RESEARCH ARTICLE

Ameliorative Effect of *Eruca sativa* Seeds and Its Rutin on Gentamicin-Induced Nephrotoxicity in Male Rats via Targeting Inflammatory Status, Oxidative Stress and Kidney Injury Molecule-1 (*KIM-1*)/*Cystatin C* Expression

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Abstract

BACKGROUND: Nephrotoxicity of Gentamicin (GM), an important aminoglycoside, is still a serious issue in clinical use. Therefore, natural products are currently being used as an alternate source of medicinal substances by researchers all over the world for new medication molecules. *Eruca sativa* shows several health benefits that appear to be associated with the content of flavonoids. Therefore, the objective of the present study was to evaluate the effect of *E. sativa* seed extract (ESE) and its active flavenol rutin (RUT) in GM-induced nephrotoxicity in adult male rats.

METHODS: The animals were divided into 10 groups: a control group, a groups administered 150 mg/kg body weight (BW) of ESE, a group administered 300 mg/kg BW of ESE, a group administered with 50 mg/kg BW of RUT, a group administered with 100 mg/kg BW of RUT, a GM-nephrotoxic group, and four GM-nephrotoxic groups treated with the same doses of ESE and RUT as previous groups. The treatments were given orally for 4 weeks. Following the treatments animals in all groups were sacrificed. The blood samples were drawn, and the kidney tissue samples were collected for further analysis.

RESULTS: ESE alleviated the nephrotoxic effects of GM as it decreased the serum levels of creatinine, urea, Na^+, K^+, tumor necrosis factor-α (TNF- α), and interleukin-1β (IL-1β). Moreover, ESE was linked with kidney injury molecule-1 (*KIM-1*) and *Cystatin C* mRNA downregulation. Although treatment with pure RUT induced the same modulation of ESE in GM- nephrotoxic rats, pure RUT was more effective than ESE in the modulation of oxidative kidney injury.

CONCLUSION: The present study revealed the health-promoting effects of ESE or RUT in the attenuation of GM-induced nephrotoxicity.

KEYWORDS: nephrotoxicity, gentamicin, *Eruca sativa*, rutin, inflammation, *KIM-1*/*cystatin C* expression

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Introduction

Nephrotoxicity induced by many synthetic drugs represents a serious problem for many populations worldwide. Gentamicin (GM) is a commonly used aminoglycoside antibiotic for treating gram-negative bacterial infections.(1) However, because of the high risk of nephrotoxicity, which affects 10% to 30% of patients, especially after long-term usage, its use is difficult.(2)

GM-induced nephrotoxicity mainly consists of increased renal oxidative stress, renal inflammatory cascades, and increases in associated pathological signaling mechanisms.(3) The underlying mechanisms of GM-induced nephrotoxicity are still unknown. Recent research suggests that GM-induced nephrotoxicity is a complicated

and multidimensional process in which GM stimulates various cellular responses that result in kidney damage and necrosis.(4) As a result, many molecular markers, such as Kidney Injury Molecule-1 (KIM-1) and Cystatin C, are employed to assess kidney injury, as well as inflammatory and oxidative stress markers.(5)

Several treatments and strategies have been attempted to ameliorate GM-induced nephrotoxicity with the major focus on the use of various antioxidant agents, such as the medicinal plants extracts having antioxidant properties. (6-8) Medicinal herbs and plants have played a significant role in preventing and treating kidney diseases in the last decades. In this concern, *Eruca sativa* (rocket) is widely used in folklore medicine to remedy renal ailments. It is a fresh peppery annual plant in the *Brassicaceae* family that is used as an edible leaf vegetable.(9) Many experimental research have proved that *E. sativa* seed extract (ESE) possesses a broad spectrum of biological activities, such as cytoprotective, antioxidant, anticancer, antiulcer, diuretic, and renal protective activities.(10-11) These biological activities may be attributed to its constituents, mainly phenolic compounds that are considered the most abundant antioxidants in medicinal plants and herbs.(12) A considerable amount of evidence suggests that flavonoids may be responsible for some of the protective benefits of phenols, which are well-known antioxidants possessing free radical scavenging and metal chelating activity.(1,13)

Quercetin-derived compounds, such as rutin (quercetin-3-O-rutinoside), was one of the most abundant flavonoids found in the rocket accessions.(14) Rutin (RUT) can be regarded as a non-toxic and non-oxidizable molecule.(15) It is a flavonol glycoside that exhibits several pharmacological activities, such as antioxidant, anticancer, anti-inflammatory, and anti-diabetic activities. It exerts renal protective effects against ischemia reperfusion (I/R)-induced renal injury.(16-18) Thus, the purpose of this research was to evaluate the therapeutic effect of ESE in comparison with its active component RUT on GM-induced nephrotoxicity in male albino rats.

