



Date palm seed extract and herbal mixture mitigate gentamicin-induced renal injury in mice: Role of protease-activated receptors (PARs) and retinoid X receptor alpha (RXR- α)

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ABSTRACT

Introduction: Gentamicin (Gen) causes renal toxicity by inhibiting protein synthesis in kidney cells, causing proximal tubule cell necrosis and renal failure. Herein, we examined the nephroprotective effect of date palm seed extract (DPSE) and one herbal mixture (HM; composed of *Tribulus terrestris*, *Aerva lanata*, *Andrographis paniculata*, and *Raphanus sativus*) against Gen-induced renal toxicity in mice with special reference to the possible role of retinoid X receptor alpha (RXR- α) and protease-activated receptor 2 (PAR-2) in this effect. **Methods:** Thirty-two male Balb/c mice divided randomly into four groups were either treated with saline, Gen (225 mg/kg/i.p., daily from day 3 to day 10), Gen (225 mg/kg i.p.) daily from day 3 to day 10 and DPSE (100 mg/kg/p.o.) daily for 10 days, or Gen (225 mg/kg i.p.) daily from day 3 to day 10 and HM (100 mg/kg/p.o., daily for 10 days). Mice were sacrificed 24 hours after the last dose administration, and kidney tissues were dissected out, weighed, and subjected to histological, immunofluorescence, and biochemical assays.

Results: The Gen-induced renal toxicity group demonstrated a significant decrease in RXR- α and a significant increase in PAR-2 protein expression. Treatment with DPSE or HM significantly improved Gen-induced effects on serum creatinine, blood urea nitrogen (BUN), white blood cells (WBCs), platelets, RXR- α extracellular matrix deposition, and PAR-2.

Conclusion: The present study stated the nephroprotective effects of DPSE and HM and revealed, for the first time, the involvement of retinoid receptors and PAR-2 in Gen-induced renal toxicity as well as in the protective effects of the two tested natural products.

Implication for health policy/practice/research/medical education:

This work provides a comprehensive perception into the possible nephroprotective effect of date palm seed extract (DPSE) and one herbal mixture (HM) against gentamycin (Gen)-induced renal toxicity in mice and the possible role of RXR- α and protease-activated receptor 2 (PAR-2) in this effect. Hence, DPSE is a promising remedy against renal toxicity.

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Introduction

The kidneys are the body organ responsible for health maintaining functions such as preserving stable levels of important molecules in the blood as well as toxin excretion. Although kidneys are efficient in clearing many

toxins from the blood, some chemicals are difficult to eliminate, resulting in their accumulation with subsequent kidney damage (1).

Gentamicin (Gen) is an aminoglycoside antibiotic with broad-spectrum activity against bacteria. Though, it

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was reported that, in up to 30% of Gen-treated patients some signs of renal toxicities are observed with the drug treatment for more than 7 days (2).

Retinoids, found in the liver or other parts of the body, are stored mainly in hepatic stellate cells (HSCs), contributing significantly to cell proliferation and differentiation (3). Retinoic acid receptors (RARs) regulate the transcription of responsive genes as heterodimers with retinoid X receptors (RXR). In contrast, RXRs play a central role in nuclear receptor signaling by forming homodimers or acting as obligatory heterodimerization partners for various nuclear receptors (e.g., RARs, peroxisome proliferator-activated receptors, vitamin D receptors). Cytosolic speckled RAR- α distribution has been observed in activated HSC *in vitro* (4) and *in vivo* in our previous publication (5).

RXR- α is predominantly found in renal tubules but lacks the glomeruli expression (6). Our preliminary data revealed translocation of RXR- α from basolateral into the apical site of distal tubules and collecting duct after monocrotalin/lipopolysaccharide (MCT/LPS) co-treatment. In addition, RXR- α translocation was a tissue factor (TF) dependent response.

The coagulation system's main role is to control the hemostasis and balance thrombus formation (7), in addition to its critical role in inflammation and angiogenesis (8). Various proteins play a part in the coagulation cascade; TF proteins' expression initiates the coagulation cascade till the accumulation of fibrin and clot formation (9). Coagulation factors have a pleiotropic effect by activating protease-activated receptors (PARs) such as activation of PAR-2 through the expression of tissue factor/VIIa complex or factor Xa in the kidney (10,11). There are various reports of exacerbation of glomerular injury by PAR-2 in diabetic kidney disease (DKD) or glomerulonephritis (11), including preeclampsia antiphospholipid syndrome kidney injury models, while its role in Gen-induced kidney injury remains controversial.

