TLR4-IN-C34 protects against acute kidney injury via modulating TLR4/MyD88/NF-κb axis, MAPK, and apoptosis

Hadeer M. Abdelsalam 1,2, Manar G. Helal 1, Nashwa M. Abu-Elsaad 1*

1 Department of Pharmacology and Toxicology, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt
2 Department of Pharmacology, Faculty of Pharmacy, Horus University, Egypt

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Objective(s): Acute kidney injury (AKI) is a major component of isoproterenol (ISO) induced cardiorenal syndrome. In this study, we investigated the effect of TLR4-IN-C34 as a toll-like receptor (TLR)-4 inhibitor on ameliorating ISO-induced AKI and the possible molecular underlying pathways.

Materials and Methods: The study included 4 groups: control group, ISO group (rats received 100 mg/kg ISO in 2 doses 24 hr apart, SC), ISO+C341 and ISO+C343 groups (rats received 1 or 3 mg/kg TLR4-IN-C34 respectively twice one hour before each ISO injection, IP).

Results: Obtained results showed that TLR4-IN-C34 injection prior to ISO decreased serum creatinine level (P<0.05). Renal tissue histopathologic changes were markedly decreased by TLR4-IN-C34. Renal relative expression of MAPK and MyD88 mRNA decreased significantly in both ISO+C34, and ISO+C34, groups compared with the ISO group (P<0.05). Furthermore, TLR4-IN-C34 lowered the inflammatory cytokines IL-8, IL-1β, and IL-12 renal levels (P<0.05). Immunostained kidney sections showed a marked decrease in NF-κb positive cells in addition to the apoptotic marker Bax (P<0.05) by the two tested doses of TLR4-IN-C34. On the other hand, the expression of the antiapoptotic marker Bcl-2 by renal cells was markedly increased.

Conclusion: It can be concluded that TLR4-IN-C34 ameliorates ISO-induced AKI through anti-inflammatory anti-apoptotic effects and modulation of TLR4 signaling pathways.

Introduction

In response to infections, xenobiotics, a variety of cytokines and environmental stresses, immune cells express toll-like receptors (TLRs) (1). Expression of these receptors is proportional to the severity of organ destruction and disease progression (2).

In the kidneys, many TLRs (TLR1 to 6) are connected with numerous renal sections and contribute to immune system inflammatory responses and renal tissue injury (3). Renal TLR signaling pathways have a clear link with acute kidney damage and have been associated with many cellular processes activated during acute kidney injury (AKI). TLR type 4 (TLR4) is a key molecule in the development of various inflammatory disorders and is best characterized during AKI development. It is considered an upstream modulator of several inflammatory pathways involved in AKI (4).

Inhibition of TLR4 can represent an interesting target to prevent AKI progression into pre-clinical renal diseases. TLR4-IN-C34 is a strong and specific inhibitor of TLR4 signaling in vivo and in vitro. It inhibits TLR4 in enterocytes and macrophages in vitro and reduces systemic inflammation in in vivo models of endotoxemia and necrotizing enterocolitis (5).

TLR4-IN-C34 introduces a novel family of small compounds with therapeutic potential in illnesses and pathological conditions characterized by overactive TLR4 signaling. It can also help to normalize pathological circumstances or disorders like a faulty electron transport chain or an excess of reactive oxygen species (ROS) (1). Zhang et al. (6) demonstrated a potential application of TLR4-IN-C34 in the treatment of central nervous system diseases resulting from neuroinflammation.

Many studies have found that isoproterenol (ISO)-induced myocardial infarction is accompanied by a cardio-renal syndrome of type 1 (7). Besides, ISO administration increases kidney oxidative stress indicators while decreasing endogenous antioxidants like catalase and glutathione levels. In ISO-treated rats, there is also a significant infiltration of inflammatory cells and marked collagen deposition in the kidneys (8).

The current study investigates the involvement of the TLR4 signaling pathway in ISO-induced nephropathy. The possible ameliorative effect of TLR4-IN-C34 on AKI development and the proposed mechanisms were inspected.

