Oxymatrine Reverses Gentamicin-Induced Nephrotoxicity via Ameliorating the Immune Response for Gentamicin Therapy

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Research

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Oxymatrine reverses Gentamicin-Induced Nephrotoxicity via ameliorating the immune response for gentamicin therapy

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Abstract

Background: The aminoglycoside antibiotic gentamicin (GM) is widely used to fight infections caused by Gram-positive and Gram-negative aerobic bacteria. However, its clinical application is limited by serious side effects. Based on this, this study aims to screen drugs that have protective effects on gentamicin-induced kidney injury.

Methods: After screening a series of candidate compounds, we found that a natural quinoline alkaloid-oxymatrine showed well protective effects on GM-induced kidney injury. We used gentamicin (100 mg/ kg/d, 7 days) treatment to establish a rat model of kidney injury, and set up control groups and oxymatrine-pretreatment groups to study the protective effect of oxymatrine on kidney injury.

Results

The results indicated that Gentamicin treatment of normal rats produced marked renal damage and resulted in significant elevation of blood urea nitrogen and creatinine, as well as N-acetyl-β-D-glucosaminidase. Oxymatrine co-administration could decrease levels of IL-1β, IL-6, and TNF-α (All P <0.01 or P <0.001), as well as N-acetyl-β-D-glucosaminidase. In addition, oxymatrine treatment significantly reduced the expression of Bax and NF-κB mRNA in the kidney (both P <0.01), and increased the expression of Bcl-2, Nrf2 and HO-1 mRNA.

Conclusions: The results demonstrate that oxymatrine down-regulates the inflammatory response and reduces the apoptosis by activating antioxidant defense, thereby reducing gentamicin-induced nephrotoxicity.

Keywords: NF-κB; Oxidative stress; Nephrotoxicity; Gentamicin; Oxymatrine;

Background

Gentamicin (GM) is an aminoglycoside antibiotic commonly used to treat severe bacterial infections, especially those caused by gram-negative and some gram-positive bacteria [1]. However, its therapeutic is limited by serious side-effects, most observably nephrotoxicity [2, 3]. It has been estimated that over 30% of patients who was treated by GM for more than 7 days showed some signs of renal impairment [4]. The activation of apoptosis was considered to be the cytotoxic mechanism of the renal toxicity of gentamicin.

The main cytotoxic mechanism of GM-induced nephrotoxicity is the activation of apoptosis, which has been reported in renal proximal tubule cells and mesangial cells [5]. It can act on mitochondria and cause oxidative stress, inducing necrosis and apoptosis. The tubular injury is the major effect of GM, with disappearance of the brush-like edge of the epithelial cells, which can develop to acute tubular necrosis [6] GM induced renal toxicity could be evaluated by histopathologic, morphometric analysis, blood urea nitrogen and serum creatinine levels. N-acetyl-β-D-glucosaminidase (NAG), a tubular lysosomal brush border enzyme, is one of the widely used proximal tubular damage biomarkers [7, 8]. Furthermore, there are some pharmacological interventions have been shown to prevent GM-induced nephrotoxicity, it is a matter of major concern to identify a promising new agent in an attempt to reduce the negative effects of gentamicin on the organism. Oxymatrine (OXY), also known as Kushenin, has a chemical formula of $C_{15}H_{24}N_2O_2$ with a molecular weight of 264.369. It is a quinolizidine alkaloid extracted from the roots of the Sophora genus plants, which has been widely reported to display a wide range of pharmacological activities [8-11] and it possesses significant anti-oxidative, anti-inflammatory and anti-apoptotic activities via regulating the related signaling pathways [10, 12]. Reports also show that OXY has potent effects on hepatitis B in mice, rheumatoid arthritis in

rats and mastitis in mice [11]. In addition, oxymatrine reduce renal interstitial fibrosis and inflammation of obstructive renal lesions by inhibiting the release of a variety of inflammatory cytokines including interleukin-6 (IL-6), interleukin-1β (IL-1β) and tumor necrosis factor alpha (TNF-α), as well as up-regulating the phosphorylated NF-κB p65 [12]. Meanwhile, OXY was found to prevent liver injure, which associated with its antioxidant and anti-apoptotic activities. A previous study has shown that OXY protected against As_2O_3-induced oxidative damage by activating Nrf2/HO-1 signaling pathway [13]. In our study, we aimed to research the protective function of oxymatrine against Gentamicin-induced renal injury.

Materials and methods

Chemicals

GM (sulphate) was purchased from Shandong lukang pharmaceutical co. LTD (10000 U/mg, Jining, China). Oxymatrine (purity≥98%) was obtained from Damas-beta Reagent Co. Ltd (Shanghai, China). All other chemicals were analytical grade.

Animals

Forty male Wistar rats (Pengyue laboratory Animal Breeding Co. Ltd. Jinan, China) (Permit Number: SYXK 2017 0005) (weight, 250-300 g) were used. All the animal experimentations were granted by the Committee of Animal Use and Protection of Qingdao Agriculture University. The rats were housed in controlled conditions at 23±2 ℃ with humidity a 60±5 % and a controlled light cycle (12-h light/12-h dark). Rats were acclimatized for 1week prior to the use and fed with water and food freely during the experiments.

Experimental design

40 rats were divided randomly into four groups: Control, OXY, GM, GM+OXY (n = 10 per group). In the GM group, rats were intraperitoneally (i.p.) injected with gentamicin (100 mg/kg). In the GM+OXY group , the rats was treated with oxymatrine (100 mg/kg, i.p.) 1 h prior to GM (100 mg/kg, i.p. , once daily) administration. The rats in the control and OXY groups were administrated with an equal amount of normal saline (1 ml/kg) or OXY (100 mg/kg). All groups were treated continuously for 7 days [12].At 12 h following the last injection, rats were euthanized with sodium pentobarbital (80 mg/kg) by intraperitoneal administration. Blood samples were centrifuged for 10 min at 3000 × g under 4 °C for 10 min, serum was then separated and stored at -80 degrees Celcius until analysis. Kidneys were divided into sections and immediately frozen (for protein, RNA and paraffin sections and subsequent biochemical)

Blood urea nitrogen (BUN) and serum creatinine (s-CRE) assays

The levels of serum BUN and CRE were examined by an automatic analyser (IDEXX Catalyst One®) using the standard diagnostic kits (IDEXX biological products trading Co., Ltd, Shanghai, China).

Measurement the Renal tissue biomarkers of oxidative stress

The homogenate (10% wt/vol) of kidney tissue was prepared in 9 volumes (9:1 w/w) of cold 50 mM Tris buffer (pH 7.4) using a mechanical grinder. The supernatants were collected by centrifugation (4000g, 15min, 4℃) and assayed for the levels of malonaldehyde (MDA), nitric oxide (NO), glutathione (GSH) and the levels of catalase (CAT), superoxide dismutase (SOD) and inducible NO synthase (iNOS) were determined using commercial assay test kits following the manufacturer's instructions (Nanjing Jiancheng, Nanjing, China). Protein concentrations were

106 determined bicinchoninic acid protein (BCA) assay kit (Beyotime Biotechnology, Haimen,
107 China).

