Pharmacogn J. 2024; 16(1): 126-130
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Research Article

Punicalagin Opposes Gentamicin Nephrotoxicity in Rats: Role of Nrf2 and NF-κB Pathways

Amr A. Fouad[1,*], Moataz Mohamedalhasan Ali[2], Mostafa Abdel-Hamid[3]

ABSTRACT

Background: Oxidative stress, inflammation, and apoptosis are implicated in gentamicin (GEN)-induced nephrotoxicity. Punicalagin (PNG) possesses antioxidant, anti-inflammatory, and antiapoptotic effects. **Objective:** The aim of the present research was to investigate the possible defensive effect of PNG against nephrotoxicity caused by GEN in male Sprague-Dawley rats. **Materials and Methods:** GEN (80 mg/kg/day, i.p.) was administered for 8 days. Treatment with PNG (25 mg/kg/day, p.o.) for 10 days, began 2 days before GEN insult. **Results:** PNG significantly decreased serum creatinine, and malondialdehyde, tumor necrosis factor-α, interleukin-6, inducible nitric oxide synthase, nuclear factor-κB p65 (NF-κB p65), and cleaved caspase-3 activity in the kidneys of GEN-challenged rats. PNG also significantly increased renal catalase, reduced glutathione, and nuclear factor erythroid 2-related factor 2 (Nrf2) in rats received GEN. Additionally, PNG markedly attenuated the histopathological kidney tissue injury caused by GEN. **Conclusion:** PNG guarded against GEN-induced kidney damage in rats through its antioxidant, anti-inflammatory, and antiapoptotic effects, and by modulating the balance between Nrf2 and NF-κB pathways.

Key words: Punicalagin, Gentamicin, Kidney, Rats.

Amr A. Fouad[1,*], Moataz Mohamedalhasan Ali[2], Mostafa Abdel-Hamid[3]

[1]Department of Pharmacology, Faculty of Medicine, Al-Baha University, Al-Baha, SAUDI ARABIA.

[2]Department of Pathology, Faculty of Medicine, Al-Baha University, Al-Baha, Saudi Arabia & Department of Pathology, Faculty of Medicine, University of Elimam Elmahdi, SUDAN.

[3]Department of Anatomy and Embryology, Faculty of Medicine, Al-Baha University, Al-Baha, SAUDI ARABIA.

Correspondence

Amr A. Fouad

Department of Pharmacology, Faculty of Medicine, Al-Baha University, Al-Baha, SAUDI ARABIA.

Tel. 0096655506196

E-mail: afouad@bu.edu.sa; amrfouad65@yahoo.com

History

- Submission Date: 15-11-2023;
- Review completed: 21-12-2023;
- Accepted Date: 28-12-2023.

DOI : 10.5530/pj.2024.16.17

Article Available online

http://www.phcogj.com/v16/i1

INTRODUCTION

Gentamicin (GEN), the aminoglycoside antibiotic, is used in clinical practice to eradicate serious Gram-negative bacillary infections. Acute kidney injury and renal impairment associated with gentamicin vary widely from 1.2% up to 55%, however most studies report rates between 8 and 26%.[1] GEN causes direct toxic effect on the proximal renal tubular cells following its preferential accumulation in these cells. The exact processes, which lead to nephrotoxic effect of GEN are not fully elucidated. Strong evidence proposes that GEN nephrotoxicity results from oxidative stress, increased reactive oxygen species (ROS) production, and exhaustion of endogenous antioxidants, as reduced glutathione (GSH) and catalase. This results in enhanced peroxidation of lipid membranes, and augmented malondialdehyde (MDA) production.[2] Nitrosative stress and enhanced generation of reactive nitrogen species (RNS) due to activation of inducible nitric oxide synthase (iNOS) are also involved in GEN nephrotoxicity.[3]

Additionally, increased production of inflammatory cytokines, as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), was reported to participate in renal tubular cell injury caused by GEN.[4] The inflammatory mediators and ROS finally up-regulate the apoptotic pathways and caspase family of proteases, which induce renal cell apoptotic death.[5]

