Gingerol Fraction from *Zingiber officinale* Protects Against Gentamicin-Induced Nephrotoxicity.


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Running Head: Gingerol Protects Against Gentamicin Nephrotoxicity

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ABSTRACT

Nephrotoxicity is the main complication of gentamicin (GM) treatment. GM induces renal damage by overproduction of reactive oxygen species and inflammation in proximal tubular cells. Phenolic compounds from ginger, called gingerols, have demonstrated to have antioxidant and anti-inflammatory effects. We investigated if the oral treatment with an enriched solution of gingerols (GF) would promote a nephroprotective effect using an animal nephropathy-model. Six groups of male Wistar rats were studied: 1) Control group (CT), 2) gingerol solution control (GF), 3) gentamicin treatment (GM) - 100 mg/kg i.p, 4-6) gentamicin plus GF at doses of 6.25, 12.5 and 25 mg/kg (GM+GF). Animals from the GM group had a significant decrease in creatinine clearance and higher levels of urinary protein excretion. This was associated with markers of oxidative stress and oxide nitric production. Also, there was activation of the mRNA levels of pro-inflammatory cytokines (TNF-α, IL-1β, IL-2 and INF-γ). The histopathological findings of tubular degeneration and inflammatory cell infiltration reinforced GM-induced nephrotoxicity. All these alterations were attenuated by previous oral treatment with GF. Animals from the GM+GF groups showed amelioration in renal function parameters, reduced lipid peroxidation and nitrosative stress, in addition to an increment in the levels of glutathione (GSH) and superoxide dismutase (SOD) activity. Gingerols also promoted significant reduction in mRNA transcription of TNF-α, IL-2 and INF-γ. These effects were dose-dependent. These results demonstrated that GF promoted a nephroprotective effect on GM-mediated nephropathy by oxidative stress, inflammatory process and renal dysfunction.
1. Introduction

Gentamicin (GM) is a typical aminoglycoside antibiotic agent widely used in clinical practice for the treatment of gram-negative infections (1, 2). However, its use is complicated by a great risk of nephrotoxicity, affecting 10 to 30% of patients, especially after long-term use (3, 4, 5).

In its elimination route, GM accumulates in the renal proximal tubular cells through the megalin/cubilin complex receptor, responsible by GM transportation inside the cell. Aminoglycoside-induced nephrotoxicity is characterized by tubular cell apoptosis and/or necrosis, predominantly in the proximal tubules (3, 6). Clinically, it results in acute kidney injury (AKI) with elevation of serum creatinine (SCr) and urea, in addition to proteinuria (5, 7). Pathological findings can be seen as proximal tubular edema, tubular necrosis and desquamation, as well as inflammation and diffuse interstitial edema.

Several studies have shown the involvement of reactive oxygen species (ROS) in gentamicin-induced AKI. Gentamicin has been shown to enhance generation of superoxide anion (O$_2^-$), peroxynitrite anion (ONOO$^-$), hydrogen peroxide (H$_2$O$_2$), and hydroxyl radical (OH) production from renal cortical mitochondria, which is associated with an increase in lipid peroxidation and decrease in antioxidant enzymes (8, 9). These ROS act directly on cells affecting their structure and functionality (2, 5).

Several phytotherapeutic agents have been used to prevent or ameliorate GM-induced AKI (5, 10, 11, 12), including Ginger extract. Ginger (Zingiber officinale) is one of the world’s best known spices cultivated in several countries. It has been used since antiquity due to its health benefits (13). Extracts obtained from its roots usually contain polyphenol compounds, such as [6]-gingerol, [8]-gingerol and [10]-gingerol,
which have been cited as the main components responsible for its pharmacological effects (14, 15). Among other potential mechanisms, Ginger has antioxidant (15, 16, 17) and anti-inflammatory properties (15, 18, 19).

Ginger has shown to protect against renal ischemia-reperfusion injury and cisplatin-related nephrotoxicity (20, 21). In the former one, its use has been associated with an antiapoptotic effect. However, ginger anti-inflammatory effects on acute nephrotoxicity have been scarcely studied. In the present study, we aimed to evaluate the effects of a gingerol-enriched fraction in a model of gentamicin-induced nephrotoxicity, focusing mainly on gentamicin oxidative and inflammatory effects (15, 18).