108 **Histopathological examination**

109 Renal tissue samples were randomly selected from 5 rats and fixed in 10% formalin, embedded in
110 paraffin, sectioned at 5 μm thickness, and placed on slides.We used hematoxylin and eosin (H&E)
111 staining to identify the pathological changes in the construction of kidney tissue in different
112 groups [14]. A semi-quantitative pathological assessment of kidney damage was performed and a
113 semiquantitative score was used to grade the severity of lesions for each kidney sample [15]. The
114 histopathological changes of tubular epithelial alterations are examined, such as desquamation,
115 vacuolization, casts, tubular dilation and inflammatory cell infiltration. Scores of 0,+1,+2,+3,+4
116 and+5 corresponded to no change, mild change, mild to moderate change, moderate change,
117 moderate to severe change and severe change, respectively. The pictures of samples (50μm) were
118 taken under a light microscope CX41 (Olympus, Hamburg, Germany) at a visual magnification of
119 20×.

120 **Measurement of caspase-9 and -3 activities and IL-6 and IL-1β and TNF-α levels**

121 The serum levels of caspase-3, caspase-9, IL-6, IL-1β and TGF-α in the renal tissue were
122 measured using ELISA kits according to the manufacturer's instructions (Jiangsu Jingmei
123 Biotechnology Co. Ltd. Jiangsu, China).

124 **Quantitative reverse-transcription (qRT) PCR**

125 Total RNA was separated from 10 mg of frozen tissue from each kidney sample using RNeasy
126 Mini Kit, according to the manufacturer's protocol (Life Technologies, Grand Island, NY). The
127 renal expression of a panel of 5 candidate genes potentially involved in tacrine's disposition based

on literature review were profiled using quantitative polymerase chain reaction, and their respective primer sequences are reported in Supporting Table 1.

PCR reactions were run under the following conditions: initial activation of Taq DNA polymerase at 95 °C for 5 min, 40 cycles of 30 s at 95 °C for denaturing, 30 s at 60 °C for annealing, and 30 s at 72 °C for elongation. RT-PCR test was analyzed by ABI QuantStudio™7 detection system (Applied Biosystem, USA). All reactions were run in triplicate. GAPDH was used as an internal control, and the ($2^{-\triangle\triangle Ct}$) method was used for the calculation of fold changes in gene expression [16].

Table 1 Primer sequences used for quantitative real-time PCR

Gene	Direction	Primer sequence (5' to 3')
Nrf2	forward	5'-CAC ATT CCC AAA CAA GAT GC-3'
	reverse	5'-TCT TTT TCC AGC GAG GAG AT-3'
HO-1	forward	5'-CGT GCT CGA ATG AAC ACT CT-3'
	reverse	5'-GGA AGC TGA GAG TGA GGA CC-3'
NF-κB	forward	5'-CAC TGT CTG CCT CTC TCG TCT-3'
	reverse	5'-AAG GAT GTC TCC ACA CCA CTG-3'
Bax	forward	5'-CCA AGA AGC TGA GCG AGT GTC-3'
	reverse	5'-TGA GGA CTC CAG CCA CAA AGA-3'
Bcl-2	forward	5'-CCG GGA GAT CGT GAT GAA GT-3'
	reverse	5'- ATC CCA GCC TCC GTT ATC CT-3'
GAPDH	forward	5'-ACA GTC CAT GCC ATC ACT GCC-3'
	reverse	5'-GCC TGC TTC ACC ACC TTC TTG-3'

Statistical analyses

The results were analyzed by GraphPad Prism 5 software and the data are expressed as mean ± standard deviation (SD). Comparisons between experimental groups were conducted by using one-way ANOVA, whereas multiple comparisons were made by using the LSD method. Statistical significance was defined as * and **and*** standing for $P < 0.05$ and $P<0.01$ and $P<0.001$.

Results

Oxymatrine Ameliorates GM-Induced nephrotoxicity in rats

After 7 days, the kidney indices of rats treated with GM (100 mg/kg) alone were higher than other groups ($P < 0.01$), and the co-treatment with OXY (100 mg/kg) significantly reduced kidney indices in **Figure. 1c**. ($P < 0.05$). The results showed that OXY could restore kidney indices. We found no differences between control and OXY group. However, both BUN and CRE levels in the GM-treated rats (group 3) were distinctly increased compared to control group ($P<0.001$ and $P<0.01$). Co-treatment with OXY (100 mg/kg) significantly decreased the levels of BUN and CRE caused by GM ($P<0.001$ and $P<0.05$) (**Figure. 1e, f**). The addition of OXY made the renal function index tend towards the normal level. In addition, serum blood NAG in group 3 was higher than it in controls ($P<0.001$), and OXY co-administration significantly reduced NAG activity ($P<0.001$) (**Figure. 1d**).

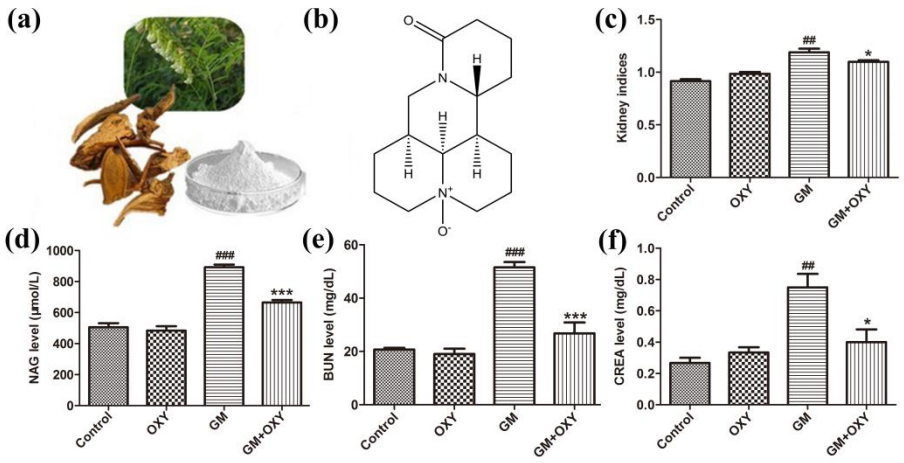


Figure. 1 (a) *Sophora flavescens ait* (the Chinese herb Kushen). (b) Chemical structure of OXY; Oxymatrine attenuates GM-induced nephrotoxicity in rats (c to f) Kidney index, urinary NAG levels, Serum BUN and CRE, respectively, in rats treated with GM in the presence or absence of oxymatrine. The results are submitted as group means± SD (n =10 in each group). ##and###, <0.01 and *P* <0.001compared to the Control group; * and ***, *P* <0.05 and *P*<0.001, respectively, compared to the GM treatment group.

Oxymatrine ameliorates gentamicin-induced oxidative stress in renal tissue

As shown in **Table 2**, GM treatment significantly increased the levels of malonaldehyde (MDA), a product of inducible nitric oxide synthase (iNOS), and nitric oxide (NO) levels to 2.38 U/mg protein, 1.81 U/mg protein, 891.8 mol/g protein (all *P*< 0.01), respectively, and significantly decreased activities of SOD and CAT and the level of GSH to 1025 U/mg of protein, 68.8 U/mg of protein and 28.3 mmol/mg of protein *P*< 0.01). OXY pre-treatment, particularly at 100 mg/kg/day, significantly attenuated all of these GM-induced biomarkers of oxidative stress. The markers did not appreciably change between the OXY and vehicle control groups.