Herbal medicines have demonstrated their potential roles in the treatment of various ailments (12,13). Several plants are used in traditional treatments systems for their nephroprotective activity, e.g., ginger, pomegranate seed oil, garlic, etc. However, most of these herbals are noted for nephroprotective effects based on old-age practices. Accordingly, extensive scientific studies are required to evaluate their pharmacological profile (14). The seeds of *Phoenix dactylifera* L. (Family Arecaceae), commonly known as date palm seeds (DPS), are an industrial by-product of date processing, commonly used in some countries as an animal feed or coffee substitute. Although considered a waste product, its high content of polyphenolic compounds suggests its biological potential. Several studies reported its antimicrobial (15), antioxidant (16), and hepatoprotective activity (17). Other reports have demonstrated the nephroprotective effect of date palm's

fruits and pits extracts by significantly reducing plasma creatinine and urea concentrations and ameliorating the proximal tubules' damage (2).

Also, previous studies have demonstrated the effect of polyherbal formulations like Sairie-to and BNO 2103 against Gen- or chromate-induced nephrotoxicity, respectively (18,19). In this context, one herbal mixture (HM) composed of *Tribulus terrestris*, *Aerva lanata*, *Andrographis paniculata*, and *Raphanus sativus* in the ratio of (3:3:3:1) is claimed to have a protective effect against kidney impairment in India. *T. terrestris*, also known as "Qutiba" or "Darisa," belonging to the family Zygophyllaceae, grows in tropical zones and survives in the desert with low-nutrient soil in Saudi Arabia, southern Europe, southern Asia, and Africa. In addition to the various medicinal uses like an aphrodisiac, analgesic, antihypertensive, diuretic, urinary anti-septic, cardiogenic, hepatoprotective, anti-cancer properties (20), and anti-parkinsonism (21), the plant is used locally in Saudi Arabia for urinary infections treatment (22). *T. terrestris* demonstrated significant protection against Gen-induced nephrotoxicity in a rat model through inhibition of blood urea nitrogen (BUN), serum creatinine (SCr), and uric acid (UA) (23). Moreover, in another study, in addition to the reduction in BUN, SCr, and UA, the study also demonstrated a significant increase in total protein (TP) and albumin along with antioxidant potential through a significant increase in catalase and superoxide dismutase as well as a decrease in oxidative stress marker malondialdehyde (MDA) levels. The study also demonstrated a significant decrease in renal biomarkers Beta 2- macroglobulin (β 2M) and kidney injury molecule-1 (KIM-1) and nitric oxide, in addition to the decrease in inflammatory marker IL-6 (24). Similarly, the ethanol extract of *A. lanata* and aqueous extracts of *A. paniculata* and *R. sativus* demonstrated nephroprotection in Gen-induced nephrotoxicity in rats (25-27). The ethanol extract of *A. lanata* demonstrated a significant decrease in BUN, Sr. and Cr. *A. paniculata* plant is used in Ayurveda for various ailments and has demonstrated immunological, antibacterial, anti-inflammatory, antithrombotic, and hepatoprotective properties. In addition, *A. paniculata* aqueous extract demonstrated a significant decrease in BUN, SCr, and UA in the Gen-induced renal toxicity model. Also, the aqueous extract of *R. sativus* demonstrated a significant decrease in BUN, SCr, UA, and oxidative stress marker MDA and increase in TP and albumin.

All the extracts in the HM are known to demonstrate nephroprotection against Gen-induced nephrotoxicity in rats, but this HM's local use for nephroprotection remains to be elucidated. Accordingly, the current study was designed to investigate the potential protective role of DPSE and the HM composed of *T. terrestris*, *A. lanata*, *A. paniculata*, and *R. sativus* in nephrotoxicity as well as to highlight the role of PAR-2 and RXR- α in this protection.

Materials And Methods

Animal

Thirty-two male Balb/c mice (5 ± 1 week old, weighing 30 ± 2 g) were used. Animals were obtained from Qassim University Animal Facility, Qassim, Saudi Arabia, housed at temperature ($25 \pm 0.5^\circ\text{C}$), relative humidity with free access to standard forage and drinking water *ad libitum*. The animals were kept in a pathogen-controlled and air-conditioned room in the animal house.

Chemicals, antibodies, and diagnostic kits

Gentamicin (Gen) was purchased from Mylan (IL, USA). Bovine serum albumin (BSA), DAPI (4, 6-diamidino-2-phenylindole), and Fluoromount were obtained from BIOMARK laboratories (India), horse serum was obtained from Sigma-Aldrich Co. (MI, USA), Dako solution was purchased from Dako (CA, USA). All other chemicals and solvents used were of analytical grade. Mouse monoclonal antibodies against PAR-2 (sc-514363) and RXR- α (sc-28358) were purchased from Santa Cruz Biotechnology (TX, USA). BUN and creatinine diagnostic kits were obtained from Crescent Diagnostic Tests (KSA). Goat anti-rabbit Alexa fluor 488 was purchased from Invitrogen (TX, United States). Cy3- conjugated Goat anti-rabbit antibody was obtained from Jackson ImmunoResearch (PA, USA).