2. Methods

Animals and experimental drug

Experiments were performed with male Wistar rats weighing 240-280 g. The animals were housed under standard laboratory conditions, maintained on a 12-hour light and dark cycle and had free access to food (Biotec®, Agua Fria, Santa Catarina – Brazil) and water. The experimental protocols and all procedures were conducted according to the norms of the National Council for Control of Animal Experimentation (CONCEA), approved by the Ethics Committee of Animal Research of the Federal University of Ceará, Fortaleza-Brazil (protocol 68/12).

Gentamicin (GM) was purchased from Sigma® and administered intraperitoneally (i.p) for seven days. The GM solution was prepared so that the maximum injected amount was 0.5–0.6 mL/rat.
Gingerol-Enriched Fraction (GF)

Nine grams of ginger extract obtained from fresh ginger rhizomes were fractionated into 14 fractions (F1-F14) by column chromatography (18.0 x 4.2 cm i.d.) on silica gel (70-230 mesh) with gradients of n-hexane/ethyl acetate. After HPLC analysis the gingerols were identified on F3 (gingerol fractions-GF). The standard gingerols used in the analysis had already been isolated and identified in previous studies (22). The gingerols present in the GF were quantified on a semipreparative HPLC system through a sample injection (50 mg of GF), collection of the fractions that contained each gingerol and further weighing (22). The GF contained: 41.7% of [6]-gingerol (the main ginger compound), 4.8% of [8]-gingerol, 2.4% of [10]-gingerol and 51.1% of other minor compounds. GF was diluted in a 2% Tween-80 solution and orally administered in a maximum amount of 2.5 – 3.5 mL per rat.

Experimental design

Animals were assigned to 6 different groups containing 7-8 animals each. The sham group (CT) received 0.4 mL i.p injections of saline solution (0.9%) for 7 consecutive days, plus oral treatment with 2% Tween-80 solution in the last 5 days. The second group (GF) also received 0.4 mL i.p injection of saline for 7 consecutive days and five days of gingerol-enriched solution (GF - 25 mg/Kg) through oral route. Another group (GM) received i.p injections of GM (100 mg/Kg) for 7 consecutive days (23) and oral treatment with 2% Tween-80 solution for 5 days. Finally, three other groups (GM+GF) received i.p injections of GM (100 mg/Kg) for 7 consecutive days and three different doses of GF (6.25, 12.5 or 25 mg/kg) for 5 days. The oral administration of saline or GF was always given on the fifth day after the first GM injection.
Evaluation of renal functions

The rats were kept individually in metabolic cages and the urine was collected for a 24-h period after the last oral administration. The animals were anesthetized and a blood sample was obtained from the abdominal aorta. Blood plasma was separated for biochemical measurements. The left kidney was removed and immediately stored at -80 °C. The right kidney was stored in 10% formalin for the histological studies. Plasma and urine samples were used for the analysis of urea, uric acid, urinary calcium and protein by standard diagnostic kits (Labtest, Fortaleza- Brasil). Creatinine was also measured spectrophotometrically in order to calculate its clearance (ClCr) and used to evaluate renal function.

Oxidative Stress Measurement

We investigated oxidative damage through the malondialdehyde (MDA) content, which is an indicative measurement of lipid peroxidation. MDA was assayed as thiobarbituric acid-reactive substances (TBARS). The reaction mixture consisted of 10% renal tissue homogenate solution added of 1% phosphoric acid (H3PO4), plus a 0.6% solution of thiobarbituric acid. The mixture of these reagents was maintained at 95 °C for 45 minutes. The mixture was cooled in running water, and n-butanol was added. The tube was vortexed for 1 min and centrifuged at 294 x g for 15 min. After centrifugation, the organic phase was removed to perform a spectrophotometer reading (520-535 nm). Protein concentration was measured using the modified Bradford method (24).
**Nitrite Measurement**

The stable nitrite levels were measured as an indirect method to detect nitric oxide using Griess reagent, based on a colorimetric assay as described by Green et al. (25). Renal tissue homogenate (100 μL) was mixed with 100 μL Griess reagent, which consists of [0.1% N-(1-naphthyl) ethylenediamine dihydrochloride and 1% sulfanilamide in 5% phosphoric acid]. After 10 minutes, absorbance was read at 540 nm. A standard curve was obtained using sodium nitrite.

