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Research

Protective Effects of Hesperetin Against Lipopolysaccharide-induced Acute Renal Injury in Rat

Hesperetinin Lipopolisakkarit-uyarımlı Sıçan Akut Böbrek Hasarına Karşı Koruyucu Etkileri

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ABSTRACT

Objective: In this study, it was aimed to investigate the effects of hesperetin on renal transforming growth factor-beta 1 (TGF-β1) expression and apoptosis in rat lipopolysaccharide (LPS)-induced acute renal injury model.

Methods: In the study, 18 adult male Wistar albino rats were used. Rats divided into three groups, respectively (n=6); control, LPS and LPS + hesperetin. Sepsis model was created with a singledose of LPS (*Escherichia coli*, O26: B6 serotype, Sigma-aldrich). LPS + hesperetin group was administered intragastrically with the aid of hesperetin oral gavage at a dose of 100 mg/kg, after LPS-induction. Twenty four hours after LPS administration, the rats were opened from the midline under ketamine-xylazine anesthesia and kidney tissue and cardiac blood were collected. Kidney tissue was examined with hematoxylin-eosin staining. TGF-β1 expression was determined by indirect immunohistochemical method. TUNEL method was used to determine renal apoptosis. Blood urea nitrogen (BUN) and creatinine levels in blood serum were determined using spectrophotometric methods.

Results: Decreased histopathological changes, TGF-β1 expression and apoptosis were determined in the LPS + hesperetin group compared to the LPS group ($p<0.05$). In addition, a significant decrease in BUN and creatinine levels was observed in the LPS + hesperetin group compared to the LPS group ($p<0.05$).

Conclusion: The data obtained show that in the LPS-induced rat sepsis model, hesperetin suppresses the expression of TGF-β1 in kidney tissue and provides a protective effect.

Keywords: Lipopolysaccharide, acute kidney injury, hesperetin, transforming growth factor-beta 1, renal apoptosis

ÖZ

Amaç: Bu çalışmada, sıçan lipopolisakkarit (LPS)-uyarımlı akut böbrek hasarı modelinde hesperetinin renal transforme edici büyüme faktör-beta 1 (TGF-β1) ifadesi ve apoptozis üzerine etkilerinin incelenmesi amaçlandı.

Gereç ve Yöntem: Çalışmada 18 adet erişkin erkek Wistar albino sıçan kullanıldı. Sıçanlar üç gruba ayrıldı (n=6); kontrol, LPS ve LPS + hesperetin. Sepsis modeli, tek doz LPS (*Escherichia coli*, O26: B6 serotipi, Sigma-aldrich) ile oluşturuldu. LPS + hesperetin grubuna, LPS uygulaması sonrası, 100 mg/kg dozda hesperetin oral gavaj yardımıyla intragastrik olarak uygulandı. LPS uygulamasını takiben 24 saat sonra, sıçanlar ketamin-ksilazin anestezisi altında orta hattan açılarak böbrek dokusu ve kardiyak kan alındı. Böbrek dokusu hematoksilen-eozin boyaması ile incelendi. Renal TGF-β1 ifadesi indirekt immünohistokimyasal yöntemle incelendi. Renal apoptozisin belirlenmesinde TUNEL metodu kullanıldı. Kan serumunda kan üre azotu (BUN) ve kreatinin seviyeleri spektrofotometrik yöntemle belirlendi.

Bulgular: LPS + hesperetin grubunda LPS grubuna kıyasla azalmış histopatolojik değişiklikler, TGF-β1 ekspresyonu ve apoptozis belirlendi ($p<0,05$). Ayrıca LPS + hesperetin grubunda LPS grubuna kıyasla BUN ve kreatinin seviyelerinde anlamlı derecede azalma gözlendi ($p<0,05$).

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Sonuç: Elde edilen veriler LPS uyarımlı sıçan sepsis modelinde hesperetinin böbrek dokusunda TGF-β1 ekspresyonunu baskılayarak koruyucu etki sağladığını göstermektedir.