Plant material

The HM was purchased from commercially available HABBA Herbal Pvt. Ltd. (Bangalore, India) and was used as it is. The HM consisted of *Tribulus terrestris*, *Aerva lanata*, *Andrographis paniculata*, and *Raphanus sativus* in the ratio of (3:3:3:1).

Date palm (*Phoenix dactylifera* L.), variety Khodary fruit samples were collected from the Hadhim farm, Qassim region, Saudi Arabia. The identity of the date palm fruit was confirmed by the Ministry of Agriculture. The voucher sample was kept in the college of pharmacy, Qassim University (DPS-3). Date seeds were removed from the fruits, adequately washed with water, dried, and powdered. The seed powder was exhaustively extracted using aqueous methanol (80%), adopting the cold maceration method. The extract was concentrated at 40°C using a rotatory evaporator, and then the concentrated extract was dried in a desiccator over anhydrous sodium sulphate to give a reddish-brown residue.

Experimental design

Mice were randomly classified into four weight-matched groups, each of 8 mice. Group 1: received saline only (control group). Group 2: received Gen only (225 mg/kg, i.p., Gen group). Gen dose was chosen based on our preliminary experiments. Group 3: mice were treated with date palm seed extract (DPSE, 100 mg/kg, P.O) daily for ten days and Gen (225 mg/kg, i.p.) starting from the third day of the experiment and continued for seven days.

Group 4: mice were treated with HM extract (100 mg/kg, P.O) daily for ten days and Gen (225 mg/kg, i.p.) starting from the third day of the experiment and continued for seven days.

Serum preparation

Mice were anesthetized using Thiopental (40 mg/kg, i.p), and blood was taken using a retro-orbital route with a non-heparinized capillary tube into EDTA tubes. Immediate estimation of the total leucocytes count and platelets was performed. Kidney function parameters: SCr and BUN were determined in plasma obtained upon blood samples' centrifugation at 4000 rpm for 20 minutes.

Assessment of hematological parameters (WBCs and Platelets)

According to the manufacturer's instructions, white blood cells (WBCs) and platelet count were performed on whole blood using VABIO360 Auto Hematology Analyzer (BIOTA, Istanbul, Turkey).

Calculation of relative kidney weight

Animals' body weight was determined prior to the sacrifice. The whole kidney tissues were carefully isolated and washed with 0.9% sterile ice-cooled saline to remove any blood from the tissues and then gently pressed between 2 filter papers to absorb the excess saline solution. Afterward, each kidney was weighed, and the relative kidney weight was calculated according to the following equation:

$$\text{Relative kidney weight} = \frac{\text{weight of kidney (gm)}}{\text{Bodyweight of mice (gm)}} \times 100$$

Histopathological study

The kidneys were fixed in Davidson's solution, followed by paraffin embedding, and tissue sections ($4 \mu\text{m}$) were stained with hematoxylin and eosin (H&E) and observed under a light microscope.

Bouin's trichrome staining

Bouin Trichrome Stain Kit was used to identify the extracellular matrix deposition in mice kidney tissues according to the manufacturer's instructions. Kidney tissues were immersed in tap water and transferred to warm Bouin's media (56°C) for 1 hour, then cooled to room temperature for 30 min. Prepared sections were then washed with tap water. Tissues were stained with Weigert's iron hematoxylin solution for 15 min then again washed with water. Finally, sections were stained with Trichrome for 20 minutes, placed in 0.5% acetic acid, and mounted with a mounting solution.

Photography

All photomicrographs were taken utilizing an Olympus (U.TV0.5XC-3) light microscope and digital camera.

Morphometric study

Using ImageJ 22 software (Version 1.52), the following items were detected in 10 non-overlapping fields in each mouse ($\times 400$) for each studied group:

- 1- The mean area percentage of collagen fibers deposition (%).
- 2- Maximal diameter of glomeruli in the mid-cortical region.

Immunofluorescence of tissue sections

Paraffin tissue sections of 4 μm thickness were deparaffinized by incubation of slides in xylene, 2 times for 15 minutes, then rehydrated through a graded ethanol series (2 x 100%, 95%, 70%, 50%, 30%, and distilled water) for 5 minutes each and washed in 10 mM phosphate-buffer 150 mM saline, pH 7.4. Antigen retrieval was performed by incubating the tissue sections in DAKO Target Retrieval Solution (10 mM Na-Citrate pH 6.0) for 20 minutes in a microwave oven (500 W). After being cooled to RT, tissue sections were treated with methanol (100%) for 30 minutes at RT then washed twice with a washing solution (0.05% tween 20/PBS). After blocking the sections with PBS containing 10% horse serum, 1% BSA in PBS for 1 hour, the slides were incubated with the primary antibody for 2 hours at 37°C and then overnight at 4°C. The slides were washed three times for 3 minutes in the washing solution and incubated directly with the fluorescence conjugated secondary antibody for 30 minutes at RT. Then, the slides were washed two times for 3 minutes in the washing solution and incubated with DAPI (diluted 1:5000 in PBS) for 3 minutes. Slides were extensively washed three times for 10 minutes in the washing solution, and the excess washing solution was gently removed. Finally, tissue sections were mounted with fluoromount G. The slides were kept at a 37°C in the oven for 2 hours, and the evaluation was performed by a Zeiss microscope coupled to a 12-bit digital image camera

Statistical analysis

Data analysis was executed by one-way ANOVA with Tukey- Kramer test for multiple comparisons using GraphPad InStat-2. A *P* value of less than 0.05 was considered significant.