Cellular responses to oxidative, inflammatory, and apoptotic insults are largely controlled by the fine balance between nuclear factor erythroid 2-related factor 2 (Nrf2), the anti-inflammatory pathway, and nuclear factor-κB (NF-κB), the pro-inflammatory pathway.[6,7] Previous investigations showed that GEN-induced renal toxicity caused by a marked disruption in the balance between Nrf2 and NF-κB pathways resulting in augmented oxidative stress, inflammatory responses, and cell apoptosis.[8,9]

Punicalagin (PNG) is a biologically active polyphenolic ingredient obtained from pomegranate (*Punica granatum*). It was reported that PNG had antioxidant, anti-inflammatory, and antiapoptotic properties.[10] PNG significantly ameliorated cisplatin-induced nephrotoxicity and endotoxemic acute kidney injury in rats,[11,12] and alleviated lupus nephritis and diabetic nephropathy induced by high-fat diet in mice.[13,14] Therefore, PNG potentially can protect against GEN-induced renal damage, and to the best of our knowledge, the nephroprotective effect of PNG in this model was not yet investigated.

MATERIALS AND METHODS

Laboratory animals

Sprague-Dawley male rats (weight 200-220 g, each) were acquired from the animal house of King Khalid University, Abha, Saudi Arabia. They were accommodated at 24°C, 45% humidity, and 12 h light/dark cycle. They were supplied with ordinary chew and tap water *ad. libitum*, and were accustomed for 1 week. The study protocol was approved by the Research Ethics Committee, Faculty of Medicine, Al-Baha University, Saudi Arabia (approval number: REC/PHA/BU-FM/2023/82). The international recommendations for dealing with laboratory animals were implemented.

Medications

PNG and GEN sulfate powders were purchased from Sigma-Aldrich Chemical Co., USA. PNG was suspended in 0.5% carboxymethylcellulose (CMC),

Cite this article: Fouad AA, Ali MM, Abdel-Hamid M. Punicalagin Opposes Gentamicin Nephrotoxicity in Rats: Role of Nrf2 and NF-κB Pathways. Pharmacogn J. 2024;16(1): 126-130.

and GEN was dissolved in normal saline. PNG and GEN doses used were selected likewise former reports.[12,15]

Study outline

Rats were randomly separated into 4 groups (n = 8, each):

- Group 1 (control) received normal saline, i.p., daily for 8 days, and daily CMC, p.o., for 10 days, starting 2 days in advance of normal saline administration.
- Group 2 received GEN (80 mg/kg/day i.p.) for 8 days, and daily CMC p.o., for 10 days, starting 2 days in advance of GEN administration.
- Group 3 received GEN (80 mg/kg/day i.p.) for 8 days, and PNG (25 mg/kg/day, p.o.) for 10 days, starting 2 days in advance of GEN administration.
- Group 4 received only PNG (25 mg/kg/day, p.o.) for 10 days

Sampling and biochemical examination

Animals were euthanized 24 h following the final GEN injection by i.p. thiopental (70 mg/kg). Blood samples were taken through cardiac punctures, and centrifuged for 10 min at 5000 rpm after clotting. Serum creatinine was measured by a colorimetric kit (Biodiagnostic, Egypt).

The kidneys were isolated, and their dry weights were determined. Homogenization of the right kidneys in cold 0.05 M K_3PO_4 buffer (pH 7.3) for 15 min at 5000 rpm was done. MDA, GSH, and catalase were determined by colorimetric kits (Biodiagnostic, Egypt), and also cleaved caspase-3 (R&D Systems, USA) in kidney homogenates. ELISA kits were used to determine iNOS (Cusabio, USA), and TNF-α and IL-6 (R&D Systems, USA), and NF-κB p65 and Nrf2 (Lifespan Biosciences, USA) in kidney homogenates.

