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THYMOQUINONE SUPPLEMENTATION PREVENTS THE DEVELOPMENT OF GENTAMICIN-INDUCED ACUTE RENAL TOXICITY IN RATS

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SUMMARY

1. The present study investigated the possible protective effects of thymoquinone (TQ), a compound derived from *Nigella sativa* with strong anti-oxidant properties, against gentamicin (GM)-induced nephrotoxicity.

2. A total of 40 adult male Wistar albino rats was divided into four groups. Rats in the first group were injected daily with normal saline (2.5 mL/kg, i.p.) for 8 consecutive days, whereas rats in the second group received TQ (50 mg/L in drinking water) for 8 consecutive days. Animals in the third group were injected daily with GM (80 mg/kg, i.p.) for 8 consecutive days, whereas animals in the fourth group received a combination of GM (80 mg/kg, i.p.) and TQ (50 mg/L in drinking water) for 8 consecutive days.

3. Gentamicin resulted in a significant increase in serum creatinine, blood urea nitrogen (BUN), thiobarbituric acid-reactive substances (TBARS) and total nitrate/nitrite (NO_x) and a significant decrease in reduced glutathione (GSH), glutathione peroxidase (GPx), catalase (CAT) and ATP levels in kidney tissues.

4. Interestingly, TQ supplementation resulted in a complete reversal of the GM-induced increase in BUN, creatinine, TBARS and NO_x and decrease in GSH, GPx, CAT and ATP to control values. Moreover, histopathological examination of kidney tissues confirmed the biochemical data, wherein TQ supplementation prevents GM-induced degenerative changes in kidney tissues.

5. Data from the present study suggest that TQ supplementation prevents the development of GM-induced acute renal failure by a mechanism related, at least in part, to its ability to decrease oxidative stress and to preserve the activity of the anti-oxidant enzymes, as well as it ability to prevent the energy decline in kidney tissues.

Key words: ATP, gentamicin, nephrotoxicity, oxidative stress, thymoquinone.

INTRODUCTION

Gentamicin (GM), an aminoglycoside antibiotic, is widely used in the treatment of severe Gram-negative infections. Nevertheless, both clinical and experimental studies report a dose-limiting nephrotoxicity, which accounts for 20% of all cases of acute renal failure (ARF) and restricts its optimal use.[1–3] Gentamicin is one of the most common causes of ARF, which results in both increased morbidity and greater health-care costs.[4] Although the changes in GM dosing from multiple-daily to once-daily doses has reduced the risk of nephrotoxicity, the incidence of GM-induced ARF remains high.[5] Several mechanisms have been suggested to account for GM-induced ARF. These include generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS),[6–8] binding to anionic phospholipids and changing the structure and function of cellular membranes,[9] and inhibition of mitochondrial oxidative phosphorylation.[10,11] In view of the importance of GM in the treatment of severe Gram-negative infections, one of the research aims being pursued most intensively is the possibility of eliminating its nephrotoxicity or reducing it to an acceptable level. In this regard, various strategies have been tried, including the use of free radical scavengers, such as curcumin,[12] caffeic acid phenethyl ester,[13] resveratrol,[14] aged garlic extract[15] and vitamins E and C.[16]

Thymoquinone (TQ), the main constituent of the volatile oil from *Nigella sativa* seeds, is reported to possess strong anti-oxidant properties.[17] Previous studies have demonstrated that TQ has a considerable protective effect against oxidative damage induced by a variety of free radical-generating agents, including doxorubicin-induced cardiotoxicity,[18] carbon tetrachloride-evoked hepatotoxicity,[19] nephropathy produced by cisplatin,[20] autoimmune as well as allergic encephalomyelitis[21,22] and gastric mucosal injury induced by ischaemia–reperfusion.[23] Although, it has been reported that TQ is effective against disease- and chemically induced nephrotoxicity,[20,24] the effects of TQ on GM-induced ARF have not been investigated to date. Therefore, the present study investigated whether oral supplementation of TQ could protect against the development of GM-induced ARF in rats.

METHODS

Animals

Adult male Wistar albino rats, weighing 230–250 g, were obtained from the Animal Care Center, College of Pharmacy, King Saud University, and were housed in metabolic cages under controlled environmental conditions (25°C and a 12 h light/dark cycle). Animals had free access to pulverized standard rat pellet food and tap water unless indicated otherwise. The protocol for the

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present study was approved by the Research Ethics Committee of College of Pharmacy, King Saud University.

Materials

Gentamicin (Gentam 80 mg vials; Spimaco; Al-Qassim Pharmaceutical Plant, Al-Qassim, Kingdom of Saudi Arabia) was a generous gift from the King Khalid University Hospital drug store, King Saud University. Thymoquinone was obtained from Sigma Chemical (St Louis, MO, USA) and all other chemicals used were of the highest analytical grade.