Results

Effect of Gen alone or with DPSE or HM on WBCs and platelet counts

In the current study, we observed that the injection of Gen significantly elevated WBCs count and significantly decreased platelets count compared to normal mice (Table 1). Pre-treatment of animals with DPSE or HM significantly decreased WBCs count compared to the Gen-induced renal toxicity group. Furthermore, both treatments renormalized platelets count compared to the Gen-induced renal toxicity mice group (Table 1).

Table 1. Effect of date palm seed extract (DPSE) or herbal mixture (HM) on hematological parameters in Gen-induced renal toxicity of mice

Groups	WBCs ($10^3/\text{mL}$)	Platelets ($10^3/\text{mL}$)
Control	5.38 \pm 0.301	768 \pm 17.9
Gen	11.03 \pm 0.72 ^a	381.06 \pm 29.87 ^a
Gen/DPSE	8.44 \pm 0.56 ^{a,b}	512.04 \pm 17.26 ^a
Gen/HM	6.64 \pm 0.46 ^b	598.03 \pm 19.05 ^b

Abbreviations: WBCs, white blood cells; DPSE, date palm seed extract; HM, herbal mixture; Gen, gentamicin.

Values are expressed as means \pm SEM (n = 6). ^a Significantly different from control group using one-way ANOVA followed by Tukey-Kramer post-test for multiple comparisons (*P* < 0.05). ^b Significantly different from Gen-treated group using one-way analysis of variance (ANOVA) followed by Tukey-Kramer post-test for multiple comparisons (*P* < 0.05).

Effect of Gen in the presence or absence of DPSE or HM on renal function parameters

In the current study, mice treated with gentamycin alone demonstrated significant elevation of Cr, and BUN levels in addition to kidney relative weight, while a significant reduction in the % change in the weight relative to the initial weight was observed compared to the normal mice (Figure 1A-D).

Mice pre-treated with DPSE or HM (100 mg/kg) displayed a significant decrease in serum levels of Cr and BUN as well as kidney relative weight. On the other hand, an increased % change in the weight was observed compared to the Gen-induced renal toxicity group. In addition, no significant difference was observed between mice treated with either DPSE and those treated with HM (Figure 1A-D).

Effect of Gen with or without DPSE or HM in kidney histological examination

The control group showed normal renal tissues, including proximal and distal convoluted tubules. Renal glomeruli comprise renal corpuscles surrounding Bowman's space and capsules that look normal size and normal structure. On the other hand, the Gen-treated group's histopathological structures showed distorted tubules with apoptotic cells lining these tubules. Furthermore, the Gen-treated group exhibited inter-tubular inflammatory cells, widening the inter-tubular spaces with apoptotic cells in renal glomeruli. Additionally, obliterated Bowman's space in renal glomeruli and marked atrophic glomeruli were seen among the sections. The tubules appeared with cytoplasmic vacuolations and apparent dilated tubules with flatted cells and dilated renal blood vessels (Figure 2A, 2B). In contrast, tissues obtained from Gen + DPSE and Gen + HM (100 mg/kg) treated mice demonstrated moderate structural changes; fewer cytoplasmic vacuolations, few atrophic glomerular cells, mild inter-tubular inflammatory cells and widening of the inter-tubular spaces (Figure 2A, 2B).