**Reduced Glutathione**

Reduced glutathione (GSH) content in renal tissue was estimated according to the method described by Sedlak and Lindsay (26) with few modifications. For the GSH assay, each kidney was homogenized in ice-cold 0.02 M EDTA solution. Aliquots (400 μL) of tissue homogenate were mixed with 320 μL of distilled water and 80 μL of 50% (w/v) trichloroacetic acid in glass tubes and centrifuged at 3000 x g for 15 min. Supernatants (400 μL) were mixed with 800 μL Tris buffer (0.4 M, pH 8.9), and 20 μL of 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB; 0.01 M) were added. After shaking the reaction mixture, the absorbance was measured at 412 nm within 5 min of 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB) addition against a blank with no homogenate. The absorbance values were extrapolated from a glutathione standard curve and GSH was expressed in grams of GSH per milligrams of protein.

**Superoxide Dismutase (SOD) Activity**

SOD activity was measured according to Sun et al. (27). The activity of this enzyme was evaluated by measuring its capacity to inhibit the photochemical reduction
of nitroblue tetrazolium (NBT). In this assay, the photochemical reduction of riboflavin generates O₂ that reduces the NBT to produce formazan salt, which has maximal absorbance at 560 nm. In the presence of SOD, the reduction of NBT is inhibited, as the enzyme converts the superoxide radical to peroxide. The results were expressed as the amount of SOD needed to inhibit the rate of NBT reduction by 50% in units of the enzyme per gram of protein. Homogenates (10% of tissue in buffer phosphate) were centrifuged (10 min, 2,608 x g, 4 °C), and the supernatant was removed and centrifuged a second time (20 min, 15,294 x g, 4 °C). The supernatant was then assayed. In a dark chamber, 1 mL of the reactant (50 mM phosphate buffer, 100 nM EDTA and 13 mM l-
-methionine, pH 7.8) was mixed with 30 μL of the sample, 150 μL of NBT (75 μM) and 300 μL of riboflavin (2 μM). The tubes containing the resulting solution were exposed to fluorescent light bulbs (15 W) for 15 min. and then read using a spectrophotometer at 560 nm.

**Inflammatory Status**

Gene expression of TNF-α, IL-1β, IL-2 and INF-γ was assayed using the CFX96 Touch Detection System (Bio-Rad, USA). Tyrosine 3-monooxygenase/tryptophan 5-
-monooxygenase activation protein zeta polypeptide (YWHAZ) was used as the reference gene (28). DNA primers for all genes (Table 1) were designed on the basis of mRNA sequences obtained from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov; accessed on February 4, 2011).

Real-time PCR assays were performed in a final volume of 25 μL containing 12.5 μL iQ SYBR Green Supermix (Bio-Rad, Hercules, USA), 200 nM primers, and 1 μL cDNA of the samples. Negative samples were also tested, with the cDNA being
replaced with autoclaved Milli-Q water. The PCR conditions were as follows: an initial
denaturation period of 3 min at 95°C, followed by 40 cycles for gene amplification.
Each cycle consisted of an initial denaturation step of 20 s at 95°C, followed by
annealing of 20 s at 60°C and extension of 45 s at 72°C. The samples were then
subjected to an extension step of 3 min at 72°C.

To measure the specificity of the applied amplifications (i.e., to determine
whether the formed products were specific for the tested genes), we performed a
melting curve analysis, in which the reaction temperature was subsequently increased
by 0.5 °C every 15 s, beginning at the annealing temperature of the tested set of primers
and ending at 95°C. Throughout the curve construction process, the changes in
fluorescence were measured, and the data obtained with the CFX Manager Software
(version 3.0; Bio-Rad) were based on the values of the threshold cycle, in which the
observed fluorescence is 10-fold higher than the basal fluorescence for each qPCR
assay. Gene expression was obtained by applying the mathematical method of 2-△△Ct
(29).

Histological and morphological analyses

The right kidneys were fixed with paraformaldehyde. Next, they were
dehydrated with increasing concentrations of 70% ethanol and processed in paraffin.
The resulting blocks were sliced in 5-μm thick sections, stained with HE and observed
under a light microscope (400×).

Statistical analysis

Values were expressed as mean ± SEM for statistical analysis. A one-way
analysis of variance (ANOVA), followed by Student–Newman–Keul’s post hoc test was
used for parametric data. Gene expression data was evaluated by Mann-Whitney test. P
values less than 0.05 were considered significant. Analysis was performed using
GraphPad Prism 5.0.

3. Results

Effect of GF on Gentamicin-induced AKI

The GM group had a significant reduction in ClCr in comparison with both CT
and GF groups. This ClCr reduction was accompanied by an increase in uric acid levels
and urine protein excretion rate – see table 2. There was a progressive amelioration of
ClCr and SCr reduction in the GM+GF groups with statistical significance at 12.5 and
25 mg/Kg doses. The 25mg/Kg dose appears to confer better protection, so it was used
in some experiments to explore GF-protective effects.