Anahtar Kelimeler: Lipopolisakkarit, akut böbrek hasarı, hesperetin, transforme edici büyüme faktör-beta 1, renal apoptozis

INTRODUCTION

Acute kidney injury (AKI) is characterized by acute tubular cell damage and renal dysfunction, and may develop due to septic, toxic or ischemic causes (1). One of the most important risk factors for the development of AKI is severe bacterial infection (2). AKI-associated Gram-negative bacterial infections continue to be a cause of high mortality in patients (3). While a specific method for the treatment of AKI has not been developed yet, the development of new agents in the treatment of septic AKI has a great potential to reduce mortality and morbidity in patients (4).

Lipopolysaccharide (LPS), which is an important pathogenic factor in the cell membrane of Gram-negative bacteria, consists of lipid and polysaccharide linked by covalent bond (5). Experimental studies have shown that it is an endotoxin that provides a strong immune response (6,7).

Various histopathological and biochemical changes have been reported in kidney tissue in LPS-induced experimental models. These are renal tubular damage, increase in blood urea nitrogen (BUN) and creatinine levels, inflammatory cell infiltration, pathological changes such as necrosis in tubular cells, and increased oxidative stress (8,9).

LPS stimulation induces the activation of nuclear factor-kappa beta and may activate the Toll-like receptor-4 pathway, which stimulates the release of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin 1-beta (IL-1β) and transforming growth factor-beta 1 (TGF-β1) (10,11). Excessive production of inflammatory cytokines may cause kidney damage by causing the migration of neutrophils and monocytes to the glomerulus (11,12). Therefore, suppression of inflammatory cytokine production is an important strategy in the prevention of kidney damage (13).

Some natural flavonoids and polyphenols have broader biological effects and targets than chemical-synthetic agents, which usually have a specific target, especially in diseases with multifactorial pathogenesis (12-14).

Hesperetin (3',5,7-trihydroxy-4-methoxyflavanone) is a natural flavonoid found abundantly in citrus fruits such as lemon, orange, grapefruit, and mandarin (15). Flavonoids are polyphenolic compounds with various biological activities, and the free hydroxyl groups in their molecular structures function as free radical scavengers (16). Previous studies have shown that hesperetin has potent anti-inflammatory and antioxidative properties, both *in vivo* and *in vitro* (17,18).

It has been reported that application of hesperetin in acrolein-induced experimental lung injury model suppresses oxidative stress and supports tissue structure by preventing apoptosis in cells (19). It has been reported that application of hesperetin in experimental rat ischemia/reperfusion-induced retinal injury model prevents retinal thinning and apoptosis in the retina (18).

These experimental studies show that hesperetin reduces damage by supporting cell and tissue structure in different tissues and organs.

When studies in which hesperetin was used as a treatment agent in different kidney damage models were examined, it was reported that it reduced lipid peroxidation, suppressed oxidative stress, decreased the release of inflammatory cytokines, and helped to preserve tissue structure by reducing histopathological changes (20,21).

The LPS-induced AKI model is the most commonly used in vivo experimental model to investigate the histopathological changes caused by sepsis in kidney tissue and the mechanisms underlying potential treatments (11). In the literature review, no study that examined the effects of hesperetin in the LPS-induced AKI model was found. In this study, it was aimed to examine the efficacy of hesperetin in the LPS-induced AKI model by histological, immunohistochemical and TUNEL methods.

METHODS

Experiment Model and Applications

The ethics committee approval required for this experimental study was obtained from the Tekirdağ Namık Kemal University Animal Experiments Local Ethics Committee (decision no: 4, date: 31.10.2018), and all applications were carried out in accordance with the Laboratory Animal Care and Guidelines. Our study was carried out at Tekirdağ Namık Kemal University Experimental Animals Application and Research Center (DHUAM) between March and May 2019. Eighteen healthy adult male Wistar albinos (3 months old, 250-300 g) were included in the study. The rats were fed ad libitum at 22±2 °C temperature, 12 hours light/12 hours dark cycle and 50-60% humidity during the experiment. Rats were divided into 3 groups, respectively (n=6), as

control, LPS, and LPS + hesperetin groups. *Escherichia coli* (O26:B6 serotype, Sigma-Aldrich) was administered intraperitoneally to the LPS group at a dose of 10 mg/kg (22). In the LPS + hesperetin group, hesperetin (SantaCruz, dissolved in 0.5 mL saline) was administered orally at a dose of 100 mg/kg, following endotoxin administration (14). At the end of 24 hours following LPS administration, the rats were opened from the midline under ketamine-xylazine (90-10 mg/kg) anesthesia. The kidney tissue was dissected and the animals were sacrificed after drawing blood from the heart. The kidney tissue was fixed in 10% formalin solution.