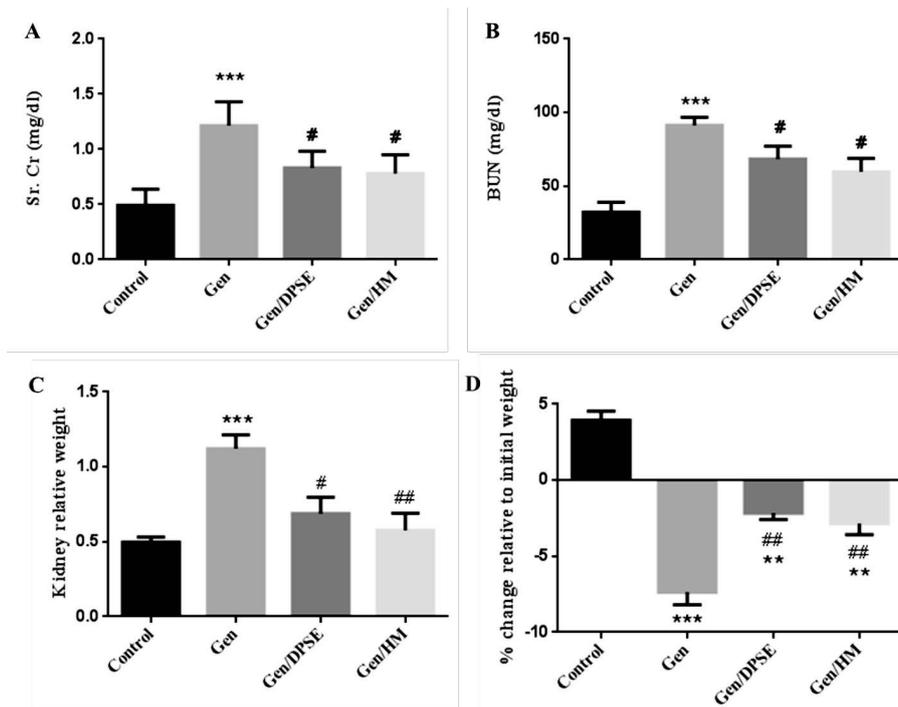


Figure 1. Effect of Gen injection in the presence or absence of DPSE or HM on serum Creatinine (A), serum blood urea nitrogen parameters (B), kidney relative weight (C), and % change relative to initial weight (D). Data are expressed as mean + SEM. ** $P < 0.01$, *** $P < 0.001$ considered statistically significant compared to the control group. # $P < 0.05$, ## $P < 0.01$ considered statistically significant compared to Gen-treated group. Abbreviations: DPSE, date palm seed extract; HM, herbal mixture; Gen, gentamicin.

Effect of Gen and DPSE or HM on kidney fibrogenesis

Sections in the renal cortex from the control group showed fewer collagen fibers surrounding the renal tubules, Bowman's capsules, and the capillary tuft of glomeruli, while gentamicin injection increased collagen deposition

around the dilated blood vessels. On the other hand, Gen + DPSE or Gen + HM (100 mg/kg) treated mice showed fewer collagen fibers around a capillary tuft of glomeruli and blood vessels (Figure 3A, 3B).

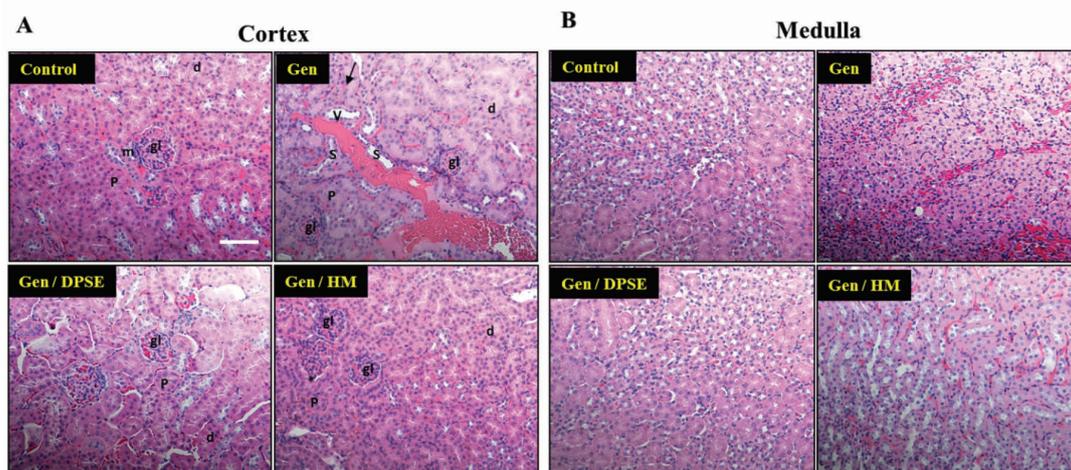


Figure 2. Histopathological examination of the control group's renal tissues, Gen-treated group, Gen + DPSE group, and Gen + HM group. Photomicrographs of tissues in the renal cortex (A) and Medullary tubules (B) from control showing renal glomeruli (gl), proximal convoluted tubules (p), distal convoluted tubules (d), Bowman's spaces (star), and macula densa (m). Gen treated mice showing atrophic glomerulus (gl). The tubule appears with cytoplasmic vacuolations (v), and the dilated tubule appears with flattened cells (arrow). Notice the widening of inter-tubular space (s). The Gen + DPSE group shows less distorted glomerulus (gl) and tubule (d). Less apoptotic cells are shown, and little cytoplasmic vacuolations are seen. Gen + HM group showing less distorted tubule (d) and glomerulus (gl); but with a slight narrowing of renal Bowman's space, fewer apoptotic cells are shown, and little cytoplasmic vacuolations are seen (H&E $\times 200$). Abbreviations: DPSE, date palm seed extract; HM, herbal mixture; Gen, gentamicin.