Methods

Drugs and Chemicals

Gentamicin sulfate was purchased from Memphis Company for Pharmaceutical and Chemical Industries (Cairo, Egypt), and RUT (3, 3′, 4′, 5, 7-pentahydroxyflavone-3-rhamnoglucoside), with a purity of 95%, was purchased from Pure Bulk (Green, OR, USA). All the other chemicals used were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA).

Preparation of ESE

E. sativa seeds were obtained from the Agricultural Research Center in Giza, Egypt. *E. sativa* seeds were ground into fine powder and used to prepare aqueous extracts according to the method described in previous publication.(19) A half gram of the fine powder was soaked in 5 mL hot distal water at 50°C for 2 h and then kept in the refrigerator with daily shaking for 5 days. The aqueous extract was obtained via filtration using double layers of gauze to get rid of herb debris and then kept in the refrigerator for further use.

High Performance Liquid Chromatography (HPLC) analysis of ESE

HPLC analysis was carried out according to previous study using Agilent Technologies 1100 series liquid chromatograph equipped with an auto sampler and a diode-array detector.(20) The analytical column was an Eclipse XDB-C18 (150 X 4.6 µm; 5 µm) with a C18 guard column (Phenomenex, Torrance, CA, USA). The mobile phase consisted of acetonitrile (solvent A) and 2% acetic acid in water (v/v) (solvent B). The flow rate was kept at 0.8 mL/min for a total run time of 60 min and the gradient programme was as follows: 100% B to 85% B in 30 min, 85% B to 50% B in 20 min, 50% B to 0% B in 5 min and 0% B to 100% B in 5 min. The injection volume was 50 µL and peaks were monitored simultaneously at 280 and 320 nm for the benzoic acid and cinnamic acid derivatives, respectively as well as 360 nm for flavonoids. All samples were filtered through a 0.45 µm Acrodisc syringe filter before injection. Peaks were identified by congruent retention times and UV spectra and compared with those of the standards.

Animals Models

A total of 60 adult male Wistar albino rats (*Rattus norvegicus*), weighing 120–130 g, were obtained from the Veterinary Serum and Vaccine Research Institute (Abbassia, Cairo, Egypt). The animals were housed in suitable cages and acclimatized to laboratory conditions for 1 week before the commencement of the experiments. They were supplied with fresh tap water and standard rodent food pellets (Agriculture-Industrial Integration Company, Giza, Egypt). The experimental protocol was approved by the Research Ethics Committee of Ain Shams University (No. 4/2013), and the experimental procedures were performed in accordance with the International Guidelines for the Care and Use of Laboratory Animals.

Design of the Experiment

The animals were randomly divided into 10 groups (n=6 in each group). Group [1] served as a control that received intraperitoneal (i.p.) injections of physiological saline. Groups [2] and [3] comprised of ESE-treated rats which orally administered low (150 mg/kg) and high (300 mg/kg) doses of ESE daily for 4 weeks. Groups [4] and [5] comprised of RUT-treated rats which orally administered low (50 mg/kg) and high (100 mg/kg) doses of RUT, respectively, daily for 4 weeks. Group [6] comprised of GM-nephrotoxic rats which injected daily with 100 mg/kg GM i.p. for 7 consecutive days to induce acute nephrotoxicity. (21) Groups [7] and [8] comprised of GM-nephrotoxic rats treated with ESE which has been injected daily with 100 mg/kg GM i.p. for 7 days, and then orally administered low (150 mg/kg) and high (300 mg/kg) doses of ESE daily for 4 weeks. Groups [9] and [10] comprised of GM-nephrotoxic rats treated with RUT which has been injected daily with 100 mg/kg GM i.p. for 7 days, and then orally administered low (50 mg/kg) and high (100 mg/kg) doses of RUT daily for 4 weeks.

Blood and Tissue Sampling

The rats in each group were sacrificed under light anesthesia, the blood samples were drawn, and the serum was centrifuged at 3500 rpm for 15 min. Serum aliquots were stored at –80°C until analysis. Immediately after sacrificing the animals, the kidneys were perfused with excess cold phosphate-buffered saline. The kidney tissue samples were then collected and cleaned, weighted, sliced, wrapped in aluminum foil, and stored at –80°C for estimation of oxidant/antioxidant status and quantitative real-time PCR (qRT-PCR) analysis, as described below.

Renal Function Analysis

The serum levels of creatinine, blood urea nitrogen (BUN), uric acid, and calcium were estimated using a commercial kit (Spectrum Diagnostics, Cairo, Egypt). The serum levels of sodium (Na^+) and potassium (K^+) were chemically determined using indirect ISE analyzer (AVL 9180 Series Electrolyte Analyzers (Roche diagnostics, Mannheim, Germany).

