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ORIGINAL ARTICLE
ORİJİNAL ARAŞTIRMA

Can Apigenin Be an Effective Therapeutic Agent Against Experimental Renal Ischemia-Reperfusion Injury?

Apigenin Deneysel Renal İskemi-Reperfüzyon Hasarına Karşı Etkili Bir Terapötik Ajan Olabilir mi?

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ABSTRACT

Aim: This study aims to reveal the effects of two doses of apigenin (API) against renal ischemia-reperfusion injury (R I/R).

Material and Method: For this purpose, 5 and 10 mg/kg doses of API were preferred in our study, and the groups were designed as sham, R I/R, 5 mg/kg API, and 10 mg/kg API groups for the implementation of the experimental protocol. In the R I/R model, 1-hour ischemia and 24-hour reperfusion periods were preferred. Oxidative and inflammatory markers were measured biochemically in samples taken at the end of the experiment.

Results: Biochemical results showed that oxidative and inflammatory markers increased significantly in the R I/R group, but antioxidant activities decreased significantly. In the 5 and 10 mg/kg API groups, R I/R damage was alleviated considerably, with these markers approaching the sham group values.

Conclusion: As a result, the study's results determined that two different doses of API were effective against R I/R-induced kidney damage.

Keywords: Apigenin, kidney, ischemia/reperfusion

ÖZ

Amaç: Bu çalışma, iki doz apigenin (API) kullanımının renal iskemi-reperfüzyon hasarına (R I/R) karşı etkilerini ortaya koymayı amaçlamaktadır.

Gereç ve Yöntem: Bu amaçla çalışmamızda API'nin 5 ve 10 mg/kg dozları tercih edilmiş ve deneysel protokolün uygulanması için gruplar sham, R I/R, 5 mg/kg API ve 10 mg/kg API grupları olarak tasarlanmıştır. R I/R modelinde 1 saatlik iskemi ve 24 saatlik reperfüzyon periyotları tercih edilmiştir. Deney sonunda alınan örneklerde oksidatif ve inflamatuvar belirteçler biyokimyasal olarak ölçüldü.

Bulgular: Biyokimyasal sonuçlar oksidatif ve inflamatuar belirteçlerin R I/R grubunda anlamlı olarak arttığını, ancak antioksidan aktivitelerin anlamlı olarak azaldığını gösterdi. 5 ve 10 mg/kg API gruplarında, R I/R hasarı önemli ölçüde hafifledi ve bu belirteçler sham grubu değerlerine yaklaştı.

Sonuç: Sonuç olarak, çalışmanın sonuçları iki farklı API dozunun R I/R ile indüklenen böbrek hasarına karşı etkili olduğunu göstermiştir.

Anahtar Kelimeler: Apigenin, böbrek, iskemi/reperfüzyon

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INTRODUCTION

In general, in various situations such as organ transplantations, heart surgeries, and vascular surgeries, ischemic damage is initiated due to obstruction of blood flow to tissues due to microcirculation disruption and obstruction in the microvascular area. However, maintaining circulation by re-establishing blood flow in the tissue causes reperfusion injury, which is a more dramatic damaging response than the ischemic period. Reperfusion injury causes the pathophysiology of many cases, such as acute renal failure, myocardial infarction, and ischemic stroke. Kidneys are one of the organs most easily affected by this damage. The pathophysiological basis of I/R is often associated with hypoxia, post-reperfusion inflammation, and oxidative stress. I/R occurring in the kidney tissue results in the accumulation of free oxygen radicals (ROS), excessive increase in cytokines that initiate the inflammatory process, cellular calcium (Ca^{2+}) accumulation, apoptosis, oxidative stress, and even autophagy. Therefore, this process may result in kidney loss (1-4). For this reason, studies have been conducted on pharmacologically active drugs and agents that can potentially reduce apoptosis, oxidative stress, and autophagy to alleviate the severity of R I/R damage (5,6).

Apigenin (API, 4,5,7-trihidroksiflavon), a natural flavonoid found primarily on plants such as chamomile and celery, has various pharmacological activities such as antioxidant, anti-carcinogenic and anti-inflammatory properties and has been used in different experimental studies (7-10). Previous studies have shown that API supports cellular antioxidant defense and increases the contractile force on the heart by reducing reactive oxygen species (ROS) (2). In two different studies, doxorubicin-induced and adriamycin-induced, it was reported that API treatment had a protective effect against cardiotoxicity by suppressing autophagy and apoptosis (8,11). Another study documented that API inhibited ethanol-induced oxidative stress and LPS-induced inflammatory cytokine production in rat hepatocytes (12).

