



Apigenin attenuates molecular, biochemical, and histopathological changes associated with renal impairments induced by gentamicin exposure in rats

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Received: 14 January 2022 / Accepted: 9 April 2022 / Published online: 29 April 2022
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Abstract

Gentamicin (GM) is an aminoglycoside antibiotic used to treat bacterial infections. However, its application is accompanied by renal impairments. Apigenin is a flavonoid found in many edible plants with potent therapeutic values. This study was designed to elucidate the therapeutic effects of apigenin on GM-induced nephrotoxicity. Animals were injected orally with three different doses of apigenin (5 mg kg⁻¹ day⁻¹, 10 mg kg⁻¹ day⁻¹, and 20 mg kg⁻¹ day⁻¹). Apigenin administration abolished the alterations in the kidney index and serum levels of kidney-specific functions markers, namely blood urea nitrogen and creatinine, and KIM-1, NGAL, and cystatin C following GM exposure. Additionally, apigenin increased levels of enzymatic (glutathione reductase, glutathione peroxidase, superoxide dismutase, and catalase) and non-enzymatic antioxidant proteins (reduced glutathione) and decreased levels of lipid peroxide, nitric oxide, and downregulated nitric oxide synthase-2 in the kidney tissue following GM administration. At the molecular scope, apigenin administration was found to upregulate the mRNA expression of *Nfe2l2* and *Hmox1* in the kidney tissue. Moreover, apigenin administration suppressed renal inflammation and apoptosis by decreasing levels of interleukin-1 β , tumor necrosis factor-alpha, nuclear factor kappa-B, Bax, and caspase-3, while increasing B-cell lymphoma-2 compared with those in GM-administered group. The recorded data suggests that apigenin treatment could be used to alleviate renal impairments associated with GM administration.

Keywords Apigenin · Gentamicin · Nephrotoxicity · Oxidative stress · Inflammation · Apoptosis

Responsible Editor: Lotfi Aleya

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Introduction

Gentamicin (GM) is an aminoglycoside antibiotic commonly used for treating severe bacterial infections (Makama et al. 2018). Among aminoglycosides, GM possesses the most adverse effects, including oxidative stress and renal toxicity (Sriram et al. 2012). Another adverse effect of GM is renal dysfunction related to GM's aggregation in the proximal tubules (Gökce et al. 2016).

Enhanced oxidative stress, inflammatory reaction, necrosis, and apoptosis in tubular cells are the most significant mechanisms in GM-induced kidney dysfunction both in vivo and in cell culture (El Mezayen et al. 2006; Hamdy and Taha 2009). Such complications resulted from the accumulation of GM in cells of the renal tubules and the interaction between GM and cellular organelles (Shehata et al. 2022).

Renal injury may be acute or chronic and is conventionally detected by measuring the serum levels of blood urea nitrogen (BUN) and creatinine, which only increase when renal damage reaches a significant degree and usually rise at least one week after GM treatment initiation (Hamdy and Taha 2009). Although serum creatinine and BUN levels are the main traditional parameters for evaluating kidney function (Xu et al. 2012), they lack sensitivity and specificity. Kidney injury molecule 1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) are recently considered more specific markers for renal injury (Tsiola et al. 2017).

GM binds to phospholipids in the renal cell membranes causing the inactivation of phospholipases, leading to the development of renal disorders, such as toxicity (Kim et al. 2013). Remarkably, flavonoid derivatives were considered nephroprotective agents because they prevent the binding of GM to the phospholipids in the cytoplasmic membranes (Jiménez-Lamana et al. 2014). In the same line, some flavonoids exert nephroprotective activities against GM nephrotoxicity (Hamdy and Taha 2009; Wen et al. 2017).

Apigenin, or 4,5,7-trihydroxyflavone, is a flavonoid naturally found in fruits and vegetables (Su et al. 2014). Apigenin has a nephroprotective effect against cisplatin nephropathy by promoting antioxidant, anti-inflammatory, and anti-apoptotic pathways (Al-Brakati et al. 2020; Tiwari et al. 2011). However, the therapeutic role of apigenin against GM-induced renal damage has not been elucidated. Therefore, this study evaluates the capability of apigenin in attenuating GM-induced nephrotoxicity.

Material and methods

Animals

This study included 42 male rats weighing approximately 200–220 g obtained from VACSERA (Cairo, Egypt). All rats were kept in cages under an appropriate environment with a mean temperature of 25 ± 5 °C and mean humidity of $50 \pm 10\%$ in a 12-h light/dark cycle. All animals were kept for one week with free water and food before the initiation of experiments that were performed according to the ethical principles of the Institutional Animal Care and Use Committee (IACUC) of Helwan University (approval no. HU2019/Z/AER919-01).

Experimental protocol

Animals were allocated into six groups with seven rats in each, as follows: In the healthy control group, the rats received saline; in the apigenin-treated group, the rats received apigenin ($20 \text{ mg kg}^{-1} \text{ day}^{-1}$); in the GM-treated group, the rats received an intraperitoneal injection of GM ($100 \text{ mg kg}^{-1} \text{ day}^{-1}$) (Sigma, St. Louis, MO, USA); in the apigenin-5-GM group, the rats orally received apigenin ($5 \text{ mg kg}^{-1} \text{ day}^{-1}$) 1 h before GM injection; in the apigenin-10-GM group, the rats orally received apigenin ($10 \text{ mg kg}^{-1} \text{ day}^{-1}$) 1 h before GM injection; and in the apigenin-20-GM group, the rats orally received apigenin ($20 \text{ mg kg}^{-1} \text{ day}^{-1}$) 1 h before GM injection. The treatments were repeated daily for seven days at the same time point. Apigenin was first dissolved in dimethyl sulfoxide (DMSO) and further diluted with physiological saline (0.9% sodium chloride). Each rat from the apigenin-treated groups received no more than 0.2% DMSO, corresponding to 10 μl . In the control and GM-treated groups, each rat received physiological saline with 10 μl DMSO. The selected doses for GM and apigenin were based on the studies by Hamdy and Taha (2009) and Al-Brakati et al. (2020), respectively.