Assessment of Oxidant or Antioxidant Activity in Kidney Tissue

Antioxidant agents included kidney glutathione reduced (GSH) level, catalase (CAT), glutathione S-transferase (GST) activities as well as oxidant agents, including malondialdehyde (MDA) and nitric oxide (NO) levels were performed using commercial assay kits (Biodiagnostic, Egyptian Company for Biotechnology, Cairo, Egypt), following the manufacturer's protocols.

Assessment of Inflammatory Markers in Serum

The serum levels of tumor necrosis factor-alpha (TNF-α) and interleukin 1 beta (IL-1β) were quantified via sandwich enzyme-linked immunosorbent assay using a commercial kit (Cusabio Technology Co. LLC, Houston, TX, USA), according to the manufacturer's protocols.

Quantitative Real-time PCR Analysis of Kidney Injury Molecule-1 (*KIM-1*) and *Cystatin C* Gene Expression in the Kidney Tissue

To assess the mRNA levels of the genes of interest, total RNA from kidney tissue samples was extracted using bioZOL reagent acquired from (BioFlux, Redwood City, CA, USA) and followed the manufacturer's protocols. The A260/A280 ratio was used to determine the purity and concentration of total RNA using a UV microplate reader (FLUOstar OPTIMA). One g of total RNA was reverse transcribed to cDNA via successive incubation with anchored 0.6-m oligo (dT) primer (Thermo Fisher Scientific Inc., Waltham, MA, USA) and RT enzyme (SibEnzyme, Ltd., Academtown, Russia) at 37°C for 10 min, 42°C for 1 h, and 70°C for 10 min using the total RNA template. The cDNA product was kept at −20°C. Reverse transcription was performed on the Biometra thermocycler (Analytik Jena Company, Göttingen, Germany).

Real-time qRT-PCR was performed using an Agilent Mx3005P QPCR system (Agilent Technologies Co., Santa Clara, CA, USA). The expression of target genes was analyzed relative to *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase) levels. The final volume for each qRT-PCR reaction was 25 µL consisting of 12.5 µL of Maxima SYBR Green qPCR Master Mix (Bioline, London, UK) and 100 pmoles of specific primers for *KIM-1* (sense: 5′-AGAGAGAGCAGGAC ACAGGCTT-3′ and antisense: 5′-ACCCGTGGTAGTCC CAAACA-3′), *Cystatin C* (sense: 5′-TCCTCTGCACCGACTCTGTC-3′ and antisense: 5′-CTGCCCTTGTT GTAC TCGCT-3′), and *GAPDH* (sense: 5-TCA AGA AGG TGG TGA AGCAG-3 and antisense 5-AGG TGG AAG AAT GGG AGT TG-3), which were obtained from Sigma-Aldrich.

Cycling was initiated with 95°C for 10 min followed by 40 cycles annealing (15 s at 95°C, 30 s at 60°C) for *KIM-1* and *GAPDH* genes, (15 s at 95°C, 30 s at 58°C) for *Cystatin C* gene, and 72°C extension for 30 s, followed by a melt curve. Triplicates of each sample were examined. The

ΔΔCt (cycle threshold, Ct) method was used to quantify differences in gene expression between the groups, which were normalized to GAPDH and represented as relative mRNA levels compared to controls.

Statistical Analysis

The data are expressed as mean±standard error of means. Statistical analysis was conducted using one-way analysis of variance (Tukey's multiple comparison test) for the significant interrelation between the version groups. GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA, Version 4) was used for the analysis.

Results

HPLC Analysis of ESE

A high- performance liquid chromatographic technique was used to identify and quantify the major phenolic compounds present in the *Eruca sativa* seed aqueous extract (ESE). To this end, a standard mixture solution of phenolic compounds was analyzed. The concentration of rutin (RUT) in the ESE was 4.955 mg/g. Another major phenolic compounds identified were Sinapic, p-hydroxybenzoic, Chlorogenic, and p-coumaric.

Effects of ESE *vs.* RUT on Kidney Function in GM-intoxicated Rats

The i.p. injection of GM at a dose of 100 mg/kg for 7 consecutive days in rats induced nephrotoxicity, as evidenced by significant increases ($p<0.001$) in the serum levels of creatinine and BUN when compared with those of healthy control rats. The doses of both ESE and RUT induced significant decreases ($p<0.001$, creatinine and BUN, respectively) in the elevated levels of creatinine and BUN when compared with those of GM-intoxicated rats, as presented in Figure 1A and 1B. Non-significant changes were reported in the serum levels of uric acid between the different experimental groups (healthy control, GM-intoxicated, and herbal extract- or RUT-treated; Figure 1C).