Histopathological examination

Left kidneys were preserved in 10% formalin solution, dehydrated in ethanol, and embedded in paraffin. Sections at 4 µm were cut and stained with hematoxylin and eosin (H&E). The pathologist who visualized the slides under light microscope did not know the slide identity. Renal tubular injury score was evaluated using a scale 0-4, as follows: normal = 0, less than 10% = 1, 10-25% = 2, 25-75% = 3, and more than 75% = 4.[16]

Statistical analysis

Data analysis by one-way ANOVA test followed by Tukey test for intergroup comparisons using GraphPad Prism Software Program (version 6.01) was done. Results showed as mean ± S.E.M., and $p < 0.05$ was the significance level.

RESULTS

Biochemical results

GEN injections (80 mg/kg/day, i.p., for 8 days) lead to a significant increment of serum creatinine ($p < 0.05$) compared to the control value. PNG (25 mg/kg/ day, p.o., for 10 days), starting 2 days prior GEN, caused a significant decrement of serum creatinine ($p < 0.05$) in rats received GEN (Figure 1).

GEN also caused a significant elevation of MDA ($p < 0.05$), and significant reductions of GSH and catalase ($p < 0.05$) in rat kidneys, opposed to control results. However, a significant reduction of MDA ($p < 0.05$), and significant increases of GSH and catalase ($p < 0.05$) in rat kidneys were noticed in PNG-treated rats (Figure 2).

As regards the inflammatory and apoptotic indicators, GEN insult gave rise to significant increases of renal NF-κB p65, TNF-α, IL-6, iNOS, and cleaved caspases-3 ($p < 0.05$), and a significant reduction of renal Nrf2 ($p < 0.05$), opposed to control values. On the contrary, PNG treatment caused significant reductions of NF-κB p65, TNF-α, IL-6, iNOS, and cleaved caspases-3 ($p < 0.05$), and a significant increase of Nrf2 ($p < 0.05$) in kidneys of rats challenged with GEN (Figures 3A-3C).

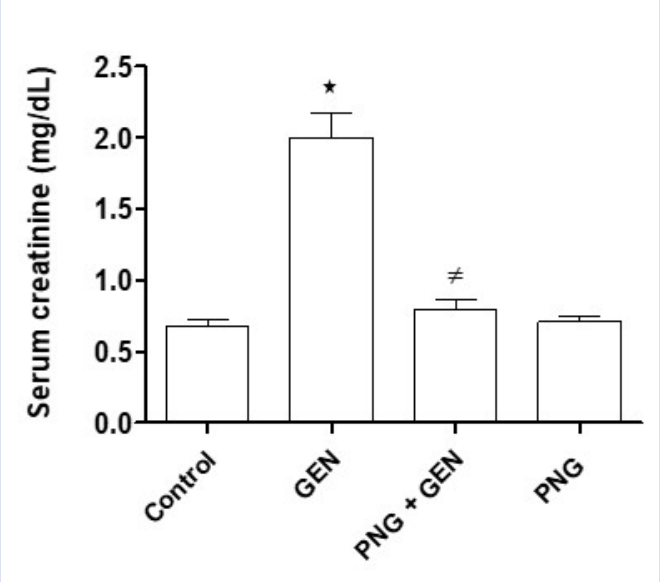


Figure 1: Effect of punicalagin (PNG) treatment on serum creatinine of gentamicin (GEN)-challenged rats. $^{*}p < 0.05$ vs. control, $^{\neq}p < 0.05$ vs. GEN group. Results are mean ± S.E.M, n = 8, in each group.