Sampling

Twenty-four hours after the experiment, all animals were euthanized by intraperitoneal injection of pentobarbital (300 mg kg^{-1}). The blood samples were collected and centrifuged for 10 min at $3000 \times g$ to separate the serum, which was then stored at -80 °C for biochemical analyses. The kidneys were removed and weighed for calculation of the renal index as follows:

$$\text{Index of the kidneys} = \frac{\text{the weight of the kidney (g)} \times 100}{\text{the final weight of the body (g)}}$$

A kidney was divided into two portions: The first portion was fixed in 10% neutral buffered formalin for histopathological examinations; the second portion was retained at -80°C for biochemical and molecular investigations.

Measurement of BUN, creatinine, sodium, and potassium

The serum levels of creatinine, BUN, sodium, and potassium were measured using colorimetric kits following the methods performed by Biodiagnostics (Giza, Egypt).

Determination of KIM-1, NGAL, and cystatin C levels

The serum levels of KIM-1, NGAL, and cystatin C were measured using an enzyme-linked immunosorbent assay (ELISA) according to the instructions by Abcam (Cambridge, UK).

Measurement of oxidative stress parameters

The kidneys were minced and homogenized in a phosphate buffer (10 mM; pH 7.4). The homogenate was centrifuged at $3000 \times g$ for 10 min. Lipid peroxide (LPO) levels were detected according to the method used by Ohkawa et al. (1975). Nitric oxide (NO) levels were investigated following the technique used by Green et al. (1982). Glutathione (GSH) level reductions were estimated using the procedure used by Ellman (1959). The activities of GSH peroxidase (GPx), GSH reductase (GR), superoxide dismutase (SOD), and catalase were analyzed using the protocol described by Paglia and Valentine (1967), De Vega et al. (2002), Nishikimi et al. (1972), and Aebi (1984), respectively.

Determination of inflammatory biomarkers

The renal levels of nuclear factor kappa B p65 subunit (NF- κ B-p65), tumor necrosis factor-alpha (TNF- α), and interleukin (IL)-1 β were assayed using ELISA kits produced by CUSABIO Life Sciences (Wuhan, China) according to the protocols of the manufacturer.

Assessment of apoptotic protein markers

The protein levels of B-cell lymphoma 2 (Bcl-2), Bax, and caspase-3 in the kidneys were measured using ELISA kits bought from CUSABIO Life Sciences (Wuhan, China).

Quantitative real-time polymerase chain reaction (PCR) technique

Total RNA was extracted from renal tissues using a TRIzol reagent kit (Qiagen, Germantown, MD, USA); then, their concentrations were measured in nanodrops. cDNA was obtained from isolated RNA using the reverse-transcription method according to the RevertAidTM H Minus Reverse Transcriptase kit provided by Fermentas (Thermo Fisher Scientific Inc., Canada). mRNA levels of *Nos2*, *Nfe2l2*, and *Hmox1* were quantitatively measured using the ViiATM 7 PCR system (Applied Biosystems, USA) using the SYBR Green PCR kit (Qiagen, Germany). The fold changes of all mRNAs were calculated using the $2^{-\Delta\Delta C_t}$ method, where they were normalized to the *Actb* acting as the internal control. Primer sequences of the selected genes are presented in Table 1.

Histological procedures

Freshly isolated kidneys were cut into small pieces and placed in 10% neutral buffered formalin. Specimens were passed through a standard alcohol dehydration-xylene sequence, embedded in paraffin, and cut into thin Sects. (5 μm in thickness). Tissue sections were stained with hematoxylin and eosin to record any histological lesions (Zhang et al. 2016). A light microscope (Nikon Eclipse, E200-LED, Tokyo, Japan) with various magnifications was used for this purpose.

Statistical analysis

All statistical analyses were performed using Statistical Package for the Social Sciences (IBM Corp., Armonk, NY, USA), and all data were presented as mean \pm standard deviation. One-way analysis of variance was used to assess the

Table 1 Primer sequences of genes analyzed in real-time PCR

Name	Accession number	Sense (5'–3')	Antisense (5'–3')
<i>Actb</i>	NM_031144.3	GCAGGAGTACGATGAGTCCG	ACGCAGCTCAGTAACAGTCC
<i>Nfe2l2</i>	NM_031789.2	CAGCATGATGGACTTGGAATTG	GCAAGCGACTCATGGTCATC
<i>Hmox1</i>	NM_012580.2	TTAAGCTGGTGATGGCCTCC	GTGGGGCATAGACTGGGGTTC
<i>Nos2</i>	NM_012611.3	GGTGAGGGGACTGGACTTTTAG	TTGTTGGGCTGGGAATAGCA

The abbreviations of the genes: *Nfe2l2*, nuclear factor-erythroid-2-related factor 2; *Hmox1*, heme oxygenase 1; *Nos2*, nitric oxide synthase 2

significant difference between different groups; *p* values less than 0.05 were used to denote statistical significance.

Results

Effects of apigenin on renal index and kidney function markers

A significant increase in the renal index was detected in the GM-treated group (*p* < 0.05) compared with the control group (Fig. 1). Conversely, apigenin-treated rats had significantly decreased renal index compared with nephrotoxic rats (*p* < 0.05); however, the renal index of rats treated with low-dose apigenin was still significantly higher than that of healthy control rats. Remarkably, the two doses (10 mg kg⁻¹

and 20 mg kg⁻¹) of apigenin significantly altered the renal index, suggesting that apigenin restrains the increment in the renal index triggered by GM in a dose-dependent manner.

Furthermore, the levels of kidney function markers, BUN, and creatinine in different animal groups are shown in Fig. 1. The GM-injected group showed a significant increase in BUN and creatinine levels compared with the control group (*p* < 0.05). Remarkably, a significant decrease (*p* < 0.05) in BUN and creatinine levels was detected in the three groups pretreated with apigenin (doses: 5 mg kg⁻¹, 10 mg kg⁻¹, and 20 mg kg⁻¹) compared with the GM-treated group. Additionally, a non-significant change in the sodium and potassium levels was observed in the GM- and apigenin-treated groups compared with the control group.