Materials and Methods

Animals

Adult male Wistar rats (n=34, 150±20 g) were bought from VACSERA (Agouza, Giza, Egypt). Animals were housed 3 weeks before starting the study for accommodation.

Chemicals and kits

DL-Isoproterenol hydrochloride (ISO), 98% crystalline powder, was bought from Alfa Aesar by Thermo Fisher Scientific (Kandel, Germany) (CAS Number: 51-30-9). TLR4-IN-C34 (C34), ≥98% (HPLC), was bought from...
Sigma Aldrich Chemical Co (St. Louis, MO, USA) (CAS Number: 40592-88-9). Urethane, 99% purity, was purchased from Sigma Aldrich Chemical Company (St. Louis, MO, USA) (CAS Number: 51-79-7).

ELISA Kits for rat interleukin (IL)-8 and IL-1β were purchased from CLOUD-CLONE CORP (USA). ELISA Kit for IL-12 was purchased from lifespan Biosciences (Vienna, Austria). A spectrophotometric kit for creatinine was purchased from Spinreact company (Barcelona, Spain). Super Script IV One-Step RT-PCR kit was purchased from Thermo Fisher Scientific (Kandel, Germany).

**Antibodies**

Rat monoclonal anti-B-cell lymphoma (Bcl)-2 was purchased from Genemed Biotechnologies Inc. (CA, USA). Nuclear factor (NF)-κB rabbit polyclonal antibodies were purchased from ABDonal Science Inc. (Wuhan, China). Anti-Bcl-2 associated X (Bax) rabbit polyclonal antibody was purchased from ServiceBio (Wuhan, China).

**Experimental design**

Thirty-four rats were divided randomly into four groups as follows: control group (n=10 rats): received normal saline (1 ml/kg, twice, 24 hr apart, SC between ears); ISO group (n=10 rats) received 100 mg/kg ISO (1 ml/kg of 10% w/v solution in normal saline; twice, 24 hr apart, SC between ears) (9), ISO+C34, (n=7 rats) and ISO+C34, (n=7 rats) groups: two groups received 1 or 3 mg/kg TLR4-IN-C34 (1 ml/kg of 0.1% or 0.3% w/v solution in water for injection respectively, IP) twice where each dose was injected one hour before each ISO injection. Doses of TLR4-IN-C34 were determined through a pilot study and in light of the previous study (5). Table 1 outlines the study’s experimental design.

**Methods**

Twenty-four hours after injecting ISO second dose, rats were anesthetized using urethane (1.8 gm/kg, intraperitoneally) (10). Myocardial infarction was ensured by recording electrocardiograms, increased heart rate, elevated myocardial troponin I, and serum CK-MB (data not shown). Blood samples were collected through retro-orbital sinus, centrifuged, and serum was separated to measure serum creatinine level spectrophotometrically using a ready-to-use kit (11).

The two kidneys were isolated and cut into 3 parts. The first part was snap-frozen in liquid nitrogen and stored at −80 °C until consequent analysis. The second part was cut longitudinally, washed in normal saline, and placed in 10% v/v neutral buffered formalin for paraffin block preparation and further immunohistochemical and histopathological examination. The last part was used for the preparation of kidney homogenate (10% w/v in phosphate buffer pH 7.4).

**Cytokines measurement**

Kidney tissue contents of IL-8, IL-12, and IL-1β were measured in the tissue homogenate using the ELISA technique as directed by the kit manufacturer.

**RNA extraction and quantitative RT-PCR**

Renal expression of mitogen-activated protein kinases (MAPK) and myeloid differentiation primary response (MyD)-88 was measured using qRT-PCR. Kidney parts frozen in liquid nitrogen were ground into powder. Total RNA was then extracted using Direct-zol RNA MiniPrep Plus (ZYMO RESEARCH CORP, USA) and its quantity and quality were measured using a Beckman dual spectrophotometer (USA) at 260 nm.

