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Aqueous garlic extract supresses experimental gentamicin induced renal pathophysiology mediated by oxidative stress, inflammation and Kim-1

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ABSTRACT

Background: Gentamicin (Gent) has rapid & high bactericidal action in addition to its cheap price. Nevertheless, 30% of gentamicin-treated patients develop nephrotoxicity.
Objective: To explore the probable nephroprotective effects of the aqueous garlic extract (AGE) & to elucidate its underlying mechanisms via monitoring proinflammatory cytokines as tumer necrosis factor (TNF-α), interleukin 6 (IL-6) and interferon-γ (INF-γ), oxidative stess markers as malondialdehyde (MDA) & superoxide dismutase (SOD) & kidney injury molecule (Kim-1) as a promising early specific biomarker of renal dysfunction.
Methods: 32 adult male rats were divided into 4 equal groups treated for 21 days as: normal control group received normal saline orally, AGE-treated group received AGE at 250 mg/kg/day orally, Gent-treated group received Gent-sulphate intraperitoneal injection at 80 mg/kg /day, and AGE & Gent cotreated group received AGE and Gent concomitantly in the same previous doses. Serum urea, creatinine, glomerular filteration rate (GFR), systolic (SBP) and diastolic blood pressure (DBP), TNF-α, IL-6, INF-γ, MDA and SOD and Kim-1 mRNA expression were evaluated in kidney tissue homogenate. Renal cortex sections stained with Haematoxylin & eosin (H&E) were examined.
Results: AGE is nephroprotective through significantly reducing serum urea, creatinine, SBP and DBP, TNF-α, IL-6, INF-γ and MDA (the main product of lipid peroxidation), decreasing expression of Kim-1 mRNA in renal tissue and increasing level of GFR, the natural antioxidant SOD and improving renal histological features of Gent-treated rats.
Conclusion: AGE normalizes Gent-induced renal dysfunction. Their co-administration is a plausible advice, although the therapeutic efficiency of Gent was not investigated.

1. Introduction

The gentamicin (Gent) family of aminoglycoside antibiotics are highly effective antimicrobial agents, particularly against severe gram-negative bacterial infections. However, Gent produces nephrotoxicity as the iceberg of a list of other serious side-effects [1]. It had been reported that up to 30% of patients receiving Gent develop symptoms of nephrotoxicity [2]. Despite such toxicities, Gent is prescribed commonly due to its rapid & high bactericidal action, low incidence of resistance, chemical stability, synergistic effect with beta-lactam antibiotics and cheap price [3]. Gent-induced kidney injury may be explicated by increased production of reactive oxygen (ROS) and nitrogen species (RNS), reduction in natural antioxidant defense and activation of inflammatory processes [2]. However, the exact mechanism of Gent-induced nephrotoxicity remains unclear and necessitates further investigations [4].

Tumor necrosis factor-alpha is a proinflammatory cytokine. Gent increases the infiltration of macrophage and stimulates the production of TNF-α that enhances the progression of nephritis [5]. Kidney injury molecule-1 is one of the biomarkers used for early discovery of renal injury [6]. It is a type-I transmembrane glycoprotein unnoticeable in healthy kidney tissue. It is coded by a gene that is a homolog of the human and mouse hepatitis A virus cellular receptor-1 gene (Havcr1). Its expression is massively increased on the luminal membrane of proximal tubular cells after ischemic and nephrotoxic injury [7] and is shed into the urine [8]. Determination

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Table 1
Primer sequences and conditions for amplifying Kim-1 mRNA. Product size is 214 bp. FP and RP = forward and reverse primers. S5'C and S3'C = Self-5'- and 3'-complementarity.

Prime	5'→3' Sequence	Start	Stop	Tm	GC%	S5'C	S3'C
FP (22 bp)	TTTGGATCTGTACCCAGTGCTT	123	144	59.62	45.45	4.0	1.0
RP (20 bp)	CAAGGCCAGCCCTCTAATGG	336	317	60.47	60.0	5.0	2.0