Daily i.p. injection of GM to rats for 7 days caused significant decreases ($p<0.001$, Na^+ and K^+, respectively) in the serum levels of Na^+ and K^+ electrolytes when compared with those of the healthy control group. Oral administration of ESE or RUT to GM-intoxicated rats caused a significant increase ($p<0.001$, Na^+ and K^+, respectively) in the serum levels of Na^+ and K^+ electrolytes when compared with those of GM-intoxicated rats (Figure 1D and 1E).

Effects of ESE *vs.* RUT on Renal Oxidative Stress Markers in GM-intoxicated Rats

MDA showed a significant increase ($p<0.001$) in the kidney tissue in the nephrotoxic group compared with the normal control. However, the nephrotoxic group treated with a low dose of ESE had a significant decrease in the MDA concentration when compared with the GM-treated group ($p<0.001$). Contrarily, high doses of ESE and pure RUT did not induce any valuable modulation with regard to the MDA concentration as compared with the GM-treated rats (Table 1).

NO, another oxidative stress indicator, showed a significant increase ($p<0.001$) in the GM-intoxicated rats compared with the normal control. However, the nephrotoxic group treated with ESE at high dose had a significant increase in NO concentration compared with the nephrotoxic group ($p<0.01$). Contrarily, the RUT-treated groups at both doses showed a significant decrease in NO concentration compared with the nephrotoxic group ($p<0.001$) (Table 1).

The GSH content significantly increased in the GM-treated group compared with the control group ($p<0.001$). The GSH level of the nephrotoxic groups treated with RUT was significantly higher than that of the GM-intoxicated animals ($p<0.001$), whereas the ESE treatment of GM-nephrotoxic rats increased the GSH level insignificantly as compared with GM-intoxicated group (Table 1). The GST activity was significantly increased in the GM-intoxicated group compared with the control group ($p<0.001$). A non-significant increase in kidney GST and CAT activities was reported between the different experimental groups (GM-intoxicated, and herb- or RUT-treated).

Effect of ESE *vs.* RUT on Serum Levels of TNF-α and IL-1β in GM-intoxicated Rats

The GM-intoxicated group showed a significant increase ($p<0.001$) in the serum levels of TNF-α and IL-1β compared with the control group. Both doses of ESE or RUT significantly modulated ($p<0.001$) the changes in the serum levels of TNF-α and IL-1β in the GM-nephrotoxic rats (Figure 2A and 2B).

Effect of ESE *vs.* RUT on mRNA Expression of Kidney *KIM-1* and *Cystatin C* in GM-intoxicated rats

The results of renal *KIM-1* and *Cystatin C* gene expression are presented in Figure 3A and 3B. The GM-intoxicated group showed a significant upregulation ($p<0.001$) in the mRNA expression of renal *KIM-1* and *Cystatin C* compared with the control group. Conversely, treatment