Biomarker	Treatment group			
	Control group	OXY group	GM group	GM+OXY group
MDA (mmol/mg of protein)	1.58±0.069	1.68±0.011	2.38±0.092$^{\#}$	1.92±0.20*
SOD (U/mg of protein)	1106±1.414	1081±6.899	1025±6.980$^{\#\#}$	1145±47.13*
CAT (U/mg of protein)	98.8±4.76	95.2±3.40	68.8±6.74$^{\#}$	96.1±2.89*
GSH (mmol/mg of protein)	36.7±2.80	36.4±2.48	28.3±2.23$^{\#}$	36.6±1.97**
iNOS (U/mg of protein)	0.811±0.027	0.764±0.028	1.81±0.21$^{\#}$	0.845±0.09**
NO (mol/g of protein)	488.1±39.86	483.4±40.07	891.8±30.28$^{\#\#\#}$	665.6±37.64***

Table. 2 Impact of oxymatrine supplementation on the levels of oxidative and nitrative stress markers in the kidney tissues of rats treated with gentamicin. Results were expressed as mean ± SD (n =10 in each group). $^{\#\#}$and$^{\#\#\#}$, P <0.01 and P <0.001compared to the control group; * and ***, P<0.05 and P<0.001, respectively, compared to the GM treatment group.

Oxymatrine attenuates GM-induced activation of caspase-9, caspase-3 and inflammatory mediators in kidney.

Compared to the control, GM treatment significantly increased the activities of caspase-9 and -3 to 1.99- and 1.72-fold (both $P < 0.001$), respectively. The administration of oxymatrine alone (group 2) did not alter caspase levels from control values (group1). Oxymatrine pre-treatment markedly attenuated GM-induced increases of caspase-9 and -3 activities (**Figure. 2a, b**). In the GM + OXY group, caspase-9 activities decreased 1.23-fold (all $P < 0.001$) and caspase-3 activities decreased 1.15-fold (all $P < 0.01$) compared to the solely GM treatment group, respectively.

Gentamicin treatment significantly increased the levels of pro-inflammatory cytokines TNF-α, IL-1β and IL-6 (all $P < 0.001$), which were markedly attenuated by oxymatrine co-administration. The significant attenuating effect was most pronounced in the GM + OXY group, where the TNF-α levels decreased from 707.3 to 628.6 pg/mL of protein ($P < 0.01$) (**Figure. 2c**) and the IL-1β levels decreased from 159.4 to 137.15pg/mL of protein ($P < 0.01$) (**Figure. 2d**) and the IL-6 levels decreased from 62.66 to 48.84 pg/mL of protein ($P < 0.001$) (**Figure. 2e**).

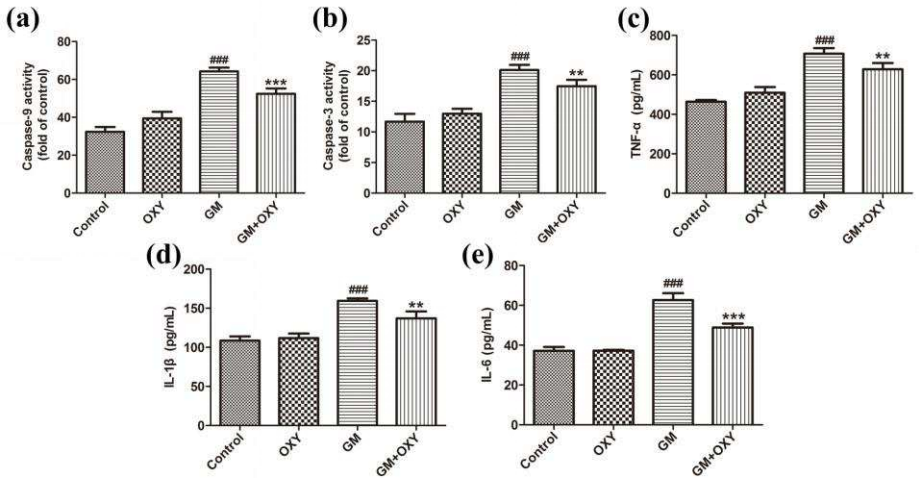
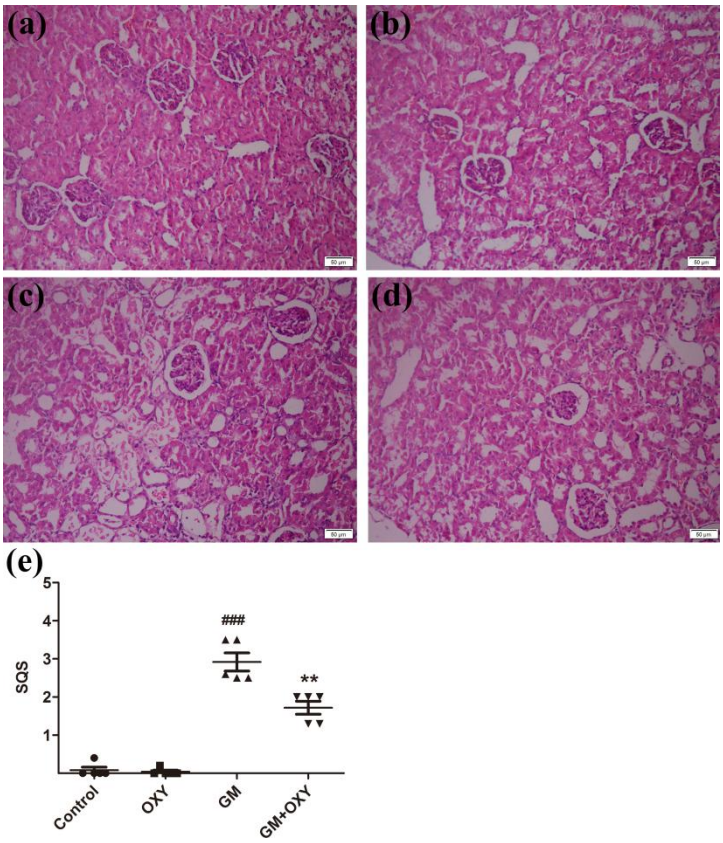


Figure. 2 Oxymatrine attenuates GM-induced nephrotoxicity by decreased levels of apoptosis markers caspase-3 (a), caspase-9 (b) and the inflammatory markers TNF-α(c), IL-1β (d) and IL-6(e) in the serum of rats treated with gentamicin. ELISA results are presented as the mean ±SD (n=10). ###, P <0.001 compared to the control group; ** and ***, P <0.01 and P<0.001, respectively, compared to the GM treatment group.

Histological examination

Microscopy was used to estimate kidney (**Figure. 3a–d**) sections from four groups of rats. Histological evaluation of the renal sections of rats in the control and OXY groups revealed a regular morphology of the glomeruli and tubuli, and there were no marked histopathological changes (**Figure. 3a, b**). Respectively, the kidney tissues of rats treated with GM alone displayed

198 extensive damage including glomeruli and tubuli morphology changes, such as degenerated
199 glomeruli with enlargement of Bowman's capsule, thickening of capsule wall, degeneration of the
200 renal tubular, tubular dilation, necrosis, cast formation, and infiltration of inflammatory cells
201 (**Figure. 3c**). Oxymatrine prophylaxis attenuated the GM-induced kidney damage, evidenced by a
202 marked attenuation of the infiltration of inflammatory cells and decrease tubular necrosis in the
203 renal cortex (**Figure. 3d**). A semiquantitative scoring reinforced these findings and revealed a
204 significant attenuation of damage by oxymatrine co-administration with GM (P<0.01) (**Figure.**
205 **3e**).