Gentamicin-induced Lipid Peroxidation is attenuated by Gingerols

Gentamicin treatment increased MDA levels in renal tissue when compared to
control group. In animals treated with GM+GF, there was a progressive reduction in
MDA levels as GF doses were increased. At the dose of 25 mg/kg, kidney MDA levels
in GM+GF group were similar to control group – figure 1. Without GM-induced renal
injury, GF alone had no effect on kidney MDA levels.

Effect of GF on Gentamicin-induced Nitrosative Stress

The serum nitrite levels were significantly elevated in kidney tissue by GM-
administration. Animals in the GM+GF25 group returned to control (CT) values of
serum nitrite levels – figure 2.
Protective Effect of Gingerols is Associated with Increase Antioxidant Enzyme Activity

The seven consecutive days of gentamicin treatment significantly reduced the levels of reduced glutathione (GSH) (Figure 3) and superoxide dismutase (SOD) activity (Figure 4). This reduction was significantly attenuated by oral treatment with GF at the dose of 25 mg/kg. GF alone, at the dose of 25 mg/kg, did not alter these parameters.

Gingerol Protective Effects are Associated with Reduced Inflammatory Gene Expression

To further explore GF protection-related mechanisms, we studied the expression of four mRNA belonging to the innate and adaptive immune response in kidney tissue samples (TNF-α, IL-1β, IL-2 and INF-γ). As stated previously, only one group taking GM and GF was evaluated (GM+GF25). In fact, TNF-α, IL-2, INF-γ and IL-1β expression increased after GM-administration (Figure 5) when compared to control (CTs, P<0.05). Animals in the GM+GF25 group had significant reduction in the expression of these genes only for TNF-α and INF-γ. However, there was a tendency in the mRNA expression of IL-1β and IL-2 to be blocked after GF treatment, but there was no statistical significance. Again, GF did not modify gene expression in animals not treated with GM.

Morphological analyses

In accordance with clearance experiments, morphological analyses demonstrated that GM+GF group had less severe hydropic degeneration of the proximal tubular epithelium than GM group. Also, inflammatory infiltrate was more often found in GM-treated animals than in GM+GF group – figure-6.
4. Discussion

In the present study, we have demonstrated the protective effects of gingerols in a gentamicin-induced nephrotoxicity model. Attenuation of GM-induced ClCr reduction by gingerols was associated with reduced oxidative stress, reduced nitric oxide metabolites and reduced gene expression of important inflammatory cytokines. Previously, ginger extract has been only fairly evaluated in GM-induced nephrotoxicity with incomplete results (i.e. no data about glomerular filtration rate – CrCl – or the mechanism associated with this protection) (30).

In a recent report, about 30% of patients treated with GM for more than 7 days showed some sign of nephrotoxicity (4, 31). The severe complications resulting from GM-induced nephropathy are limiting factors for its clinical use (32, 33). Gentamicin-induced kidney injury is characterized by renal tubular epithelial cell necrosis, apoptosis, inflammatory response, oxidative stress, and vascular contraction (34, 35).

Gingerols are the most abundant pungent compounds in fresh ginger roots. These phenolic compounds have been cited in several studies as the main responsible for the pharmacological effects of ginger, with the most abundant being 6-gingerol (36). These compounds have, among others, anti-inflammatory and anti-oxidant properties. These effects have been demonstrated in various organs submitted to diverse injury models. In renal tissue, it has been studied in ischemia-reperfusion injury, cisplatin nephrotoxicity, renal damage caused by carbon tetrachloride and diabetic nephropathy (37, 38, 39, 40).

In our model of GM-induced nephrotoxicity, the beneficial effects of gingerols were remarkable, in which almost complete restoration of ClCr was found using a GF dose of 25mg/Kg. This prevention of ClCr decrease was accompanied by less cell
damage, as seen in the histological analysis and by a reduction in protein excretion rate. In proximal tubular damage, the renal tubule capacity to reabsorb normally filtered low-weight proteins is impaired. The almost normalization of urine protein excretion seen in animals treated with GF is a marker of functional preservation of this tubular segment.