Experimental design

The GM treatment protocol used in the present study to develop ARF has been reported previously.[3,25] A total of 40 male Wistar albino rats was used and divided at random into four groups of 10 animals each. Rats in the first group were injected daily with normal saline (2.5 mL/kg, i.p.) for 8 consecutive days, whereas rats in the second group received TQ (50 mg/L in drinking water) for 8 consecutive days. The calculated doses of TQ, based on the average daily water intake, were 4 mg/kg per day according to Badary *et al*.[20] Animals in the third group were injected daily with GM (80 mg/kg, i.p.) for 8 consecutive days, whereas animals in the fourth group received a combination of GM (80 mg/kg) and TQ (50 mg/L in drinking water) for 8 consecutive days. Twenty-four hours after the last dose of the specific treatment, animals were anaesthetized with ether and blood samples were obtained by heart puncture. Serum was separated for the measurement of blood urea nitrogen (BUN) and creatinine, thiobarbituric acid-reactive substances (TBARS), an index of lipid peroxidation, and total nitrate/nitrite (NO_x), an index of nitric oxide (NO) production. Animals were then killed by decapitation after exposure to ether in a dessicator kept in a well-functioning hood and both kidneys were isolated. The right kidneys were excised quickly, washed with saline, blotted with a piece of filter paper, decapsulated and homogenized in normal saline or 6% perchloric acid as, indicated in the procedures given for the measurement of each parameter, using a Branson sonifier (250; VWR Scientific, Danbury, CN, USA). The left kidneys from each group were removed for histopathological examination. Kidneys were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned at 3 μm and stained with haematoxylin and eosin (H&E) for light microscopic examination. Slides were coded and were examined by a histopathologist who was blinded to the treatment groups.

Assessment of BUN and serum creatinine

Blood urea nitrogen and serum creatinine concentrations were measured spectrophotometrically according to the methods of Tobacco *et al*.[26] and Fabiny and Ertingshausen,[27] respectively.

Determination of GSH and lipid peroxidation in serum and kidney tissues

Tissue levels of the acid soluble thiols, mainly GSH, were assayed spectrophotometrically at 412 nm, according to the method of Ellman,[28] using a Shimadzu (Tokyo, Japan) spectrophotometer. The GSH content was expressed as μmol/g wet tissue. The degree of lipid peroxidation in serum and kidney tissues was determined by measuring TBARS in the supernatant from tissue homogenate.[29] Homogenates were centrifuged at 1500 *g* and the supernatant was collected and used for the estimation of TBARS. Absorbance was measured spectrophotometrically at 532 nm.

Determination of total NO_x concentrations in serum and kidney tissues

Total NO_x, an index of NO production, in serum and kidney tissues was measured as nitrite according to the method of Miranda *et al*.[30] The assay is based on the reduction of nitrate by vanidium trichloride combined with detection by the acidic Griess reaction. The diazotization of sulphanilic acid with nitrite at acidic pH and subsequent coupling with *N*-(10 naphthyl)-ethylenediamine produced an intensely coloured product that is measured spectrophotometrically at 540 nm.

Determination of glutathione peroxidase and catalase activity in kidney tissues

The activity of glutathione peroxidase (GPx) was determined according to the method of Lawrence and Burk.[31] Changes in absorbance at 340 nm were recorded at 1 min intervals for 5 min and the results are expressed as μmol min/g tissue. Catalase (CAT) activity was determined spectrophotometrically according to the method of Higgins *et al*.,[32] which is the assay of hydrogen peroxide (H_2O_2). Catalase activity is expressed as μmol/min per g tissue using a molar absorbance of 43.6 for H_2O_2.

Determination of ATP and ADP in kidney tissues

Adenosine triphosphate and ADP were determined in kidney tissues using HPLC according to the method reported by Botker *et al*.[33] In brief, kidney tissues were homogenized in ice-cold 6% perchloric acid, centrifuged at 110 *g* for 15 min at 0.5°C and the supernatant injected onto the HPLC after neutralization to pH 6–7. Chromatographic separation was performed at a flow rate of 1.2 mL/min, using ODS-Hypersil, 150 × 4.6 mm I.D. 5 μm column (Supelco SA, Gland, Switzerland) and 75 mmol/L ammonium dihydrogen phosphate as the mobile phase. The peak elution was followed at 254 nm.

Statistical analysis

Differences between values obtained (mean±SEM; $n = 10$) were evaluated by one-way analysis of variance (ANOVA) followed by the Tukey–Kramer multiple comparison test. $P \leq 0.05$ was taken to indicate statistical significance.

RESULTS

Table 1 shows the effects of GM on serum creatinine and BUN in normal and TQ-supplemented rats. Administration of GM (80 mg/kg) for 8 successive days resulted in highly significant 139 and 258% increases in serum creatinine and BUN, respectively, whereas oral administration of TQ alone for 8 successive days showed no significant changes. Interestingly, oral supplementation of TQ to GM-treated rats for 8 successive days resulted in a complete reversal of the GM-induced increase in BUN and serum creatinine to control values.

Figure 1 shows the effects of GM, TQ and their combination on the ATP levels (Fig. 1a) and the ATP/ADP ratio (Fig. 1b), an index of mitochondrial function and energy production, in rat kidney tissues. Administration of GM for 8 successive days resulted in significant 69 and 46% decreases in ATP and the ATP : ADP ratio, respectively, compared with the control group. Supplementation of TQ alone for 8 successive days induced a significant 114% increase in the ATP : ADP ratio. Moreover, TQ supplementation to GM-treated rats completely reversed the decrease in ATP and the ATP : ADP ratio induced by GM to control values.

