Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Ethanolic Extracts of Grape and Guava Seeds Ameliorate Gentamicin-Induced Acute Renal Injury in Rats

Shaimaa A. Abd Elwahab [1], Awatif M. Abdel-Maksoud [2], Eman M. Sadek [3] and Maha M. Mohamed [4]

[1]The Blood Laboratory Department, National Nutrition Institute, Cairo, Egypt
[2] Nutritional Needs and Growth Department, National Nutrition Institute, Cairo, Egypt.
[3] Histology Department, Faculty of Medicine, Cairo University, Cairo, Egypt.
[4] Home Economic Department, Faculty of Women for Arts, Science, and Education, Ain Shams University, Cairo, Egypt

Abstract

Objective: This investigation was designed to analyze the phytochemicals of the ethanolic extracts of grape and guava seeds and to assess their possible ameliorating effects on gentamicin (GM)-induced nephropathy in rats. **Material and methods**: Sixty-four male Sprague Dawley rats were randomly allocated into eight groups and treated for ten consecutive days as follows: Group 1 (control group) was injected intraperitoneally with saline solution; Groups 2, 3, and 4 received a daily oral dosage of grape seed extract (GrSE, 40 mg/kg b.wt /day, *p.o*), guava seed extracts (GuSE, 300 mg/ b.wt /day, *p.o*) or a combination of both, respectively. Group 5 was intoxicated with GM (100 mg/kg/ b.wt /day, *i.p*). Groups 6, 7, and 8 received an oral dose of GrSE and/or GuSE, along with an intraperitoneal injection of GM. **Results:** Chemical analysis by high-performance liquid chromatography with diode-array detection (HPLC-DAD) showed that the ethanolic extracts of GrSE and GuSE are rich in phenolic compounds such as gallic acid, chlorogenic acid, 3-hydroxycinnamic acid, ferulic acid, caffeic acid, ellagic acid, daidzein, apigenin, syringic acid, naringenin, p- coumaric acid, quercetin, rutin, and vanillic acid. GrSE or GuSE oral administration did not cause any considerable biochemical or histological alterations in the normal rats. Rats treated with GM showed significant elevation in serum concentration of urea and creatinine and a significant reduction in serum levels of total proteins, sodium, and potassium. In contrast, serum alkaline phosphatase activity was significantly elevated. Additionally, GM injection resulted in a significant rise in the serum levels of triacylglycerol (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL), and a significant decrement in serum high-density lipoprotein cholesterol (HDL). Furthermore, GM-injected rats displayed a substantial decline in the activity of erythrocyte copper, zinc superoxide dismutase (Cu, Zn-SOD), and blood and renal levels of reduced glutathione (GSH) along with elevation in the renal level of malondialdehyde (MDA). Up-regulation of kidney injury molecule-1 (KIM-1) and nuclear factor kappa B p65 subunit (NF-κB p65) gene expression was also observed in GM-injected rats compared with control. Simultaneous administration of GrSE and/or GuSE with gentamicin attenuated the nephrotoxic effects of GM as indicated by improvement in renal function parameters, normalization of serum electrolytes, restoration of serum lipid profile, reduction in renal lipid peroxidation, enhancement of the antioxidant status and down-regulation of KIM-1 and NF-κB p65 gene expression. The results of the histopathological evaluation confirmed these biochemical findings. **Conclusion:** Treatment with grape and guava seed extracts ameliorated gentamicin-induced nephrotoxicity via hypolipidemic, antioxidant, and anti-inflammatory effects.

Keywords: *gentamicin, nephrotoxicity, grape seeds extract, guava seeds extract, rats*

1. Introduction

Gentamicin (GM), an aminoglycoside antibiotic, is employed to treat infections that seriously threaten health. It is extensively used due to its broad-spectrum actions against gram-negative and gram-positive bacteria, chemical stability, and rapid bactericidal effects [1]. Nonetheless, there are numerous nephrotoxic adverse effects of GM. This

*Corresponding author e-mail: maha.mahmoud.2591975@gmail.com; (Maha M. Mohamed).
Receive Date: 04 October 2022, **Revise Date:** 29 October 2022, **Accept Date:** 06 November 2022,
First Publish Date: 06 November 2022
DOI: 10.21608/EJCHEM.2022.166876.7056

occurs at minimal therapeutic doses and contributes to 10%-15% of all episodes of acute renal failure [1, 2]. GM-induced nephropathy is attributed to its buildup in the proximal convoluted tubules [4]. The cationic aminoglycosides interact with anionic phospholipids of the proximal tubular cells to cause phospholipidosis and trigger necrosis [5, 6]. The experimental data point to oxidative stress as a pivotal factor in GM-induced nephrotoxicity. GM intensifies the generation of reactive oxygen species (ROS) that cause cytotoxic consequences and promote lipid peroxidation, protein oxidation, and apoptosis [7, 8]. Furthermore, the amplified ROS generation brought on by GM plays a crucial role in the onset of inflammation through NF-κB activation [9, 10, 11], which activates the target genes implicated in the inflammatory process, such as adhesion molecules, cytokines (TNF-α and IL-6), and inducible nitric oxide synthase (iNOS) [12].

Fruit by-products, like seeds, are good sources of bioactive compounds. The pharmaceutical and food industries could benefit from using these abundant, affordable renewable resources by introducing new pharmacological and/or nutritional products, lowering costs and industrial waste, and eventually positively affecting the economy and the environment [13, 14]. Seeds, as the by-product of the fruit processing industry, have been found to contain phenolic compounds [15]. Phenolic compounds exhibit numerous physiological characteristics, such as antioxidant, anti-allergenic, anti-atherogenic, anti-inflammatory, antimicrobial, anti-thrombotic, cardioprotective, and vasodilatory effects [16, 17, 18].

Grapes (Vitis vinifera L.) are the fruit crops grown the most widely in the world. Grape seeds comprise 38%–52% of the dry mass [19], a sizeable portion of the industrial by-product. Grape seeds constitute a low-cost source of antioxidant compounds [20, 21]. Tang et al. [22] found that grape seeds have up to ten times more total flavonoid concentration than grape peel, highlighting the significance of this grape-derived by-product. Proanthocyanidins (PACs) from grape seeds, in particular, are more effective free radical scavengers than vitamins C, E, and β-carotene. [23, 24].

Guava (Psidium guaijava L.) is one of the most common tropical and semitropical fruits. This fruit's excellent nutritional value and significant therapeutic capabilities make it desirable [25]. Some studies found that guava has anti-inflammatory, analgesic, antipyretic, and antibacterial effects [26]. The main processed products of guava fruit products are beverages, juices, and canned slices. The seeds are the waste product of these industries. Guava seeds are 6-12 % of the fruit weight. The seeds have been reported to contain 14% oil, 15% protein, and 13% starch [27]. Additionally, phenolic chemicals with antioxidant properties are abundant in guava seeds [25-27]. Michael et al. [28] identified ten phenolic and flavonoid compounds in guava seeds. Guava seeds had higher concentrations of ß-carotene, lycopene, anthocyanins, and yellow flavonoids than the fruit pulps. Furthermore, it is considered a rich natural source of resveratrol and coumarin [28]. The presence of these bioactive phenolic compounds suggests that guava seeds could be an interesting source of antioxidant and anti-inflammatory substances.

Thus, this investigation aimed to determine whether grape seed and/or guava seed ethanolic extracts had nephro-protective effects on gentamicin-induced nephrotoxicity in rats.

2. Material and methods

2.1. Materials

2.1.1. Chemicals

Gentamicin sulfate pure powder was obtained from CID- Chemical Industries Development Company, Giza, Egypt. It was dissolved freshly in saline before each treatment. The following materials were bought from Sigma-Aldrich Co. (USA): carboxymethyl cellulose, diethyl ether, hematoxylin, eosin, xylene, streptavidin-biotin, and diaminobenzidine. Methanol (95%), ethanol, petroleum ether (60-80°C), and hydrogen peroxide were provided by El-Nasr Pharmaceutical Co. in Egypt.

2.1.2. Animals

Sixty-four male adult albino Sprague-Dawley rats were purchased from the Medical Research Center, Al-Demerdash Hospital, Cairo, Egypt. Their body weight was 200-250 grams. The animals were kept in metallic cages in a healthy environment. Water and basal diet [29] were provided ad-libitum during the trial (ten days) and the week-long adaptation phase.

2.2. Methods

2.2.1. Grape and guava seeds extract

Grape and guava seeds were separated from the pulp and peel, and the collected seeds were crushed into powder after being shade dried. For every 50 g of the powder, 500 ml of 70% ethanol was added, and the mixture was then rotary-mixed at ambient temperature for 24 hours. The resulting mixtures were filtered, and the solvent was evaporated at 40 °C in a rotary evaporator while under vacuum. The residues left over were dissolved in water (by concentration 2 g /100 ml for GrSE and 15 g / 100 ml for GuSE) and stored in a freezer [30].

2.2.2. Quantification of the bioactive compounds using high-performance liquid chromatography (HPLC)

An Agilent 1260 series was used for HPLC analysis. The separation was accomplished using an Eclipse C18 column (4.6 mm x 250 mm i.d., 5 m). At

a rate of flow of 0.9 ml/min, the mobile phase was composed of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B). The mobile phase was programmed in a linear gradient in the following order: 0 minutes (82% A); 0-5 minutes (80% A); 5-8 minutes (60% A); 8-12 minutes (60% A); 12-15 minutes (82% A); 15-16 minutes (82% A); and 16-20 minutes (82% A). At 280 nm, the multi-wavelength detector was monitored. The injection volume for each of the sample solutions was five µL. The column temperature was kept constant at 40 °C.

2.2.3. Experimental protocol

The Review and Approval Committee at National Nutrition Institute reviewed and approved the study (protocol IN0009, approved March 2017). Eight groupings of the animals (eight rats/group) were selected at random and treated for ten days as follows: Group-I: served as negative control and received 1 ml of saline (0.9%; *i.p.*); Groups II, III, and IV received GrSE (40 mg/kg b.wt /day /day *p.o.*)[31], GuSE (300 mg/kg b.wt /day, *p.m.*,) [32] and a mixed dose of both, respectively. Group V (positive control) animals were injected with 100 mg/kg body weight of gentamicin (*i.p.*), according to Dhanarajan et al. [33]. Groups VI, VII, and VIII received GrSE, GuSE, and a mixture of both, respectively, along with gentamicin for ten days.