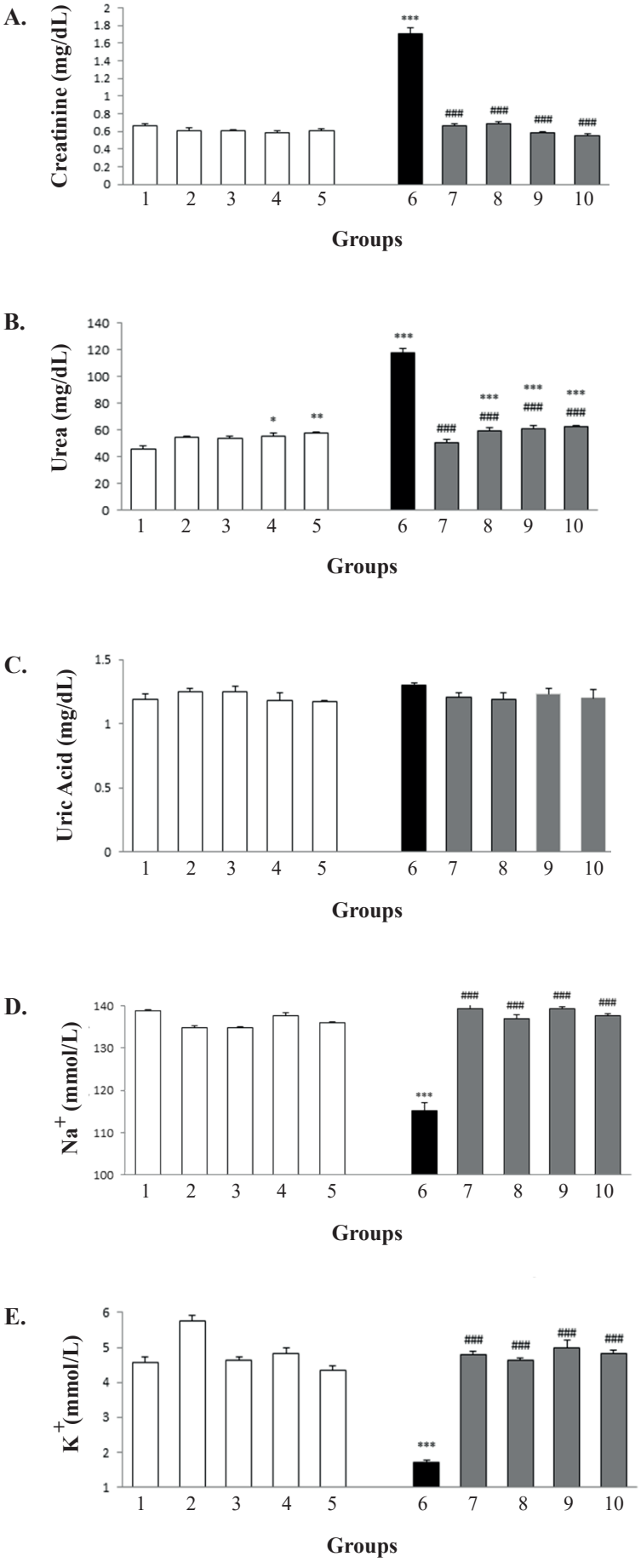

Figure 1. Effect of GM, ESE, and RUT in male albino rats, administered separately. A: serum creatinine level; B: serum urea level, (c) serum uric acid level; D: serum Na^+ concentration; E: serum K^+ concentrations. Data expressed in terms of mean±SEM (n=6). ***(p<0.001): statistically significant compared with control group, ###(p<0.001): statistically significant compared with gentamicin injected group. 1: Control group; 2: ESE 150 mg/kg BW; 3: ESE 300 mg/kg BW; 4; RUT 50 mg/kg BW; 5: RUT 100 mg/kg BW; 6: GM-nephrotoxic rats; 7: GM+ESE 150 mg/kg BW; 8: GM+ESE 300 mg/kg BW; 9: GM+RUT 50 mg/kg BW; 10: GM+RUT 100 mg/kg BW.

Table 1. Oxidative stress markers and antioxidant defense system in kidney tissues of control and GM-nephrotoxic male albino rats treated with either ESE or RUT.

Parameters	Control	ESE		RUT	
		150 mg/kg bw	300 mg/kg bw	50mg/kg bw	100 mg/kg bw
MDA (nmol/g tissue)	123.63±4.02	138.40±2.90	207.89±7.2*	254.62±14.64*	246.04±7.74*
NO (µmol/g tissue)	16.49±0.42	16.34±1.31	15.38±0.56	8.61±0.31*	9.50±0.79*
GSH (mmol/g tissue)	43.60±1.2	38.50±1.6	44.70±1.7	143.00±5.2*	119.30±1.5*
CAT activity (U/g tissue)	8.87±0.06	9.06±0.04	8.96±0.09	9.12±0.03	9.01±0.21
GST activity (U/g tissue)	4.43±0.11	5.81±0.16*	2.93±0.17*	2.42±0.12*	1.11±0.06*

Parameters	GM	GM+ESE		GM+RUT	
	100 mg/kg bw	150 mg/kg bw	300 mg/kg bw	50mg/kg bw	100 mg/kg bw
MDA (nmol/g tissue)	248.69±2.09*	187.83±6.13$^{\#}$	268.92±3.86	268.49±4.30	276.78±5.24
NO (µmol/g tissue)	26.64±0.55*	29.78±0.96	31.03±1.10$^{\$}$	8.13±0.56$^{\#}$	11.31±0.56$^{\#}$
GSH (mmol/g tissue)	65.20±1.2*	75.40±1.7	75.40±1.4	106.20±4.14$^{\#}$	92.30±1.0$^{\#}$
CAT activity (U/g tissue)	9.15±0.05	8.91±0.11	8.86±0.06	9.14±0.04	8.99±0.03
GST activity (U/g tissue)	5.87±0.07*	5.46±0.23	5.26±0.14	5.36±0.27	5.32±0.13

MDA: malondialdehyde; NO: nitric oxide; GSH: reduced glutathione; CAT: catalase; GST: glutathione S-transferase. Data expressed in terms of mean±SEM (n=6). Means were compared by one-way analysis of variance where $p<0.001$ (*) *vs.* control, while $p<0.01$ ($) and $p<0.001$ (#) *vs*. GM-treated group of rats.

of the nephrotoxic group with both doses of ESE or RUT significantly decreased ($p< 0.001$) renal *KIM-1* and *Cystatin C* mRNA expression in the GM-nephrotoxic rats.