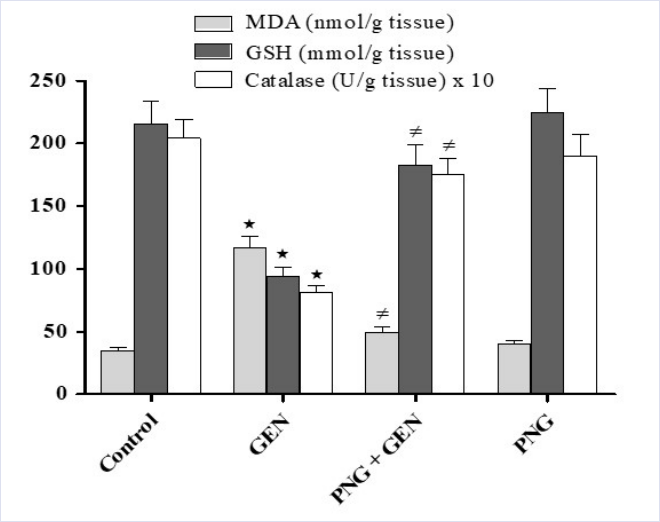


Figure 2: Effects of punicalagin (PNG) treatment on malondialdehyde (MDA), reduced glutathione (GSH), and catalase in kidneys of gentamicin (GEN)-challenged rats. $^{*}p < 0.05$ vs. control, $^{\neq}p < 0.05$ vs. GEN group. Results are mean ± S.E.M, n = 8, in each group.

Histopathological results

GEN administration resulted in an obvious distortion of the normal histological picture of the kidney. Renal tubular epithelium show necrosis and desquamation in the tubular lumen. Tubular dilatation, areas of coagulative necrosis, and infiltration with inflammatory cells were also detected. On the opposite, PNG treatment mitigated renal tissue injury, preserved normal renal histology, and significantly reduced the tubular injury score in rats received GEN (Figure 4).

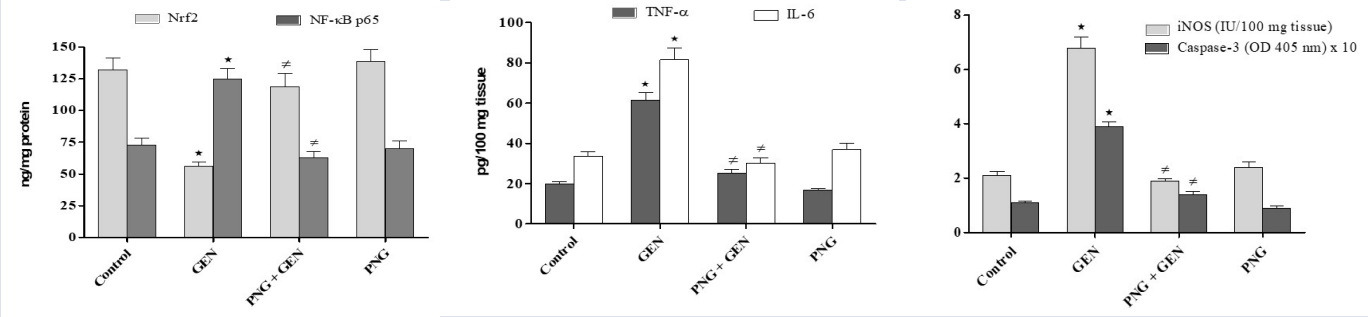


Figure 3: Effects of punicalagin (PNG) treatment on: (A) nuclear factor erythroid 2-related factor 2 (Nrf2) and nuclear factor- κB p65 (NF-κB p65); (B) tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6); (C) inducible nitric oxide synthase (iNOS) and cleaved caspases-3 in kidneys of gentamicin (GEN)-challenged rats. $^{*}p < 0.05$ vs. control, $^{\neq}p < 0.05$ vs. GEN group. Results are mean ± S.E.M, $n = 8$, in each group.