Effect of Gen with or without DPSE or HM pre-treatment on RXR- α proteins expression

Immunofluorescence staining showed a constitutive protein expression of RXR- α in the glomeruli, distal tubules, proximal tubule, collecting ducts, and early part of Henle's loops in the control group. On the other hand, mice injected with gentamycin alone showed reduced expression of RXR- α in the glomeruli, distal tubules, proximal tubule, collecting ducts, and early part of Henle's

loops compared to control mice. Mice treated with Gen + DPSE or Gen + HM displayed significant restoration in RXR- α protein expression in all renal parts types compared to Gen treated mice (Figure 4A, B, C).

Effect of Gen alone or with DPSE or HM on the expression of PAR-2

We identified negative or basal expression of PAR-2 proteins in renal tubules and glomeruli in control animals

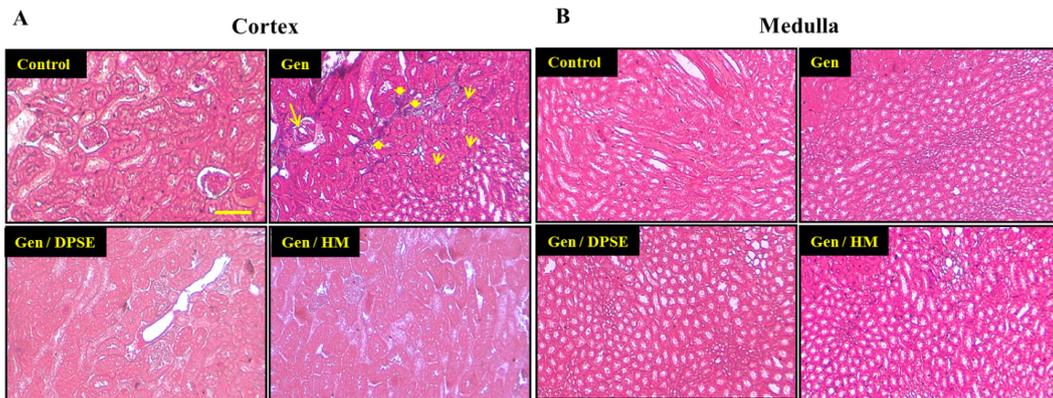


Figure 3. Gomori's trichrome stain renal tissues from control, Gen, Gen + DPSE and Gen + HM treated mice in the cortex (A) and medulla (B) showing collagen deposition in the tubular interstitial spaces (arrowhead), apical part of the distal, proximal tubules (short arrows) and glomeruli (long arrow) in Gen-treated animals compared to control mice. Mice treated with DPSE or HM in Gen's presence showing less collagen deposition than Gen treated mice. The pictures were taken under 200 X magnification. Abbreviations: DPSE, date palm seed extract; HM, herbal mixture; Gen, gentamicin.

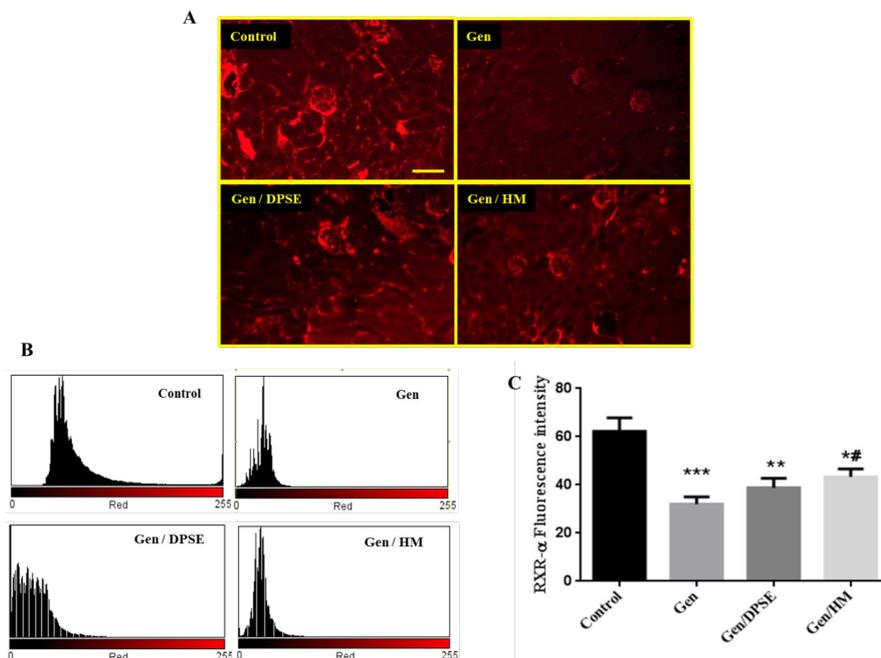


Figure 4. Photomicrographs of kidney sections showing the effect of Gen or DPSE or HM on renal cortex using immunofluorescence analysis showing RXR- α expression (A), histogram showing the effect of treatments on different treated groups (B), and quantitative analysis of immunofluorescence staining of RXR- α (C). The intensity of fluorescence was quantified utilizing Image-J/ NIH software. Values are expressed as mean + SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ considered statistically significant compared to control group. # $P < 0.05$ considered statistically significant compared to Gen-treated group. Abbreviations: DPSE, date palm seed extract; HM, herbal mixture; Gen, gentamicin.