The body weight of each animal in a particular group was measured before and after the experiment. Following the trial period, animals were sacrificed by decapitation under anesthesia. The blood samples were collected in dry centrifuge tubes for the separation of serum and in heparinized tubes. Serum was isolated by centrifugation at 3000 r.p.m for 15 minutes. Serum aliquots were frozen at -20ºC to determine creatinine, urea, uric acid, total proteins, alkaline phosphatase (ALP), sodium, potassium, and lipid profile later. The heparinized blood sample was utilized for the determination of reduced glutathione. The erythrocytes were washed two times with cold saline and stored for Cu, Zn-SOD estimation at -20ºC. After the rats had been sacrificed, both kidneys were quickly removed and weighed. For histological investigation, one of the two kidneys was submerged in a neutral 10% formaldehyde solution. The other was divided equally into two longitudinal sections. One half was used to prepare 10% (w/v) homogenate in phosphate buffer (0.1 M, pH 7.4) using a homogenizer. The kidney homogenate was centrifuged at 10,000 g for 15 min, and the supernatant was used to estimate renal GSH and MDA. The remaining half was kept at -80 °C to assess the levels of NF-κB p65 and KIM-1 gene expression using quantitative real-time polymerase chain reaction (qPCR).

2.2.4. Biochemical analysis

2.2.4.1. Renal function parameters

Standard spectrophotometric techniques were used to quantify serum levels of urea [34], creatinine [35], and uric acid [36] using the auto-analyzer model. The serum total protein level was measured using Stanbio kits [37]. The activity level of serum ALP was estimated by photometric techniques using an auto-analyzer model [38]. Serum potassium concentration was quantified by the turbidimetric tetraphenylborate (TPB) method without deproteinization [39]. Sodium concentration was measured by the uranyl thioglycolate method with precipitation [40].

2.2.4.2. Serum lipid profile

Concentration levels of serum TG, TC, and HDL were evaluated enzymatically through colorimetric techniques of Fossati & Prencipe [41], Guder et al. [38], and Rifle et al. [42], respectively. The equation proposed by Friedwald et al. [43] was used to compute the serum LDL value. [LDL=TC-(HDL + TG/5)]

2.2.4.3. Determination of oxidative stress markers

Renal and blood GSH contents were measured using 5,5′-dithionitrobenzoic acid to produce the yellow-colored 5-thio-2-nitrobenzoic acid, which can be quantified colorimetrically at 412 nm [44]. Malondialdehyde (MDA), the by-product of lipid peroxidation, was determined by the thiobarbituric acid assay. MDA and thiobarbituric acid (TBA) interact in an acid medium to form a pink-colored TBA complex that can be extracted with n-butanol and quantified colorimetrically at 535 and 520 nm [45]. The determination of Cu, Zn-superoxide dismutase activity is based on the ability of the enzyme to inhibit the reduction of nitro blue tetrazolium (NBT) / (prevent formazon formation) by the superoxides produced by the interaction of photo-reduced riboflavin and oxygen [46].

2.2.4.4. Gene expression of NF-κB p65 and KIM-1 by real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR)

Total RNA was extracted from kidney tissue utilizing a miRNeasy Mini Kit (Qiagen, USA) following the manufacturer's instructions. The purity and the concentration of RNA obtained were determined spectrophotometrically at 260/280 nm using a dual-wavelength Beckman Spectrophotometer (USA). Afterward, the extracted RNA was reverse-transcribed into complementary DNA (cDNA) using a high-capacity cDNA reverse transcription kit (Fermentas, USA). Real-time qPCR amplification and analysis were carried out using an Applied Biosystem with software version 3.1 (StepOne™, USA). Sequences of primers (Invitrogen Co., USA) used for

RT-qPCR were as follows: KIM-1 gene forward primer: (5'- TTTGGATCTGTACCCAGTGCTT-3'), reverse primer: (5'- CAAGGCCAGCCCTCTAATGG -3'); NF-κB p65 gene forward primer: (5'-GAACTTGTGGGGAAGGACTG-3'), reverse primer: (5'-GGGGTTATTGTTGGTCTGGA-3'); and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) forward primer: 5′-ATCACCATCTTCC AGGAGCG -3′, reverse primer 5′-CCTGCTTCACC ACCTTCTTG-3′. The PCR program was adjusted to start with initial activation for 2 min at 94°C, followed by 45 cycles (94°C for 5 sec, 62°C for 10 sec, and 72°C for 20 sec) using an Applied Biosystem with software version 3.1 (Step One™, USA). Each sample was evaluated, its data adjusted to the reference gene level (GAPDH), and its relative copy number (RCN) expressed. The samples' cycle threshold (Ct) estimates were computed, and transcript levels were evaluated by the $2^{-\Delta\Delta Ct}$ technique [47].

2.2.5. Histological analysis

For light microscopic analysis, the right kidney was fixed in a neutral 10% formalin solution; the specimens were embedded in paraffin wax, divided into sections, and stained with hematoxylin-eosin [48].

2.2.6. Statistical analysis

Statistical analysis was carried out using the software Statistical Packages for Social Science [49], and values were compared using one-way analysis of variance (ANOVA) followed by Tukey post hoc multiple comparisons. All values were represented as mean ± standard error of the mean, and a p-value <0.05 was chosen as the significance threshold.

3. Results

3.1. Phytochemical analysis of ethanolic extracts of grape and guava seeds

Figure 1 demonstrates a chromatogram of standard polyphenolic compounds by using high-performance liquid chromatography. The results of HPLC-DAD analysis revealed the presence of many phenolic compounds in the GrSE (Table 1 and Figure 2a). The concentration of chlorogenic acid in the GrSE was comparatively high, up to 3173.9 µg/mL, followed by methyl gallate (633 µg/ml), syringic acid (440 µg/ml), gallic acid (261 µg/ml), caffeic acid (229 µg/ml), kaempferol (57.88 µg/ml) and naringenin (38.1 µg/ml). However, the lowest amount was found for quercetin (4.92 µg/ml). On the other hand, Table (1) and Figure (2b) demonstrate that GuSE contained syringic acid (228.2 µg/ml), quercetin (126.8 µg/ml), caffeic acid (60.15 µg/ml), daidzein (53.87 µg/ml), ferulic acid (42.08 µg/ml), chlorogenic acid (41.65 µg/ml), cinnamic acid (29.4 µg/ml), gallic acid (27.2 µg/ml), methyl gallate (23.48 µg/ml) and catechin (8.85 µg/ml).

Table 1. HPLC–DAD data of the polyphenolic compounds detected in grape seeds and guava seeds ethanolic extract

Molecules	Standard		Grape Seeds Extract		Guava Seeds Extract	
	Peak Area	Conc. (µg/mL)	Peak Area	Conc. (µg/mL)	Peak Area	Conc. (µg/mL)
Gallic acid	15	190.27	165.55	261.03	17.25	27.20
Chlorogenic acid	50	330.35	1048.49	3173.90	13.76	41.65
Catechin	75	316.49	0.00	0.00	1.87	8.85
Methyl gallate	15	259.01	633.01	733.19	20.27	23.48
Coffeic acid	18	245.39	156.52	229.63	41.00	60.15
Syringic acid	17.2	201.20	257.43	440.16	133.48	228.22
Rutin	26	202.57	2.41	6.20	0.00	0.00
Ellagic acid	120	353.61	93.98	637.83	3.33	22.59
Coumaric acid	20	703.65	19.51	11.09	9.37	5.33
Vanillin	12.9	373.12	51.47	35.59	13.69	9.47
Ferulic acid	20	359.23	6.99	7.78	37.79	42.08
Naringenin	30	323.82	20.56	38.10	6.34	11.76
Daidzein	35	547.09	7.62	9.75	42.10	53.87
Querectin	40	331.69	4.39	10.58	52.60	126.88
Cinnamic acid	10	501.92	12.34	4.92	73.38	29.24
Apigenin	50	780.29	6.13	7.86	3.93	5.04
Kaempferol	30	191.49	18.47	57.88	0.00	0.00