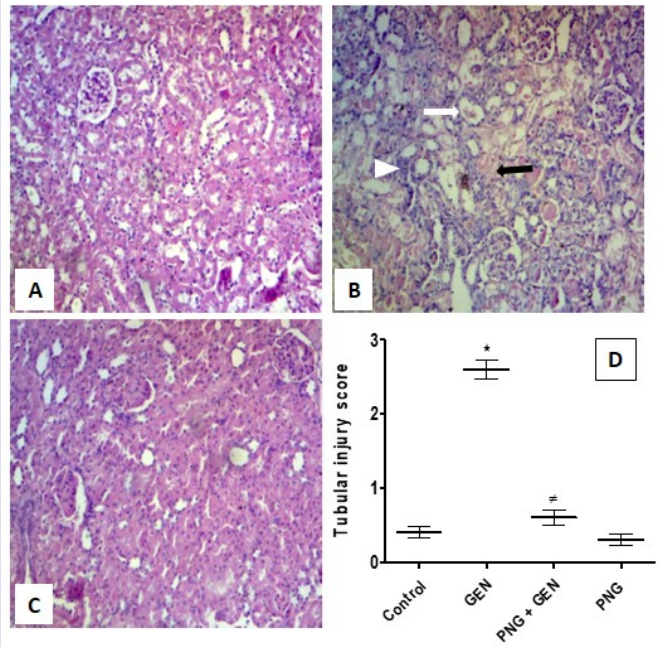


Figure 4: H&E (200×) of rat kidneys from: (A) control showing normal renal histology; (B) gentamicin (GEN) group demonstrating marked distortion of kidney architecture, renal tubular necrosis and dilatation, desquamation of lining epithelium (white arrow), interstitial edema, areas of coagulative necrosis (black arrow), and inflammatory cell infiltration (white head); (C) punicalagin (PNG) + GEN showing preservation of the normal kidney architecture; (D) tubular injury score. $^{*}p < 0.05$ vs. control, $^{\neq}p < 0.05$ vs. GEN. Results are mean ± S.E.M., $n = 8$, in each group.

DISCUSSION

Previous reports, similar to the current study, revealed that renal injury and dysfunction induced by GEN were largely related to oxidant stress and increased generation of ROS. Subsequent depletion of endogenous antioxidant defenses and enhanced peroxidation of lipid biomembranes lead to damage of renal proximal tubular cells.[2,12] It was also reported that GEN caused nitrosative stress by activating iNOS, and increased production of RNS, as various nitric oxide-derived compounds, which caused nitration of cellular macromolecules, mitochondrial dysfunction, and altered structure and function of critical proteins.[3] In addition, prior investigations showed that oxidative stress upregulated various inflammatory cascades, notably the NF-κB pathway leading to further tissue injury. Phosphorylation of the cytoplasmic inhibitor of nuclear factor-κB (IκB) results in release of the active NF-κB p65 subunit, which translocate to the nucleus and activate gene transcription of inflammatory cytokines, as TNF-α and IL-6.[8]

This study showed that PNG significantly blocked oxidative stress and preserved the natural antioxidants, as evidenced by the reduction in MDA, and maintenance of GSH and catalase activity in kidneys of GEN-challenged rats. PNG also prevented kidney iNOS activation and nitrosative stress in rats received GEN. The main factor responsible for the antioxidant effect of PNG is that it can inhibit NADPH oxidase-4, which is the major source of oxidative stress and ROS generation, as demonstrated in a model of diabetic nephropathy caused by high-fat diet/streptozotocin in mice.[17] In addition, the antinitrosative effect of PNG is due to suppression of iNOS expression, and decreased production of RNS, as shown in kidneys of cisplatin-challenged rats,[12] and joints of mice with collagen-induced rheumatoid arthritis.[18] In consistent with the present investigation, the GEN-induced generation of ROS, RNS, and inflammatory cytokines lead to upregulation of apoptotic mediators, which eventually activate caspase-3-dependent execution phase apoptosis.[5] PNG inhibited the production of cleaved caspase-3 and provided a significant antiapoptotic effect in kidneys of rats received GEN. This is similar to prior studies, which showed the PNG suppressed cell apoptosis through its antioxidant, antinitrosative, and anti-inflammatory effects.[12,19]

The fine balance between the Nrf2 anti-inflammatory pathway and the NF-κB pro-inflammatory pathway is the main factor regulating the homeostasis of cellular responses to oxidative stress, inflammation, and apoptosis. Nrf2 is a transcription factor, which activates cellular defenses against oxidative and inflammatory insults.[7] On the other hand, NF-κB is a family of inducible transcription operators, which is responsible for upregulation of inflammatory responses.[6] In agreement with previous studies, the current investigation demonstrated that GEN insult resulted in a significant decrease of Nrf2, associated with a significant increase of NF-κB p65 in rat kidneys.[8,9] The current study also revealed that PNG treatment significantly restored the balance between the two pathways, as indexed by the significant increment of Nrf2, and the significant decrement of NF-κB p65 in kidneys of GEN-challenged rats. Similarly, prior investigations showed that PNG protected against oxidative stress, inflammation, and apoptosis through upregulation of Nrf2 pathway and inhibition of NF-κB pathway. This was evidenced in studying the defensive effect of PNG against cisplatin nephrototoxicity in rats,[12] methotrexate hepatotoxicity in mice,[19] and obesity-induced oxidant and inflammatory responses in mice fed high-fat diet.[20]