Figure 2 shows the effects of GM, TQ and their combination on TBARS levels, an index of lipid peroxidation, in the serum (Fig. 2a) and kidney tissues (Fig. 2b) of rats. Gentamicin treatment resulted in significant 113 and 41% increases in TBARS in serum and kidney tissues, respectively. Concomitant administration of TQ plus GM produced a marked normalization of TBARS in kidney tissues, whereas TQ failed to decrease serum TBARS to control values.

The effects of GM on the GSH content and GPx and CAT activity, indices of anti-oxidant defence mechanisms, in kidney tissues from

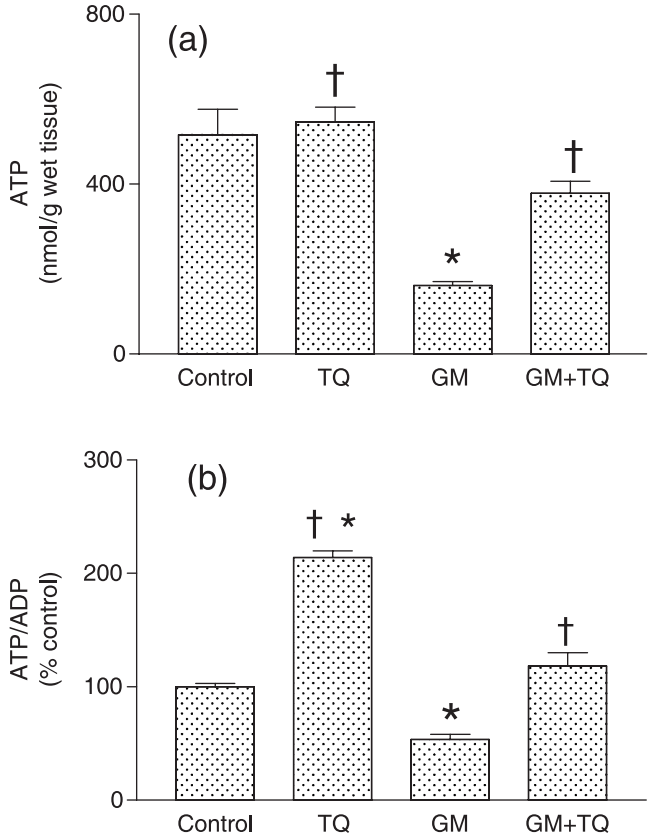

Fig. 1 Effect of gentamicin (GM), thymoquinone (TQ) and their combination on (a) ATP levels and (b) the ATP : ADP ratio in rat kidney tissues. Data are presented as the mean±SEM ($n = 10$). *$P < 0.05$ compared with control; †$P < 0.05$ compared with the GM group (ANOVA followed by the Tukey–Kramer test).

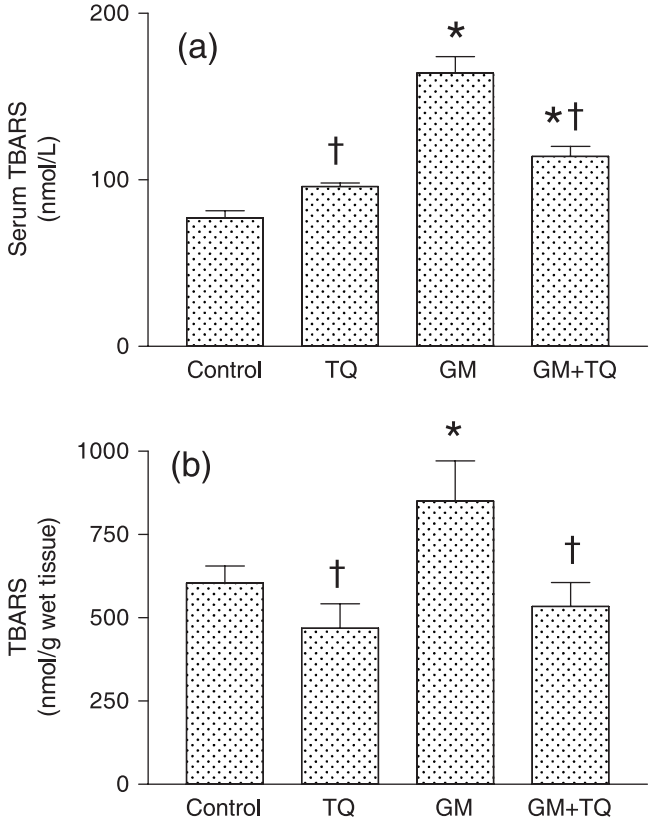

Fig. 2 Effect of gentamicin (GM), thymoquinone (TQ) and their combination on thiobarbituric acid-reactive substances (TBARS) levels in (a) serum and (b) kidney tissues of rats. Data are presented as the mean±SEM ($n = 10$). *$P < 0.05$ compared with control; †$P < 0.05$ compared with the GM group (ANOVA followed by the Tukey–Kramer test).

Table 1 Effects of gentamicin, thymoquinone and their combination on serum levels of blood urea nitrogen and creatinine in rats

Groups	Blood urea nitrogen (mmol/L)	Serum creatinine (μmol/L)
Control	8.47 ± 0.38	83.09 ± 9.72
TQ	7.55 ± 0.46†	6.01 ± 2.65†
GM	30.3 ± 4.1*	98.88 ± 26.52*
TQ + GM	8.33 ± 0.40†	3.97 ± 6.18†

Data are presented as the mean±SEM ($n = 10$). *$P < 0.05$ compared with control; †$P < 0.05$ comparedwith the GM group (ANOVA followed by the Tukey–Kramer test).