Figure 1 also displays the concentrations of KIM-1, NGAL, and cystatin C in the different groups. Serum KIM-1, NGAL,

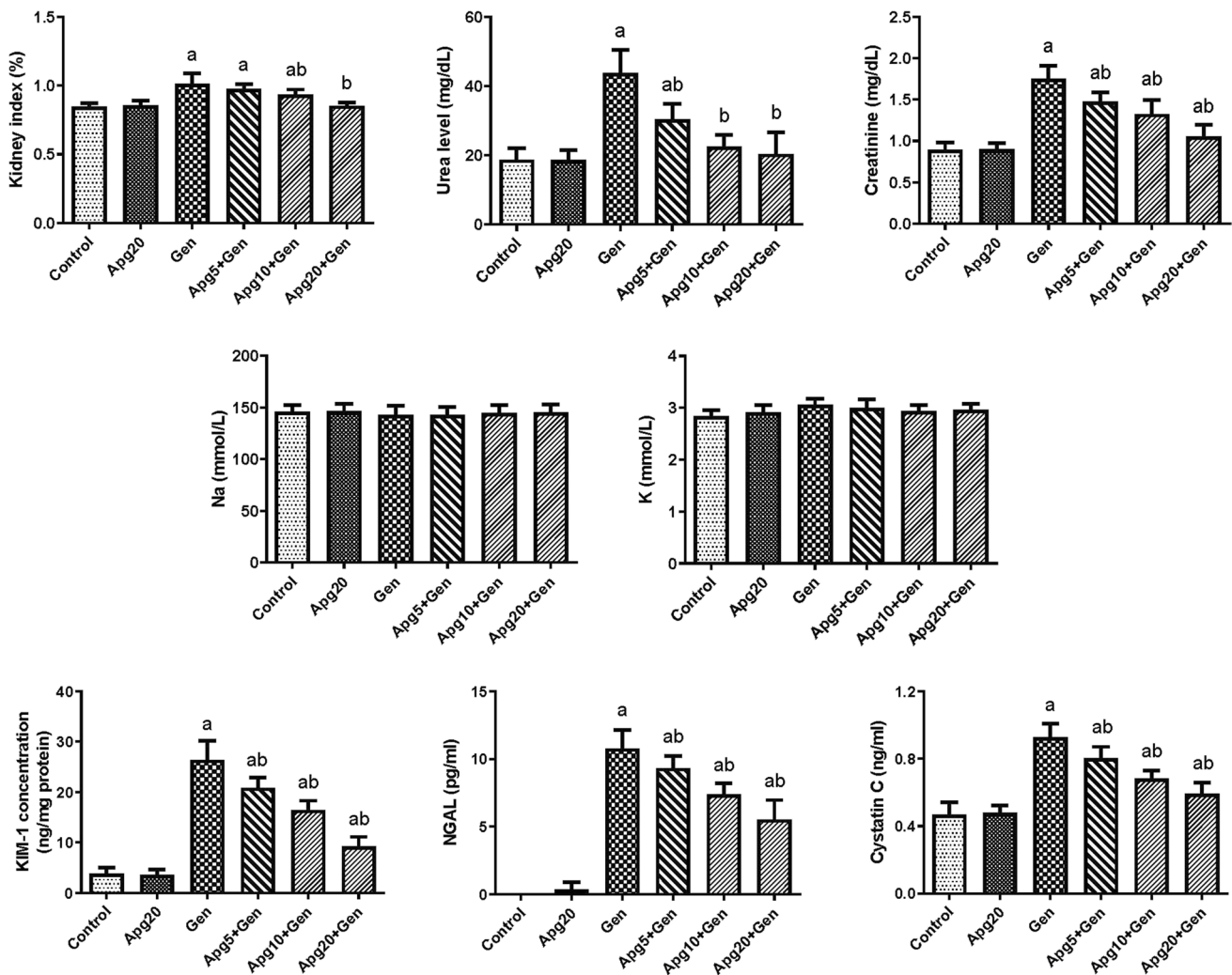


Fig. 1 The renal index and blood urea nitrogen, creatinine, sodium, potassium, kidney injury molecule-1, neutrophil gelatinase-associated lipocalin, and cystatin C levels following apigenin treatment (5 mg kg⁻¹, 10 mg kg⁻¹, and 20 mg kg⁻¹) in rats with gentamicin

(GM)-induced nephrotoxicity. Data are expressed as mean ± standard deviation (*n* = 7). ^aIt represents the statistical significance relative to that of the control group at *p* < 0.05. ^bIt represents the statistical significance relative to that of the GM-treated group at *p* < 0.05

and cystatin C concentrations significantly increased in the GM-treated group compared with the control group. However, treatment with the three doses of apigenin resulted in a significant decrease in the serum concentrations of KIM-1, NGAL, and cystatin compared with the GM-treated group.

Apigenin stimulates renal antioxidant mechanisms in GM-treated animals

The injection of GM-induced oxidative stress in renal tissues is evidenced by the rising levels of NO and LPO and decreasing levels of SOD, CAT, GPx, GR, and GSH (Fig. 2). However, apigenin (5 mg kg^{-1} , 10 mg kg^{-1} , and 20 mg kg^{-1}) possessed antioxidant properties, as shown by the elevated protein levels of SOD, CAT, GPx, GR, and GSH, along with a significant reduction in the levels of LPO, NO, and *Nos2* compared with the GM-injected group.

Furthermore, to test the effect of apigenin on the Nrf2/HO-1 pathway in the renal tissues of GM-treated rats, we examined the mRNA expression of *Nfe2l2* and *Hmox1* in the renal tissues of the different animal groups. GM induced a significant downregulation in the mRNA expression of *Nfe2l2* and *Hmox1* (Fig. 3). Apigenin per se caused a significant upregulation in *Nfe2l2* expression. Moreover, pretreatment with apigenin restrained the harmful effects of GM, and their expressions were upregulated after apigenin administration.