206 **Figure. 3** Representative histopathological changes in kidneys of rats treated with GM and
207 oxymatrine. (a) Control group, (b) Oxymatrine group, (c) Gentamicin group, (d)
208 Gentamicin+Oxymatrine group, (e) Semiquantitative scores of kidney damage (group the means
209 ±SD, n=5) . Semiquantitative scores of kidney damage (group means ±the SD, n =5). ###, P

<0.001compared to the control group; ** and ***, P <0.01 and P<0.001, respectively, compared to the GM treatment group. OXY inhibited gentamicin-induced renal inflammatory responses.

Oxymatrine Down-Regulates the Expression of Bcl-2, NF-κB mRNAs and Up-Regulates the Expression of Bcl-2, Nrf2 and HO-1 mRNAs

To examine the effects of oxymatrine more closely, we measured steady-state mRNA levels of genes which were induced in kidneys by GM. For the biomarkers of apoptosis, OXY pre-treatment further up-regulated the expression of Bcl-2 level but reduced Bax level (***P<0.001). In addition, GM treatment increased the expression of Nrf2 and HO-1. funnily, these levels were even greater in all oxymatrine-treated groups compared to that in the saline-treated control rats (**Figure. 4**). Instead, the expression of NF-κB mRNA was significantly down-regulated in the GM+OXY group compared to the GM group.

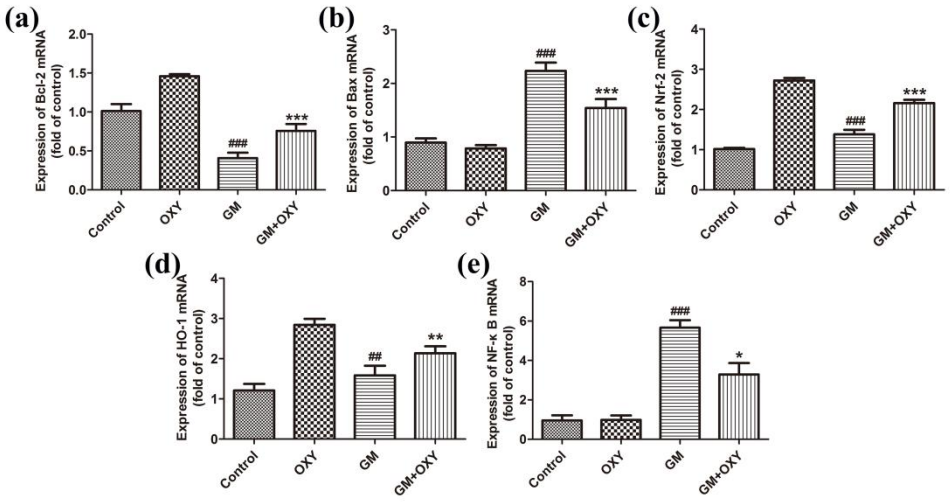
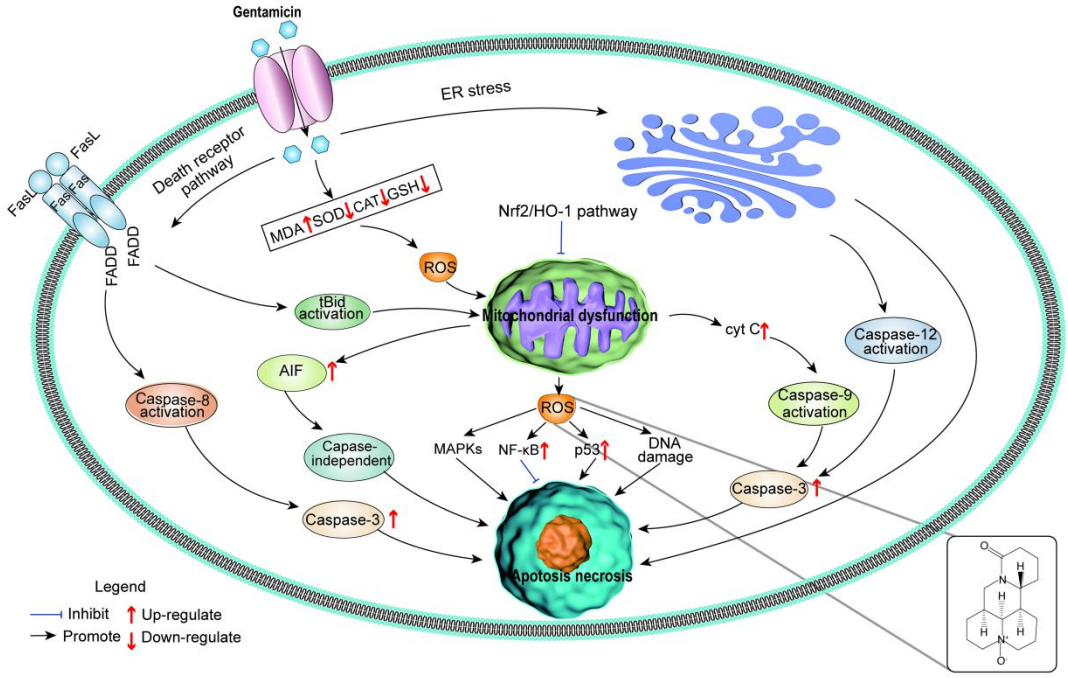


Figure. 4 Effect of oxymatrine on GM-induced expression of Bcl-2 (a), Bax (b)，Nrf-2(c), HO-1(d) and NF-KB (e) mRNAs. Data are presented as mean ±SD (n = 10). (n=10 in each group) *P < 0.05, ** P < 0.01and ***P<0.001 compared to the control group; $^{\#\#}$ P < 0.01 and $^{\#\#\#}P$<0.001, compared to the GM treatment group



225

226 **Figure. 5** Model of oxymatrine renoprotection against gentamicin-induced nephrotoxicity.
227 Oxymatrine inhibits intracellular ROS formation and increases Nrf2 antioxidant signaling pathway
228 to reduce cell apoptosis. Oxymatrine also reduces inflammatory damage by inhibiting the
229 activation of the nuclear factor-kappa B (NF-κB) signaling pathway.

230 **Discussion**

231 Gentamicin (GM) is an effective aminoglycoside antibiotic used worldwide to treat severe
232 infections. However, nephrotoxicity which manifests as acute tubular necrosis and impairment in
233 renal function , and oxidative damage limit its long term clinical use [17, 18]. The underlying
234 mechanisms of gentamicin-induced nephrotoxicity are still unclear. Accordingly, ameliorating this
235 adverse side-effect and thereby promotion of gentamicin therapy is of the utmost importance.
236 Oxymatrine is a major alkaloid found in radix Sophorae flavescent and has been reported to
237 exhibit numerous pharmacological and biological activities [17, 19]. The aim of this study is to
238 explore protective effect of oxymatrine on kidney damage by gentamicin in rats.
239 Co-administration of oxymatrine (100mg/kg, i.p.) and GM for 7 days effectively reversed

GM-induced acute kidney damage, which was demonstrated by the decrease of serum BUN and CRE levels. These findings indicate that oxymatrine reduced the functional impairment caused by high-dose GM. This was confirmed by a decrease in damaged kidney (**Figure. 1e, f**). In addition, N-acetyl-beta-glucosaminadase (NAG) is a renal enzyme which is an early, sensitive and reliable indicator of renal damage [20]and the NAG excretion showed significant increase in serum after GM administration and it was neutralized by oxymatrine co-administration (**Figure. 1d**).