(Figure 5A, B, C). Gen-treated mice displayed a significant increase in the protein expression of PAR-2 in the luminal part of renal tissue tubules. Pre-treating the mice with DPSE or HM decreased PAR-2 protein expression in the basolateral and apical sites of mice kidney sections (Figure 5A, B, C).

Discussion

The kidney's main role is blood filtration as well as water and electrolytes balance. One of the renal dysfunction results is its inability to filter the blood by the glomeruli or the renal tubules' inability to keep water and electrolytes balanced. Although Gen is considered a low-cost and effective antibacterial drug, its clinical use is limited due to its nephrotoxicity (28), which is demonstrated by cortical and medullary tubular toxicities as well as a reduction in glomerular filtration. It was stated that a single dose of Gen caused nephrotoxicity in 10%-25% of patients (29). In the current work, injection of mice with Gen (225 mg/kg, i.p.) for seven days significantly increased the plasma levels of BUN, Cr, and total WBCs, concomitant with a significant decrease in platelets count compared to the control group. These findings confirmed the kidney tubular dysfunction after Gen administration. Several previous studies have similarly concluded that Gen administration increases renal toxicity markers, including serum levels of BUN and Cr (30,31). On the other hand, Gen pre-treated

mice with DPSE or HM significantly decreased BUN, Cr, and total WBCs, and significantly elevated platelets counts compared with the Gen group. These results are in accordance with a previous study that reported the significant effects of date palm's flesh and seed extracts on reducing the elevated plasma levels of urea and Cr in Gen-induced nephrotoxicity. This effect was suggested to be attributed to the antioxidant phytoconstituents of date palm's flesh and seed (2). Considering previous results, that discussed the effect of each of the individual components of HM, *T. terrestris* was previously reported to ameliorate Gen-induced nephrotoxicity (24). *A. lanata* is commonly employed in Siddha system of medicine and is stated to exhibit a marked protective effect against Gen-induced nephrotoxicity in the rat (32,33). Furthermore, the nephroprotective potential of *A. paniculata* and *R. sativus* has been confirmed by several studies (26,27,34,35).

Additionally, the current results demonstrated that Gen caused distorted tubules with apoptotic cells lining these tubules. Furthermore, Gen group exhibited inter-tubular inflammatory cells, widening of the inter-tubular spaces with apoptotic cells in the renal glomeruli. Also, obliterated Bowman's space in the renal glomeruli and marked atrophic glomeruli were also observed. The tubules appeared with cytoplasmic vacuolations, apparent dilated tubules with flatted cells, and dilated renal blood vessels. These findings agree with the previous studies,