Side Effects of ESE *vs.* RUT in Non-nephrotoxic Rats

The high dose of ESE and both doses of RUT significantly decreased ($p<0.001$) the GST activity (Table 1) and significantly increased ($p<0.001$) the renal MDA concentration in non-nephrotoxic rats. In addition, the effects of both doses of ESE on other parameters in non-nephrotoxic rats in the present study were beneficial.

Discussion

Aminoglycoside antibiotics are known to induce significant nephrotoxicity; therefore, their therapeutic applications are restricted.(22) GM, a typical aminoglycoside, is commonly used for gram-negative bacterial infections. A specific GM renal toxicity may be related to its accumulation in the renal proximal tubules.(23) Moreover, GM damages the kidneys by causing structural and functional alterations in the plasma membrane, mitochondria, and lysosomes.(3) As a result, lowering this risk will lead to greater usage of GM.

The current investigation showed that treatment with GM (100 mg/kg, i.p., for 7 consecutive days) was successful in inducing experimental nephrotoxicity in rats by causing a considerable increase in the serum levels of creatinine and BUN, associated with the decreases in the serum levels of Na^+ and K^+ electrolytes. Similarly, various studies reported that treatments with GM induce nephrotoxicity, as evidenced by the deterioration of renal functions, characterized by an increase in the serum levels of creatinine and BUN, accompanied by impairment in glomerular functions. (24,25)

Over the last decade, there has been a lot of attention in preventing aminoglycoside nephrotoxicity. Thus, to modulate the nephrotoxicity effects of GM, the nephrotoxic rats were treated with ESE or RUT. Both ESE and pure RUT improved nephrotoxicity by significantly reducing the serum levels of BUN and creatinine coupled with a significant elevation of the serum levels of Na^+ and K^+. A finding consistent with the results obtained by the previous study, which revealed the ESE's nephroprotective and diuretic properties in rats with GM-induced nephrotoxicity. (26) Another study reported that treatment with RUT (150 mg/kg/day orally) ameliorated nephrotoxicity in GM-induced nephrotoxic rats by reducing the serum levels of creatinine, urate, and BUN.(27) This indicated that ESE or RUT decreased the overall renal damage induced by GM.

Moreover, the most important pathways of GM-induced nephrotoxicity are the production of free radicals and oxidative stress induction. Furthermore, the overproduction of free radicals in conjunction with the depletion of the proximal renal tubule antioxidant potential are the most critical mechanisms of GM-induced nephrotoxicity.