Previous studies have indicated that one of the mechanisms described for the pathogenesis of GM-induced nephrotoxicity was related to oxidative stress and it has been suggested that ROS was the central key in the mechanisms that leads to tubular necrosis and the decrease of glomerular filtration rate [2, 21]. In line with these findings, decreased superoxide dismutase (SOD)，catalase (CAT) activities and low levels of glutathione (GSH) were detected in the kidney tissues of the GM-treated rats (Table 2). Notably, treatment in vivo with GM showed significant decreased total antioxidant capacity and significant elevated lipid peroxides (MDA), iNOS and NO levels, which are markers of oxidative stress.Pretreatment with oxymatrine strongly diminished these adverse oxidative/nitrative changes, indicating that oxymatrine was administered by intraperitoneal injection had a nephroprotective effect (Table 2). Nuclear respiratory factor 2 (Nrf2) is a transcription factor is an important transcriptional factor which can reduce the oxidative stress by increasing antioxidant defense[22]. In our study, we found that oxymatrine treatment increased the nuclear factor (erythroid-derived-2)-like 2 (Nrf2) and its downstream targets HO-1 mRNA expression. Oxymatrine administration also increased SOD and CAT activity levels depleted by Gentamicin (Table 2). These data indicated that the Nrf2/HO-1 pathway played a vital role in the nephroprotective effect of oxymatrine against GM. Previous studies have shown

that OXY preprocessing deduced level of ROS production in renal tissue caused by ischemia/reperfusion and activated serum antioxidant enzyme activities. Oxymatrine also ameliorated pathological damage in myocardial tissue induced by doxorubicin with anti-oxidative and anti-inflammatory effects[23, 24]. Furthermore, oxymatrine has been shown to improve microcirculation and liver function [24].

Conversely, the NF-κB represses Nrf2-antioxidant signalling at the transcription level by depriving for transcription co-activator CREB binding protein from Nrf2 and facilitating recruitment of histone deacetylase 3 to MafK [25]. Our results indicated that the up-regulation of the signaling pathway of Nrf2/HO-1 contributed to the ability of oxymatrine to inhibit the NF-κB-mediated inflammatory response (**Figure. 5**). Furthermore, the results also revealed that OMT post-treatment ameliorated hypoxic-ischemic-induced oxidative stress in neonatal rats through inhibiting the accumulation of MDA content and enhancing the antioxidant enzymes activities (SOD, GSH-PX, CAT, T-AOC), which suggested that OMT could alleviate HIBD in neonatal rats by decreasing lipid peroxide and improving the antioxidant defense system [26]. Overall, the radical scavenging activity of oxymatrine and its ability to improve the resistance of the kidney by activating their intrinsic antioxidant defense mechanisms are major factors that are responsible for the observed dose-dependent reduction of GM-induced nephrotoxicity.

Previous studies identified that the mitochondrial dysfunction [27, 28], apoptosis/necrosis [29, 30] and inflammation [31] are also included in gentamicin-induced kidney injury in rats and human renal proximal tubular cells (HKC). The Bcl-2 family proteins are well-characterized regulators of the mitochondrial pathway, including pro-and anti-apoptotic members such as Bax and Bcl-2, which can regulate the activation of caspases that cleave and activate downstream

284 caspases such as caspase-3, -6, and -7 [32]. Since caspase-3 is a central protein in apoptosis
285 pathways in higher eukaryotes, it can be activated via both the intrinsic (mitochondrial) and the
286 extrinsic (death receptor) pathways. Caspase-9 (CASP-9) is an initiator CASP in the
287 apoptosome-driven apoptosis pathway and plays a central role in the mitochondrial apoptosis
288 pathway, which is engaged in response to numerous apoptotic stimuli [33]. We have demonstrated
289 that oxymatrine supplementation significantly attenuated GM-induced increased activity of
290 caspase-9 and -3 in serum (**Figure. 2a, b**), OXY-treatment significantly increased the expression
291 of Bcl-2 and decreased the expression of Bax mRNAs (**Figure. 4a, b**). Indeed, recent studies have
292 shown that the majority of pharmacological activities of oxymatrine are closely linked to its role
293 in modulating mitochondrial function and dynamics [34, 35].

294 Inflammation plays another major role in the pathogenesis of GM-induced nephrotoxicity
295 [35]. The nuclear factor kappa B (NF-κB) signaling pathway is known to regulate the expression
296 of numerous genes involved in inflammation adhesion and survival. Thus, it is thought to be a key
297 transcription factor that can mediate acute and chronic inflammation by transcription of genes
298 encoding cytokines, chemokines, adhesion molecules, pro-inflammatory enzymes and
299 apoptosis-regulating proteins and consequently cause apoptosis and interstitial fibrosis in
300 progressive renal disease [36, 37]. In the current study., we found that GM-evoked inflammatory
301 responses in kidneys resulted in infiltration of inflammatory cells and improved NF-κB expression,
302 as well as the production of the pro-inflammatory cytokines TNF-α, IL-1β and IL-6 (2 c to e).
303 Oxymatrine supplementation markedly diminished NF-κB expression and significantly decreased
304 pro-inflammatory cytokines like TNF-α, IL-1β and IL-6 generation to control levels (**Figure. 2c to**
305 **e**).

306 Coincidently, oxymatrine suppressed the production of nuclear factor-κB (NF-κB) to tumor
307 necrosis factor-α (TNF-α), interleukin-6 (IL-6), and inter-cellular adhesion Molecule-1 (ICAM-1).
308 This study shows that oxymatrine could be a promising candidate for using as an adjuvant drug in
309 clinical rodent colitis therapy [28]. In order to improve anti-inflammatory activity of the
310 oxymatrine, HSPC liposomes were selected， using pH gradient loading method, OMT HSPC
311 liposomes were stable and reliable liver target carrier to prolong OMT retention time and maintain
312 high therapeutically level in liver [40]. We used a high doses of the nephrotoxic agent GM, Such
313 doses would be certainly not relevant to the doses used in clinic. Our data shows that oxymatrine
314 provided valuable protection against GM nephrotoxicity and there is a critically need to develop a
315 new drug delivery system to improve the bioavailability of oxymatrine. Notably, various
316 formulations such as an oxymatrine-loaded nanostructured lipid carrier have improved the
317 stability and bioavailability of oxymatrine. Therefore, it is time to sum up the theoretical results.

318 **Conclusions**

319 In conclusion，the results of this study suggest that oxymatrine had significant effect in reducing
320 GM-induced nephrotoxicity in rats by inhibiting oxidative/nitrative stress, apoptosis and
321 inflammation in kidney tissue. Oxymatrine can work as a candidate of novel agent
322 supplementation for the prevention of nephrotoxicity in patients receiving gentamicin therapy.