Reverse transcription of extracted RNA was carried out using the SuperScript IV One-Step RT-PCR kit (Thermo Fisher Scientific, Waltham, MA, USA). Designed primers are shown in Table 2 (Vivantis, CA, USA). Briefly, 10 µl of purified RNA was mixed with 40 µl one-step RT-PCR mix containing 25 µl of 2× Platinum™ SuperFi™ RT-PCR Master Mix, 2.5 µl of forward primer (10 µM), 2.5 µl of reverse primer (10 µM), 0.5 µl of SuperScript™ IV RT Mix, and 9.5 µl of nuclease-free water. Components were mixed gently and placed in a pre-heated thermal cycler (Step One Applied Biosystem, Foster City, USA). The following protocols were carried out: cDNA reverse transcription protocol (1 cycle at 55 °C for 10 min) followed by RT Enzyme inactivation protocol (1 cycle at 95 °C for 2 min). Thereafter, cDNA amplification protocol was applied: denaturation (40 cycles at 95 °C for 10 sec), annealing (40 cycles at 55 °C for 10 sec), and extension (40 cycles at 72 °C for 30 sec) followed by the final extension (1 cycle at 72 °C for 5 min). After running

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### Table 1. Schematic representation of the study experimental design

| Group          | 0    | 23   | 24   | 48   |
|----------------|------|------|------|------|
| Control        | ☐    | ☐    | ☐    | ☐    |
| ISO            | ☐    | ☐    | ☐    | ☐    |
| ISO+C34        | ☐    | ☐    | ☐    | ☐    |
| ISO+C34        | ☐    | ☐    | ☐    | ☐    |

- ☐ Normal saline (0.9% w/v, 1 ml/kg, SC)
- ☐ Isoproterenol (ISO) (100 mg/kg, 1 ml/kg of 10% w/v solution in normal saline, SC)
- ☐ C34 (1 mg/kg, 1 ml/kg of 0.1% w/v solution in water for injection, IP)
- ☐ C34 (3 mg/kg, 1 ml/kg of 0.3% w/v solution in water for injection, IP)

### Table 2. Primers designed for qRT-PCR of MAPK, MyD88 and GAPDH

| Primer | Forward sequence | Reverse sequence |
|--------|------------------|------------------|
| MAPK   | 5’-ATGGGAGAAACAAACAAACCTCACTG-3’ | 5’-TTGCTCCCCATGTATGCTTCA-3’ |
| MyD88  | 5’-ATGTGGTGCCGTTGGGCAATGTTGC-3’ | 5’-CCAGTTATACCCAGAGTG-3’ |
| GAPDH  | 5’-TGGCATTGGGCACTTGC-3’ | 5’-TTTGCACCTGAGTCNATGAT-3’ |

MAPK: Mitogen-activated protein kinases; MyD88: Myeloid differentiation factor 88
RT-PCR cycles, obtained data were stated in Cycle threshold (Ct) for both MAPK and MyD88 and conforming to the housekeeping gene GAPDH. The RQ of each target gene is counted and standardized to housekeeping gene rendering to the calculation of delta-delta Ct ($\Delta\Delta$Ct) (12).

**Histopathology and Immunohistochemistry**

Paraffin wax blocks were processed and 4-μm-thick kidney sections were stained with hematoxylin-eosin stain and examined for tubular injury and interstitial fibrosis. The severity of the renal injury was assessed according to the degree of congestion, interstitial inflammation, and tubular necrosis. The injury was graded semi-quantitatively as follows: score 0 was considered normal, score 1 for mild changes, score 2 for moderate changes, and score 3 for severe changes (13).

