



OPEN Trinitroglycerine-loaded chitosan nanoparticles attenuate renal ischemia-reperfusion injury by modulating oxidative stress

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Renal ischemia-reperfusion (I/R) injury is a common clinical factor for acute kidney injury (AKI). A current study investigated the renoprotective effects of the trinitroglycerine (TNG) combination with chitosan nanoparticles (CNPs) on renal I/R-induced AKI. Rats were randomly assigned to five groups (n = 8/group): Sham, I/R, TNG (50 mg/kg) + I/R, CNPs (60 mg/kg) + I/R, and TNG-CNPs + I/R. Bilateral renal pedicles were occluded for 60 min to induce ischemia. TNG, CNPs, or TNG-CNPs were administered intraperitoneally 30 min before renal ischemia. After 24 h of reperfusion, blood samples were collected, and both kidneys were removed. The left kidney was used for oxidative stress analysis. The right kidney was preserved in 10% formalin for histopathological examination via H&E staining. After renal ischemia-reperfusion injury, there was an observed increase in plasma creatinine (Cr) and blood urea nitrogen (BUN), accompanied by a decrease in glomerular filtration rate (GFR) in rats. Total oxidative stress (TOS) levels were also significantly higher in the I/R group, whereas total antioxidative capacity (TAC) was reduced. Histopathological examination revealed damage in the kidneys of rats in the I/R group. Pretreatment with the TNG-CNP formulation before I/R increased plasma and tissue TAC levels in rats. It also corrected the renal histopathological changes and functional disorders induced by I/R injury, as evidenced by reduced Cr and BUN, increased GFR, and attenuated oxidative stress. The results suggest that the TNG-CNP combination provides renoprotective effects against I/R-induced AKI by improving antioxidant status and minimizing renal injury.

Keywords Nanoparticle, Chitosan, Trinitroglycerine, Renal ischemia-reperfusion, Oxidative stress, Renoprotective

Acute kidney injury (AKI) induced by renal ischemia/reperfusion (I/R) injury is an emergency and severe clinical disorder with high morbidity and mortality¹. Renal ischemia has a complex pathophysiology². Ischemia triggers kidney injury, impairing the oxygen and nutrient supply to tubular cells and their ability to excrete waste products. Although reperfusion restores blood flow, it can paradoxically promote glomerular, vascular, and tubular disturbances. Renal I/R stimulates the excessive production of reactive oxygen species (ROS) and inflammatory cytokines, leading to structural and functional dysfunction^{3,4}.

AKI induced by renal I/R is a severe clinical problem without specific medicine now except for expensive hemodialysis treatment⁵. Kidney-targeted drugs are not more, and small molecules are excreted too rapidly to reach effective drug concentrations in damaged kidneys^{6,7}. Based on these problems, the management of AKI is still limited in scope today. Nanotherapeutics based on nanotechnology aim to design and prepare nanoparticles (NPS) from the molecular and atomic scale to bulk form, which may solve the pharmacological therapy problem^{8–10}.

More and more nanoparticle-based drugs with various physical and chemical properties are being developed to effectively distribute drugs, increase accumulation, and regulate the release of drugs in injured kidneys^{11,12}. The medications based on chitosan NPS (CNPs) are tested for their ability to selectively scavenge excess ROS and