323 **Abbreviations**

324 GM:Gentamicin; OXY:Oxymatrine; BUN:Serum blood urea nitrogen; CRE:Serum creatinine
325 MDA:Malondialdehyde; NO:Nnitric oxide; GSH:glutathione; SOD:Superoxide Dismutase:
326 CAT:Catalase; iNOS: inducible NO synthase; ROS:Reactive oxygen; H&E:Hematoxylin and
327 eosin; Caspase-3:Cysteine aspartic acid protease-3; Caspase-9:Cysteine aspartic acid protease-9

IL-6:Interleukin-6; IL-1β:Interleukin-1β; TGF-α:Transforming growth factor alpha; qRT-PCR：Quantitative reverse-transcription PCR; Nrf2:Nuclear factor (erythroid-derived-2)-like 2; HO-1:Heme oxygenase-1; NF-κB:Nuclear factor kappa B; Bax:Bcl-2 Associated X Protein Bcl-2:B-cell leukemia-lymphoma; GAPDH:Glyceraldehyde phosphatedehydrogenase; CREB:Transcription co-activator; MafK:A small Maf protein; HIBD:Hypoxia-ischemia brain damage; GSH-PX:Glutathione-peroxidase; T-AOC:Total antioxidant capacity; HKC:Human embryonic kidney epithelial cells; ICAM-1:Intercellular adhesion molecule-1.

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Not applicable.

Authors' contributions

Zhihui Hao and Songyao Kang conceived and designed the research; Songyao Kang, Fengting Lang and Hongxia Hao performed the experiments; Yuwei Chen, Tianshi Xiao assisted in data collection and Songyao Kang analyzed the data and statistical analysis, prepared the figures,drafted, edited, and revised manuscript; Chen Gao critically revised the intellectual content of the manuscript and Zhihui Hao approved the final version of the manuscript submitted.

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Availability of data and materials

The datasets used in this study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

The Research work involve animals are received ethical approval from theAnimal Use and rotection of Qingdao Agriculture University.

Consent for publication

Not applicable.

Competing interests

All authors declare that they have no confict of interests.

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458

Figures

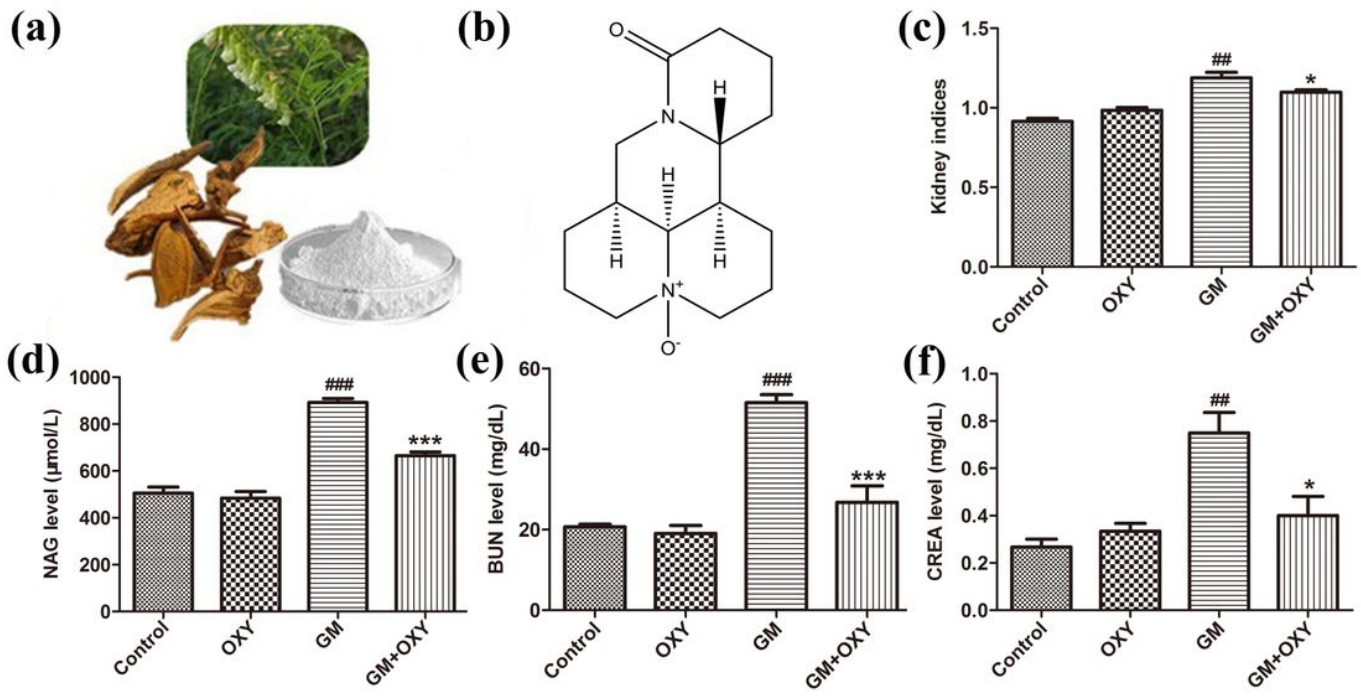

Figure 1

(a) Sophora flavescens ait (the Chinese herb Kushen). (b) Chemical 155 structure of OXY; Oxymatrine attenuates GM-induced nephrotoxicity in rats (c to f) Kidney index, urinary NAG levels, Serum BUN and CRE, respectively, in rats treated with GM in the presence or absence of oxymatrine. The results are submitted as group means± SD (n =10 in each group). ##and###, <0.01 and P <0.001compared to the Control group; * and ***, P <0.05 and P<0.001, respectively, compared to the GM treatment group.