Apigenin administration mitigates GM-induced renal inflammation and apoptosis

GM induced a pro-inflammatory response evidenced by a significant increase ($p < 0.05$) in the protein levels of NF κ B-p65, IL-1 β , and TNF- α in the kidneys compared with those of the control group (Fig. 4). However, apigenin treatment diminished renal inflammation, as shown by a significant decrease in the protein levels of IL-1 β , NF κ B-p65, and TNF- α compared with those in the GM-treated group ($p < 0.05$).

In relation to the results in the control group, GM mediated apoptosis in the kidneys of rats, as evidenced by a significant rise ($p < 0.05$) in the protein levels of Bax and caspase-3 along with a decline in Bcl-2 level (Fig. 4). Conversely, in the three apigenin-treated groups, apigenin could promote anti-apoptotic mechanisms by increasing Bcl-2 and decreasing Bax and caspase-3 protein levels compared with the GM-treated group.

Apigenin protects renal tissue following GM administration

To evaluate the nephroprotective effect of apigenin on the renal histological alternations, the renal tissues were

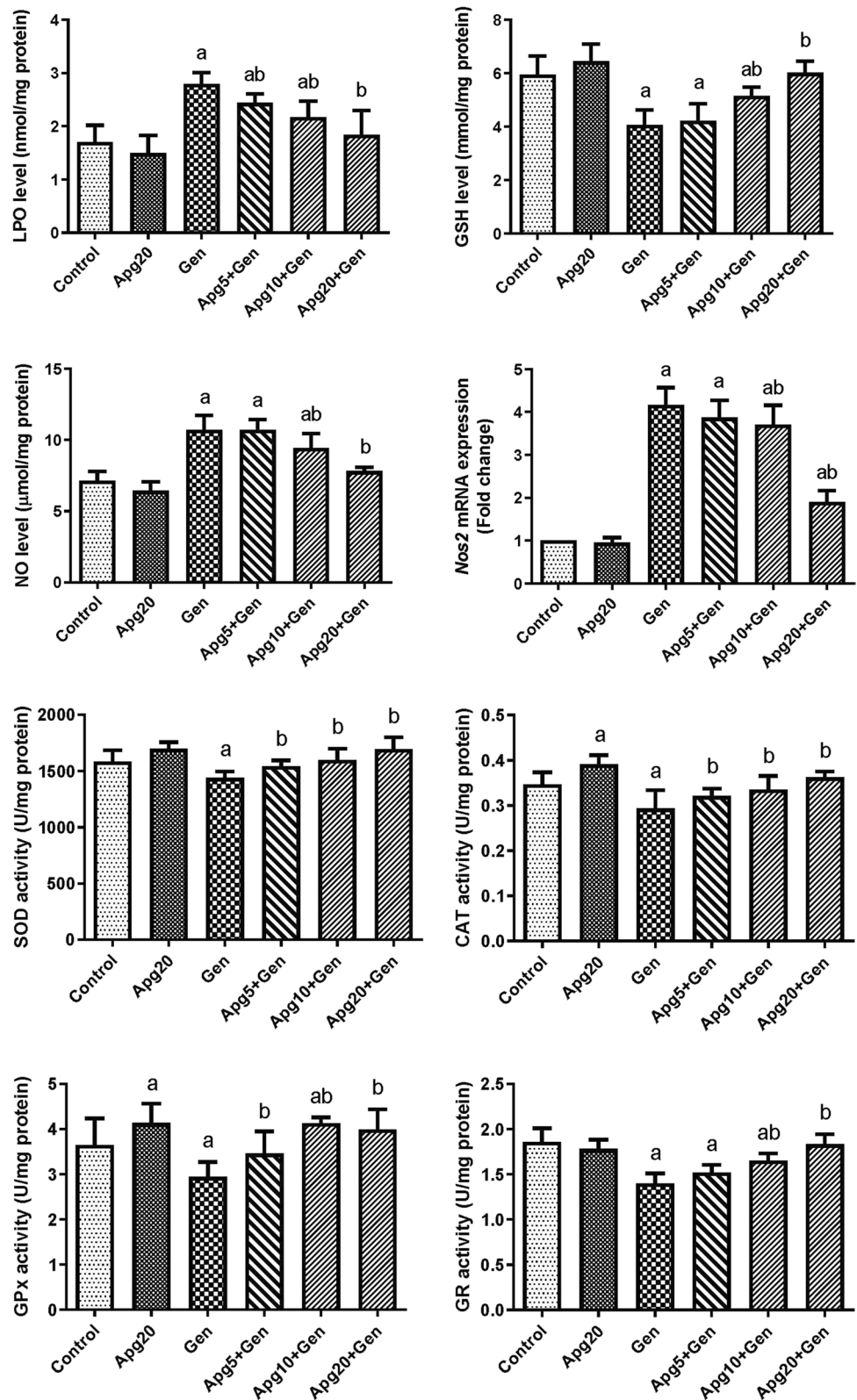
examined using hematoxylin and eosin (H&E) staining. As a result, the GM-treated group showed edema in the renal corpuscles with congested glomeruli, severe infiltration of inflammatory leukocytes, cytoplasmic vacuolation, and tubular epithelial injury (Fig. 5C). Pretreatment with apigenin attenuated glomerular and tubular injuries in rats with GM-induced nephrotoxicity (Fig. 5D-F). The nephroprotective effects of apigenin were supported by a decrease in inflammatory cell infiltration and preservation of the glomerulus. The control and rats treated with apigenin alone showed normal morphology of the glomeruli and tubular cells (Fig. 5A, B).