In the present study, we aimed to study several mechanism associated with gingerol-induced nephroprotection. According to previous reports on the anti-oxidant effects of ginger extract, rats receiving GM and gingerols show a reduction in GM-induced oxidative stress. Lipid peroxidation is an initial event in the GM-induced nephrotoxicity injury cascade. This view is supported by an increase in MDA level, an index of LPO, depletion of kidney GSH content and decrease in SOD. In a recent study (41), aminoglycoside and other bactericidal antibiotics induced oxidative damage to DNA, proteins and membrane lipids. The antioxidant N-acetyl-L-cysteine ameliorates the oxidative stress-related effects. Oxidative substances have been implicated in GM-associated mitochondrial lesions in renal proximal tubular cells, being the possible mechanism through which gingerol and other anti-oxidative substances ameliorate GM-induced nephrotoxicity. Other substances with antioxidant properties have been tested in aminoglycoside-induced nephrotoxicity (42, 43, 44). In the present study, we used a model that approximates from clinical practice: in vivo study, protective substance administration was initiated after toxic agent; and functional parameters with clinical relevance (ClCr) were evaluated besides histopathology, inflammatory gene expression and oxidative status.

Vasodilator effects of NO appear to play a role in GM-induced nephrotoxicity (45). Moreover, NO seems to increase renal injury through its reaction with superoxide radical (O$_2^-$), generating the very cytotoxic peroxynitrite (46). However, the detrimental effect of NO in GM-induced nephrotoxicity appears to be mediated only by inducible
oxide nitric-synthase (iNOS) and not by its constitutive form (eNOS). While treatment
with a specific inhibitor of iNOS, aminoguanidine, reduces GM-induced oxidative
damage, eNOS inhibition can indeed aggravate it (47, 48). Unfortunately, we were not
able to determine which NOS form was attenuated by gingerol administration. We
suggest that its inhibitory effect is similar to that attributed to [6]-gingerol, as it
inhibited NO production in LPS-activated J774.1 macrophages and reduced iNOS
protein levels in these cells (49). Reduction of NO synthesis can ameliorate GM-
induced nephrotoxicity, by itself, but it can also act through the reduction of oxidative
stress caused by gingerols.

An increased or unbalanced ROS production and oxidative stress mediate the
inflammatory response unleashed by GM. Superoxide anion and hydrogen peroxide
activate nuclear factor NF-KappaB (NFκB) (6, 50), a key mediator of several
inflammatory pathways, which induces the expression of pro-inflammatory cytokines
and iNOS (51,52,53).

Gentamicin was associated with an increase in inflammatory gene expression of
TNF-α, IL-2, IL-1β and INF-γ. This up regulation was attenuated by gingerols, except
for IL-1β and IL-2, in which there was no statistical significance. It has been previously
demonstrated that gingerols can inhibit TNF-α expression in liver, nervous system and
others (16, 54). Also, it is known that gingerols reduce the expression of IL-2 and INF-γ
by T-cells (55, 56). These GM-induced inflammatory molecules participate in the
pathogenesis of tubulointerstitial impairment through the promotion of leukocyte
attraction and adhesion to inflamed renal tubular cells. In our study, the reduction in
inflammatory gene expression is in accordance with the reduced inflammatory infiltrate
seen in GM+GF25 animals.
Our data demonstrate a dose-dependent protective effect in all evaluated parameters. Although we were unable to determine low or up threshold of dose-effect curve, this relationship is relevant to future clinical studies and to understanding the effects of these substance in oxidative stress. We have used a gingerol extract fraction with a predominance of [6]-gingerol just as reported by others. Although comprising more than 85% of all gingerol administered, we cannot attribute all beneficial effects to [6]-gingerol. Recently, was demonstrated that the less abundant [10]-gingerol and not [6]-gingerol was responsible for the anti-inflammatory effects on neuronal cells (57).

In conclusion, a Gingerol-enriched fraction reduced GM-induced nephrotoxicity and this protection is associated with a reduction in oxidative stress, NO production and inhibition of inflammatory gene expression.

Acknowledgments

Many thanks for The Department of Chemistry at Federal University of Ceara, which provided the GF. We are also thankful for the financial support of CAPES and INCT-IBISAB.
REFERENCES


Table 1. Oligonucleotide sequence of primers used in the qPCR.