Based on this and similar studies in the literature, no study has been found investigating the effects of API treatment against R I/R damage. For this reason, this study was considered to evaluate the potential antioxidant and anti-inflammatory activities of API against R I/R-induced renal tissue damage.

MATERIAL AND METHOD

Experimental Protocol

All animal experiments were carried out at Atatürk university's Animal Experiments Research Center (ATADEM). All animals were kept at 55±5% humidity, 12h/12h, day/night, and an average room temperature of 25±2°C, and all animals were fed tap water and standard laboratory chow. 24 female Wistar albino rats were weighed (210-220 g) and randomly divided into 4 groups. Groups were defined as Sham, R I/R, 5 mg/kg API, and 10 mg/kg API. Sham group rats were anesthetized with ketamine/xylazine (100/15 mg/kg, intraperitoneally "i.p.") mixture. The back area was shaved and cleaned with an antiseptic solution (povidone-iodine), and an incision was made and closed again without any other procedure. In the R I/R group, bilateral renal artery and veins were clamped with a microvascular clamp to prevent blood flow for 1 hour. Subsequently, the clamps were opened for reperfusion, blood circulation was continued for 24 hours, and the incision was sutured with 3/0 silk. During reperfusion, the animals were sacrificed, and kidney tissues were removed. A single dose of API was given i.p. at doses of 5 mg/kg and 10 mg/kg, and then the I/R model was created as defined in the R I/R group. API (Apigenin; 95.0% (HPLC) | CAS No. 520-36-5) was purchased from Sigma Aldrich Co. (Missouri, USA). Xylazine hydrochloride (Rompun, Bayer, Istanbul) and ketamine (Ketalar, Pfizer, Istanbul) anesthesia were provided for the experiment. In the final stage, the kidney tissues taken at the end of the experiment were preserved in appropriate conditions for biochemical and histopathological studies.

Biochemical Analysis

Testicular tissues were homogenized using tissue lyser to perform biochemical analyses. Total antioxidant status (TAS) and total oxidant status (TOS) values (Elabscience Wuhan, China) were measured in the resulting homogenates using ELISA. The OSI value was also calculated as the TOS/TAS ratio (6). In addition, myeloperoxidase (MPO) (13) activity, malondialdehyde (MDA) (14) level, and superoxide dismutase activity (SOD) (15) analyses were performed according to the methods specified in studies in the literature.

Statistical Analysis

SPSS 20 (SPSS Corporation, Chicago, IL, USA) statistical program was used for data analysis. The results were expressed as mean±standard error (deviation) (SD), and $p<0.05$ was considered statistically significant. One-way analysis of variance was used for statistical analysis, and the Tukey post hoc test was applied to determine the difference between groups

RESULTS

Considering the Sham group results, MPO activity, OSI, TOS, and MDA levels were significantly increased in the R I/R group ($p<0.001$). In addition, it was observed that these results decreased statistically significantly in the 5 and 10 mg/kg API groups. When antioxidant

levels were evaluated, TAS level and SOD activity decreased significantly in the R I/R group (p<0.001). It was determined that the antioxidant system was strengthened in the API groups compared to the R I/R group decreased (p<0.001). API pretreatments increased SOD levels compared to the R I/R group. These data show that API can increase antioxidant enzyme levels and suppress oxidative stress (**Table 1**). TNF-α and IL-1β levels in the R I/R group (p < 0.001) were significantly increased compared to the sham group. However, in comparison with the R I/R group (p < 0.001), TNF-α and IL-1β levels significantly decreased in 5 and 10 mg/kg API groups (p < 0.001).

DISCUSSION

Oxidative stress is the disruption of the balance between endogenous antioxidants and oxidants in favor of oxidants. Oxidants, defined as ROS, interact with cellular molecules, causing peroxidation and disrupting homeostasis. ROS are responsible for lipid peroxidation, DNA, and mitochondria damage by primarily attacking membrane lipids in the tissue (16-18). The antioxidant system provides the first defense against ROS accumulating in the tissue. In particular, SOD, an endogenous antioxidant, is the first defense against attack by ROS (18,19).