Other sections were used for immunohistochemical assessment of NF-κB p-65, BAX, and Bcl-2 protein expression by immunohistochemistry technique. Briefly, after deparaffinization and rehydration antigen retrieval was performed. Sections were incubated with rabbit anti-NF-κB p-65 monoclonal antibodies (A19653), mouse monoclonal anti-Bcl-2 (61-0005), and anti-Bax rabbit polyclonal antibodies (GB11007-1) overnight at 4 °C. After washing, samples were incubated with goat anti-rabbit secondary antibody as a secondary antibody [HRP Goat Anti-Rabbit IgG (H+L) (AS014)] at 1:10000 dilution for 2 hr at room temperature and then visualized with dianaminobenzidine. Sections were examined under a light microscope “40x lens” (Leica). Appropriate controls were made to exclude background or non-specific binding (14).

**Statistical analysis**

Data except (histopathologic scores) are expressed as mean ± standard deviation (SD) and analyzed using One-way analysis of variance (ANOVA) test followed by post-hoc Tukey-Kramer test. Statistical significance was accepted at ($P<0.05$). Histopathological scores are expressed as median and interquartile range and analyzed using the Kruskal-Wallis test followed by post-hoc Dunn’s multiple comparison tests. Graphs were constructed and statistical analysis was carried out using GraphPad Prism V5.01 (GraphPad Software Inc, San Diego, CA, USA).

**Results**

*Effect on serum creatinine level*

Serum creatinine was significantly ($P<0.001$) elevated by ISO when compared with the control group. In ISO+C34$^1$ and ISO+C34$^3$ groups, creatinine level was significantly ($P<0.001$) low compared with the ISO group level and became comparable with the control group level (Figure 1).

*Effect on kidney tissue content of IL-1β, IL-8, and IL-12*

The group that received ISO alone showed a significantly ($P<0.001$) high renal content of IL-1β compared with the control group. In the ISO-C34$^3$ group, renal IL-1β content was significantly ($P<0.001$) decreased when compared with the ISO group but remained significantly ($P<0.001$) higher than the control group renal content. The higher dose of TLR4-IN-C34 in the ISO+C34$^3$ group significantly ($P<0.001$) decreased IL-1β compared with ISO injection alone and the smaller dose in the ISO-C34$^1$ group (Figure 2a).

Injection of ISO alone showed a significantly ($P<0.001$) high renal content of IL-8 compared with the control group. In the ISO-C34$^3$ group, renal IL-8 content was significantly ($P<0.001$) decreased when compared with the ISO group. Likewise, C34 (3 mg/kg) decreased IL-8 renal concentration Figure 1. Effect of TLR4-IN-C34 on serum creatinine level in isoproterenol-injected rats. *** $P<0.001$ compared with control group, ### $P<0.001$ compared with isoproterenol group; (n=6/group)

**TLR: Toll-like receptors**

Figure 2. Effect of TLR4-IN-C34 on interleukin (IL)-1β (a), IL-8 (b), and IL-12 (c) levels in isoproterenol injected rats. *** $P<0.001$ compared with control group, ### $P<0.001$ compared with isoproterenol group, and $\omega$ $P<0.001$ compared with TLR4-IN-C34 1 mg/kg; (n=6/group)

**TLR: Toll-like receptors**
significantly compared with groups that received ISO or ISO+C34_1 (P<0.001). There was no significant difference in IL-8 renal content between the control group and the ISO+C34_1 group (Figure 2b).

The renal concentration of IL-12 was significantly (P<0.001) elevated after ISO injection. Injection of C34 (1 mg/kg) before ISO significantly (P<0.001) decreased IL-12 compared with injection of ISO solely but the cytokine content did not reach that of the control group. In the ISO+C34_1 group, IL-12 concentration was significantly (P<0.001) lower than either the ISO group or ISO-C34_1 group concentrations(Figure 2c).