Discussion

GM-induced renal injury has been considered the most well-defined model for studying drug-associated nephrotoxicity (Hamdy and Taha 2009). A significant increase in the renal index was observed in the current study after GM treatment, which conforms to Abdelrahman and Abdelmageed (2020) and Feyzi et al. (2020). This increase may be related to histopathological alterations, as Udupa and Prakash (2019) reported. Moreover, Karadeniz et al. (2008) have attributed this increase to the generation of inflammatory mediators and interstitial infiltration of immune cells, followed by edema development in GM-treated animals. Interestingly, apigenin administration reduced the increased renal index in response to GM exposure.

Creatinine and BUN are considered the main traditional parameters for evaluating kidney function (Kim et al. 2010). In this study, a significant increase in creatinine and BUN serum levels was observed in the GM-treated group compared with the control group. These results conform to many studies that have demonstrated that over-discharge of creatinine and urea into the serum could indicate reduced glomerular filtration rate (GFR) and impaired renal function, especially proximal tubule function (Abd-Elbaset et al. 2017; Liu et al. 2020; Talib and AbuKhader 2013). Moreover, elevated creatinine and BUN levels were considered the most important hallmarks of GM-induced renal toxicity (Al-Malki and Sayed 2014; Shehata et al. 2022). Upraised creatinine and BUN levels in this study may be explained by efficient reabsorption of GM in the proximal renal tubule, and GM's accumulation in tubular cells disturbed the renal circulation, reducing the GFR and subsequently raising serum creatinine and BUN levels (Al-Malki and Sayed 2014). On the other hand, apigenin administration caused a noticeable reduction in the serum levels of creatinine and BUN and restored their normal levels as compared to GM-treated rats. The renoprotective effect of apigenin was shown by a reduction in the serum levels of BUN, and creatinine could be due to

Fig. 2 The renal levels of lipid peroxidation, nitric oxide (NO), NO synthase 2 (*Nos2*) expression, glutathione, superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase following apigenin treatment (5 mg kg⁻¹, 10 mg kg⁻¹, and 20 mg kg⁻¹) in rats with gentamicin (GM)-induced nephrotoxicity. Data are expressed as mean ± standard deviation (SD) (*n* = 7). mRNA expression results are expressed as mean ± SD of three assays in duplicate references to *Actb* and represented as fold changes (log2 scale) compared with the mRNA levels of the control group. ^aIt represents the statistical significance relative to that of the control group at *p* < 0.05. ^bIt represents the statistical significance relative to that of the GM-treated group at *p* < 0.05



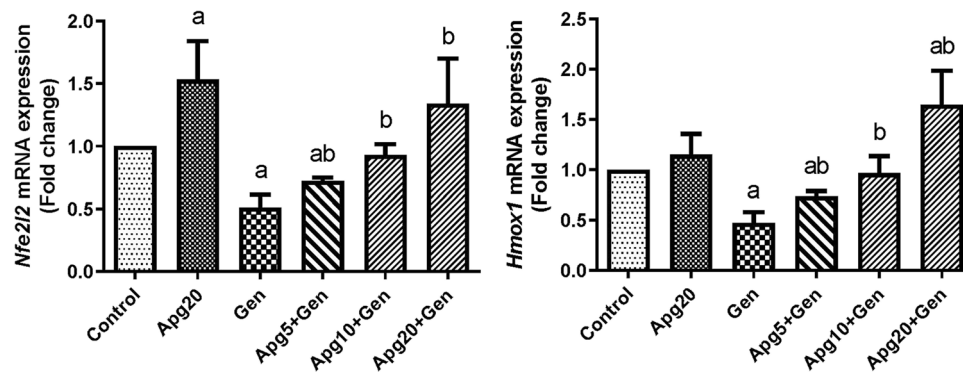


Fig. 3 Renal mRNA expression of nuclear factor-erythroid-2-related factor 2 and heme oxygenase 1 following apigenin treatment (5 mg kg⁻¹, 10 mg kg⁻¹, and 20 mg kg⁻¹) in rats with gentamicin (GM)-induced nephrotoxicity. mRNA expression results are expressed as mean ± standard deviation of three assays in duplicate

references to *Actb* and represented as fold changes (log₂ scale) compared with the mRNA levels of the control group. ^aIt represents the statistical significance relative to that of the control group at $p < 0.05$. ^bIt represents the statistical significance relative to that of the GM-treated group at $p < 0.05$