Biochemical Analysis

The blood samples were centrifuged at 4500 rpm for 5 minutes and serum samples were obtained. BUN and creatinine levels were determined from the obtained serum samples with the help of a biochemistry autoanalyzer (Cobas-501, RocheDiagnostic).

Histopathological Examination

Following the 24-hour fixation of the tissues in formalin, they were washed by keeping them under running tap water for a night. After washing, kidney tissues were exposed to alcohol series with increasing concentrations (60-70-80-90-96-100) and tissue blocks were obtained by embedding kidney tissues first in a soft paraffin-toluene mixture and then in hard paraffin (Slee, MPS). These blocks were used in histological, immunohistochemical and TUNEL examinations.

Hematoxylin-eosin (H&E) staining method was used for histopathological examination. Sections (5 µm) obtained using a semi-automatic microtome (Sleecut, 5061) were kept in an oven (37 °C) overnight to ensure complete adhesion to the slide surface. These sections were taken in toluene and dewaxed (5 min x 2 times). Afterwards, they were put into water after passing through alcohol series at decreasing concentrations (100-96-90-80-70-60%). After the hydration process, they were stained with hematoxylin for 3 minutes and after washing, with eosin for 5 minutes.

Afterwards, the slides were passed through a series of increasing concentrations of alcohol and placed in toluene. They were covered with enthallan following the transparentizing process.

Obtained H&E stained kidney tissue sections were examined using a light microscope (Olympus CX40) and image analysis program (Kameram, GEN III). The histopathological changes that occurred were detected and photographed.

TGF-β1 Immunohistochemical Staining

Avidin-biotin peroxidase complex technique was used for TGF-β1 immunohistochemical marking. Paraffin-free slides were passed through an alcohol series at decreasing concentrations and washed with phosphate buffer. Tissue areas on the slide were delimited with a hydrophobic pen. For antigen retrieval, sections were boiled in a microwave oven for 5 min in citrate buffer. The procedure of endogenous peroxide suppression was performed with hydrogen peroxide. In order to prevent non-specific binding, primary antibody application (TGF-β1, sc-52893) was carried out in a humid box for one hour at 37 °C, following a one-hour block serum step at laboratory temperature. After the application of secondary antibodies with biotin and streptavidin, which were applied for 20 minutes after the primary antibody application, the coloring process was carried out with chromogen (AEC; 3-amino-9-ethyl carbazole, Thermoscientific). Sections stained with Mayer's hematoxylin were examined under a light microscope. TGF-β1 staining intensity was graded semiquantitatively (0; no staining, 1; weak, 2; moderate, 3; strong).

Apoptotic Examination

TUNEL (Terminal deoxynucleotidyltransferase-mediated dUTP nickend-labelling) method was used to determine apoptosis in kidney tissue. The procedure for using the kit was applied to the deparaffinized slides. DAB (3,3'-diaminobenzidine tetrahydrochloride) was used as chromogen. After ground staining with Mayer's hematoxylin, the sections were examined under a light microscope. The apoptotic index was calculated by measuring the number of TUNEL-positive cells stained dark brown in the cell nucleus in photographs obtained from 10 different areas of each kidney section (TUNEL positive cell number/total number of cells counted x 100) (23).

Statistical Analysis

The obtained data were analyzed using SPSS (PASW, 18.0.0) statistical program. The data of the groups were evaluated using the non-parametric Kruskal-Wallis test. Differences between the groups were measured with the Mann-Whitney U test. When the difference between the groups was less than $p<0.05$, it was considered statistically significant.