**Effect on renal histopathology:**

Histopathological examination of kidney sections stained with hematoxylin-eosin stain (Figure 3a and 3b) revealed marked tissue damage in the ISO group. Sections from the ISO group showed multifocal areas of inflammation in the cortex and medulla with perivascular edema. Desquamation and separation of tubular epithelium, tubular necrosis, and extensive fibrosis were observed markedly. In the ISO+C34_1 group, stained sections showed less renal tissue damage with decreased areas of fibrosis, perivascular edema, and tubular dilation. The higher dose of C34 received by the ISO+C34_1 group retained normal tubular and glomerular structures with no fibrosis and only mild perivascular edema. Renal injury score in ISO and ISO+C34_1 was significantly (P<0.001) higher than the control group score. A significant (P<0.05) decrease in injury score was obtained in the ISO+C34_1 group compared with the ISO group (Figure 3c).

**Effect on renal expression of NF-κB p-65, Bax, and Bcl-2**

Immuno-stained sections (Figure 4a) revealed a marked increase in positive brown tubular reaction against NF-κb in the ISO group compared with the control group. The Renal sections stained against Bcl-2 [Figure 6a] showed a strong positive brown tubular reaction in the control group. This reaction was markedly less in the ISO group compared with the control group and was slightly higher in ISO+C34_1. In the ISO+C34_1 group, Bcl-2 positive cells were reaction decreased in the ISO+C34_1 group and decreased much more in the ISO+C34_3 group compared with the ISO group. The percentage of NF-kb positive cells in ISO and ISO+C34_1 groups was significantly high compared with the control group (P<0.001, 0.01, respectively). Positive cell percentage was significantly (P<0.001) less in ISO+C34_3 compared with the ISO group (Figure 4b). Similarly, the proapoptotic marker Bax expression was markedly increased in stained renal sections [Figure 5a] isolated from the ISO group. Expression of Bax decreased in groups that received C34 and more notably in the ISO+C34_1 group that showed a significantly (P<0.001) low percentage of Bax-positive cells compared with the ISO group (Figure 5b).
significantly (P<0.01) increased compared with the ISO group (Figure 6b).

**Effect on renal expression of MAPK and MyD88**

Relative expression of MAPK and MyD88 is represented in (Figure 7a). Injection of ISO significantly increased MAPK expression when compared with the control group. Low dose TLR4-IN-C34 (1 mg/kg) significantly (P<0.001) decreased MAPK expression in renal tissue compared with both ISO and C34 low dose (Figure 7b). Additionally, the ISO group showed high (P<0.001) expression of MyD88 in the kidney compared with the control group. The group that received ISO+C34, showed a significantly (P<0.001, 0.01) low MyD88 expression when compared with the ISO group or ISO+C34 group, respectively (Figure 7c).

**Discussion**

AKI is a progressive renal complication that significantly affects the patient’s life with huge economic burden. Untreated AKI eventually progresses to a chronic form and end-stage renal disease. Increasing evidence shows that TLR4-mediated inflammatory response plays a pivotal role in the pathogenesis of AKI (15).

The current study investigated the role of TLR4 in the management of ISO-induced AKI through the effect of using TLR4-IN-C34, a new selective inhibitor of TLR4. The study involved studying two doses of TLR4-IN-C34 in rats, measurement of serum creatinine level, and examination of renal tissue for histopathological changes. In addition, the effect on cytokines engaged in inflammatory pathways of AKI and apoptotic markers Bax/Bcl2 were evaluated. Targeting TLR4/MyD88/NF-κb and MAPK expression was evaluated as well.

Injection of ISO in large doses resulted in significant damage in renal tissue integrity indicated by histopathologic
changes as multifocal areas of inflammation in the renal cortex and medulla, perivascular fibrosis, desquamation, and separation of tubular epithelium. These nephrotic effects were accompanied by an increase in creatinine serum level indicating altered renal function. These effects may be explained by activation of the MAPK signaling pathway, reactive oxygen species, and collagen deposition (16). In ISO received group, NF-kB positive cells were markedly increased proposing that ISO increases renal TLR4/NF-kB signaling pathway proteins similar to cardiac tissue as reported by (17).