CONCLUSION

This research work detected that PNG significantly protected against kidney toxicity induced by GEN in rats. The PNG-related nephroprotective effect by hindering oxidant/nitrosative stress, inflammation, and apoptosis resulted from GEN. Resetting the balance

between Nrf2 and NF-κB pathways probably plays a crucial role by which PNG provides its significant renoprotective effect in GEN-challenged rats.

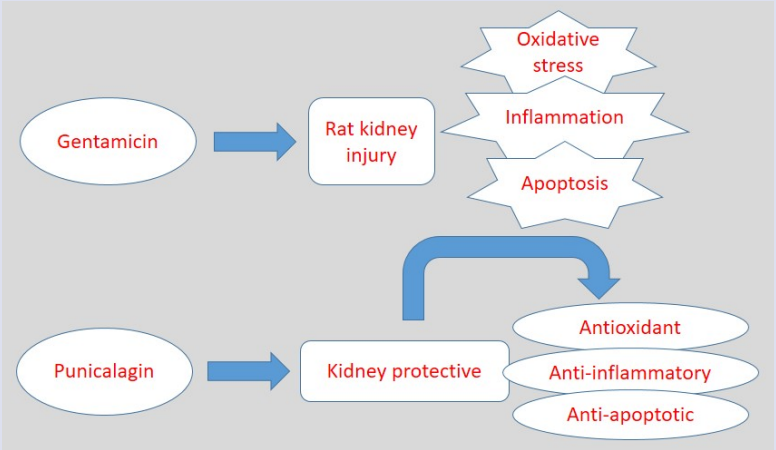
CONFLICTS OF INTEREST

No conflicts to disclose.