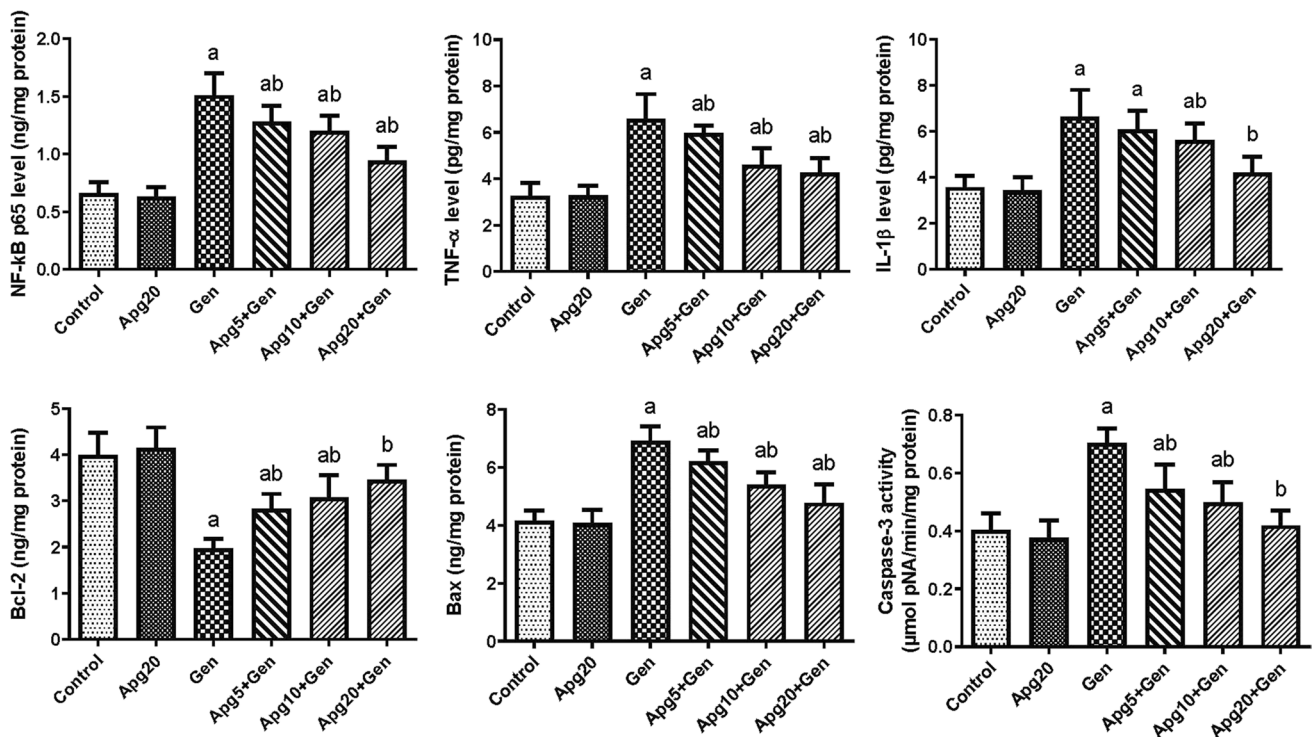


Fig. 4 The renal levels of nuclear factor kappa B p65 subunit, tumor necrosis factor-alpha, interleukin-1β, and apoptotic-related protein levels (Bcl-2, Bax, and caspase-3) following apigenin treatment (5 mg kg⁻¹, 10 mg kg⁻¹, and 20 mg kg⁻¹) in rats with gentamicin

(GM)-induced nephrotoxicity. Data are expressed as mean ± standard deviation ($n = 7$). ^aIt represents the statistical significance relative to that of the control group at $p < 0.05$. ^bIt represents the statistical significance relative to that of the GM-treated group at $p < 0.05$

its antioxidant capabilities, as reactive oxygen species (ROS) might be responsible for reducing GFR (Mansour 2000).

KIM-1 and NGAL are recently considered more specific and sensitive renal injury markers (Tsiola et al. 2017; Yin et al. 2019). KIM-1 elevation may indicate renal damage, as demonstrated by Hansen et al. (2001), who reported that KIM-1 is a membranous glycoprotein unnoticeable in normal

renal tissue or the urine but is overexpressed in the proximal renal tubules after renal injury. Besides, KIM-1 is used for the early sensitive and specific detection of nephrotoxicity induced by chemicals (Al-Brakati et al. 2021a; Albrahim 2020). NGAL is a glycoprotein found at low concentrations in the proximal tubule and neutrophils (Wang et al. 2015). A recent study detected a significant increase in NGAL in

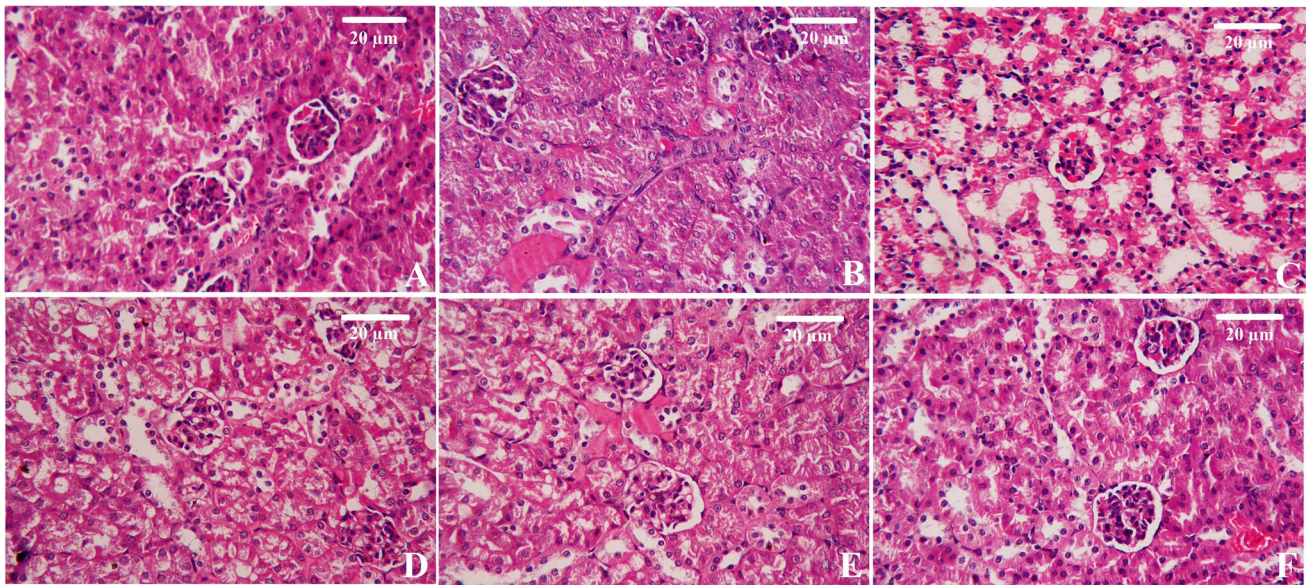


Fig. 5 Photomicrographs of kidney tissues from the control and apigenin-treated groups (A and B, respectively) show a normal kidney structure. In the gentamicin (GM)-treated rats (C), severe inflammation, cytoplasmic vacuolation, severe tubular necrosis and apopto-

sis, and congested glomeruli are shown. Pretreatment with apigenin (5 mg kg^{-1} , 10 mg kg^{-1} , and 20 mg kg^{-1}) (D, E, and F, respectively) markedly attenuated all renal damages caused by GM. Hematoxylin and eosin, scale bar = $20 \mu\text{m}$

the GM-treated group compared with the healthy control group. Similar to our results, a highly expressed NGAL was observed within the thick ascending loop of Henle, distal renal tubule, and collecting tubule of rats and mice with renal toxicity (Zhang and Sun 2015). Likewise, *in vivo* studies have shown that the levels of KIM-1 and NGAL altered before the tubular renal injury, and these changes are coupled with the severe histopathological changes in the renal tubules proposing their efficiency in predicting GM-induced acute renal damage (Shehata et al. 2022).