Toll-like receptor-4 has been implicated in the pathophysiology of a variety of inflammatory illnesses (18). It is a well-known lipopolysaccharide pathogen recognition receptor that plays an important role in the innate immune system. Death-associated molecular patterns (DAMPs) are also recognized by TLR4 and substantially activate its signaling (19). Nephrotoxins result in renal cell damage, and in turn the release of certain DAMPs as HMGB1, triggering the activation of the pattern recognition receptor TLR4 among others and an inflammatory response (20).

Inhibition of TLR4 by TLR4-IN-C34 may halt multiple pathways underlying ISO-induced nephrotoxicity. Promotion of MyD88 is one of the TLR4 signaling pathways that activate NF-kB-mediated cytokine release (21).

Interleukin-8 is a multifunctional chemokine that can proceed as both an anti-inflammatory myokine and a pro-inflammatory cytokine and it has a strong link with AKI in multiple experimental studies (22). It has been applied as a biomarker for AKI following cardiopulmonary surgery (23). Higher content of IL-8 may account for renal parenchymal and tubular damage. This cellular damage results from the chemotactic effect of IL-8 on neutrophils releasing lysosomal enzymes and up-regulating endothelial adhesion molecules (24, 25).

In inflammatory responses, production of IL-8 may be enhanced via the MyD88-dependent TLR4 signaling pathway (26). TLR4-IN-C34 suppressed IL-8 renal overproduction indicating reduced neutrophils chemotaxis and subsequently renal tissue damage. Similar effects were obtained by other TLR4 inhibitors in some inflammatory diseases (27, 28).

TLR4 is a key molecule that could mediate the NF-kB inflammatory cascade leading to AKI (19). Activation of TLR4/p38MAPK signaling stimulates the inflammatory NF-kB pathway and production of inflammatory cytokines among which are tumor necrosis factor (TNF)-α, IL-1, and IL-6 (30). Our study verified a decrease in NF-kB target gene expression in renal tissue damaged by ISO, proposing that ISO increases renal TLR4/NF-kB signaling thereby attenuating inflammation, oxidative stress, and apoptosis.

In injured kidneys, inflammatory reaction induces apoptosis and promotes renal epithelial loss, which is a characteristic of AKI (32). After injury initiation, numerous renal tubular epithelial cells in the nephrons become apoptotic leading to cell necrosis. Damage to the mitochondrial outer membrane also occurs causing increased production of TNF-α, Bax, and caspase, all of which trigger apoptosis. A decreased expression of Bcl-2 with overproduction of Bax is considered a hallmark in apoptosis upstreaming (33).

Conclusion
We believe that TLR4-IN-C34 is a novel therapeutic strategy that decreases ISO-related AKI by modulating TLR4/NF-kB/MAPK/MYD88 signaling thereby attenuating inflammation, oxidative stress, and apoptosis.

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Authors' Contributions
MGH and NMA Designed the study; HMA Funded the experiment; HA, MH, and NA Approved the final study plan; HA, MH, and NA Managed the manuscript draft; MH and NA Supervised, directed, and interpreted data; MGH and NMA Prepared and visualized the data; Adegoke EO, Adeniran SO, Zeng Y, Wang H, Wang C, et al. Pharmacological inhibition of TLR4/NF-κB with TLR4-IN-C34 attenuated microcystin-leucine arginase toxicity in bovine Sertoli cells. J Appl Toxicol 2019; 39: 832-843.

References
1. Adegoke EO, Adeniran SO, Zeng Y, Wang X, Wang H, Wang C, et al. Pharmacological inhibition of TLR4/NF-κB with TLR4-IN-C34 attenuated microcystin-leucine arginase toxicity in bovine Sertoli cells. J Appl Toxicol 2019; 39: 832-843.
2. Sivak KV, Stosman KI, Rassokha TA, Aleksandrov AG, Kurzmich NN, Orshanskaya YR, et al. Mesenteric lymphatic alterations observed during DSS induced intestinal inflammation.
are driven in a TLR4-PAMP/DAMP discriminative manner. Front Immunol 2019; 26: 557-568.