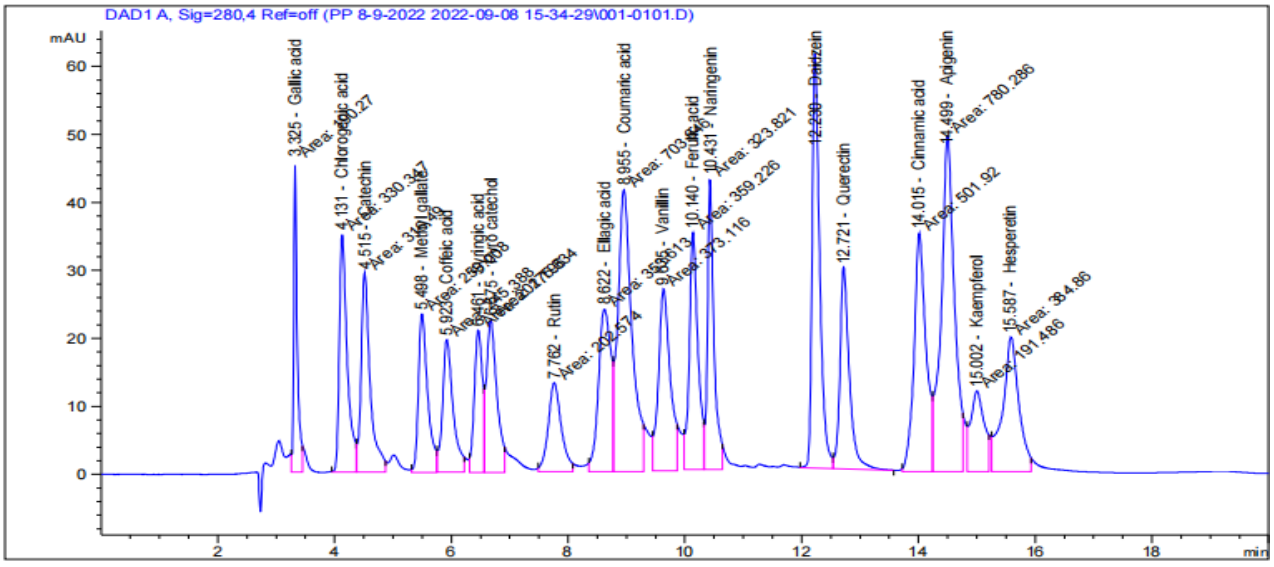

Figure 1. HPLC–DAD chromatograms at 280 nm of standard polyphenolics compounds

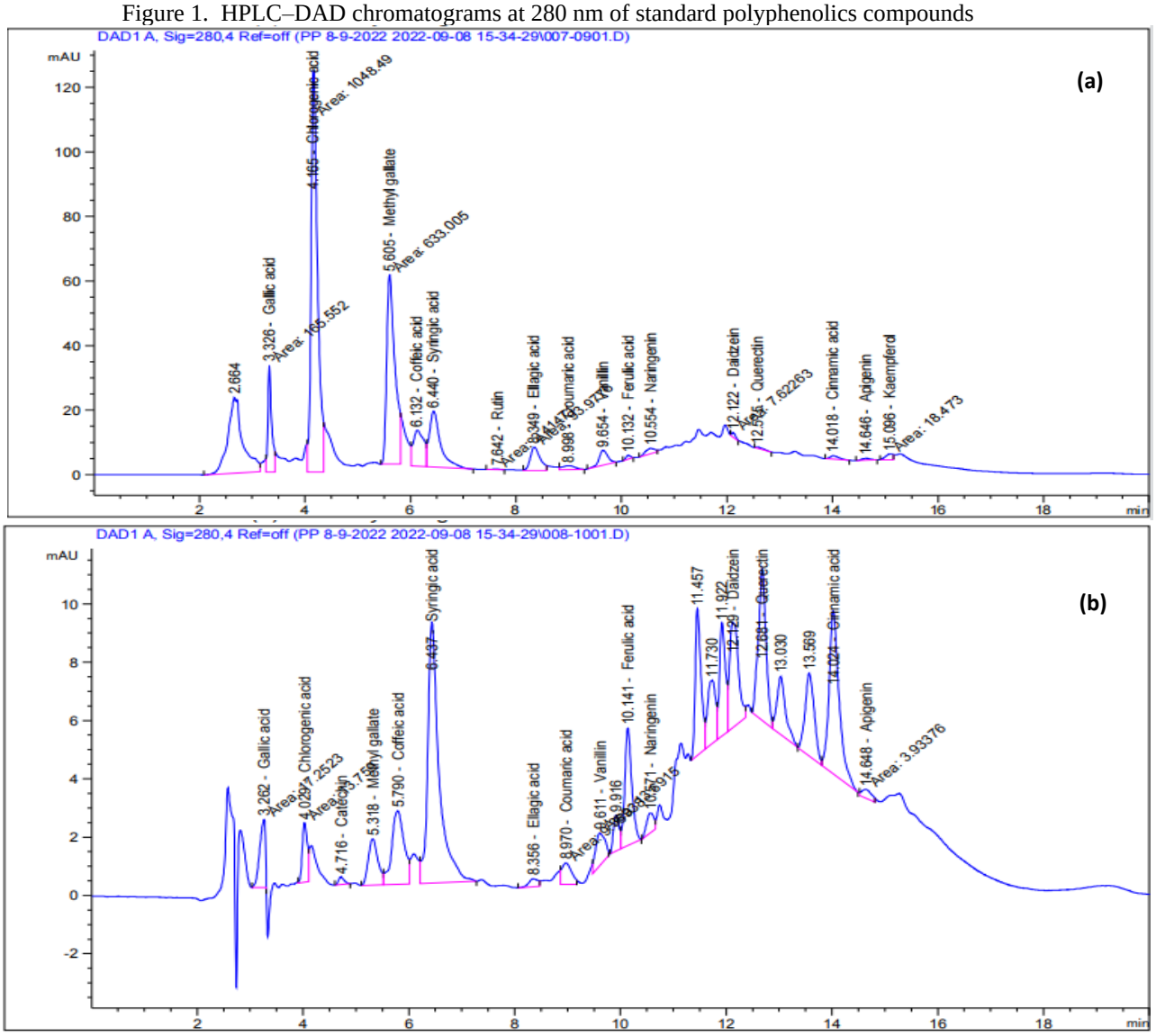

Figure 2. HPLC–DAD chromatograms at 280 nm of the detected polyphenolics compounds in the ethanolic extracts of grape seeds (a) and guava seeds (b)

3.2. Biochemical results

3.2.1. Effects of grape seed and/or guava seed extracts on normal rats

Administration of GrSE, GuSE, or their combination to normal rats did not significantly change the measured parameters compared to the negative control ($p > 0.05$).

3.2.2. Effects of grape seed and/or guava seed extracts on GM-injected rats

Gentamicin injection resulted in a significant reduction ($p < 0.05$) in the percentage of increase in body weight and a significant rise ($p < 0.05$) in absolute and relative kidney weights by 43.39% and 65.21%, respectively, as compared to the negative control. Treatment of gentamicin-injected rats with GrSE, GuSE, and a mixture of both caused a non-significant ($p > 0.05$) increase in body weight and a significant ($p < 0.05$) decrease in the absolute (12.6%, 19.73%, and 18.4%) and relative (18.42%, 15.17%, and 21.05%) weights of the kidney as compared to the positive control (Table 2).

As shown in Table (3), ten days of GM injection significantly ($p < 0.05$) elevated serum levels of urea and Cr (8.8-fold and 10.2-fold, respectively) and significantly ($p < 0.05$) decreased total serum proteins (by 8.8%) as compared to the control group. Concurrent administration of seed extracts of grape, guava and their combination with GM significantly ($p < 0.05$) attenuated the increment of serum urea (50.6%, 44.9%, and 53.8%, respectively) and Cr (57.5%, 67.7%, and 56.94%, respectively) compared to the positive control. Serum total protein level was significantly ($p < 0.05$) raised by 11.69% in the group treated with GrSE but not significantly ($p > 0.05$) in other groups. Treatment of GM-injected rats with GrSE and/or GuSE did not show appreciable changes in serum uric acid ($p > 0.05$) compared to GM-injected rats.

According to Figure (3a), rats injected with GM had considerably ($p < 0.05$) higher serum ALP activity (45.3%) than the control group. This rise was significantly ($p < 0.05$) decreased in the groups that received oral administration of GrSE, GuSE, and their combination by 17.06%, 11.9%, and 15.2%, respectively, compared with the positive control. KIM-1 was highly ($p < 0.05$) up-regulated (5.47 folds) in the group injected with GM in comparison with the negative control. Oral administration of GrSE, GuSE, or their combination significantly ($p < 0.05$) downregulated this intense elevation by 56.1%, 67.4 %, and 59.78%, respectively, as compared to the positive control (Figure 3b).

GM injection significantly ($p < 0.05$) reduced serum sodium by 10.6% and potassium by 10.2% compared with the negative control. Simultaneous treatment of GM-intoxicated rats with extracts of grape and/ or guava seeds nearly normalized serum sodium. Serum potassium level was significantly ($p < 0.05$) reduced in gentamicin-injected rats that received guava seed extract by 17.8% as compared to the positive control. However, no considerable alterations were observed in serum potassium levels in nephrotoxic rats that received GrSE or both extracts compared to the positive control ($p > 0.05$).

Rats treated with gentamicin had significant ($p < 0.05$) higher TG, TC, and LDL serum levels while having lower HDL level than the control animals. Oral administration of the tested extracts significantly ($p < 0.05$) improved the serum lipid profile in GM-intoxicated rats (Table 4).

Data represented in Figure (5) demonstrate that administration of GM caused a significant ($p < 0.05$) decrement in blood and renal GSH levels (20% and 33.7%) along with the decline in the activity of erythrocyte Cu, Zn-SOD activity by 20% as compared to the negative control. This impairment in renal antioxidant status was accompanied by a considerable elevation in renal lipid peroxidation, as shown by a significant ($p < 0.05$) rise in the level of renal MDA by 36% in GM-intoxicated animals as compared to the negative control (Figure 6a). The oxidative stress resulting from GM injection was improved with the administration of GrSE, GuSE, or their mixture, as the activity of erythrocyte SOD was significantly ($p < 0.05$) increased by 45.6%, 67.3%, and 29.9%, respectively (Figure 5a). Also, the blood GSH increased ($p < 0.05$) by 41.7%, 48.7%, and 21.4%, respectively (Figure 5b), while renal GSH level increased ($p < 0.05$) by 68.7%, 41.06%, and 38.7%, respectively (Figure 5c) as compared to the positive control. On the other hand, renal MDA was significantly ($p < 0.05$) decreased by 38.5%, 40%, and 41.7%, respectively, as compared to GM-treated rats (Figure 6a).

Gene expression examination revealed that GM injection induced a substantial (6.57-fold) increase in renal NF-κB p65 mRNA expression compared to the negative control ($p < 0.05$). Contrarily, treatment with GrSE, GuSE, or their mixture resulted in a meaningful downregulation ($p < 0.05$) of NF- κB mRNA expression levels by 57.8%, 60.1%, and 63.9%, respectively, as compared to the positive control (Figure 6b).

Table 2. Effects of grape seeds and guava seeds extracts on the percent increase in body weight and kidney weights (absolute and relative) in normal and gentamicin-treated rats

Groups	Percent increase in body weight (g%)	Kidney Weight*	
		Absolute (g)	Relative (g%)
Negative control	24.5±1.2[a]	0.53±0.02[a]	0.23±0.01[a]
Grape seed	24.1±2.3[a]	0.5±0.02[a]	0.21±0.01[a]
Guava seed	23.1±2.2[a]	0.54±0.009[a]	0.23±0.006[a]
Mixed dose	22.9±1.98[a]	0.58±0.05[a]	0.28±0.01[ac]
Positive control (GM)	14.6±1.21[b]	0.76±0.03[b]	0.38±.007[b]
GM + Grape seeds	15.4±1.09[b]	0.61±.01[ac]	0.31±0.007[c]
GM + Guava seeds	16.28±1.17[b]	0.69±0.05b[c]	0.32±0.02[c]
GM + Mixed dose	14.98±1.31[b]	0.62±0.06[c]	0.30±0.01[c]

These values are expressed as mean ±SEM (n=8 rats /group). Values with a different superscript letter in the same column differ significantly at $p < 0.05$ (one-way ANOVA followed by Tukey's multiple comparisons test)
*Mean of two kidneys

3. Effects of grape and /or guava seed extracts on kidney function tests in normal and gentamicin-treated rats

Groups	Serum urea (mg/dL)	Serum creatinine (mg/dL)	Serum uric acid (mg/dL)	Serum total Protein (mg/dL)
Negative control	33.66±2.8[a]	0.70±0.02[a]	1.38±0.77[a]	6.66±0.11[a]
Grape seeds	38.5±1.19[a]	0.650±0.02[a]	1.3±0.04[a]	6.62±0.11[a]
Guava seed	42.5±2.72[a]	0.7±0.0[a]	1.42±0.06[a]	6.62±0.19[a]
Mixed dose	36.25±1.43[a]	0.7±0.04[a]	1.5±0.07[a]	6.6±0.12[a]
Positive control (GM)	296.5±25.5[c]	7.2±0.82[c]	1.64±.08[a]	6.07±0.17[b]
GM + Grape seeds	146.33±11.07[b]	3.06±0.26[b]	1.31±0.10[a]	6.78±0.24[a]
GM + Guava seeds	163.25±26.4[b]	2.32±0.19[b]	1.28±0.04[a]	6.49±0.08[ab]
GM + Mixed dose	136.75±44.7[b]	3.1±0.81[b]	1.25±0.06[a]	6.55±0.06[ab]

These values are expressed as mean ±SEM (n=8 rats /group). Values with a different superscript letter in the same column differ significantly at $p < 0.05$ (one-way ANOVA followed by Tukey's multiple comparisons test)

Table 4. Effects of grape and\or guava seed extracts on serum lipid profile in normal and gentamicin-treated rats