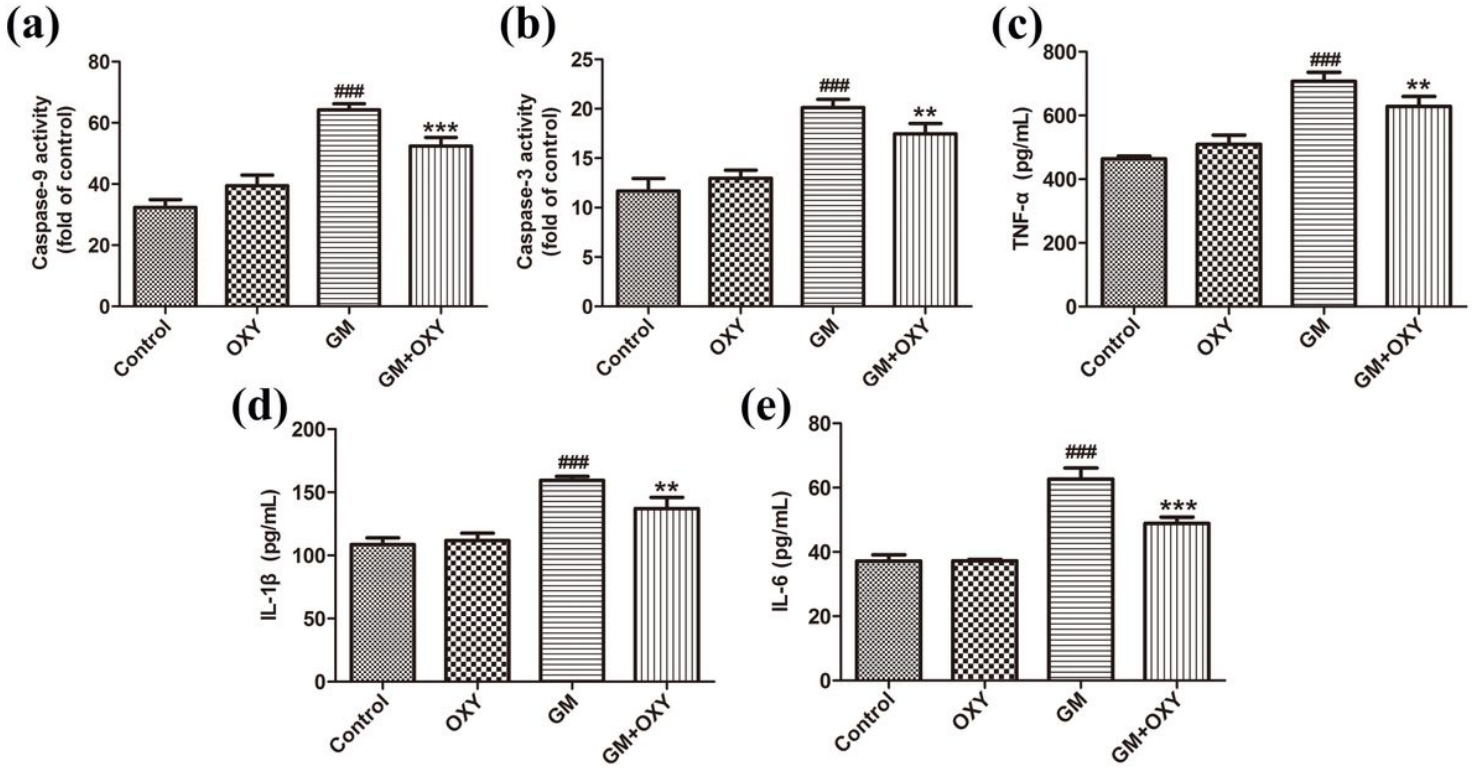

Figure 2

Oxymatrine attenuates GM-induced nephrotoxicity by decreased levels of apoptosis markers caspase-3 (a), caspase-9 (b) and the inflammatory markers TNF-α(c), IL-1β (d) and IL-6(e) in the serum of rats treated with gentamicin. ELISA results are presented as the mean ±SD (n=10). ###, P <0.001 compared to the control group; ** and ***, P <0.01 and P<0.001, respectively, compared to the GM treatment group.

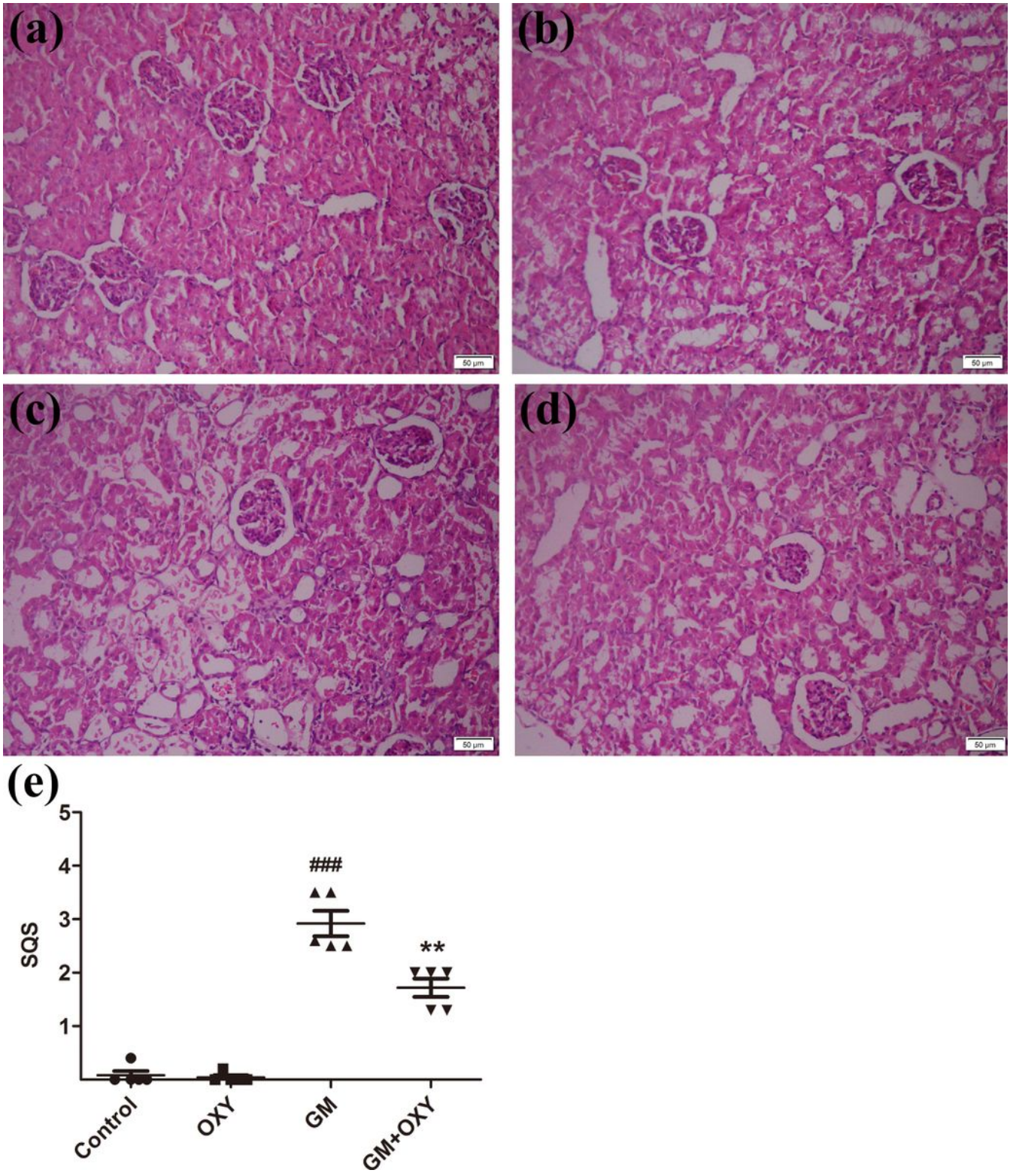

Figure 3

Representative histopathological changes in kidneys of rats treated with GM and oxymatrine. (a) Control group, (b) Oxymatrine group, (c) Gentamicin group, (d) Gentamicin+Oxymatrine group, (e) Semiquantitative scores of kidney damage (group the means ±SD, n=5) . Semiquantitative scores of kidney damage (group means ±the SD, n =5). ###, P 13 <0.001compared to the control group; ** and ***,

P <0.01 and P<0.001, respectively, compared to the GM treatment group. OXY inhibited gentamicin-induced renal inflammatory responses.