3. Farrar A. Acute Kidney Injury. Nurs Clin 2018; 53:499-510

4. Vázquez-Carballo C, Guerrero-Hue M, García-Caballero C, Rayego-Mateos S, Opazo-Ríos L, Morgado-Pascual JL, et al. Toll-like receptors in acute kidney injury. Int J Mol Sci 2021; 22: 816-852.

5. Neal MD, Jia H, Eyer B, Good M, Guerrero CJ, Sodhi CP, et al. Discovery and validation of a new class of small molecule Toll-like receptor 4 (TLR4) inhibitors. PLoS one 2013; 8: e65779.

6. Zhang SS, Liu M, Liu DN, Yang YL, Du GH, Wang YH. TLR4-IN-C34 inhibits lipopolysaccharide-stimulated inflammatory responses via downregulating TLR4/MyD88/NF-κB/NLRP3 signaling pathway and reducing ROS generation in BV2 cells. Inflammation 2022; 45: 838-850.

7. Ghartavoli MM, Gholizadeh-Ghaleh Aziz S, Babaei G, Hossein Farjah G and Hassan Khadem Ansari M. The protective impact of betaine on the tissue structure and renal function in isoproterenol-induced acute kidney injury in rats. Mol Genet Genomic Med 2019; 7: e00579.

8. Hasen R, Lasker S, Hasan A, Zerin I, Zamila M, Parvez F, et al. Canagliflozin ameliorates renal oxidative stress and inflammation by stimulating AMPKAkt-eNOS pathway in the isoproterenol-induced oxidative stress model. Sci Rep 2020; 10: 14659.

9. Toutouchi NS, Afrozoyan A, Rameshrad M, Rezakabbsh A, Vaez H, Hamedeyazdan S, et al. Cardioprotective effects of rosmarinic acid on isoproterenol-induced myocardial infarction in rats. Pharm Sci 2017; 23: 103-11.

10. Pistas M, Perra S, Pillolla G, Melis M, Muntoni AL, and Gessa GL. Adverse exposure to cannabinoids induces long-lasting changes in the response to drugs of abuse of rat midbrain dopamine neurons. Biol Psychiatry 2004; 56: 94-96.

11. Young DS, Pestaner L and Gibberman V. Effects of drugs on cellular laboratory tests. Clin Chem 1975; 21:1D-432D.

12. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 2001; 29: e45-49.

13. Aslan A, van den Heuvel MC, Stegeman CA, Popa ER, Leliveld AM, Molema G, et al. Kidney histopathology in lethal human sepsis. Crit Care 2018; 22: 359-370.

14. Masuda T, Okawa H, Sato S, Satodate R, Suzuki K and Sato SJ/HC. Distinguishing small lymph vessels in the portal tracts of human liver from portal veins by immunohistochemistry for a smooth muscle actin. Hepatol Commun 1996; 4: 277-282.

15. Jha AK, Gairola S, Kundra S, Doye P, Syed AM, Ram C, et al. Toll-like receptor 4: An attractive therapeutic target for acute kidney injury. Life Sci 2021; 271: 119155.

16. Naseroleslami M, Aboutaleb N, Mokhtari B. Anmiotic membrane mesenchymal stem cells labeled by iron oxide nanoparticles exert cardioprotective effects against isoproterenol (ISO)-induced myocardial damage by targeting inflammatory MAPK/NF-κB pathway. Drug Deliv Transl Res 2021; 11: 242-254.

17. Qi C, Shao Y, Liu X, Wang D and Li X. The cardioprotective effects of icariin on the isoproterenol-induced takotsubo-like rat model: Involvement of reactive oxygen species and the TLR4/NF-κB signaling pathway. Int Immunopharmacol 2019; 74: 105733.

18. Stephens M, Liao S and von der Weid PY. Mesenteric lymphatic alterations observed during DSS induced intestinal inflammation are driven in a TLR4-PAMP/DAMP discriminative manner. Front Immunol 2019; 10: 557-568.