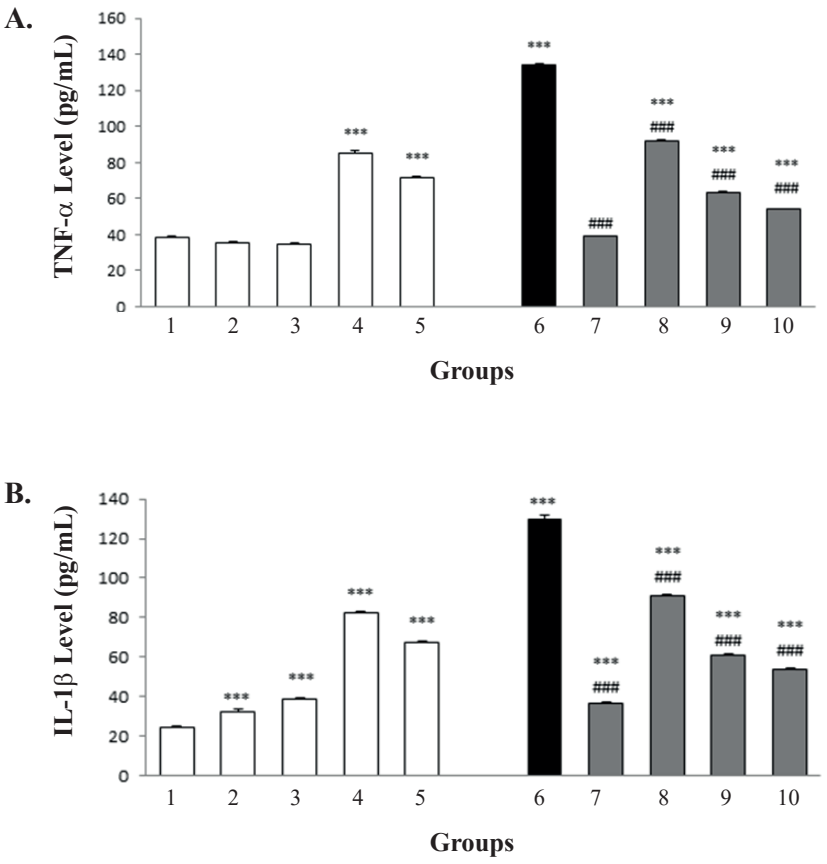

Figure 2 Effect of GM, ESE, and RUT in male albino rats, administered separately. A: Serum TNF-α level; B: Serum IL-1β level. Data expressed in terms of mean±SEM (n=6). ***(p<0.001): statistically significant compared with control group, ###(p<0.001): statistically significant compared with gentamicin injected group.1: Control group; 2: ESE 150 mg/kg BW; 3: ESE 300 mg/kg BW; 4; RUT 50 mg/kg BW; 5: RUT 100 mg/kg BW; 6: GM-nephrotoxic rats; 7: GM+ESE 150 mg/kg BW; 8: GM+ESE 300 mg/kg BW; 9: GM+RUT 50 mg/kg BW; 10: GM+RUT 100 mg/kg BW.

The development of oxidative stress results in lipid peroxidation, which is the first step in the nephrotoxicity injury cascade, as well as tubular damage caused by GM.(22) To support this, we found a significant elevation in the peroxidized lipid decomposition marker MDA after GM administration, indicating renal injury in GM-intoxicated rats. In addition to this, the production of renal NO increased after GM treatment. The findings confirm the hypothesis that oxidative stress was the primary cause of organ damage following GM treatment. Treatment with a low dose of ESE ameliorates the oxidative stress-related effects by reducing lipid peroxidation, whereas a high dose induces the production of renal NO in GM-nephrotoxic rats. It is well known that ESE contains high yields of polyphenolic compounds, including a large class of flavonoids that have a powerful antioxidant capacity, anti-lipid peroxidation, reducing power, and DPPH radical scavenging effect.

Conversely, despite the fact that flavonoids are generally categorized as antioxidants and this activity is commonly associated with beneficial effects on human health, it become observed that, quercetin, kaempferol, and its glycoside extracted from *E. sativa* may be very elusive via acting on different cell types.(28) Moreover, another study showed that a hot aqueous extract of *E. sativa* leaves had some unfavourable effects on kidneys, as evidenced by moderate degenerative alterations in the renal tubules, indicating that this extract has a minor adverse effect on kidneys.(29)

RUT ameliorated GM toxicity by significantly decreasing the production of renal NO and increasing renal GSH levels at both low and high doses. The health benefits of RUT in modulating the NO level in GM-nephrotoxic rats may be due to its antioxidant action and the inhibition of xanthine oxidase enzyme that plays a critical role in NO production in mammalian hypoxic tissue.(30)

Another mode through which GM exerts its nephrotoxicity is by stimulating the generation of pro-inflammatory cytokines in the region around the renal tissues.(31) The current investigation established that GM suggestively augmented TNF-α and IL-1β production in GM-nephrotoxic rats. This finding confirmed that the inflammatory cytokines might be important in the sequence of events leading to GM-induced proximal tubule dysfunction and nephrotoxicity progression.

Contrarily, the present data indicated that ESE at both low and high doses expressively reduced the downstreaming inflammatory cascade in GM-nephrotoxic rats. Thus, the reduction in the serum levels of TNF-α and

