell death and inflammation. They participate in ordered processes such as apoptosis and inflammation. Caspases are classified according to their roles in apoptosis; caspase-3...
Fig. 5. Caspase-3 immunostaining. a) In the control group, negative caspase-3 expression was observed in cells in the glomerular structure while caspase-3 expression was observed in mild tubular cells. b) In the ischemia group, caspase-3 expression was positive in blood vessel endothelial cells, tubular cells and intertubular connective tissue cells in visceral cells in the glomerular area. c) In the ischemia-reperfusion group, in the glomerular structure, most of the visceral and parietal cells, an increase in tubule cells and an increase in inflammatory cells, and caspase-3 expression were observed. d) While in the ischemia-reperfusion + Potentilla fulgens group, glomerular cells and tubule cells were positive in some of the caspase-3, in the majority of them, the expression of caspase-3 was negative.

Potentilla fulgens acts as an executioner caspase (McIlwain et al., 2013). In the ischemia (Fig. 5b) and ischemia-reperfusion group (Fig. 5c), most of the visceral and parietal cells in glomerular structure, caspase-3 expression was observed with an increase in apoptosis in tubule cells. In the Potentilla fulgens-treated group, the expression of caspase-3 was positive in some of the glomerular cells and tubule cells, whereas caspase-3 expression was negative in most (Fig. 5d).

Potentilla fulgens are thought to stop apoptotic cell development at a certain stage, which affects the cytokine mechanism and plays an important role in the reduction of inflammatory cells and angiogenic regulation.
días antes del procedimiento de isquemia + reperfusión. Se realizó análisis bioquímicos (MDA, GSH y MPO) de muestras. Los tejidos renales se fijaron con formalina neutra al 10 % y se aplicó el protocolo de seguimiento de tejido de parafina de rutina y teñido con hematoxilina y eosina. Se aplicó inmunotinocimiento de ADAMTS-5 y Caspasa-3 para inmunohistoquímica y se examinó con un microscopio óptico. En el grupo de isquemia, se observó inflamación y congestión en los vasos y el aumento de la expresión de ADAMTS-5 en células glomerulares y células tubulares. En la reperfusión, se observó un aumento en la expresión de ADAMTS-5 de células glomerulares degenerativas, células tubulares y tejido conjuntivo intertubular y células inflamatorias. En el grupo de Potentilla fulgens, la degeneración y la inflamación disminuyeron y se observó expresión positiva de ADAMTS-5. En el grupo de isquemia y reperfusión de isquemia, aumentó la aparición apoptótica y expresión positiva de Caspasa-3 en células glomerulares y tubulares, y expresión negativa en la mayoría de las células del grupo de Potentilla fulgens. Se cree que Potentilla fulgens detiene el desarrollo de las células apoptóticas en una determinada etapa, lo que afecta el mecanismo de las citocinas y juega un papel importante en la reducción de las células inflamatorias y la regulación angiogénica.

PALABRAS CLAVE: Potentilla fulgens; Isquemia renal; Rata; ADAMTS-5; Caspasa-3

REFERENCES