TQ, thymoquinone; GM, gentamicin.

Table 2 Effects of gentamicin, thymoquinone and their combination on the level of reduced glutathione and the activity of glutathione peroxidase and catalase in rat kidney tissues

Groups	Reduced glutathione (μmol/g wet tissue)	Glutathione peroxidase (U/g wet tissue)	Catalase (U/g wet tissue)
Control	3.39 ± 0.20	45.2 ± 1.5	22.1 ± 2.3
TQ	3.12 ± 0.13†	48.2 ± 1.7†	19.6 ± 1.2†
GM	2.45 ± 0.05*	23.5 ± 1.5*	9.9 ± 0.8*
TQ + GM	3.12 ± 0.13†	43.4 ± 2.0†	19.4 ± 0.5†

Data are presented as the mean±SEM ($n = 10$). *$P < 0.05$ compared with control; †$P < 0.05$ compared with the GM group (ANOVA followed by the Tukey–Kramer test).

TQ, thymoquinone; GM, gentamicin; U, enzyme activity (μmol/min).

normal and TQ supplemented rats are given in Table 2. Treatment with GM resulted in significant 28, 48 and 55% decreases in GSH, GPx and CAT, respectively, compared with the control group. Oral supplementation of TQ in combination with GM resulted in a complete reversal of GM-induced decreases in GSH, GPx and CAT to control values.

Figure 3 shows the effects of GM on NO_x concentrations, an index of NO production, in serum (Fig. 3a) and kidney tissues (Fig. 3b) in normal and TQ-supplemented rats. Treatment with GM resulted in a significant 66% increase in NO_x concentrations in kidney tissues, whereas TQ alone showed no significant effect (a 10% decrease). Oral administration of TQ to GM-treated rats resulted in complete reversal of the GM-induced increase in NO_x in kidney tissues to control values. Worth mentioning is that none of these treatments showed any significant effects on NO_x concentrations in the serum.

Histopathological examination of kidney specimens from control rats revealed normal renal glomeruli surrounded by capsule and normal proximal, distal and convoluted tubules (Fig. 4a). Sections from rats treated with GM alone showed clear signs of glomerular and tubular

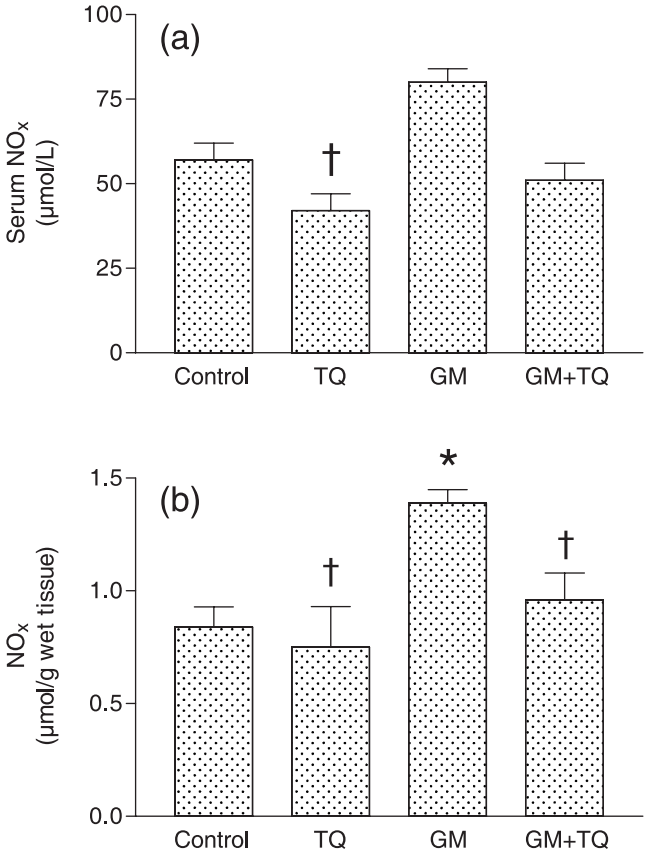

Fig. 3 Effect of gentamicin (GM), thymoquinone (TQ) and their combination on total nitrate/nitrite (NO_x) concentrations in (a) serum and (b) kidney tissues of rats. Data are presented as the mean±SEM ($n = 10$). $^*P < 0.05$ compared with control; $^\dagger P < 0.05$ compared with the GM group (ANOVA followed by the Tukey–Kramer test).

necrosis, interstitial nephritis and desquamation of the tubular epithelial cells in the renal cortex (Fig. 4b). In addition, in GM-treated rats, most tubules showed vacuolated cytoplasm and dilatation of the tubular lumen with intraluminal blood stagnation (Fig. 4c). Interestingly, kidney specimens from rats treated with TQ and GM revealed significant improvement in glomeruli and renal tubules, evidenced by less vacuolation and more preservation of tubular histology compared with the GM-treated group (Fig. 4d).