Groups	Triacyglycerols (mg/dL)	Total Cholesterol (mg/dL)	HDL-Cholesterol (mg/dL)	LDL-Cholesterol (mg/dL)
Negative control	45.66±2.6[a]	59±2.36 [a]	22.33±1.1[a]	28±1.23[ad]
Grape seed	56.25±3.49[ac]	55.5±1.65[c]	37.75±1.6[c]	26.25±0.94[ac]
Guava seed	52.25±11.1[a]	61.75±1.65[ab]	31.5±1.19[b]	23.5±0.86[c]
Mixed dose	51±6.67[bc]	62.5±4.13[ab]	22.5±3.0[a]	25.25±1.65[ac]
Positive control (GM)	79.3±5.05[b]	75.8±1.166[b]	19.8±0.59[a]	31.6±1.09[b]
GM + Grape seeds	66.33±2.76[b]	68.33±2.92[bc]	27.16±1.66[d]	27±0.93[acd]
GM + Guava seeds	63.5±4.66[bc]	66.25±3.47[ab]	22.25±2.01[a]	28±1.22[ad]
GM + Mixed dose	66.5±3.12[b]	61±1.58[ab]	20.75±0.47[a]	25±0.81[ac]

These values are expressed as mean ±SEM (n=8 rats /group). Values with a different superscript letter in the same column differ significantly at $p < 0.05$ (one-way ANOVA followed by Tukey's multiple comparisons test)

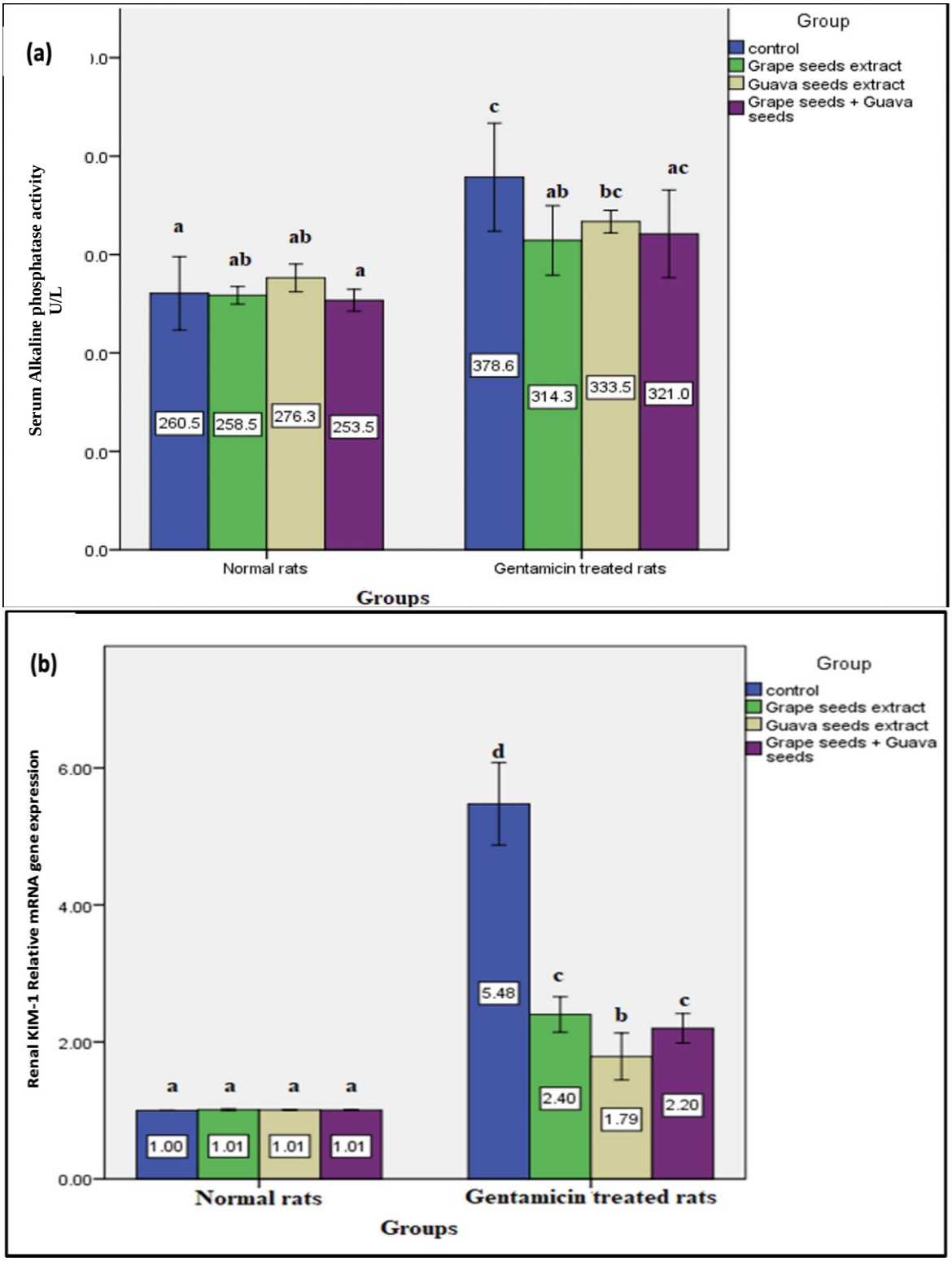

Figure 3. Effects of grape and guava seeds extracts on (a) serum alkaline phosphatase activity and (b) renal KIM-1 gene expression in normal and gentamicin-treat**ed** rats

These values are expressed as mean ±SEM (n=8 rats /group). Columns with different superscript letters differ significantly at $p < 0.05$ (one-way ANOVA followed by Tukey's multiple comparisons test)

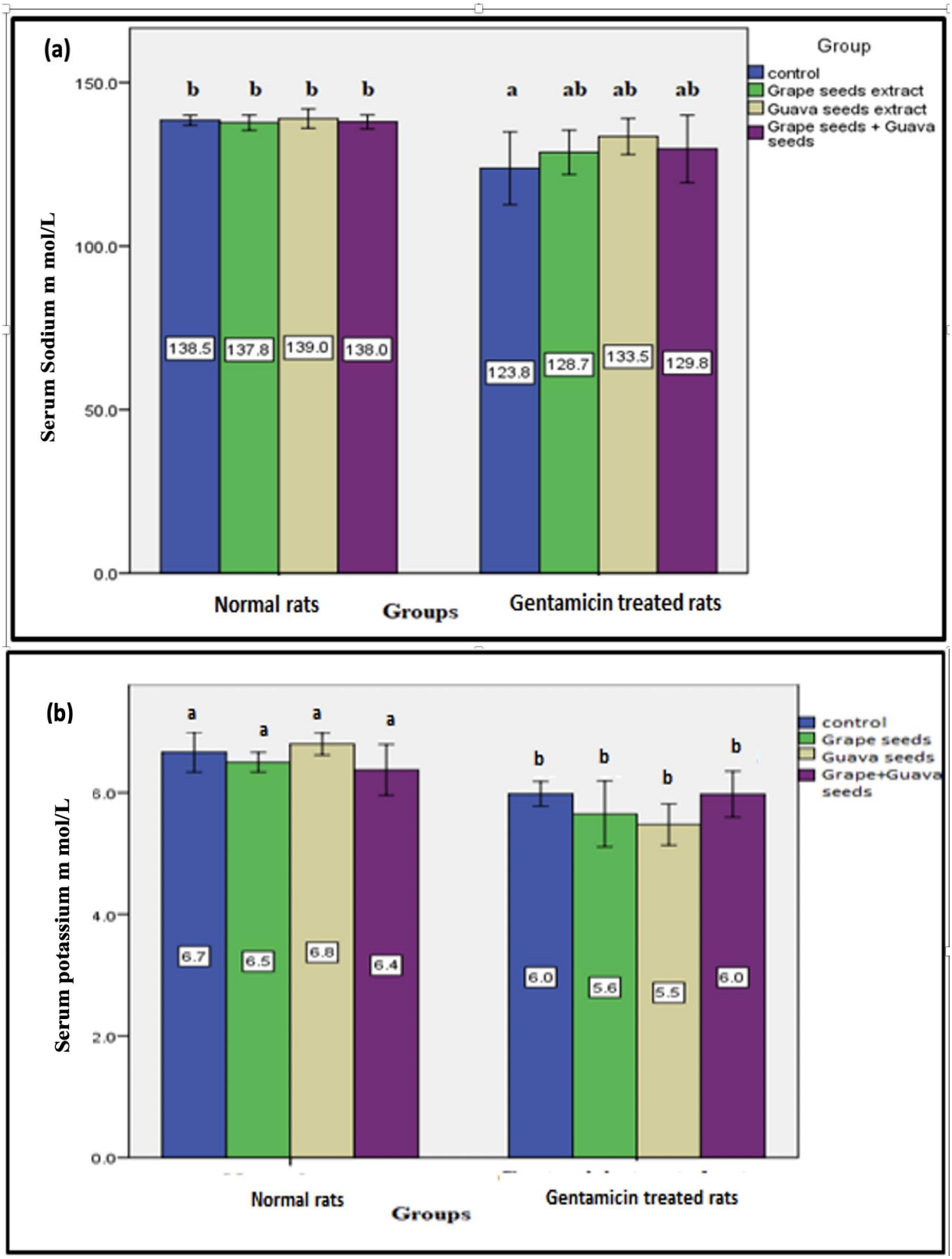

Figure 4. Effects of grape and/or guava seeds ethanolic extracts on serum (a) sodium and (b) potassium in normal and gentamicin-treated rats

These values are expressed as mean ±SEM (n=8 rats /group). Columns with different superscripts differ significantly at $p < 0.05$ (one-way ANOVA followed by Tukey's multiple comparisons test)