REFERENCES

1. Selby NM, Shaw S, Woodier N, Fluck RJ, Kolhe NV. Gentamicin-associated acute kidney injury. QJM. 2009;102:873-80.
2. Yue L, Yang YR, Ma WX, Wang HY, Fan QW, Wang YY, *et al.* Epigallocatechin gallate attenuates gentamicin-induced nephrotoxicity by suppressing apoptosis and ferroptosis. Molecules. 2022;27:8564.
3. Tomșa AM, Răchișan AL, Pandrea SL, Benea A, Uifălean A, Toma C, *et al.* Curcumin and vitamin C attenuate gentamicin-induced nephrotoxicity by modulating distinctive reactive species. Metabolites. 2022;13:49.
4. Abd-Eldayem AM, Dahpy MA, Badary DM, Alnasser SM, Hareedy MS. Celecoxib has preventive and therapeutic benefits against nephrotoxicity caused by gentamicin in mice. Drug Res (Stuttg). 2022;72:259-67.
5. Abdelrahman RS, Abdelmageed ME. Renoprotective effect of celecoxib against gentamicin-induced nephrotoxicity through suppressing NF κ B and caspase-3 signaling pathways in rats. Chem Biol Interact. 2020;315:108863.
6. Liu T, Zhang L, Joo D, Sun SC. NF- κ B signaling in inflammation. Signal Transduction and Targeted Ther. 2017;2:e17023.
7. Wardyn JD, Amy H. Ponsford AH, Sanderson CM. Dissecting molecular cross-talk between Nrf2 and NF- κ B response pathways. Biochem. Soc. Trans. 2015;43:621-6.
8. Nadeem RI, Aboutaleb AS, Younis NS, Ahmed HI. Diosmin mitigates gentamicin-induced nephrotoxicity in rats: insights on miR-21 and -155 expression, Nrf2/HO-1 and p38-MAPK/NF- κ B pathways. Toxics. 2023;11:48.
9. Zaky HS, Abdel-Sattar SA, Allam A, Ahmed HI. Further insights into the impact of rebamipide on gentamicin-induced nephrotoxicity in rats: modulation of SIRT1 and β -catenin/cyclin D1 pathways. Drug Chem Toxicol. 2023;46:851-63.
10. Venusova E, Kolesarova A, Horky P, Slama P. Physiological and immune functions of punicalagin. Nutrients. 2021;13:2150.
11. Fouad AA, Qutub HO, Al-Melhim WN. Nephroprotection of punicalagin in rat model of endotoxemic acute kidney injury. Toxicol Mech Methods. 2016;26:538-43.
12. Aladaileh SH, Al-Swailmi FK, Abukhalil MH, Ahmeda AF, Mahmoud AM. Punicalagin prevents cisplatin-induced nephrotoxicity by attenuating oxidative stress, inflammatory response, and apoptosis in rats. Life Sci. 2021;286:120071.
13. Seo Y, Mun CH, Park SH, Jeon D, Kim SJ, Yoon T, *et al.* Punicalagin ameliorates lupus nephritis via inhibition of PAR2. Int J Mol Sci. 2020;21:4975.
14. Hua Q, Han Y, Zhao H, Zhang H, Yan B, Pei S, *et al.* Punicalagin alleviates renal injury via the gut-kidney axis in high-fat diet-induced diabetic mice. Food Funct. 2022;13:867-79.
15. Abdelsameea AA, Mohamed AM, Amer MG, Attia SM. Cilostazol attenuates gentamicin-induced nephrotoxicity in rats. Exp Toxicol Pathol. 2016;68:247-53.
16. Ramesh G, Reeves WB. p38 MAP kinase inhibition ameliorates cisplatin nephrotoxicity in mice. Am J Physiol Renal Physiol. 2005;289:F166-74.
17. An X, Zhang Y, Cao Y, Chen J, Qin H, Yang L. Punicalagin protects diabetic nephropathy by inhibiting pyroptosis based on TXNIP/NLRP3 pathway. Nutrients. 2020;12:1516.
18. Ge G, Bai J, Wang Q, Liang X, Tao H, Chen H, *et al.* Punicalagin ameliorates collagen-induced arthritis by downregulating M1 macrophage and pyroptosis via NF- κ B signaling pathway. Sci China Life Sci. 2022;65:588-603.
19. Al-Khawalde AAA, Abukhalil MH, Jghef MM, Alfwuaires MA, Alaryani FS, Aladaileh SH, *et al.* Punicalagin Protects against the development of methotrexate-induced hepatotoxicity in mice via activating Nrf2 signaling and decreasing oxidative stress, inflammation, and cell death. Int J Mol Sci. 2022;23:12334.
20. Kang B, Kim CY, Hwang J, Jo K, Kim S, Suh HJ, *et al.* Punicalagin, a pomegranate-derived ellagitannin, suppresses obesity and obesity-induced inflammatory responses via the Nrf2/Keap1 signaling pathway. Mol Nutr Food Res. 2019;63:e1900574.

GRAPHICAL ABSTRACT



ABOUT AUTHORS

Amr A. Fouad
Professor of Pharmacology & Therapeutics, Faculty of Medicine, Al-Baha University, Al-Baha, Saudi Arabia.

Moataz Mohamedalhasan Ali
Associate Professor of Pathology, Faculty of Medicine, Al-Baha University, Al-Baha, Saudi Arabia & Associate Professor of Pathology, Faculty of Medicine, University of Elimam Elmahdi, Sudan.

Mostafa Abdel-Hamid
Associate Professor of Anatomy and Embryology, Faculty of Medicine, Al-Baha University, Al-Baha, Saudi Arabia.

Cite this article: Fouad AA, Ali MM, Abdel-Hamid M. Punicalagin Opposes Gentamicin Nephrotoxicity in Rats: Role of Nrf2 and NF-κB Pathways. Pharmacogn J. 2024;16(1): 126-130.