DISCUSSION

Although the aminoglycoside antibiotic GM can cause nephrotoxicity and, despite the availability of other antibacterial drugs with equal or better sensitivity and safety profiles than aminoglycosides, aminoglycosides remain an important group of antibiotics against several Gram-negative life-threatening infections.[7] Therefore, the goal of reducing or preventing the development of GM-induced nephrotoxicity has attracted considerable efforts. Several studies have reported that oxygen free radicals are considered to be important mediators of GM-induced ARF.[6,34,35] Accordingly, among the main approaches used to ameliorate GM-induced nephrotoxicity is the use of agents with powerful anti-oxidant properties. A recent study has reported that the oil of *N. sativa* may be useful in ameliorating signs of GM nephrotoxicity.[36] However, the efficacy and safety of the oil in this model of ARF has not been investigated to date. Therefore, the present study selected to evaluate the effects of TQ, the main constituent of *N. sativa* oil, on the nephrotoxicity induced by GM and to determine whether TQ supplementation could prevent the development of GM-induced ARF.

The results of the present study demonstrate that GM increases nephrotoxicity indices, namely serum creatinine (139%) and BUN (258%), and induces clear signs of glomerular and tubular necrosis, interstitial nephritis and dilatation of the tubular lumen with intraluminal blood stagnation, indicating ARF. The present results confirm those reported previously.[3,12,35] Interestingly, oral TQ supplementation completely prevented the increase in nephrotoxicity indices and histopathological lesions induced by GM, suggesting that TQ may have a potential protective effect against GM-induced ARF. Previous studies have demonstrated that TQ attenuates cardiotoxicity and nephrotoxicity induced by other chemotherapeutic agents, including doxorubicin,[18] cisplatin[20] and ifosfamide.[24]

In the present study, the marked decrease in ATP and the ATP : ADP ratio in GM-treated rats paralleled the marked increase in nephrotoxicity indices (serum creatinine and BUN) and the marked degenerative changes in kidney tissues, which may point to the contribution of mitochondrial dysfunction and energy depletion in GM-induced ARF. Interestingly, TQ supplementation completely reversed GM-induced decreases in ATP and the ATP : ADP ratio to control values. Moreover, TQ significantly increased the ATP : ADP ratio, which is essential for mitochondrial function. These results suggest that TQ may enhance substrate utilization and/or oxidative phosphorylation with a consequent increase in energy production and mitochondrial function. However, no previous studies have investigated the effects of TQ on ATP production and mitochondrial function.

Data from the present study revealed that GM significantly increased NO_x and TBARS in serum and kidney tissues, suggesting that oxidative stress and NO production induced by GM play a role in GM-induced kidney damage. A strong relationship between nephrotoxicity and oxidative stress has been confirmed in many experimental models.[12,13,15,34] Nitric oxide is known to inhibit DNA repair proteins, thereby inhibiting the ability of the cell to repair damaged DNA.[37] Moreover, Pedraza-Chaverri *et al*.[8] reported that GM-related nephrotoxicity was due to GM-induced increases in hydroxyl and superoxide anion radicals, markers of lipid peroxidation, and nitrotyrosine, a marker of protein peroxidation. The authors added that the GM-generated superoxide anion radical and NO interact to form peroxynitrite, which exerts harmful effects on kidney tissues.

In the present study, GM significantly decreased the anti-oxidant mechanisms, including GSH, GPx and CAT, thus leaving the kidney tissues with no known defence against ROS. Therefore, kidneys from GM-treated rats are more vulnerable to both ROS and RNS. Similar results have been reported in recent studies, which have demonstrated decreases in GPx and CAT activity in kidney tissues from GM-treated rats.[16,35] It is well documented that anti-oxidant enzymes, including GPx and CAT, are inactivated by ROS, including superoxide anion radical and hydrogen peroxide.[38,39] Therefore, one can anticipate that ROS and RNS generated by GM inhibit the anti-oxidant enzymes, which is consistent with the theory of oxidative stress as an important event in GM-induced kidney damage. Interestingly, TQ supplementation completely reversed the observed decreases in GSH, GPx and CAT by GM to control values. This effect is consistent with the data presented by Kruk *et al*.,[40] who reported