RESULTS

In the histopathological evaluation, normal histological structure was observed in the kidney tissue of the control group. The structure of proximal and distal tubules, interstitial tissue and kidney bodies were found to be normal (Figure 1a). In the LPS group, shedding especially in

tubular epithelial cells and pycnotic nuclei in some tubular epithelial cells were observed (Figure 1b). Although the tubule epithelial shedding continued in the group treated with hesperetin, it was found to be less frequent compared to the LPS group (Figure 1c).

When immunohistochemical TGF-β1 staining was examined, it was observed that positive immune reactivity was intense in tubular epithelial cells (Figure 2a-c). TGF-β1 semiquantitative staining scores were 0.3±0.5, 2.5±0.54, and 1.66±0.51 in the control, LPS and LPS + hesperetin groups, respectively. It was determined that there was an increase in TGF-β1 immune reactivity in the LPS group compared to the control group ($p<0.05$). It was found that hesperetin administration provided a significant decrease in TGF-β1 immune reactivity ($p<0.05$).

In our study, apoptosis occurring in kidney tissue was determined by the TUNEL method based on the detection of apoptotic cells by marking broken DNA ends (Figure 3). In the control, LPS and LPS + hesperetin groups, the apoptotic index was calculated as 5.3±1.8, 29.5±5.2, and 15.8±5.3, respectively. While a significant increase was observed in the LPS group compared to the control group ($p<0.05$), a significant decrease was detected in the LPS + hesperetin group compared to the LPS group ($p<0.05$).

Serum BUN and creatinine levels are presented in Table 1. LPS administration caused a significant increase in serum BUN and creatinine levels compared to the control group ($p<0.05$). It was determined that hesperetin administration provided a significant decrease in kidney function tests BUN and creatinine levels compared to the LPS group ($p<0.05$).

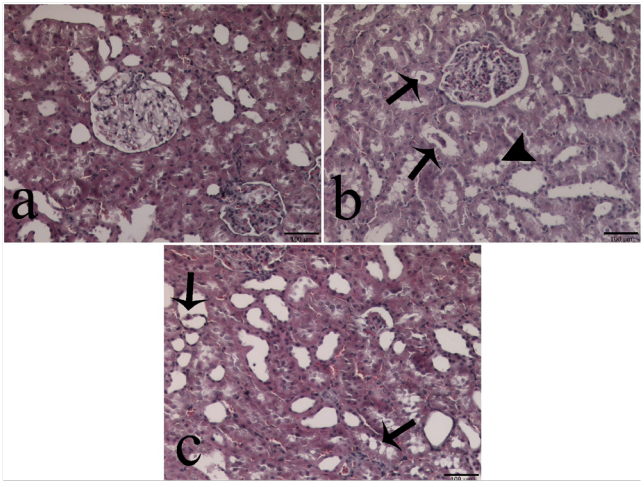

Figure 1. Histological structure of hematoxylin-eosin (H&E) stained kidney tissue (a; Control, b; LPS, c; LPS + hesperetin, bar; 100 µm, magnification; 200x, arrow; epithelial cell shedding, arrowhead; pycnotic cells, staining; H&E)
LPS: Lipopolysaccharide

DISCUSSION

Although the LPS-induced sepsis model does not represent all the features of sepsis encountered in the clinic, it is frequently used in the literature to create an AKI model because the findings of AKI caused by it are similar (24).

Although septic AKI is a complication that can be seen frequently in intensive care units and has a high mortality rate, there is no specific treatment method yet (25). In these patients, especially kidney transplantation is an effective treatment method (26). Today, studies examining the possible treatment efficacy of agents with known anti-inflammatory, anti-apoptotic and antioxidant properties against AKI damage are frequently encountered in the literature.