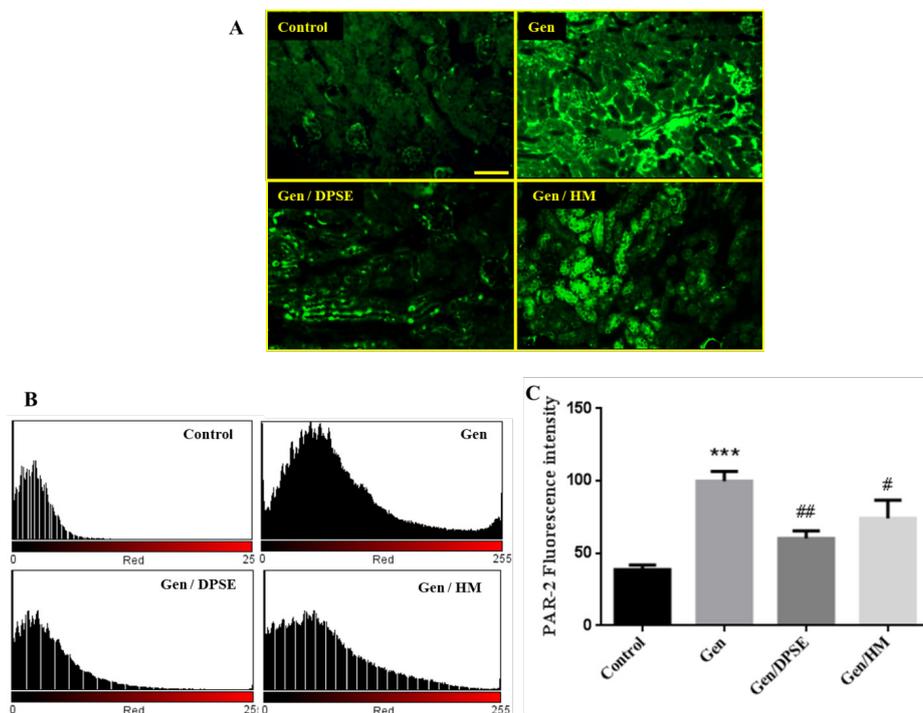


Figure 5: Photomicrographs of kidney sections showing the effect of Gen or DPSE or HM on renal cortex using immunofluorescence analysis showing PAR-2 expression (A), histogram showing the effect of treatments on different treated groups (B), and quantitative analysis of immunofluorescence staining of PAR-2 (C). The intensity of fluorescence was quantified utilizing Image-J/ NIH software. Values are expressed as mean \pm SEM. *** $P < 0.001$ considered statistically significant compared to control group. # $P < 0.05$, ## $P < 0.01$ considered statistically significant compared to Gen-treated group. Abbreviations: DPSE, date palm seed extract; HM, herbal mixture; Gen, gentamicin.

which reported elevated renal toxicity biomarkers levels as well as massive damage to glomerular and tubular structure upon treatment with Gen (100 or 200 mg/kg, i.p.) (36,37). In this regard, pre-treatment with DPSE or HM markedly improved the obliterated histopathological features distorted by Gen alone. These findings add more evidence for the possible nephroprotective effect of DPSE and HM. Few previous studies concluded similar findings of *T. terrestris* and *A. lanata* on histopathological features (24,32).

Moreover, the current results displayed a significant increase in PAR-2 protein expression in Gen-injected mice compared to the control group. This nicely matches the earlier reports that stated increased expression of PAR-2 in unilateral ureteral obstruction in mouse model (38) and in IgA nephropathy patients (39). Also, PAR-2 gene expression was found to be elevated in the kidney tissues of STZ-treated mice compared to normal rats (40). On the other side, pre-treatment of mice with DPSE or HM showed a significant decrease of PAR-2 compared to the Gen-treated group suggesting that the observed nephroprotective effect of both extracts might be mediated by inhibition of PAR-2. Interestingly, this is the first report showing the involvement of PAR-2 in Gen-induced renal toxicity in mice as well as in the protective effect observed for DPSE and HM.

Retinoids are important agents that keep the cell's regular function, such as a healthy immune system, differentiation, proliferation, normal male and female reproduction (3). Retinoic acid (RA) mediates these activities by binding to a family of nuclear receptors, the retinoid X receptors (RXRs), which involve three isotypes (α , β and γ) that affect transcription of several genes during vertebrate development (41). No report was found discussing the possible role of RXR- α in Gen-induced nephrotoxicity. However, a previous report revealed the loss of RXR- α from renal tubules in MCT/LPS-induced renal injury and concluded that RXR- α and the coagulation factor, TF, may be considered as important regulatory mediators in MCT/LPS renal toxicity (42). Herein, results showed a significant reduction in RXR- α protein expression in Gen-treated mice compared to its constitutive expression in the cortical and medullary tubules and the glomeruli of the control mice. Mice groups pretreated with DPSE or HM exhibited a significant increase of RXR-proteins expression compared to the Gen-only treated group. These findings added another proof for the nephroprotective effect of DPSE and HM. Notably, this is the first report discussing the effect of DPSE or HM on RXR- α proteins expression in renal toxicity.

Plants have already been stated as a generous and renewable source of medicinally useful agents due to their astonishing metabolic system. Accordingly, the recorded protective effect of the employed extracts was suggested to be attributable to their rich metabolic content, especially of polyphenolic compounds.

Conclusion

The current study displayed that the elevation of PAR-2 and reduction of RXR- α protein expression have an essential role in developing nephrotoxicity after Gen administration. Moreover, DPSE and HM extracts could significantly reduce Gen-induced nephrotoxicity in mice. This effect might be attributable to the valuable secondary metabolites content of the tested extracts. Hence, more phytochemical studies are recommended to address the main phytoconstituents responsible for the observed effect.

Authors' contributions

SS: Methodology, data analysis, writing, and original draft. SAAM: Writing, editing, and supervising the manuscript. EA: Methodology, writing, manuscript & editing the manuscript. HMA: Methodology, formal analysis, and Investigation. MSAB: Conceptualization, methodology, writing, editing, and supervision.

Conflict of interests

The authors declare no conflict of interest.

Ethical considerations

The institutional Research Ethics Committee, College of Pharmacy, Qassim University, Saudi Arabia, approved the animal experimental procedure and care (Approval ID 2020 - CP- 2). All practical experiments were carried out according to NIH Guidelines for the Care and Use of Laboratory Animals.

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