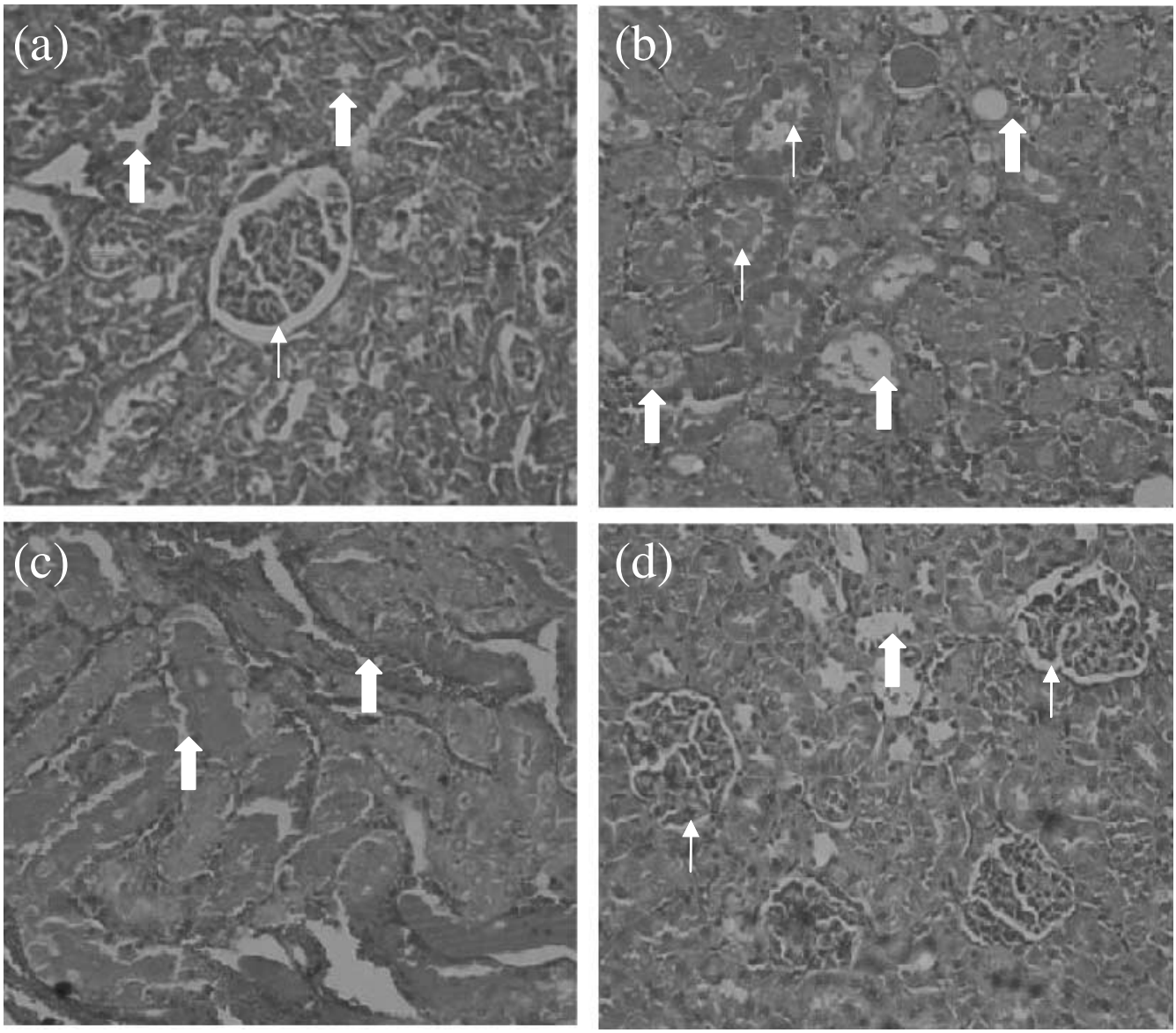

Fig. 4 Photomicrographs of kidney specimens stained with haematoxylin and eosin (original magnification ×200). (a) Kidney from a control rat showing normal glomeruli surrounded by capsule (thin arrow) and normal renal tubules (thick arrow). (b) Kidney from a rat treated with gentamicin (GM) alone showing glomerular (thin arrow) and tubular necrosis and interstitial nephritis (thick arrow). (c) Kidney from a rat treated with GM alone showing dilatation of tubular lumen and red blood cells between the tubules, which denote massive bleeding (thick arrow). (d) Kidney from a rat treated with TQ and GM showing minimal residual damage in the glomeruli (thin arrow) and renal tubules (thick arrow).

that TQ acted as a scavenger of superoxide, hydroxyl radical and singlet molecular oxygen.

In the present study, the observed increase in NO_x and its contribution to GM-related nephrotoxicity disagree with the results presented by Ghaznavi *et al*.,[41] who reported that increased NO formation prevents GM-induced nephrotoxicity. They reported that L-arginine (an NO donor) prevented, whereas the inducible nitric oxide synthase (iNOS) inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) enhanced, GM-induced nephrotoxicity. However, that study was performed *in vitro* using rat isolated perfused kidney, in which the entire biological environment and the role of substrate competition were lacking. It is most likely that GM-induced oxidative stress may lead to inflammation, with the consequent upregulation of iNOS and overproduction of NO. Data presented herein showed that TQ supplementation prevented NO production in kidney tissues; an effect that may be useful in ameliorating GM-induced ARF. Thus, TQ may reduce oxidative stress-induced inflammation, with a consequent decrease of iNOS upregulation. The inhibitory effect of TQ against NO production has been reported in a previous study, where TQ inhibited iNOS protein synthesis and iNOS mRNA expression in rat lipopolysaccharide-stimulated peritoneal macrophage cells.[42] More recently, El-Mahmoudy *et al*. have reported that the protective value of TQ against the development of streptozotocin-induced diabetes mellitus was via an NO inhibitory mechanism.[43] Moreover, *in vitro* and *in vivo* studies have reported that TQ inhibits the endogenous synthesis of many inflammatory mediators secondary to the inhibition

of both cyclo-oxygenase[44] and 5-lipoxygenase.[45] In conclusion, data from the present study suggest that TQ supplementation prevents the development of GM-induced ARF by a mechanism related, at least in part, to its ability to decrease oxidative stress and to preserve the activity of anti-oxidant enzymes, as well as it ability to prevent the energy decline in kidney tissues.