Cystatin C is a lysosomal enzyme that constrains the degradation of specific proteins within and outside a cell. This enzyme is expressed by all cells, except for nucleated ones, and is formed and carried into the plasma at a stable level in healthy conditions (Choi et al. 2010). In this study, a substantial upregulation of cystatin C was observed in GM-treated rats compared with the healthy control group. These observations conform to the study of Udupa and Prakash (2019), who have attributed this increase to the cellular degradation of the proximal tubules and diminished reabsorptive ability. Furthermore, cystatin C was used for detecting acute damage in the renal tubules and glomeruli in experimental animals, and it was considered a highly sensitive biomarker (Park et al. 2010).

It was indicated that treatment of experimental animals with GM elicited renal dysfunction depending on its accumulation in cells of the renal tubules (Gökce et al. 2016). Several studies have proposed that ROS is known to be the master mediator of GM-induced acute renal injury (Hamdy

and Taha 2009). GM induces nephrotoxicity by overproducing ROS, triggering lipid peroxidation of cytoplasmic membranes and protein degradation. These severe changes consequently resulted in improper enzymatic activation, mitochondrial function, and cellular damage (Al-Malki and Sayed 2014; Shehata et al. 2022; Wen et al. 2017).

NO acts as a signal substance and guardian of cell functions as it plays an important role in maintaining the normal physiology of the kidney by controlling both blood flow in the renal cortex and the function of the renal tubules (Kassab et al. 2021; Korani et al. 2013). In addition, NO regulates signal transduction pathways, cells energetics, host immunoreactions, and the pathology of kidney failure (Gaiser et al. 2013). In this study, the level of NO increased in the kidneys of GM-treated rats, and these results conform to other studies (Darakhshan et al. 2015). In this study, the overproduction of NO may be due to a hyperactive inducible NO synthase (iNOS) that induces NO production (Piao et al. 2011). NO overproduction can induce cell damage by reacting with superoxide anion O_2^- generating cytotoxic peroxynitrite. In other words, NO and peroxynitrite act as key mediators of oxidative stress and pathophysiology in GM-induced nephrotoxicity (Akter et al. 2018). They cause several adverse effects, such as protein degradation, enzymatic inactivation, peroxidation of lipids, disruption of the respiratory chain in cells, and impairment of DNA repair systems (Piao et al. 2011). Our data showed that NO levels decreased after apigenin treatment. This observation was confirmed by Al-Brakati et al. (2021b), who have reported

that flavonoid-derived compounds prevent the expression of *Nos2* and its isoforms, which are responsible for the production of NO and pro-inflammatory cytokines.

The formation of renal LPO in this study conforms to previous studies (Sethi et al. 2008; Wen et al. 2017). LPO increases cytoplasmic membranes' permeability, resulting in ion-exchange imbalance (Sethi et al. 2008). In normal conditions, antioxidant enzymes, such as SOD, CAT, GPx, and GR, can reduce renal damage by scavenging free radicals or ROS and preventing GM-induced apoptosis (El-Ghany et al. 2009; Sethi et al. 2008). In this study, the decreased levels of SOD, CAT, GR, and GPx observed in GM-treated rats may be attributed to GM-induced ROS generation that inhibits the defense mechanisms elicited by these enzymes (El-Ghany et al. 2009; Sethi et al. 2008). GM-induced oxidative injury in this study conforms to those indicated by several studies (Al-Quraishy et al. 2020; Dkhil et al. 2019), which have clarified that disruption between massive free radicals and deficient degradation of these radicals by antioxidant defenders might be the reason for GM-induced renal injury.

Remarkably, the increased LPO levels and decreased antioxidants concentrations were noticeably retained by apigenin treatment. These results conform to several studies (Tiwari et al. 2011; Wang et al. 2014), which have indicated that apigenin exerts a nephroprotective effect by presenting antioxidant properties, allowing it to scavenge several free radicals, preventing LPO formation, and restoring antioxidant enzyme levels to normal.

Heme oxygenase 1 (HO-1) is a rate-limiting protein in the catabolic pathway of heme, which converts into bilirubin by liberating iron and carbon monoxide. HO-1, with other antioxidant enzymes, limits redox imbalance; thus, it is activated to respond to oxidative stress (Abraham and Kappas 2008). *Hmox1* is a target gene of nuclear factor-erythroid-2-related factor 2 (*Nfe2l2* or Nrf2) that regulates its expression (Piao et al. 2011). This regulation can be explained as follows: Nrf2 is considered a cell defender factor that is enhanced in response to ROS through translocation into the nucleus and binding to an antioxidant response element (ARE) that in turn triggers the transcription of *Hmox1* and other antioxidant genes (Mills et al. 2018; Dkhil et al. 2019; Al-Quraishy et al. 2020).

In the same line, Nrf2 is involved in various cellular protective mechanisms, such as mediating antioxidative mechanisms in various renal diseases (Nezu and Suzuki 2020). The upregulation of *Hmox1* and *Nfe2l2* mRNAs after apigenin treatment observed in this study was also observed in rat primary hepatocytes, as shown by Huang et al. (2013), who have reported that the overexpression of these genes could inhibit oxidative stress induced by tert-butyl hydroperoxide. Additionally, Yang et al. (2018a) have indicated that treatment of high-fructose diet-fed mice with apigenin enabled the translocation of *Nfe2l2* into the nucleus and

the subsequent rise of *Hmox1* expression led to the alleviation of oxidative stress. Similar results were observed in ischemic male rats treated with apigenin in a study by Zhang et al. (2019), who concluded that apigenin acts as a powerful antioxidant.