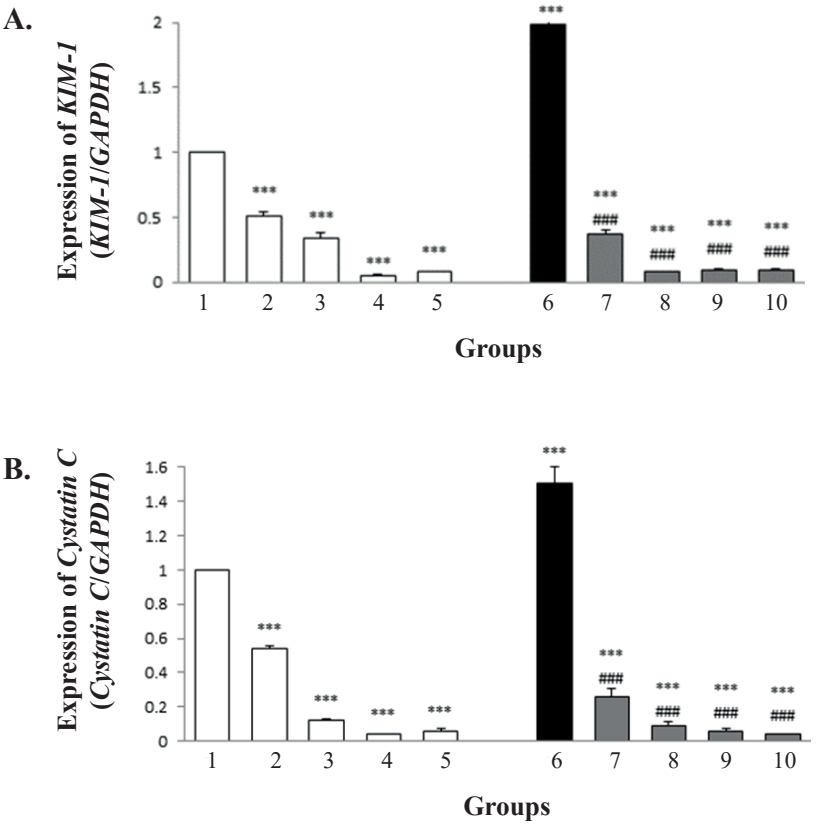

Figure 3 Effect of GM, ESE, and RUT in male albino rats, administered separately. A: mRNA expression of *KIM-1*; B: *Cystatin C* in kidney tissue. Values were presented as fold changes relative to control and GM injected rats after normalization with the expression of a house-keeping gene (*GADPH*). Data expressed in terms of mean±SEM (n=6). ***(p<0.001) statistically significant compared with control group, ###(p <0.001) statistically significant compared with gentamicin injected group. 1: Control group; 2: ESE 150 mg/kg BW; 3: ESE 300 mg/kg BW; 4; RUT 50 mg/kg BW; 5: RUT 100 mg/kg BW; 6: GM-nephrotoxic rats; 7: GM+ESE 150 mg/kg BW; 8: GM+ESE 300 mg/kg BW; 9: GM+RUT 50 mg/kg BW; 10: GM+RUT 100 mg/kg BW.

IL-1β is another piece of evidence confirming the renal protective effect of ESE. The anti-inflammatory effect of seeds is possibly mediated through flavonoids that discourage pro-inflammatory gene expression, TNF-α, and some other ILs.

Furthermore, the inflammatory markers levels in RUT-treated animals restored to the normal levels as compared to control animals, indicating the efficiency of RUT in counteracting inflammation, thus suppressing the release of TNF-α and IL-1β. This is confirmed by previous study which also reported that RUT supplementation significantly decreased the NF-kB expression and attenuated IL-1β and TNF-α generation to control levels.(32)

On the alternative view, previous studies demonstrated that the kidney injury markers, such as *KIM-1* and *Cystatin C*, were independently associated with pro-inflammatory cytokines, especially TNF-α.(33,34) KIM-1, a type 1 transmembrane glycoprotein, is known to be highly upregulated in the proximal tubule epithelial cells' apical membrane of both human and rodent kidneys after nephrotoxicity.(35) On the other hand, *Cystatin C* is formed at a steady flow by the nucleated cells. It is a small protein molecule that has been expressed in the proximal tubule, where its reabsorption and metabolism occur and freely filters via the glomeruli. *Cystatin C* mRNA was selected as a marker as it is expressed in healthy rats in adequate amounts and showed a marked upregulation after podocyte damage.(36) Consistent with the previous findings, we also observed a progressive increase of *KIM-1* mRNA and *Cystatin C* mRNA expression in the kidneys of GM-induced nephrotoxic rats, suggesting the occurrence of tubular injury. Similarly, another study reported a significant increase in *KIM-1* and *Cystatin C* in GM-treated animals.(37) Enhanced *KIM-1* mRNA expression might be associated with regeneration or proliferation of cells in response to toxic abuse.(38) Furthermore, another publication revealed that the induction of podocyte injury led to a *de novo* expression of *Cystatin C* in the podocytes.(36) In addition, the cells in the glomerular tuft coexpress both PEC and podocyte proteins markers in a variety of experimental nephropathies.(39)

Previously, it has been shown that ESE and RUT might have a renoprotective prosperities. Moreover, if renal damage is reduced with renoprotective therapy, *KIM-1* expression is also reduced.(40) In line with previous findings, we also reported a nephroprotective effect of ESE or RUT through a significant downregulation of the expressions of *KIM-1* mRNA and *Cystatin C* mRNA in GM-induced nephrotoxicity. However, the pathway through which ESE or RUT results in this nephroprotective effect is unknown.

Conclusion

This study discovered that ESE and pure RUT used in the study were very effective in attenuating GM-induced nephrotoxicity by reducing the inflammatory cytokines and mRNA expressions kidney injury markers such as *KIM-1* and *Cystatin C*, in addition to modulating biochemical renal markers. On the other hand, the nephroprotective activity of RUT has a remarkable association with antioxidant capacity enhancement. Therefore, pure RUT is superior in the amelioration of GM-induced nephrotoxicity, followed by ESE. Accordingly our results encourage use of ESE or RUT as a dietary supplement for patients suffering from kidney diseases and those undergoing GM therapy; further studies are required to investigate that.

Authors Contribution

All authors contribute equally in data collection, experimental design, interpretation, statistical analysis, literature review, manuscript preparation, and review.

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