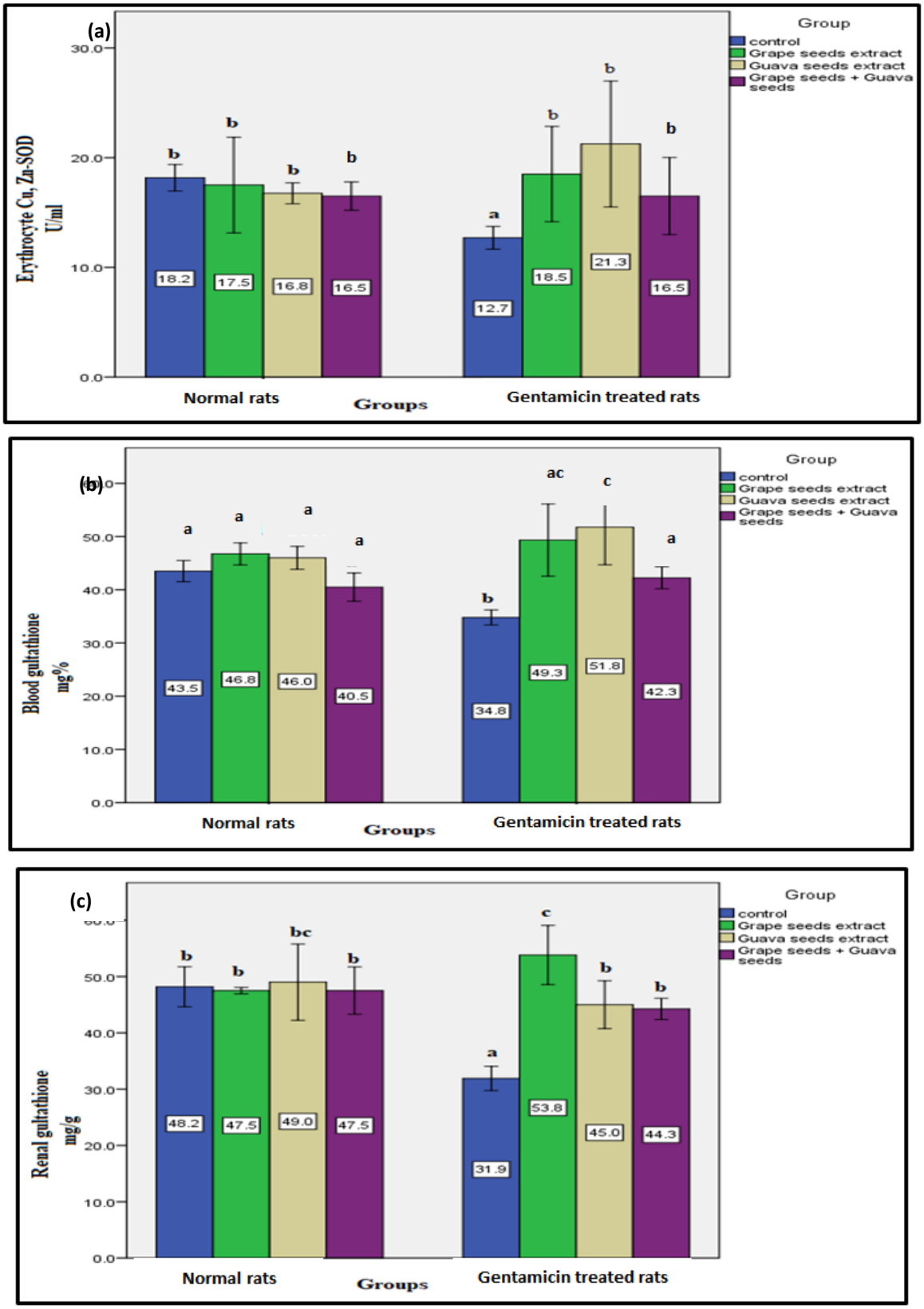

Figure 5. Effects of grape and/or guava seeds ethanolic extract on (a) erythrocytes SOD, (b) blood GSH and (c) renal (GSH) in normal and gentamicin-treated rats

These values are expressed as mean ±SEM (n=8 rats /group). Columns with different superscript letters differ significantly at $p < 0.05$ (one-way ANOVA followed by Tukey's multiple comparisons test)

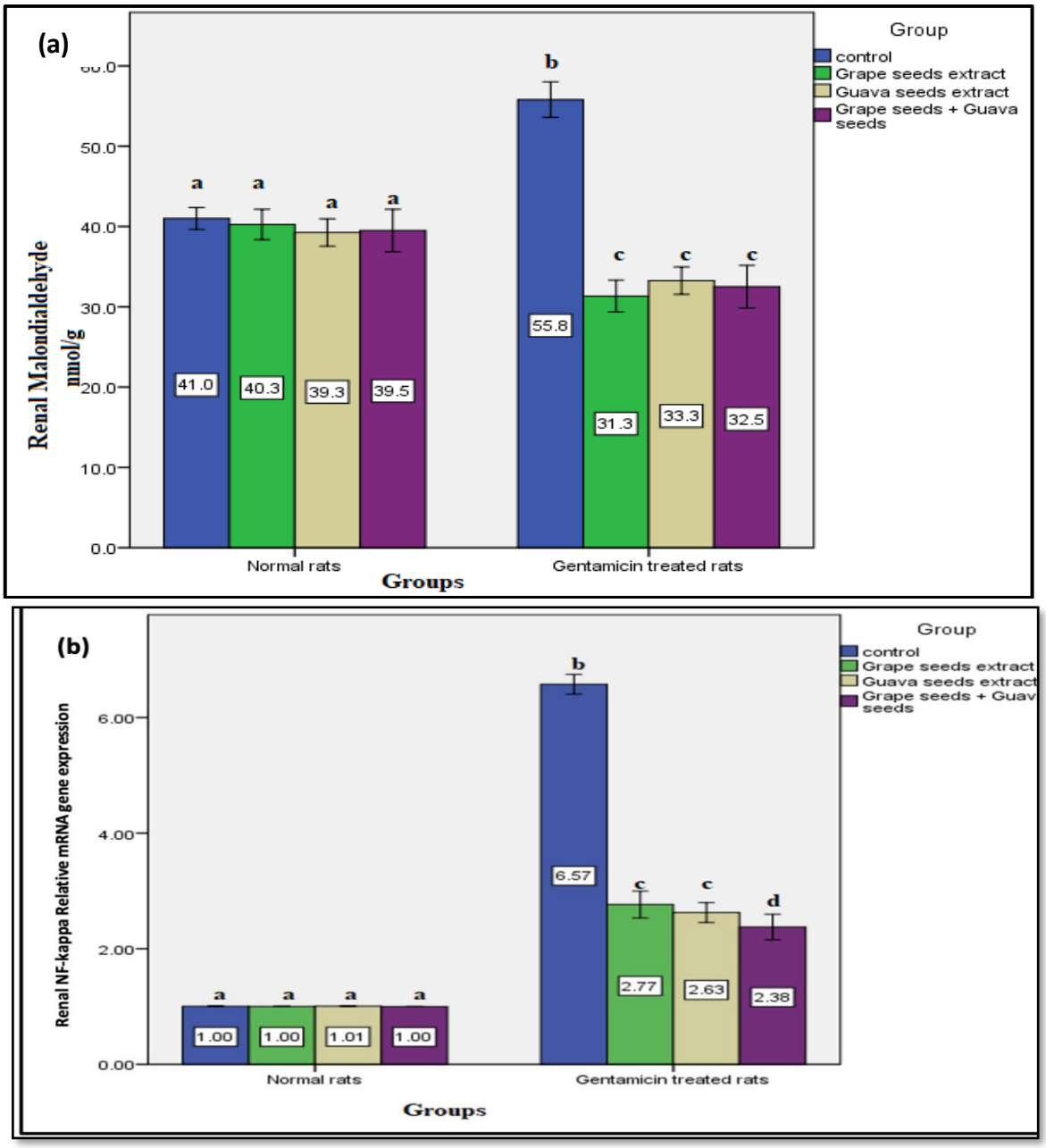

Figure 6. Effects of grape and/or guava seeds ethanolic extracts on (a) renal MDA and (b) renal Nuclear Factor Kappa (NF- kappa) gene expression in normal and gentamicin-treated rats

These values are expressed as mean ±SEM (n=8 rats /group). Columns with different superscripts differ significantly at $p < 0.05$ (one-way ANOVA followed by Tukey's multiple comparisons test)

3.3. Histological results

The results of microscopic examination of the kidney cortex and medulla sections in normal and GM-injected rats treated with GrSE and/or GuSE are presented in Figures 7 and 8, respectively.

4. Discussion

Gentamicin (GM)-induced nephrotoxicity is a well-known experimental model of drug-induced renal damage. In this study, we planned to induce renal dysfunction to evaluate the nephroprotective efficacy of grape and/or guava seed extracts treatment. Thus, GM was injected intraperitoneally at 100 mg/kg for ten successive days, which is known to cause significant nephrotoxicity [50].

In this experiment, the percent increase in body weight was significantly reduced. In contrast, the absolute and relative weights of kidneys were increased in GM-intoxicated rats compared to the control group. These results agree with the results of other investigators, who also reported a decline in the body weight of rats after GM treatment [51, 52].

Weight loss of GM-treated rats may be due to loss of appetite and elevation of energy expenditure caused by GM. Acute renal failure causes acidosis due to an increment in the catabolism that is associated with anorexia. Thus, there is a decrease in food consumption, which results in weight loss [53]. In this study, the significant rise in kidney weights of GM-treated rats might be due to the inflammation and edema that followed GM treatment and might be explained by an increase in renal cortical phospholipids brought on by GM-induced lysosomal phospholipidosis [50-53].

In the current study, GM-induced decline in renal function was manifested by a significant rise in serum levels of urea and creatinine along with the increment in the activity of ALP beyond normal limits and increased gene expression of renal KIM-1. Elevated levels of urea and creatinine in serum might be related to a low glomerular filtration rate (GFR), which is associated with renal dysfunction [3-6]. It has been proposed that GFR may decrease as a result of GM-induced necrosis of tubules, which may result in fewer functional nephrons. It is also possible that the tubular casts, which are most likely the result of peeling off tubular epithelial cells and shedding brush borders, impair renal excretory function by obstructing urine flow and may leave gaps along the tubular epithelial cells whereby glomerular filtrate can re-enter the circulation with further exacerbation of the GFR decline [6,9]. Renal KIM-1 gene upregulation in this study agrees with the results of previous studies in which the elevation in the expression of this protein was observed at very high levels on the apical membrane of proximal tubule cells after ischemic and nephrotoxic injury [54, 55]. KIM-1, a type I transmembrane glycoprotein, is undetectable in the healthy kidney; nevertheless, upon renal injury, proximal convoluted tubules have a dramatically increased expression of KIM-1 [54]. The increment in serum activity of ALP observed in this study revealed that GM-induced oxidative stress damages in rats [7, 8, 10]. ALP is an endoplasmic reticulum marker. It is often used to evaluate plasma membranes' integrity [9]. GM toxicity causes cell membrane injuries and increases the serum activity of ALP [56]. In this study, the low levels of both serum electrolytes Na+ and K+ after GM intoxication is in harmony with the observations of Bencheikh et al. [52] and could be attributed to the kidney's inability to conserve sodium and potassium. Govindappa et al. [55] suggested that GM blocks several basolateral and brush border membrane transporters, resulting in electrolyte imbalances.

Data from this study indicate that GM treatment increased TG, TC, and LDL serum levels while decreasing HDL levels. These results agree with the data from previous studies in which GM-induced nephrotoxicity was accompanied by secondary hyperlipidemia [57-59]. The increase in serum cholesterol in GM-intoxicated rats could be attributed to the liver's enhanced cholesterol synthesis because of the elevated bioavailability of cholesterol precursor mevalonate because of decreased mevalonate catabolism in the injured kidney [58, 60]. The hypertriglyceridemia related to renal damage could be explained by the delay in the clearance of the circulating TG-rich lipoproteins due to a decrease in lipoprotein lipase activity [57]. The role of hyperlipidemia in the pathophysiology of renal damage has been elucidated in many reports [57-60]. For instance, hyperlipidemic obese Zucker rats were found to develop albuminuria and spontaneous focal glomerulosclerosis, while the onset of glomerulosclerosis was reduced when plasma lipids were decreased with hypolipidemic drugs [60]. This points to a strong possibility that the development of hyperlipidemia during GM-induced nephrotoxicity may also be crucial for the development of renal injury. [59].