The findings obtained in our study demonstrated the renal protective effect of hesperetin in the rat LPS-induced AKI model with histological and biochemical data for the first time in the literature. Preservation of the renal histological structure by hesperetin administration may have been achieved by inhibiting TGF-β1 immunoreactivity and the activity of the inflammatory cytokine cascade and preventing

Table 1. Serum BUN and creatinine levels of the groups

	BUN (mg/dL)	Creatinine (mg/dL)
Control	12.1±2.3	0.7±0.1
LPS	42±5.8*	3.7±0.5*
LPS + hesperetin	27.1±5.7**	2.3±0.3**

BUN: Blood urea nitrogen, LPS: Lipopolysaccharide, *$p<0.05$ compared to the control group, **$p<0.05$ compared to the LPS group

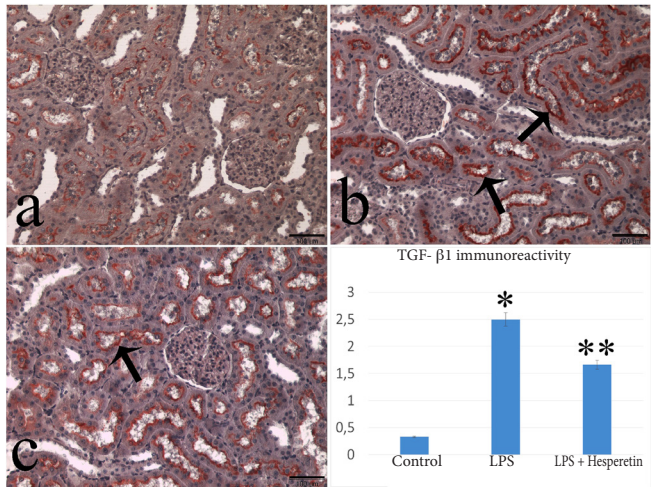

Figure 2. TGF-β1 immunohistochemistry findings (a; Control, b; LPS, c; LPS + hesperetin, bar; 100 µm, magnification; 200x, counterstaining; Mayer's hematoxylin, arrows; TGF- β1 immunoreactivity, *$p<0.05$ compared to control, **$p<0.05$ compared to LPS)
LPS: Lipopolysaccharide

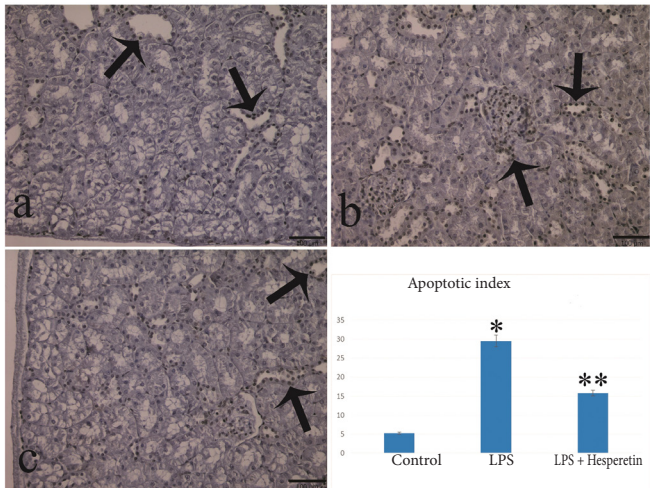

Figure 3. TUNEL staining findings of kidney tissue (a; Control, b; LPS, c; LPS + hesperetin, bar; 100 µm, magnification; 200x, counterstaining; Mayer's hematoxylin, arrows; TUNEL-positive apoptotic cells, *; p<0.05 compared to control, **; p<0.05 compared to LPS)
LPS: Lipopolysaccharide

apoptosis. There are many studies in the literature showing that hesperetin reduces/inhibits tissue damage in different experimental models due to its antioxidative, anti-inflammatory and anti-apoptotic properties (27,28).

Duran and Karaboğa (28) reported that administration of hesperetin prevented liver damage following blunt chest trauma. In the study of Trivedi et al. (29), it was revealed that heart damage caused by doxorubicin can be alleviated by the administration of hesperetin.

Kumar et al. (20) reported the therapeutic effect of hesperetin against kidney damage caused by cisplatin, a frequently used anticancer drug. In the aforementioned study, hesperetin treatment provided a significant decrease in histopathological changes caused by cisplatin application, serum BUN and creatinine levels, oxidative stress markers of superoxide dismutase, glutathione peroxidase, malondialdehyde, nitric oxide and inflammatory cytokines of TNF-α, IL-1β and IL-6 levels in kidney tissue.