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REFERENCES

1. Humes HD, Weinberg JM. Toxic nephropathies. In: Brenner MB, Rector Jr FE (eds). *Kidney.* Saunders, Philadelphia. 1986; 453–91
2. Eisenberg JM, Koffer H, Glick RA *et al.* What is the cost of nephrotoxicity associated with aminoglycosides? *Ann. Intern. Med.* 1987; **107**: 900–9.
3. Al-Majed AA, Mostafa AM, Al-Rikabi AC, Al-Shabanah OA. Protective effects of oral arabic gum administration on gentamicin-induced nephrotoxicity in rats. *Pharmacol. Res.* 2002; **46**: 445–51.
4. Kaloyanides GJ, Bosmans JL, De Broe ME. Antibiotic- and immunosuppression-related renal failure. In: Schrier RW, Gottschalk CW (eds). *Diseases of the Kidney*, Vol. 2. Little, Brown & Co., Boston, MA. 1997; 1115–51.
5. Ferriols-Lisart R, Alos-Alminana. Effectiveness and safety of once-daily aminoglycosides: A meta-analysis. *Am. J. Health Syst. Pharm.* 1996; **53**: 1141–50.
6. Walker PD, Barri Y, Shah SV. Oxidant mechanisms in gentamicin nephrotoxicity. *Renal Fail.* 1999; **21**: 433–42.
7. Ali BH. Agents ameliorating or augmenting experimental gentamicin nephrotoxicity: Some recent research. *Food Chem. Toxicol.* 2003; **41**: 1447–52.
8. Pedraza-Chaverri J, Barrera D, Maldonado PD *et al*. S-Allylmercaptocysteine scavenges hydroxyl radical and singlet oxygen *in vitro* and attenuates gentamicin-induced oxidative and ntrosative stress and renal damage *in vivo*. *BMC Clin. Pharmacol.* 2004; **4**: 5–18.
9. Ramsammy LS, Kaloyanides GJ. Effect of gentamicin on the transition temperature and permeability to glycerol of phosphatidylinositol-containing liposomes. *Biochem. Pharmacol.* 1987; **36**: 1179–81.
10. Weinberg JM, Humes HD. Mechanisms of gentamicin-induced dysfunction of renal cortical mitochondria. 1. Effects on mitochondrial respiration. *Arch. Biochem. Biophys.* 1980; **205**: 222–31.
11. Kopple JD, Ding H, Letoha A *et al.* L-Carnitine ameliorates gentamicin-induced renal injury in rats. *Nephrol. Dial. Transplant.* 2002; **17**: 2122–31.
12. Ali BH, Al-Wabel N, Mahmoud O, Mousa HM, Hashad M. Curcumin has a palliative action on gentamicin-induced nephrotoxicity in rats. *Fundam. Clin. Pharmacol.* 2005; **19**: 473–7.
13. Vardi N, Parlakpinar H, Ozturk F, Acet A. Gentamicin-induced nephrotoxicity and protective effect of caffeic acid phenethyl ester in rats. *Fundam. Clin. Pharmacol.* 2005; **19**: 173–7.
14. Morales AI, Rodriquez-Barbero A, Vicente-Sanchez C, Mayoral P, Lopez-novoa JM, Perez-Barriocanal F. Resveratrol inhibits gentamicin-induced mesangial cell contraction. *Life Sci.*2006; **78**: 2373–7.
15. Velasco-Velazquez MA, Maldonado PD, Barrera D, Torres V, Zentella-Dehesa A, Pedraza-Chaverri J. Aged garlic extract induces proliferation and ameliorates gentamicin-induced toxicity in LLC-PK1 cells. *Phytother. Res.* 2006; **20**: 76–8.
16. Kadkhodaee M, Khastar H, Faghihi M, Ghaznavi R, Zahmatkesh M. Effects of co-supplementation of vitamins E and C on gentamicin-induced nephrotoxicity in rat. *Exp. Physiol.* 2005; **90**: 571–6.
17. Houghton PJ, Zarka R, de las Heras B, Hoult JR. Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. *Planta Med.* 1995; **61**: 33–6.
18. Nagi MN, Mansour MA. Protective effect of thymoquinone against doxorubicin-induced cardiotoxicity in rats: A possible mechanism of protection. *Pharmacol. Res.* 2000; **41**: 283–9.
19. Nagi MN, Alam K, Badary OA, Al-Shabanah OA, Al-Sawaf HA, Al-Bekairi AM. Thymoquinone protects against carbon tetrachloride hepatotoxicity in mice via an antioxidant mechanism. *Biochem. Mol. Biol. Int.* 1999; **47**: 153–9.
20. Badary OA, Nagi MN, Al-Shabanah OA, Al-Sawaf HA, Al-Sohaibani MO, Al-Bekairi AM. Thymoquinone ameliorates the nephrotoxicity induced by cisplatin in rodents and potentiates its antitumor activity. *Can. J. Physiol. Pharmacol.* 1997; **75**: 1356–61.
21. Mohamed A, Shoker A, Bendjelloul F *et al.* Improvement of experimental allergic encephalomyelitis (EAE) by thymoquinone; an oxidative stress inhibitor. *Biomed. Sci. Instrum.* 2003; **39**: 440–5.
22. Mohamed A, Afridi DM, Garani O, Tucci M. Thymoquinone inhibits the activation of NF-kappaB in the brain and spinal cord of experimental autoimmune encephalomyelitis. *Biomed. Sci. Instrum.* 2005; **41**: 388–93.
23. El-Abhar HS, Abdallah DM, Saleh S. Gastroprotective activity of *Nigella sativa* oil and its constituent, thymoquinone, against gastric mucosal injury induced by ischaemia/reperfusion in rats. *J. Ethnopharmacol.* 2003; **84**: 251–8.
24. Badary OA. Thymoquinone attenuates ifosfamide-induced fanconi syndrome in rats and enhances its antitumour activity in mice. *J. Ethnopharmacol.* 1999; **67**: 135–42.
25. Erdem A, Gundogan NU, Usubutum A *et al.* The protective effect of taurine against gentamicin-induced acute tubular necrosis in rats. *Nephrol. Dial. Transplant.* 2000; **15**: 1175–82.
26. Tobacco A, Meiattini F, Moda E, Tarii P. Simplified enzymic/colorimetric serum urea nitrogen determination. *Clin. Chem.* 1979; **25**: 336–7.
27. Fabiny DL, Ertingshausen G. Automated reaction-rate method for determination of serum creatinine with the Centrifichem. *Clin. Chem.* 1971; **17**: 696–700.
28. Ellman GL. Tissue sulfahydryl groups. *Arch. Biochem. Biophys.* 1959; **82**: 70–7.
29. Ohkawa H, Ohish N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid. *Anal. Biochem.* 1979; **95**: 351–8.
30. Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Ntric Oxide* 2001; **5**: 62–71.
31. Lawrence RA, Burk RF. Glutathione peroxidase activity in selenium-deficient rat liver. *Biochem. Biophys. Res. Commun.* 1976; **71**: 952–8.
32. Higgings C, Baehner RL, McCallister J, Boxer L. Polymorphnuclear leukocytes species differences in the disposal of hydrogen peroxides (H_2O_2). *Proc. Soc. Exp. Biol. Med.* 1978; **158**: 478–81.
33. Botker HE, Kimose M, Helligso P, Nielsen TT. Analytical evaluation of high-energy phosphate determination by high performance liquid chromatography in myocardial tissue. *J. Mol. Cell. Cardiol.* 1994; **26**: 41–8.
34. Parlakpinar H, Tasdemir S, Polat A *et al.* Protective role of caffeic acid phenethyl ester (CAPE) on gentamicin-induced acute renal toxicity in rats. *Toxicology* 2005; **207**: 169–77.
35. Karahan I, Atessahin A, Yilmaz S, Ceribasi AO, Sakin F. Protective effect of lycopene on gentamicin-induced oxidative stress and nephrotoxicity in rats. *Toxicology* 2005; **215**: 198–204.
36. Ali BH. The effect of *Nigella sativa* oil on gentamicin nephrotoxicity in rats. *Am. J. Clin. Med.* 2004; **32**: 49–55.
37. Watanabe K, Hess A, Bloch W, Michel O. Expression of inducible nitric oxide synthase (iNOS/NOS II) in the vestibule of guinea pigs after the application of cisplatin. *Anticancer Drugs* 2000; **II**: 29–32.
38. Halliwell B, Gutteridge JM. Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem. J.* 1984; **219**: 1–14.
39. Pigeolet E, Corbisier P, Houbion A *et al.* Glutathione peroxidase, superoxide dismutase, and catalase inactivation by peroxides and oxygen derived free radicals. *Mech. Ageing Dev.* 1990; **51**: 283–97.