A substantial body of research suggests that partially reduced oxygen metabolites are key mediators of GM-induced nephropathy. GM increases the production of superoxide anion and hydrogen peroxide by renal cortical mitochondria. In the presence of a metal catalyst, the interaction between superoxide anion and hydrogen peroxide can form hydroxyl radicals. It has been reported that GM increases the production of hydroxyl radicals and causes the release of iron from renal cortical mitochondria [7-11]. Consistent with different previous investigations, the results of the present study show that GM caused a significant elevation in renal MDA levels and a noticeable drop in the activity of erythrocyte SOD and in the levels of blood and renal GSH. GM-induced elevation in renal MDA, a biomarker of endogenous lipid peroxidation, is indirect evidence of an increase in a free radical generation [59, 61-62]. GSH can protect cells from injury caused by oxygen free radicals as it is one of the most significant endogenous antioxidants that scavenge singlet oxygen and hydroxyl radicals. Additionally, SOD could catalyze the mutation of superoxide radicals. So, it is considered the main enzyme to react with oxygen radicals. It is proposed that antioxidant enzymes are suppressed by ROS, including O_2^-, H_2O_2, and OH^- radicals. The suppressive effects of GM on the enzymatic antioxidants lead to ROS generation, which is considered one of the factors associated with GM-induced renal toxicity and dysfunction [8,50].

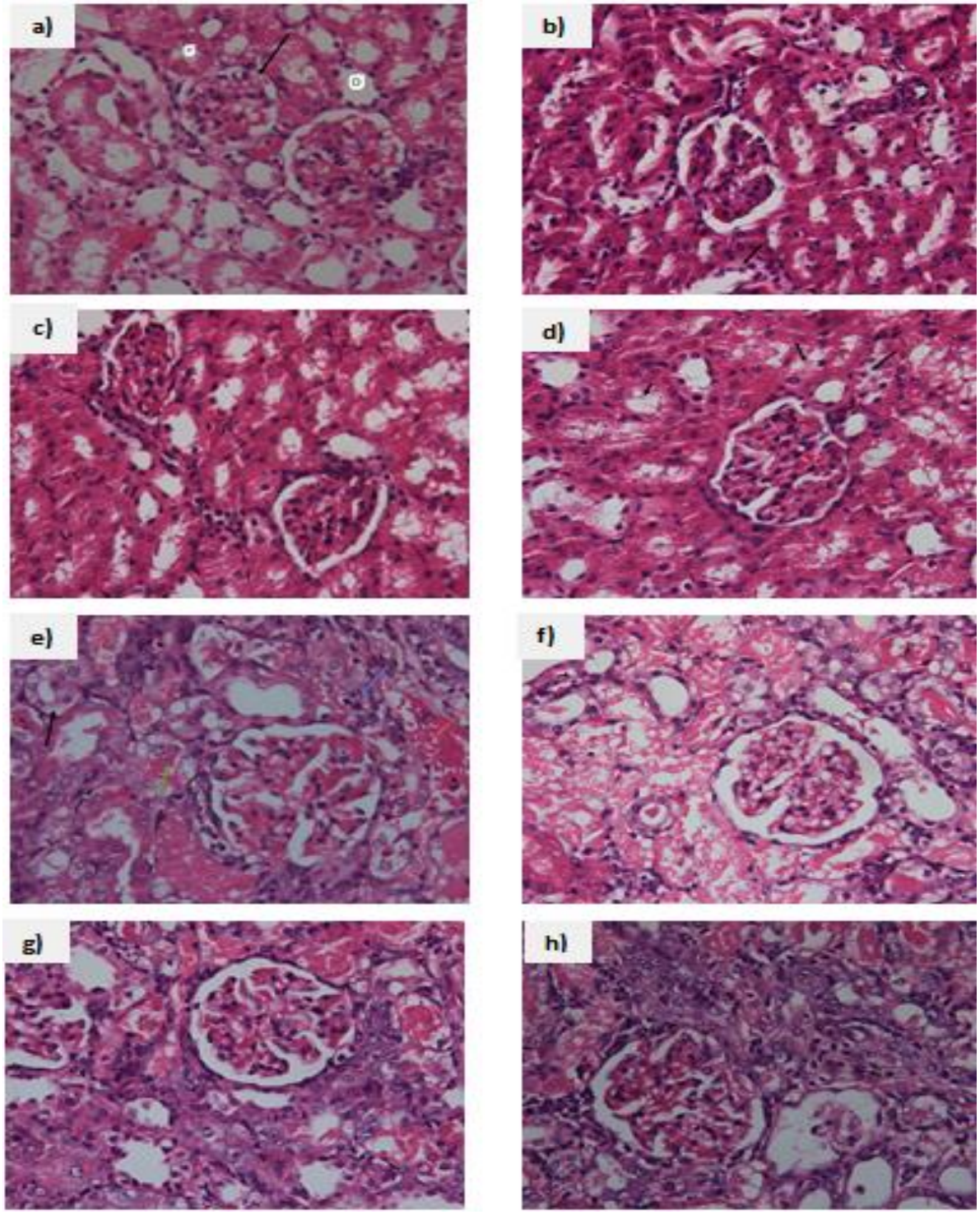

Figure 7. A photomicrograph of a section in the kidney cortex in: a) normal control showing normal glomeruli (black arrow), proximal convoluted tubules (P), and distal convoluted tubules (D), b) normal rats that received GrSE showing almost normal appearance of glomeruli and tubules, c) normal rats that received GuSE showing normal appearance of glomeruli and tubules, d) normal rats that received grape and guava seed ethanolic extracts showing hydropic degeneration in some tubules e) GM-intoxicated rats showing hydropic degeneration in tubules (black arrow), acidophilic casts in tubules (green arrow), congestion of blood vessels (red arrow) and lymphocytic infiltration (blue arrow), f) GM-injected rats treated with GrSE showing less damage in renal tubules, g) GM-treated rats that received GuSE showing normal glomeruli and less damage, h) GM-injected rats that received the combined extracts showing normal glomeruli and less damage in renal tubules H & E X400

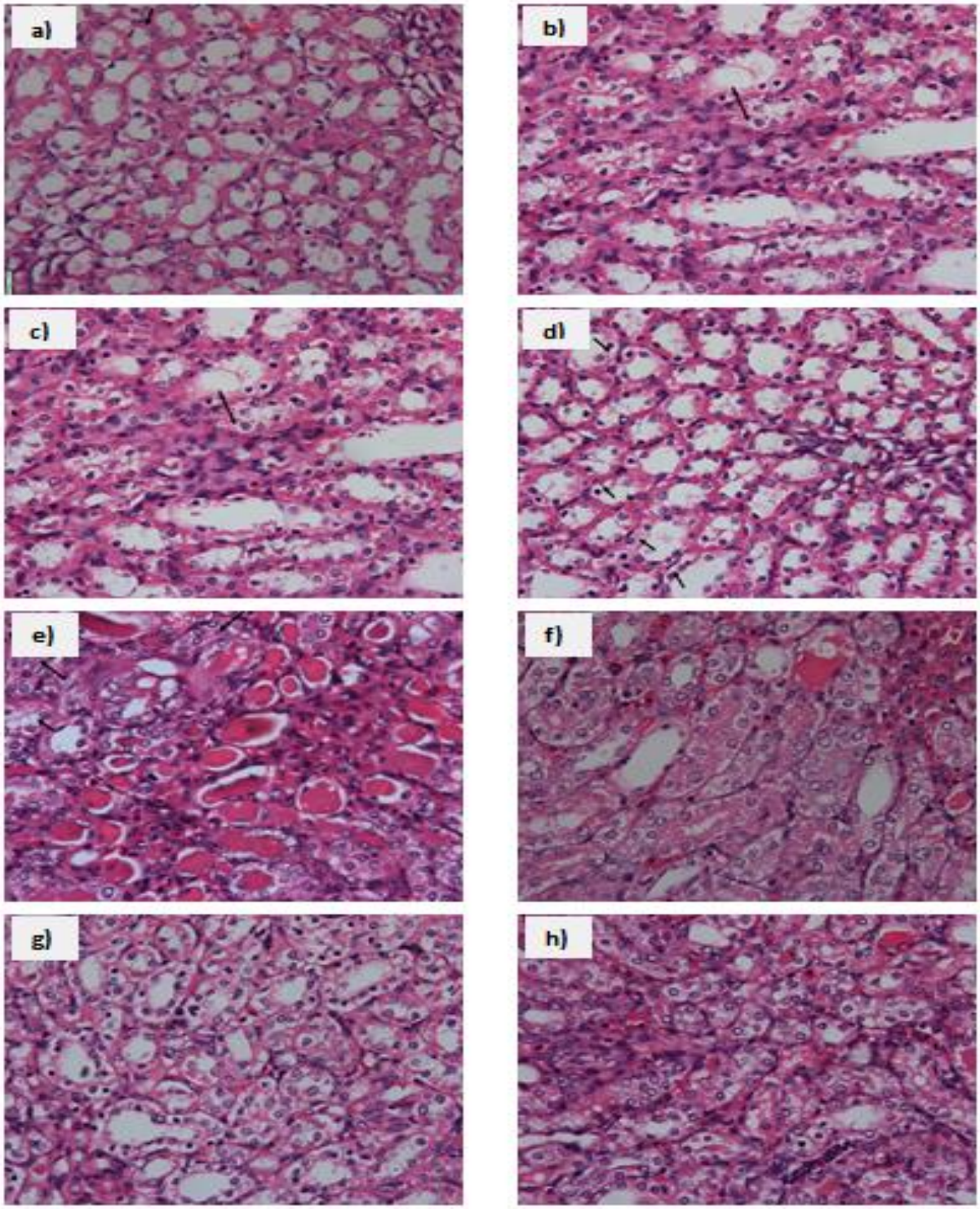

Figure 8. A photomicrograph of a section in the kidney medulla in: a) normal control showing normal collecting tubules (black arrow) and blood vessels (red arrow), b) normal rats that received GrSE showing hydropic degeneration in few tubules (arrow), c normal rats that received GuSE showing minimal hydropic degeneration in some tubules (arrow), d) in normal rats that received grape and guava seed ethanolic extracts showing hydropic degeneration in some tubules (arrows), e) GM-intoxicated rats showing wide spread acidophilic casts in tubules with or without loss of lining epithelium in addition to hydropic degeneration in some tubules (arrows), f) GM-injected rats treated with GrSE showing less damage in renal tubules, g) GM-treated rats that received GuSE showing less damage in renal tubules compared positive control h) GM-injected rats that received the combined extracts showing less damage in renal tubules compared to positive control. H & E X400

In this investigation, the upregulation of NF-κB in renal tissue of GM-treated rats is in accordance with the results of previous studies and reinforces the role of GM-derived inflammation as an underlying cause of alteration of the kidney, favoring the development of nephrotoxicity [10-11]. Inflammation and oxidative stress are inextricably related because each enhances the other. In this context, oxidative stress invariably recruits inflammation through the activation of NF-κB, which is the general transcription factor for various proinflammatory cytokines, chemokines, and adhesion molecules [8-10]. A previous study demonstrated that increased NF-κB activation in GM-treated rats was followed by increased inflammatory substance synthesis. Moreover, TNFα also activates the NF-κB pathway, thus amplifying the inflammatory response [11-12].