It was determined that AKI-like findings occurred in the endotoxemia model created using LPS. Hesperetin administration can prevent the development of kidney damage by inhibiting the activation of the proinflammatory cytokine cascade and the expression of TGF-β1 and also by suppressing apoptosis in tubular epithelial cells.

There are some limitations in our study. Hesperetin treatment was applied following LPS administration and its effect was evaluated for a short time (24 h). This is not consistent with patient management in a clinical setting. In addition, hesperetin treatment was administered as a single dose. These limitations can be improved in new studies evaluating the effect of hesperetin application at different doses and longer than our study on kidney damage.

CONCLUSION

The results of our study show that hesperetin, a natural flavonoid, should be evaluated not only as a preventative but also for its therapeutic potential in diseases with kidney damage. More extensive *in vivo* and *in vitro* studies are needed to fully explain the treatment mechanism of hesperetin in the LPS-induced AKI model.

ETHICS

Ethics Committee Approval: The ethical aproval required to conduct this study was obtained from Tekirdağ Namık Kemal University Animal Experiments Local Ethics Committee (HADYEK- T2018-115) (decision no: 4, date: 31.10.2018).

Informed Consent: Animal experiment study.

Authorship Contributions

Surgical and Medical Practices: S.K., İ.K., Concept: S.K., İ.K., Design: S.K., İ.K., Data Collection or Processing: S.K., İ.K., Analysis or Interpretation: S.K., İ.K., Literature Search: S.K., İ.K., Writing: S.K., İ.K.

Conflict of interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Xin SB, Yan H, Ma J, Sun Q, Shen L. Protective Effects of Luteolin on Lipopolysaccharide-Induced Acute Renal Injury in Mice. Med Sci Monit 2016;22:5173-80.
2. Gursel G, Demir N. Incidence and risk factors for the development of acute renal failure in patients with ventilator-associated pneumonia. Nephrology (Carlton) 2006;11:159-64.
3. Zhang C, Walker LM, Mayeux PR. Role of nitric oxide in lipopolysaccharide-induced oxidant stress in the rat kidney. Biochem Pharmacol 2000;59:203-9.
4. Xu D, Chen M, Ren X, Ren X, Wu Y. Leonurine ameliorates LPS-induced acute kidney injury via suppressing ROS-mediated NF-κB signaling pathway. Fitoterapia 2014;97:148-55.
5. Bosshart H, Heinzelmann M. Targeting bacterial endotoxin: two sides of a coin. Ann N Y Acad Sci 2007;1096:1-17.
6. Abdelmageed ME, El-Awady MS, Suddek GM. Apocynin ameliorates endotoxin-induced acute lung injury in rats. Int Immunopharmacol 2016;30:163-70.
7. Xie X, Liu H, Wu J, Chen Y, Yu Z, De Isla N, et al. Rat BMSC infusion was unable to ameliorate inflammatory injuries in tissues of mice with LPS-induced endotoxemia. Biomed Mater Eng 2017;28(s1):S129-38.