Moreover, SOD and Glutathione peroxidase enzymes are reliable indicators of oxidative stress damage. MPO is a peroxidase enzyme found in large amounts in neutrophils and monocytes. In cases of I/R, drug toxicities, and sepsis, MPO levels increase due to neutrophil migration and ROS accumulation (20-23). I/R induces sterile inflammation, including neutrophil stimulation, cytokine production, and other proinflammatory processes. As it is known, TNF-α and interleukin-1 (IL-1) are pro-inflammatory cytokines (24). In a study evaluating the effect of vitamin C on R I/R injury, t lipid peroxidation in the kidney tissue decreased due to I/R, and accordingly, MPO activity increased, SOD activity decreased, and even TNF-α and IL levels increased (25). In a different study on R I/R, it was reported that oxidant and pro-inflammatory cytokines increased in the kidney tissue, antioxidant defense was inadequate, and in this way, in vivo and in vitro apoptosis and oxidative stress developed (26). In their study evaluating kidney damage caused by I/R, Wong et al. documented that pro-apoptotic proteins increased and anti-apoptotic proteins decreased, resulting in an apoptotic response (27). Current approaches have increased interest in natural agents that have as few side effects as possible, have a low toxic reaction in tissue, and are natural in various pathological cases. In a study evaluating the anti-inflammatory effects of apigenin and genistein on the rat intestinal epithelial cells with TNF-α stimulation in response to heat treatment, It has been observed that API suppresses inflammation in the tissue by showing anti-inflammatory effects (28). In a study evaluating the impact of API against cyclosporine-induced free radical-induced renal damage. It has been detected that API protects tissue against nephrotoxicity by reducing the lipid hydroperoxides and increasing the total antioxidants (29).

When we examine the literature for the protective effects of API against R I/R, various research studies consider several parameters. Liu et al. discovered the impact of API in R I/R via activation of the JAK2/STAT3 pathway (30). API prevented apoptosis through PI3K/Akt mediated mitochondria-dependent apoptosis signaling pathway in a R I/R model (31). Another API-R I/R study demonstrated the effects of API on the expression levels of B-cell lymphoma-2, Fas, and Fas ligand (32). All these research approaches the impact of API in R I/R. Different from the literature; our study focused on the effects of API for oxidative stress and anti-inflammatory mechanisms by investigating related parameters, which makes the current data a contribution to the literature and supporting it.

CONCLUSION

The results obtained in this study revealed the tissue protective effect of API against oxidative stress and inflammatory response in R I/R damage. However, the critical point to be noted here is that further experimental and clinical research is needed on this subject in light of the results of API in this study.

Table 1. Kidney tissue SOD (U/mg protein), MDA (nmol/g protein), MPO (U/mg protein), TAS (mmol Trolox Eq/mg protein), TOS (μmol H2O2 Eq/mg protein), OSI, TNF-α (pg/mg protein), IL-1β (pg/mg protein) results of all groups.

	Sham	R I/R	5mg/kg API	10mg/kg API
TNF-α (pg/mg protein)	23847.05±4159.65	37750.80±4269.17	25428.02±3426.39	22488.35±3024.37
IL-1β (pg/mg protein)	26525.48±2356.75	72800.17±4996.07	37700.13±6381.10	28098.01±3627.29
SOD (U/mg protein)	516.71±85.69	276.35±14.70	448.46±35.29	481.32±26.34
MDA (nmol/g protein)	72.92±5.73	113.96±7.91	79,85±6.29	76.17±7.30
MPO (U/mg protein)	38574.75±4973.37	76829.40±12252.96	45795,08±3319,54	40052.15±2069.50
TAS Level (mmol Trolox Eq/mg protein)	2.32±0.37	1.33±0.11	1.93±0.21	2.18±0.19
TOS Level (μmol H2O2 Eq/mg protein)	6.3±0.28	9.46±0.60	7.03±0.59	6.5±0.51
OSI Level (Arbitrary units)	0.27±0.04	0.71±0.05	0.36±0.05	0.29±0.04

ap<0.001 Compared to the Sham group, bp<0.001 Compared to the R I/R group, and *p<0.05 Compared to 5 mg/kg and 10 mg/kg API groups. Data are presented as Mean±SD.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was approved by the Atatürk University Animal Experiments Local Ethics Committee (Date:28.07.2017, Decision No:81).

Informed Consent: Not necessary

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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