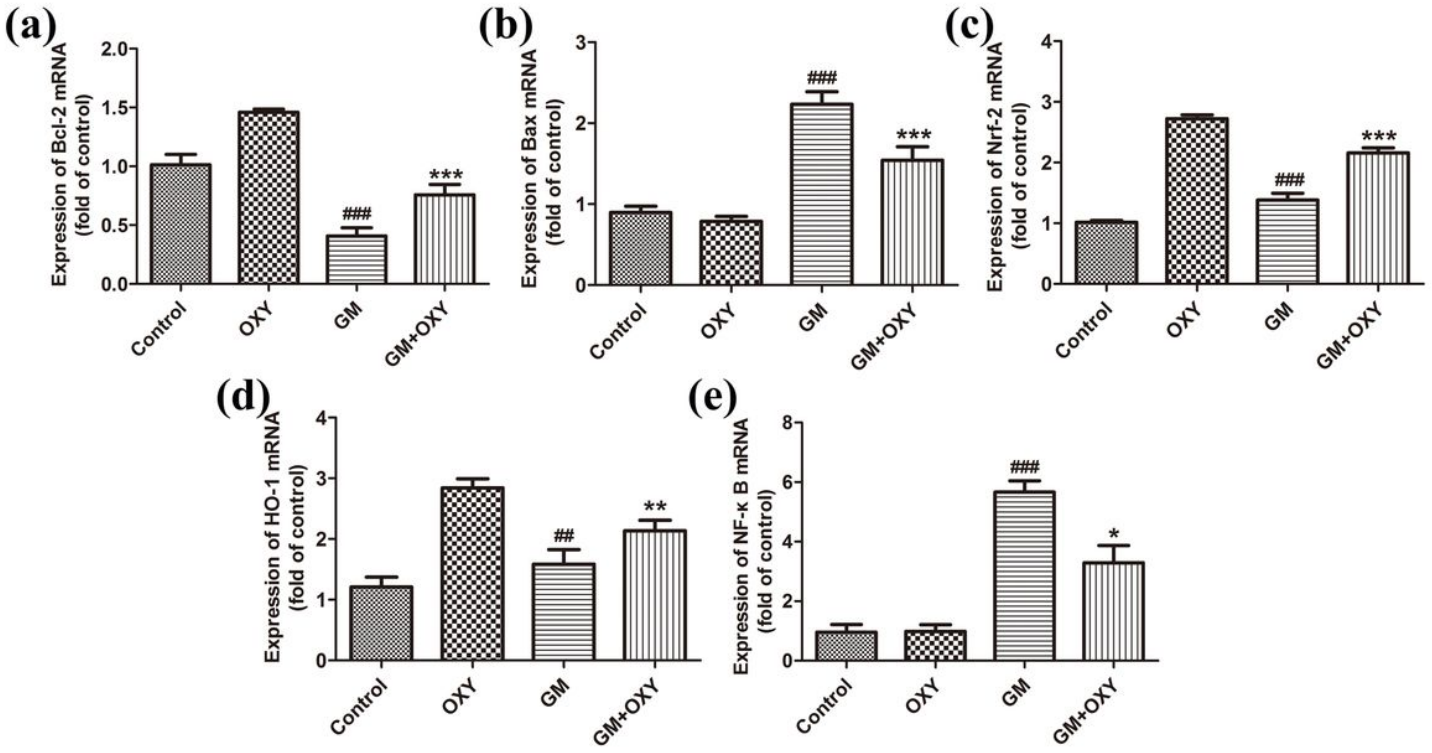

Figure 4

Effect of oxymatrine on GM-induced expression of Bcl-2 (a), Bax (b)Nrf-2(c), HO-1(d) and NF-KB (e) mRNAs. Data are presented as mean ±SD (n = 10). (n=10 in each group) *P < 0.05, ** P < 0.01and ***P<0.001 compared to the control group; ## P < 0.01 and ###P<0.001, compared to the GM treatment group

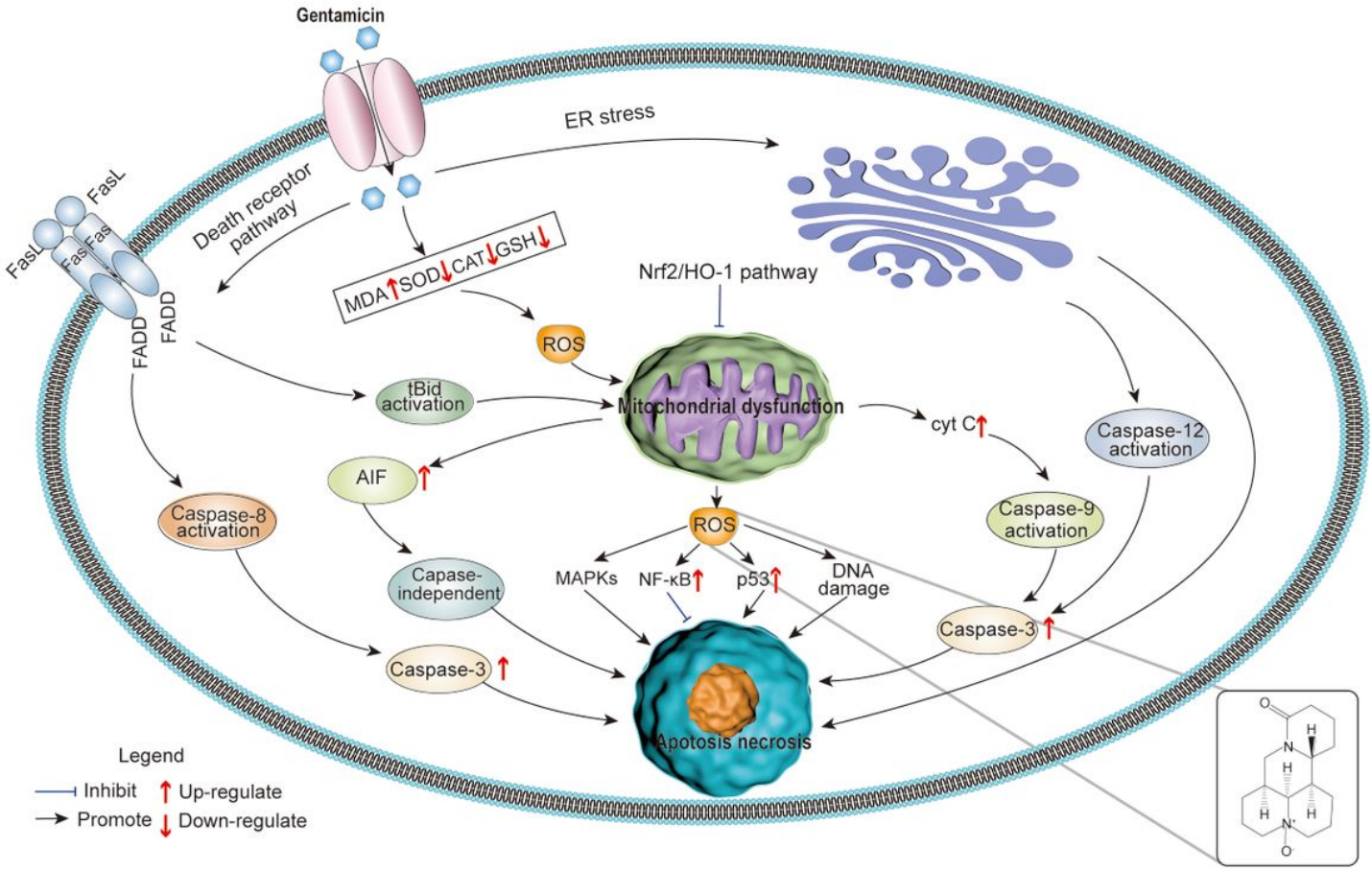

Figure 5

Model of oxymatrine renoprotection against gentamicin-induced nephrotoxicity. Oxymatrine inhibits intracellular ROS formation and increases Nrf2 antioxidant signaling pathway to reduce cell apoptosis. Oxymatrine also reduces inflammatory damage by inhibiting the activation of the nuclear factor-kappa B (NF-κB) signaling pathway.