The findings of this study suggest that hyperlipidemia, oxidative stress, and inflammation resulting from GM injection may all be factors that contribute to the kidney's alteration, promoting the development of nephrotoxicity [8,59,63]. Consequently, there is much interest in using plants rich in phenols and flavonoids because of their hypolipidemic characteristics, capacity to scavenge free radicals and antioxidant and anti-inflammatory properties. [16-18]. In the current investigation, rats given grape and/or guava seed extracts along with GM demonstrated moderation of the nephrotoxic insult as evidenced by restoration of the kidney function parameters towards the expected levels and improvement in blood lipid profile accompanied by a decline in lipid peroxidation, enhancement in the antioxidant status and diminished expression of NF-κB.

According to the HPLC-DAD study, the ethanolic extracts of grape and guava seeds are rich in polyphenolic compounds such as ferulic acid, hydroxytyrosol, gallic acid, methyl gallate, catechin, vanillic acid, quercetin, p-coumaric acid, naringenin, rutin, and 3-hydroxycinnamic acid. In agreement with the results of this investigation, it was reported that GrSE is a complex mixture of polyphenols containing dimers, trimers, and other oligomers of catechin and epicatechin and their gallate derivatives that are together called proanthocyanidins (PACs) [15-22]. PACs from grape seeds decreased lipid peroxidation by the chelation of ROS and enhancement of the antioxidant defense system [23]. In addition to the free radical scavenging and antioxidant activity [24], PACs also exhibit anti-inflammatory effects. PACs and anthocyanidins can inhibit cyclooxygenase and inducible nitric oxide synthase. As a result, these ingredients interfere with the inflammation signaling pathway [21-24]. Furthermore, Bashir et al. [64] showed that GrSE pre-administration activates the expression of the transcription factor nuclear factor-erythroid 2-related factor 2 (Nrf2), decreases the NF-κB activities, indicating its inhibitory effect on the inflammatory response by utilizing NF-κB-dependent pathway. Additionally, Nrf2 is regarded as one of the most crucial cellular defense systems to battle oxidative stress, particularly in regulating Phase II detoxifying enzymes and antioxidant status. Nrf2 activates several antioxidant genes, including heme oxygenase-1 (HO-1), and catalyzes the breakdown of heme to yield CO and biliverdin, which serves as an antioxidant substance in the biological system [64]. Moreover, previous studies have shown that PACs regulate lipid metabolism and are potent hypolipidemic agents [65-66]. It was hypothesized that PACs cause hypolipidemia by influencing the miRNAs that regulate lipid metabolism [65].

In the same context, guava seeds are a promising source of bioactive substances and potential antioxidant, antimicrobial, and anticarcinogenic compounds [28]. The results of this study confirmed the results of previous studies where HPLC identified chlorogenic acid, caffeic acid, ferulic acid, and O-coumaric acid in guava seed extract [68-70]. Similarly, Flores et al. [70] reported that this fruit contains phenolic components (flavonoids and derivatives of ellagic acid) that have antioxidant properties and inhibitory activity on interleukin-8 production and matrix metalloproteinase-1 expression. Furthermore, novel guava seed polysaccharides have been studied for their potential to be anti-inflammatory and anti-PC-3 prostate cancer [69].

Based on these results, the presence of the bioactive polyphenolic compounds in GrSE and GuSE may be responsible for the nephroprotective effects observed in this study.

In conclusion, the present study confirmed that GM-induced kidney injury, as indicated by the elevation of serum urea, creatinine, and ALP, increased expression of KIM-1 in the kidney besides disturbance in serum electrolytes such as Na+ and K+ and hyperlipidemia along with elevation of oxidative stress and inflammation. Grape and guava seed extracts ameliorated GM-induced kidney injury in rats by reducing hyperlipidemia and lipid peroxidation, potentiating endogenous antioxidant capacity, and suppressing inflammatory pathways.

6. References

1. Jian, X., Deng, Z. and Sun, Y. (2015): Aminoglycoside gentamicin research: fundamental progress and new application prospects. Sheng Wu Gong Cheng Xue Bao. 31; 829-44
2. Bayomy, N.A., Elbakary, R.H., Ibrahim, M.A.A. and Abdelaziz, E.Z. (2017): Effect of Lycopene and Rosmarinic Acid on Gentamicin Induced Renal Cortical Oxidative Stress, Apoptosis, and

Autophagy in Adult Male Albino Rat. Anat Rec (Hoboken). 300:1137-1149.
3. Hayward, R.S., Harding, J., Molloy, R., Land, L., Longcroft-Neal, K., Moore, D. and Ross, J.D.C. (2018): Adverse effects of a single dose of gentamicin in adults: a systematic review. Br J Clin Pharmacol. 84:223-238
4. Garg, B.D., Olson, M.J., Li, L.C. and Roy, A.K. (1989): Phagolysosomal alterations induced by unleaded gasoline in epithelial cells of the proximal convoluted tubules of male rats: effect of dose and treatment duration. J Toxicol Environ Health. 26: 101-18.
5. Ramsammy, L.S., Josepovitz, C., Lane, B. and Kaloyanides, G.J. (1989): Effect of gentamicin on phospholipid metabolism in cultured rabbit proximal tubular cells. Am J Physiol. 256(1 Pt 1):C204-13.
6. Nagai, J. and Takano, M. (2014): Entry of aminoglycosides into renal tubular epithelial cells via endocytosis-dependent and endocytosis-independent pathways. Biochem Pharmacol. 90: 331-7.
7. Zaidi, S.N.F. and Usman S.M. (2021): Salicylic acid attenuates gentamicin-induced nephrotoxicity in rabbits. Pak J Pharm Sci. 34:165-170.
8. Pakfetrat, Z., Janfeshan, S., Masjedi, F., Rafiei, M. and Karimi, Z. (2021): Involvement of oxidative stress and toll-like receptor-4 signaling pathways in gentamicin-induced nephrotoxicity in male Sprague Dawley rats. Drug Chem Toxicol. 19:1-8.
9. Paszti-Gere, E., Barna, R.F., Kovago, C., Szauder, I., Ujhelyi, G., Jakab, C., Meggyesházi, N., Szekacs, A. (2015): Changes in the distribution of type II transmembrane serine protease, TMPRSS2 and in paracellular permeability in IPEC-J2 cells exposed to oxidative stress. Inflammation. 38:775-83.
10. Wu, D., Luo, N., Wang, L., Zhao, Z., Bu, H., Xu, G., Yan, Y., Che, X., Jiao, Z., Zhao, T., Chen, J., Ji, A., Li, Y. and Lee, G.D. (2017): Hydrogen sulfide ameliorates chronic renal failure in rats by inhibiting apoptosis and inflammation through ROS/MAPK and NF-κB signaling pathways. Sci Rep. 7: 455.
11. Vysakh, A., Abhilash, S., Kuriakose, J., Midhun, S.J., Jyothis, M. and Latha, M.S. (2018): Protective effect of Rotula aquatica Lour against gentamicin induced oxidative stress and nephrotoxicity in Wistar rats. Biomed Pharmacother. 106:1188-1194.
12. Pflug, K.M. and Sitcheran, R. (2020): Targeting NF-κB-Inducing Kinase (NIK) in Immunity, Inflammation, and Cancer. Int J Mol Sci. 21:8470.
13. Chaouch, M.A. and Benvenuti, S. (2020): The Role of Fruit by-Products as Bioactive Compounds for Intestinal Health. Foods. 9:1716.
14. Machado, A.P.D.F., Geraldi, M.V., do Nascimento, R.P., Moya, A.M.T.M., Vezza, T., Diez-Echave, P., Gálvez, J.J., Cazarin, C.B.B. and Maróstica Júnior, M.R. (2021): Polyphenols from food by-products: An alternative or complementary therapy to IBD conventional treatments. Food Res Int. 140:110018.
15. Peixoto, C.M., Dias, M.I., Alves, M.J., Calhelha, R.C., Barros, L., Pinho, S.P. and Ferreira, I.C.F.R. (2018): Grape pomace as a source of phenolic compounds and diverse bioactive properties. Food Chem. 253: 132-138.
16. Zhu, F., Du, B. and Xu, B. (2018): Anti-inflammatory effects of phytochemicals from fruits, vegetables, and food legumes: A review. Crit Rev Food Sci Nutr. 58:1260-1270.
17. Nikawa, T., Ulla, A. and Sakakibara, I. (2021): Polyphenols and Their Effects on Muscle Atrophy and Muscle Health. Molecules. 26:4887.
18. Zhang, Q., Cheng, Z., Wang, Y. and Fu, L. (2021): Dietary protein-phenolic interactions: characterization, biochemical-physiological consequences, and potential food applications. Crit Rev Food Sci Nutr. 61:3589-3615.
19. Thorsten, M., Andreas, S., Dietmar, R. K. and Reinhold, C. (2013): Residues of grape (Vitis vinifera L.) seed oil production as a valuable source of phenolic antioxidants [2009] Food chemistry, 112:551-559
20. Lucarini, M., Durazzo, A., Romani, A., Campo, M., Lombardi-Boccia, G. and Cecchini, F. (2018): Bio-Based Compounds from Grape Seeds: A Biorefinery Approach. Molecules. 23:1888.
21. Sochorova, L., Prusova, B., Jurikova, T., Mlcek, J., Adamkova, A., Baron, M. and Sochor J. (2020): The Study of Antioxidant Components in Grape Seeds. Molecules. 25:3736.
22. Tang, G.Y.; Zhao, C.N.; Liu, Q.; Feng, X.L.; Xu, X.Y.; Cao, S.Y.; Meng, X.; Li, S.; Gan, R.Y and Li, H.B. (2018): Potential of grape wastes as a natural source of bioactive compounds, Molecules, 23, 2598.
23. Rodríguez-Pérez, C., García-Villanova, B., Guerra-Hernández, E. and Verardo, V. (2019): Grape Seeds Proanthocyanidins: An Overview of In Vivo Bioactivity in Animal Models. Nutrients. 11:2435.