Apoptosis plays a key role in the normal physiology of renal functions and in drug-induced nephrotoxicity, where dysregulation of apoptotic mechanisms results in various renal disorders (Li et al. 2018). Apoptosis is initiated by a cascade of caspase-1, caspase-8, and caspase-9 that trigger caspase-3, whose activation is the key mediator of GM-induced renal cell death (Chen et al. 2011). Bcl-2 is the primary apoptotic inhibitor that is considerably underexpressed in injured renal tissues (Meier et al. 2000).

Currently, the GM-treated group displayed an elevation in caspase-3 and Bax levels and a decrease in Bcl-2 levels; our data conform to other studies (Liu et al. 2020; Yang et al. 2018b). Apoptosis detected in GM-treated rats in this study may be due to several mechanisms, including the accumulated GM within the endoplasmic reticulum preventing RNA translation into proteins and constrained the posttranslational processing of proteins (Horibe et al. 2004). These mysterious events put the endoplasmic reticulum under stress and stimulated cell death (Peyrou et al. 2007). Additionally, GM may interfere with the transport function of some transmembrane proteins, such as the sodium–potassium pump (Sassen et al. 2006), resulting in improper tubular reabsorption, cell swelling, and substantial necrosis or programmed cell death (Sriram et al. 2012).

In addition, apoptosis observed after GM administration in this study may be due to the overproduction of ROS, as was indicated in other studies (Bustos et al. 2016; Kim et al. 2010) that recorded a significant relationship between oxidative stress and the induction and progression of renal cell death in many experimental animals. This relationship can be explained as follows: GM-induced ROS can activate intrinsic pathways of mitochondria by disturbing cellular respiration machinery and reducing ATP formation and trigger the liberation of cytochrome C and the overexpression of other pro-apoptotic proteins, such as Bax, causing cleavage of key proteins, nuclear envelope, and DNA, resulting in apoptosis (Bustos et al. 2016). Importantly, in this study, apigenin could attenuate GM-induced apoptosis by decreasing renal caspase-3 and Bax levels and increasing Bcl-2 levels. Similarly, in vivo studies, apigenin treatment could reverse renal alterations caused by apoptosis in animals with cisplatin-induced nephrotoxicity and renal ischemia–reperfusion injury (Liu et al. 2017; Tiwari et al. 2011).

Cytokines are inflammatory mediators that regulate normal cellular physiology and are associated with tissue damage and repair (Ramesh and Reeves 2004). GM-induced nephrotoxicity has also been associated with the activation and excessive release of inflammatory cytokines,

predominantly IL-1 β and TNF- α (Mahmoud 2017; Salama et al. 2018). In this study, the significant increase in the renal levels of NF κ B-p65 and TNF- α observed in the GM-treated group was also indicated in other studies revealing that NF κ B-p65 overstimulation is accompanied by an aggregation of TNF- α (Darakhshan et al. 2015; Sahu et al. 2014). Additionally, NF- κ B is considered a principal initiator of inflammatory reactions, especially renal inflammation, in GM-induced renal toxicity (Bae et al. 2014). Furthermore, TNF- α is the main regulator of the renal immune response triggered by many drugs by amplifying renal pathophysiological mechanisms elicited by drugs (Fredriksson et al. 2011).

The increased level of IL-1 β recorded in this study was also observed by Bae et al. (2008), who have elucidated that GM activates NF κ B-p65, which subsequently resulted in the overproduction of pro-inflammatory cytokines. The reduction of the protein levels of NF κ B-p65, IL-1 β , and TNF- α after apigenin treatment recorded in this study could be attributed to the anti-inflammatory ability of apigenin, which was also observed in cisplatin-induced nephrotoxicity models in a study by Funakoshi-Tago et al. (2011), who have observed that apigenin could overwhelm the inflammatory response immunomodulation in the kidneys by diminishing NF κ B-p65, IL-1 β , and TNF- α levels.

The anti-inflammatory effect exerted in the apigenin-treated groups may be due to apigenin's capability of interfering with the NF- κ B signaling pathway through several molecular mechanisms, such as direct inhibition of signal transducer and activator of transcription 3, blockage of the phosphorylation and degradation of inhibitor of NF- κ B, and inactivation of inhibitor of NF- κ B kinase (Shukla et al. 2015). These adverse events, in turn, resulted in the inactivation of NF- κ B (Qin et al. 2016), improper translocations to the nucleus, and loss of DNA-binding activity (Wang et al. 2014).

Although TQ administration mitigated AgNPs-mediated renal injury significantly, further mechanistic studies are required to investigate its anti-apoptotic, antioxidant, and anti-inflammatory activities in other organs.

Conclusion

Collectively, apigenin administration normalized kidney index and kidney function markers following GM exposure. Additionally, apigenin prevented renal oxidative damage by decreasing pro-oxidant and enhancing Nrf2 and its downstream antioxidant proteins. Moreover, apigenin suppressed the inflammatory and apoptotic cascades induced by GM. These results suggest that apigenin could use to alleviate the renal impairments associated with GM application.

Acknowledgements The authors thank the Taif University Researchers Supporting Program (Project number: TURSP-2020/151), Taif University, Saudi Arabia, for supporting this study.

Author contribution Animal treatments, molecular and biochemical methodologies were performed by M.M.H., R.B.K., A.E.A., and M.S.L.; histological methodology and investigation were performed by A.A. and A.H.M.; data analysis, software, data curation, and visualization were performed by H.A.A., F.A., K.F.A., and A.T.; writing – reviewing and editing manuscript was performed by M.A., R.S.B., R.B.K., and M.M.H. All authors participated in the design and interpretation of the study and approved the final manuscript.

Data availability All relevant data are within the paper.

Declarations

Ethics approval All procedures were performed according to the ethical principles of the Institutional Animal Care and Use Committee (IACUC) of Helwan University (approval no. HU2019/Z/AER919-01).

Consent to participate Not applicable.

Consent for publication Consented.

Conflict of interest The author declares no competing interest.

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