40. Kruk I, Michalska T, Lichszteld K, Kladna A, Aboul-Enein HY. The effect of thymol and its derivatives on reactions generating reactive oxygen species. *Chemosphere* 2000; **41**: 1059–64.
41. Ghaznavi R, Faghihi M, Kadkhodaee M, Shams S, Khastar H. Effects of nitric oxide on gentamicin toxicity in isolated perfused rat kidneys. *J. Nephrol.* 2005; **18**: 548–52.
42. El-Mahmoudy A, Matsuyama H, Borgan MA *et al.* Thymoquinone suppresses expression of inducible nitric oxide synthase in rat macrophages. *Int. Immunopharmacol.* 2002; **11**: 1603–11.
43. El-Mahmoudy A, Shimizu Y, Shiina T, Matsuyama H, El-Sayed MG, Takewaki T. Successful abrogation by thymoquinone against induction of diabetes mellitus with streptozotocin via nitric oxide inhibitory mechanism. *Int. Immunopharmacol.* 2005; **5**: 195–207.
44. Marsik P, Kokoska L, Landa P, Nepovim A, Soudek P, Vanek T. *In vitro* inhibitory effects of thymol and quinones of *Nigella sativa* seeds on cyclooxygenase-1- and -2-catalyzed prostaglandin E2 biosyntheses. *Planta Med.* 2005; **71**: 739–42.
45. El-Dakhakhny M, Madi NJ, Lembert N, Ammon HP. *Nigella sativa* oil, nigellone and derived thymoquinone inhibit synthesis of 5-lipoxygenase products in polymorphonuclear leukocytes from rats. *J. Ethnopharmacol.* 2002; **81**: 161–4.