24. Gupta, M., Dey, S., Marbaniang D, Pal P, Ray S, Mazumder B. Grape seed extract: having a potential health benefit. J Food Sci Technol. 2020 Apr;57(4):1205-1215
25. Radha, M., Rais, N., Satankar, V., Pandiselvam, R., Sayed, A.A.S., Senapathy, M., Anitha, T., Singh, S., Tomar, M., Dey, A., Zengin, G., Amarowicz, R. and Bhuyan D.J. (2022): Guava (Psidium guajava L.) seed: A low-volume, high-value byproduct for human health and the food industry. Food Chem. 386:132694.
26. Jamieson, S., Wallace, C.E., Das, N., Bhattacharyya, P. and Bishayee, A. (2021): Guava (Psidium guajava L.): a glorious plant with cancer preventive and therapeutic potential. Crit Rev Food Sci Nutr. 21:1-32.
27. Liu, X., Yan, X., Bi, J., Liu, J., Zhou, M., Wu, X. and Chen Q. (2018): Determination of phenolic compounds and antioxidant activities from peel, flesh, seed of guava (Psidium guajava L.). Electrophoresis. 39:1654-1662.
28. Michael, H.N., Salib J.Y. and Ishak M.S. (2002): Acylated flavonol glycoside from Psidium guajava L. seeds, Pharmazie, 57:859- 60,
29. Reeves, P.G., Nielsen, F.H. and Fahey, G.C. Jr. (1993): AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr. 123:1939-51.
30. Enginar, H., Cemek, M., Karaca, T. and Unak, P. (2007): Effect of grape seed extract on lipid peroxidation, antioxidant activity and peripheral blood lymphocytes in rats exposed to x-radiation.International Devoted to Pharmacological,21:1029-1035.
31. Safa, J., Argani, H., Bastani, B., Nezami, N., Rahimi, A. B., Ghorbani, H. A., Kalagheichi, H., Amirfirouzi, A. and Mesgari, M., J. (2010): Protective effect of grape seed extract on gentamicin-induced acute kidney injury, Iranian Journal of Kidney Diseases ,1735-8582.
32. Muruganandan, S., Srinivasan, K., Tandan, S., Lal, J., Chandra, S. and Raviprakash, V. (2001): Anti-inflammatory and analgesic activities of some medicinal plants. Anti-inflammatory analgesic activities of some medicinal plants. 22: 56-58
33. Dhanarajan, R., Abraham, P. and Isaac, B. (2006): Protective effect of ebselen, a selenoorganic drug, against gentamicin-induced renal damage in rats. Basic clinical pharmacology toxicology. 99: 267-272.
34. Thomas, L., Tranchand, P., Berard, F., Secchi, T., Colin, C. and Moulin, G. (1998): Semiological value of ABCDE criteria in the diagnosis of cutaneous pigmented tumors. Dermatology. 197: 11-17.
35. Soldin, S. J., Brugnara, C. and Wong, E. (2007): Pediatric reference intervals, American Association for Clinical Chemistry, Incorporated.
36. Young, S. D., O'byrne, S. F., Young, D. S., Young, S. and Stephen, O. B. (2000): EVA and value-based management, McGraw-Hill Professional Publishing.
37. Tietz, N.W. (1994): Determination of total protein in serum and urine. Fundamentals of clinical chemistry, 2 nd ed. (Tietz, N.W. ed). pp 692.
38. Guder, W., da Fonseca-Wollheim, F., Heil, W., Schmitt, Y., Töpfer, G., Wisser, H. and Zawta, B. (2000): The haemolytic, icteric and lipemic sample recommendations regarding their recognition and prevention of clinically relevant interferences. Journal of Laboratory Medicin.24: 357-364.
39. Tietz, N. w. (1976): Determination of potassium by turbidity measurement without deproteinization, Fundamentals of Clin Chem 876.
40. Trinder, P. (1951): Determination of sodium by colorimetric measurement with sodium ion precipitation. Analyst. 76: 595.
41. Fossati, P. and Prencipe, L. (1982): Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin. Chem. 28: 2077-80.
42. Rifal, N., Warnick, G.R. editors: (1994): Laboratory measurement of lipids, lipoproteins and apolipoproteins. Washington DC: American Association of Clinical Chemistry (AACC) p. 21 – 42.
43. Friedewald, W.T., Levy, R.I. and Frerickson, D.S. (1972): Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin. Chem. 18:499-502.
44. Beutler, E., Duron, O. and Kelly, B. M. (1963): Improved method for the determination of blood glutathione. Lab. Clin. Med. 61: 882-888.
45. Uchiyama, M. and Mihara, M. (1978): Determination of malondialdehyde precursor in tissues by thiobarbituric acid test. Anal. biochem. 86: 271-278.
46. Winterbourne, C. C., Howkins, R. E., Brain, M. and Carrell, R. W. (1975): The estimation of red

cell SOD activity. Laboratory and clinical medicine. 85: 337-34

47. Rao, X., Huang, X., Zhou, Z. and Lin, X. (2013): An improvement of the 2ˆ(-delta delta CT) method for quantitative real-time polymerase chain reaction data analysis, Biostat Bioinforma Biomat 3:71-85.
48. Bancroft, J.D., Stevens, A., Turner, D.R., (1996): Theory and Practice of Histological Techniques, Fourth ed. Churchill Livingstone, New York.
49. IBM corp. released 2012.IBM SPSS Statistics for Windows, Version 2012. Armonk, NY: IBM corp.
50. Medić, B., Stojanović, M., Rovčanin, B., Kekić, D., Škodrić, S. R., Jovanović, G. B., Vujović, K. S., Divac, N., Stojanović, R. and Radenković, M. (2019): Pioglitazone attenuates kidney injury in an experimental model of gentamicin-induced nephrotoxicity in rats. Scientific reports 9:1-10
51. Nale, L. P., More, P. R., More, B. K., Ghumare, B. C., Shendre, S. B., and Mote, C. S. (2012): Protective effect of Carica papaya L. seed extract in gentamicin induced hepatotoxicity and nephrotoxicity in rats. Int J Pharm Bio Sci, 3: 508-515.
52. Bencheikh, N., Bouhrim, M., Kharchoufa, L., Al Kamaly, O.M., Mechchate, H., Es-Safi, I., Dahmani, A., Ouahhoud, S., El Assri, S., Eto, B., Bnouham, M., Choukri, M. and Elachouri M. (2021): The Nephroprotective Effect of Zizyphus lotus L. (Desf.) Fruits in a Gentamicin-Induced Acute Kidney Injury Model in Rats: A Biochemical and Histopathological Investigation. Molecules, 26:4806.
53. Deboer, M.D. (2009): Animal models of anorexia and cachexia. Expert Opin Drug Discov., 4(11):1145-1155.
54. Luo, Q.H., Chen, M.L., Chen, Z.L., Huang, C., Cheng, A.C., Fang, J., Tang, L. and Geng, Y. (2016): Evaluation of KIM-1 and NGAL as Early Indicators for Assessment of Gentamycin-Induced Nephrotoxicity Kidney Blood Press Res 41:911-918.
55. Govindappa, P.K., Gautam, V., Tripathi, S.M., Sahni, Y.P. and Raghavendra, H.L.S. (2019): Effect of Withania Somnifera on Gentamicin Induced Renal Lesions in Rats. Braz. J. Pharmacogn., 29: 234–240.
56. Khan, M. R., Badar, I. and Siddiquah, A. (2011): Prevention of hepatorenal toxicity with Sonchus asper in gentamicin treated rats. BMC complementary alternative medicine. 11: 1-9.
57. Abdel-Gayoum, A. A., Ali, B. H., Ghawarsha, K. and Bashir, A. A. (1993): Plasma lipid profile in rat with gentamicin-induced nephrotoxicity. Human & experimental toxicology, 12: 371-375.
58. Ahmadvand, H., Dehnoo, M.G., Dehghani, A., Bagheri, S. and Rooh Angiz, R. (2014): Serum paraoxonase 1 status and its association with atherogenic indexes in gentamicin-induced nephrotoxicity in rats treated with coenzyme Q10, Cheraghi Ren Fail., 36: 413–418
59. Babaeenezhad, E., Negar Nouryazdan, N., Nasri, M., Ahmadvand, H. and Sarabi M.M. (2021): Cinnamic acid ameliorate gentamicin-induced liver dysfunctions and nephrotoxicity in rats through induction of antioxidant activities, Heliyon 7 e07465
60. Kisiske, B.L., Clearly, M.P. and Keane, W.F. (1988): Treatment of hyperlipidaemia reduces glomerular injury in obese Zucker rats, Kidney International' 33: 667-72.
61. Salem, N. A. and Salem, E. A. (2011): Renoprotective effect of grape seed extract against oxidative stress induced by gentamicin and hypercholesterolemia in rats, Renal Failure. 33: 824-832.
62. Laorodphun, P., Cherngwelling, R., Panya, A. and Arjinajarn, P. (2022): Curcumin protects rats against gentamicin-induced nephrotoxicity by amelioration of oxidative stress, endoplasmic reticulum stress and apoptosis. Pharm Biol. 60:491-500
63. Ismaiel E.A.M., Abd El-Latif H.A., Mohammed H.E., Abd El Fattah M.A., Rashed L., Abdel-Wahhab M.A.(2020): Modulation of nephrotoxicity induced by gentamicin with bone marrow mesenchymal stem cells and moringa oleifera extract, Egyptian Journal of Chemistry, 63 , pp. 751-763
64. Bashir, N., Manoharan, V. and Miltonprabu, S. (2016): Grape seed proanthocyanidins protects against cadmium induced oxidative pancreatitis in rats by attenuating oxidative stress, inflammation and apoptosis via Nrf-2/HO-1 signaling, The Journal of Nutritional Biochemistry. 32:128-141
65. Escudero, L.B., Bladé, C., Ribas-Latre, A., Casanova, E., Salvadó, M. J., Arola, L., and Arnal, A.A. (2012): Grape seed proanthocyanidins repress the hepatic lipid regulators miR33 and miR122 in rats. Molecular nutrition & food research, 56: 1636-1646.

66. Razavi, S. M., Gholamin, S., Eskandari, A., Mohsenian, N., Ghorbanihaghjo, A., Delazar, A. andArgani, H. (2013): Red grape seed extract improves lipid profiles and decreases oxidized low-density lipoprotein in patients with mild hyperlipidemia. Journal of Medicinal Food, 16: 255-258.
67. El Anany, A.M. (2015): Nutritional composition, antinutritional factors, bioactive compounds and antioxidant activity of guava seeds (Psidium Myrtaceae) as affected by roasting processes. J Food Sci Technol. 52:2175-83
68. Mohamed, G.F, Mohamed, S.S. and Fakhriya , S.T. (2011): Antioxidant, antimicrobial, and anticarcinogenic properties of Egyptian guava seed extracts. Nat. and Sci., 9 (11): 32- 41
69. Lin, H.C. and Lin, J.Y. (2020): GSF3, a polysaccharide from guava (*Psidium guajava* L.) seeds, inhibits MCF-7 breast cancer cell growth via increasing Bax/Bcl-2 ratio or Fas mRNA expression levels. Int. J. Biol. Macromol. 161:1261–1271.
70. Flores, G., Dastmalchi, K., Wu, S.-B., Whalen, K., Dabo, A.J., Reynertson, K.A., Foronjy, R.F., D'Armiento, J.M. and Kennelly E.J. (2013): Phenolic-rich extract from the Costa Rican guava (*Psidium friedrichsthalianum*) pulp with antioxidant and anti-inflammatory activity. Potential for COPD therapy. Food Chem; 141:889–895.