8. Chunzhi G, Zunfeng L, Chengwei Q, Xiangmei B, Jingui Y. Hyperin protects against LPS-induced acute kidney injury by inhibiting TLR4 and NLRP3 signaling pathways. Oncotarget 2016;7:82602-8.
9. Tan F, Chen Y, Yuan D, Gong C, Li X, Zhou S. Dexmedetomidine protects against acute kidney injury through downregulating inflammatory reactions in endotoxemia rats. Biomed Rep 2015;3:365-70.
10. Nair AR, Masson GS, Ebenezer PJ, Del Piero F, Francis J. Role of TLR4 in lipopolysaccharide-induced acute kidney injury: protection by blueberry. Free Radic Biol Med 2014;71:16-25.
11. Ye HY, Jin J, Jin LW, Chen Y, Zhou ZH, Li ZY. Chlorogenic Acid Attenuates Lipopolysaccharide-Induced Acute Kidney Injury by Inhibiting TLR4/NF-κB Signal Pathway. Inflammation 2017;40:523-9.
12. Kiyonaga N, Moriyama T, Kanmura Y. Effects of landiolol in lipopolysaccharide-induced acute kidney injury in rats and in vitro. Shock 2019;52:117-23.
13. Zhao H, Zheng Q, Hu X, Shen H, Li F. Betulin attenuates kidney injury in septic rats through inhibiting TLR4/NF-κB signaling pathway. Life Sci 2016;144:185-93.
14. Polat FR, Karaboga I, Polat MS, Erboğa Z, Yılmaz A, Güzel S. Effect of hesperetin on inflammatory and oxidative status in trinitrobenzene sulfonic acid-induced experimental colitis model. Cell Mol Biol (Noisy-le-Grand) 2018;64:58-65.
15. Shirzad M, Heidarian E, Beshkar P, Gholami-Arjenaki M. Biological Effects of Hesperetin on Interleukin-6/Phosphorylated Signal Transducer and Activator of Transcription 3 Pathway Signaling in Prostate Cancer PC3 Cells. Pharmacognosy Res 2017;9:188-94.
16. Arul D, Subramanian P. Inhibitory effect of naringenin (citrus flavonone) on N-nitrosodiethylamine induced hepatocarcinogenesis in rats. Biochem Biophys Res Commun 2013;434:203-9.
17. Lee JH, Kim GH. Evaluation of antioxidant and inhibitory activities for different subclasses flavonoids on enzymes for rheumatoid arthritis. J Food Sci 2010;75:H212-7.
18. Kara S, Gencer B, Karaca T, Tufan HA, Arikan S, Ersan I, et al. Protective effect of hesperetin and naringenin against apoptosis in ischemia/reperfusion-induced retinal injury in rats. ScientificWorldJournal 2014;2014:797824.
19. Park JH, Ku HJ, Park JW. Hesperetin mitigates acrolein-induced apoptosis in lung cells in vitro and in vivo. Redox Rep 2018;23:188-93.
20. Kumar M, Dahiya V, Kasala ER, Bodduluru LN, Lahkar M. The renoprotective activity of hesperetin in cisplatin induced nephrotoxicity in rats: Molecular and biochemical evidence. Biomed Pharmacother 2017;89:1207-15.
21. Wang J, Zhu H, Yang Z, Liu Z. Antioxidative effects of hesperetin against lead acetate-induced oxidative stress in rats. Indian J Pharmacol 2013;45:395-8.
22. Kaya S, Albayrak Kaya S, Polat E, Fidanol Erboğa Z, Duran Y, Polat FR, et al. Protective effects of hesperetin on lipopolysaccharide-induced acute lung injury in a rat model. Turk Gogus Kalp Damar Cerrahisi Derg 2020;28:359-68.
23. Aksu F, Aksu B, Unlu N, Karaca T, Ayvaz S, Erman H, et al. Antioxidant and renoprotective effects of sphingosylphosphorylcholine on contrast-induced nephropathy in rats. Ren Fail 2016;38:1089-98.
24. Han M, Li Y, Liu M, Li Y, Cong B. Renal neutrophil gelatinase associated lipocalin expression in lipopolysaccharide-induced acute kidney injury in the rat. BMC Nephrol 2012;13:25.
25. Verbrugge FH, Dupont M, Shao Z, Shrestha K, Singh D, Finucan M, et al. Novel urinary biomarkers in detecting acute kidney injury, persistent renal impairment, and all-cause mortality following decongestive therapy in acute decompensated heart failure. J Card Fail 2013;19:621-8.
26. Lattanzio MR, Kopyt NP. Acute kidney injury: new concepts in definition, diagnosis, pathophysiology, and treatment. J Am Osteopath Assoc 2009;109:13-9.
27. Kumar B, Gupta SK, Srinivasan BP, Nag TC, Srivastava S, Saxena R, et al. Hesperetin rescues retinal oxidative stress, neuroinflammation and apoptosis in diabetic rats. Microvasc Res 2013;87:65-74.
28. Duran Y, Karaboğa İ. Effect of hesperetin on systemic inflammation and hepatic injury after blunt chest trauma in rats. Biotech Histochem 2020;95:297-304.
29. Trivedi PP, Kushwaha S, Tripathi DN, Jena GB. Cardioprotective effects of hesperetin against doxorubicin-induced oxidative stress and DNA damage in rat. Cardiovasc Toxicol 2011;11:215-25.