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<tr>
<th>Genes</th>
<th>Primer Sequences [5'-3' sense (S) and antisense (AS)]</th>
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| NF-α      | S – GTACCCACTCGTAGCAAAC  
           | AS – AGTTGGTTGTCTTTTGAGATCCATG |
| IL-1β     | S – GACCTGTTCTTTGAGCTGACA  
           | AS – CTCATCTGGACAGCAGGCAAAGTC |
| IL-2      | S – CAAGCAGGCCACAGAATTGA  
           | AS – CCGAGTTCATTTTCAGCGCA     |
| INF-γ     | S – ACAGTAAAGCAAAAAGGATGCA  
           | AS – GCTGGATCTGTTGGGTTGTTTC   |
| YWHAZ     | S – GCTACTTGCTTAGGTGTGCT  
           | AS – TGGCTGTGACTGTCAGGCAAT    |

Table 2. Effect of GF on parameters of overall renal function in GM-induced nephrotoxicity

<table>
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<th>CT</th>
<th>GM</th>
<th>CT-GF (25 mg/kg)</th>
<th>GM + GF (6.25 mg/kg)</th>
<th>GM + GF (12.5 mg/kg)</th>
<th>GM + GF (25 mg/kg)</th>
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<td>Serum creatinine</td>
<td>0.62±0.03</td>
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<td>0.51±0.01#</td>
<td>0.87±0.06</td>
<td>0.78±0.07#</td>
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<tr>
<td>Creatinine clearance</td>
<td>1.02±0.18</td>
<td>0.41±0.08*</td>
<td>0.97±0.1#</td>
<td>0.55±0.08</td>
<td>0.68±0.09</td>
<td>0.88±0.14#</td>
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<td>(ml/min)</td>
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<tr>
<td>Urea (mg/dl)</td>
<td>47.3±2.61</td>
<td>80.6±7.77*</td>
<td>37.9±2.8#</td>
<td>62.5±6.7</td>
<td>53.8±6.9#</td>
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<td>Urinary protein</td>
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<td>93.5±9.28*</td>
<td>44.5±4.82#</td>
<td>78.3±9.93</td>
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<td>(mg/dl)</td>
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<td>3.74±0.52*</td>
<td>1.57±0.29#</td>
<td>2.5±0.5</td>
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Values were expressed as mean ± SEM.*Statistical significance with P < 0.05 as compared to control (CT) and #Statistical significance with P <0.05 as compared to Gentamicin (GM).
Legends

**Figure 1.** Effects of GF on lipid peroxidation (MDA) in the gentamicin-induced nephrotoxicity. The results were expressed as mean ± SEM (n=7). Statistical analysis was performed by ANOVA followed by Newman–Keuls as the post hoc test where * shows a significant difference compared to saline (CT) and # represents a significant difference compared to gentamicin (GM) with P < 0.05.

**Figure 2.** Effect of GF on serum nitrite levels in gentamicin-induced nephrotoxicity. The results were expressed as mean ± SEM (n=6). Statistical analysis was performed by ANOVA followed by Newman–Keuls as the post hoc test where * shows a significant difference compared to saline (CT) and # represents a significant difference compared to gentamicin (GM) with P < 0.05.

**Figure 3.** Effect of GF on the amount of glutathione (GSH) in gentamicin-induced nephrotoxicity. The results were expressed as mean ± SEM (n=7). Statistical analysis was performed by ANOVA followed by Newman–Keuls as the post hoc test where * shows a significant difference compared to saline (CT) and # represents a significant difference compared to gentamicin (GM) with P < 0.05.

**Figure 4.** Effect of GF on the superoxide dismutase (SOD) activity in gentamicin-induced nephrotoxicity. The results were expressed as mean ± SEM (n=7). Statistical analysis was performed by ANOVA followed by Newman–Keuls as the post hoc test where * shows a significant difference compared to saline (CT) and # represent a significant difference compared to gentamicin (GM) with P< 0.05.
Figure 5. Effect of GF on the relative expression of TNF-α IL-1β IL-2- INF-γ α genes in gentamicin-induced nephrotoxicity. Statistical analysis was performed by Mann-Whitney test (n= 6) where * shows a significant difference compared to saline (CT) and # represents a significant difference compared to gentamicin (GM) with P < 0.05.

Figure 6. Photomicrograph depicting sections of kidneys from rats receiving saline (control) (A) GM (Gentamicin 100 mg/kg) (B and C) saline plus oral treatment with GF at a dose of 25 mg/Kg (D) and gentamicin plus GF 25 mg/kg (E and F). There is vacuolar degeneration in proximal tubule cells (B) and inflammatory infiltrate (C) associated with GM-administration. These features were attenuated by GF-concomitant treatment (E and F). (HE ×400).