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22. Kim, K. H., Kerndt, C. C., Adnan, G., Schaller, D. J. & Nitroglycerin StatPearls. Treasure Island (FL) companies. Disclosure: Connor Kerndt declares no relevant financial relationships with ineligible companies. Disclosure: Ghufuran Adnan declares no relevant financial relationships with ineligible companies. StatPearls Publishing Copyright ©. StatPearls Publishing LLC.; 2024. (2024).
23. Uhlig, K. et al. Inotropic agents and vasodilator strategies for the treatment of cardiogenic shock or low cardiac output syndrome. *Cochrane Database Syst. Rev.* **11** (11), Cd009669 (2020).
24. Twiner, M. J., Hennessy, J., Wein, R. & Levy, P. D. Nitroglycerin use in the Emergency Department: current perspectives. *Open. Access. Emerg. Medicine: OAEM.* **14**, 327–333 (2022).
25. Cantow, K., Flemming, B., Ladwig-Wiegand, M., Persson, P. B. & Seeliger, E. Low dose nitrite improves reoxygenation following renal ischemia in rats. *Sci. Rep.* **7** (1), 14597 (2017).
26. Andrabi, S. M. et al. Nitric Oxide: Physiological Functions, Delivery, and Biomedical Applications. *Advanced science* (Weinheim, Baden-Wurttemberg, Germany). ;10(30):e2303259. (2023).
27. Asadi, K. et al. Trinitroglycerin-loaded chitosan nanogels accelerate angiogenesis in wound healing process. *Int. J. Biol. Macromol.* **278**, 134937 (2024).
28. Yohan, D. & Chithrani, B. D. Applications of nanoparticles in nanomedicine. *J. Biomed. Nanotechnol.* **10** (9), 2371–2392 (2014).
29. Sancaktutar, A. A. et al. The protective effects of pomegranate extracts against renal ischemia-reperfusion injury in male rats. *Urol. Annals.* **6** (1), 46–50 (2014).
30. Li, J. et al. Resveratrol alleviates inflammatory responses and oxidative stress in rat kidney ischemia-reperfusion Injury and H₂O₂-Induced NRK-52E cells via the Nrf2/TLR4/NF- κ B pathway. *Cell. Physiol. Biochem.* **45** (4), 1677–1689 (2018).
31. Ge, X., Cao, Z. & Chu, L. The antioxidant effect of the metal and metal-oxide nanoparticles. ;11(4):791. (2022).
32. Nelson, B. C., Johnson, M. E., Walker, M. L., Riley, K. R. & Sims, C. M. *Antioxid. Cerium Oxide Nanopart. Biology Med.* ;5(2):15. (2016).
33. Asadi, K. et al. Trinitroglycerin-loaded chitosan nanogels: shedding light on cytotoxicity, antioxidantivity, and antibacterial activities. *Int. J. Biol. Macromol.* **265** (Pt 2), 130654 (2024).
34. Najafi, H. et al. Nitric oxide releasing nanofibrous fmoc-dipeptide hydrogels for amelioration of renal ischemia/reperfusion injury. *J. Control Release.* **337**, 1–13 (2021).
35. Forghani, N., Karimi, Z., Mokhtari, M., Shariati, M. & Masjedi, F. Association of Oxidative Stress with Kidney Injury in a hyperandrogenemic female rat model %J. *Iran. J. Med. Sci.* **48** (2), 187–197 (2023).
36. Aksu, U. et al. Fluoxetine ameliorates imbalance of redox homeostasis and inflammation in an acute kidney injury model. *J. Physiol. Biochem.* **70** (4), 925–934 (2014).
37. Katsumi, H. et al. S-nitrosylated l-serine-modified dendrimer as a kidney-targeting nitric oxide donor for prevention of renal ischaemia/reperfusion injury. *Free Radic Res.* **54** (11–12), 841–847 (2020).
38. Yu, H. et al. Size and temporal-dependent efficacy of olipraz-loaded PLGA nanoparticles for treatment of acute kidney injury and fibrosis. *Biomaterials* **219**, 119368 (2019).
39. Gao, J. et al. The use of Chitosan based hydrogel for enhancing the therapeutic benefits of adipose-derived MSCs for acute kidney injury. *Biomaterials* **33** (14), 3673–3681 (2012).

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Author contributions

Z.K and A.GH conceptualization of the study, funding acquisition, methodology, and visualization. Z.K, P.GH, A.GH, and KH.A carried out the literature research performed the experimental parts, and prepared the figures and tables. Z.K, A.GH, and KH.A participated in software and formal analysis parts, writing - review & editing. All authors read, edited, and approved the final manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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