19. Shirali AC. Goldstein DR Tracking the toll of kidney disease. J Am Soc Nephrol 2008; 19: 1444-1450.

20. Chen Q, Guan X, Zuo X, Wang J, Yin W. The role of high mobility group box 1 (HMGB1) in the pathogenesis of kidney diseases. Acta Pharm. Sin. B 2016; 6: 183–188.

21. Zhang B, Zeng M, Li M, Kan Y, Li B, Xu R, et al. Proteopine protects mice against LPS-induced acute kidney injury by inhibiting apoptosis and inflammation via the TLR4 signaling pathway. Molecules 2019; 25: 15-28.

22. Alimo A, Castiglione V, Borrelli C, Saccaro LF, Franzini M, Masi S, et al. Oxidative stress and inflammation in the evolution of heart failure: From pathophysiology to therapeutic strategies. Eur J Prev Cardiol 2020; 27:494-510.

23. Gorjipour F, Totonchi Z, Gholampour Dehaki M, Hosseini S, Tirgarfakheri K, Mehrabanian M, et al. Serum levels of interleukin-6, interleukin-8, interleukin-10, and tumor necrosis factor-α, renal function biochemical parameters and clinical outcomes in pediatric cardiopulmonary bypass surgery. Perfusion 2019; 34: 651-659.

24. Mukaida N. Interleukin-8: an expanding universe beyond neutrophil chemotaxis and activation. Int J Hematol 2000; 72: 391-398.

25. García-Covarrubias L, Cedillo IS, Morales L, Fonseca-Sanchez MA, García-Covarrubias A, Villanueva-Ortega E, et al. Interleukin 8 Is overexpressed in acute rejection in kidney transplant patients. Transplant Proc 2020; 52: 1127-1131.

26. Hasan A, Akhter N, Al-Roub A, Thomas R, Kochumon S, Wilson A, et al. TNF-α in combination with palmitate enhances IL-8 production via the MyD88-independent TLR4 signaling pathway: potential relevance to metabolic inflammation. Int J Mol Sci 2019; 20: 4112-4133.

27. Saenger T, Vordenbäumen S, Genich S, Haidar S, Schulte M, Nienberg C, et al. Human αs1-casein induces IL-8 secretion by binding to the ecto-domain of the TLR4/MD2 receptor complex. Biochim Biophys Acta 2019; 1863: 632-643.

28. Samaripita S, Kim JY, Rasool MK, Kim KS. Investigation of toll-like receptor (TLR) 4 inhibitor TAK-242 as a new potential anti-rheumatoid arthritis drug. Arthritis Res Ther 2020; 22: 16-25.

29. Nair AR, Masson GS, Ebenzer 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.

30. Verma VK, Malik S, Narayanan SP, Mutneja E, Sahu AK, Bhattachar J, et al. Role of MAPK/NF-κB pathway in cardioprotective effect of Morin in isoproterenol induced myocardial injury in rats. Mol Biol Rep 2019; 46: 1139-1148.

31. Yang J, Wang Z, and Chen D-L. Shikonin ameliorates isoproterenol (ISO)-induced myocardial damage through suppressing fibrosis, inflammation, apoptosis and ER stress. Biomed Pharmacother 2017; 93: 1343-1357.

32. Gao L, Wu WF, Dong L, Ren GL, Li HD, Yang Q, et al. Protocatechuic aldehyde attenuates cisplatin-induced acute kidney injury by suppressing nox-mediated oxidative stress and renal inflammation. Front Pharmacol 2016; 7: 479-476.

33. Havasi A, Borkan SC. Apoptosis and acute kidney injury. Kidney Int 2011; 80: 29-40.

34. de Ponte MC, Casare FAM, Costa-Pessoa JM, Cardoso VG, Malnic G, Mello-Aires M, et al. The Role of β-Adrenergic Overstimulation in the Early Stages of Renal Injury. Kidney and Blood Press Res 2017; 42: 1277-1289.