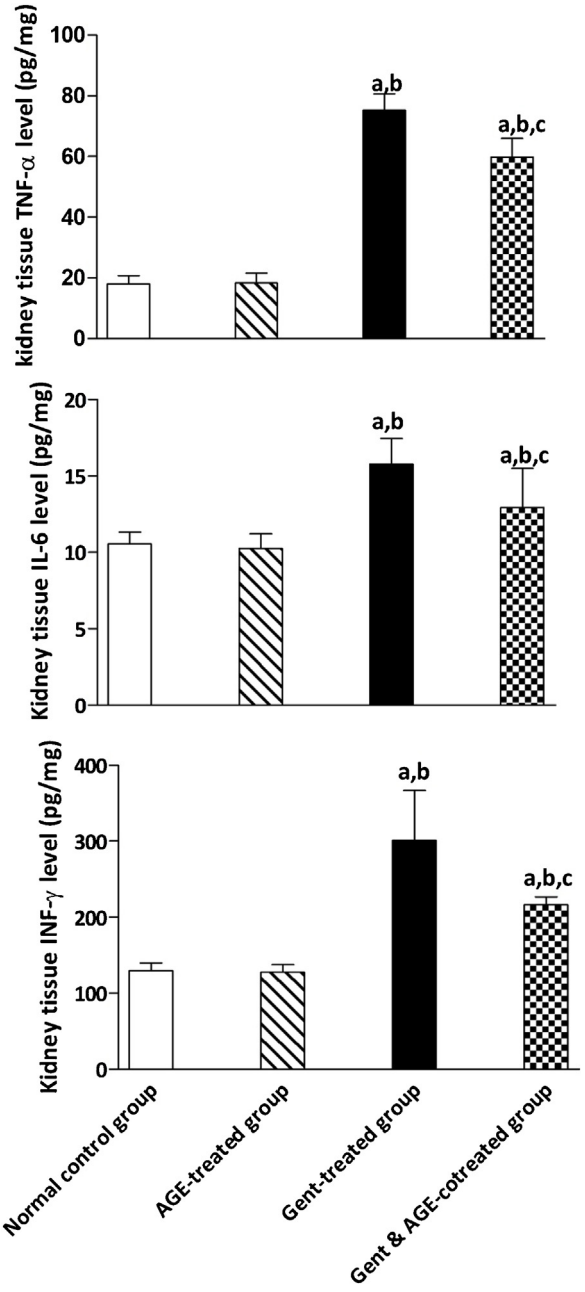

Fig. 1. Kidney tissue homogenate levels of inflammatory markers: TNF-α, IL-6, INF-γ in the different studied groups. Data are mean ± SD (n = 8 for each group). aP: significant vs. the normal control group. bP: significant vs. AGE-treated group; cP: significant vs. Gent-treated group. ($P \leq 0.05$).

of urinary Kim-1 enables sensitive, specific and accurate prediction of human nephrotoxicity in preclinical drug screening [9].

Garlic (Allium sativum) is a member of Alliaceae family, broadly cultivated in the northern hemisphere, contains more than 200 active chemical compounds [10]. It is commonly investigated in its aqueous homogenate extract. Allicin (diallyl-thiosulfinate) is the principal bioactive compound present in the extract. Alliinase is activated by garlic cloves chopping or crushing to convert alliin into allicin. In addition, allyl-methyl-thiosulfonate, 1-propenyl-allyl-thiosulfonate, and γ-L-glutamyl-S-alkyl-L-cysteine are important sulfur-containing compounds present in garlic homogenate [11]. These volatile sulfur-containing thiosulfinates are responsible for the pungent aroma, taste and biological effects of garlic [10]. Moreover, garlic has large amount of antioxidants and flavonoids which can be utilized in detoxification systems [12]. Garlic has numerous pharmacological actions and medical applications, namely: lowers serum cholesterol levels, decreases oxidative stress, stimulates the immune system, enhances detoxification of foreign compounds and has antibacterial & antifungal actions [13].

The aim of this experimental study is to investigate the nephroprotective effects of AGE in a rat model of Gent-induced nephrotoxicity and its potential ameliorating effect on changes induced in arterial blood pressure (ABP) and expression of Kim-1, inflammatory and oxidative stress markers. In addition, traditional renal dysfunction biomarkers and renal tissue histopathological examination will be done.

2. Materials and methods

2.1. Gentamicin and aqueous garlic extract preparation

Gentamicin sulphate (Epigenet; EIPICO, 6th of October City, Egypt) 80 mg/2 mL ampoules were used. Fresh local cultivar garlic was bought from the local market, Assiut, Egypt. AGE was prepared from 50 g of peeled cloves after chopping, homogenized in 75 mL of cold sterile 0.9% NaCl, filtered thrice, and centrifuged at 2000 rpm for 10 min. The clear supernatant was recovered and made up to 100 mL with the saline. The concentration of this garlic preparation is 500 mg/mL depending on the weight of the starting material [14].

2.2. Animals

A total of 32 adult male rats belonging to Wistar albino strain and weighing 190–250 g were used in this study. The rats were housed in clean capacious cages in the animal house of the Faculty of Medicine, Assiut University, Assiut, Egypt. They were maintained on a natural 12:12-h light-dark cycle, in an aerated room, temperature (25 ± 5 °C), with ad libitum water and food (standard rat pellets). Before the beginning of the experiment, animals were adapted to the laboratory conditions for one week. This study design was permitted by the Animal Bioethics Committee of the Faculty of Medicine, Assiut University, Assiut, Egypt.

2.3. Experimental design and collection of samples

Animals were randomly divided into 4 groups (8 animals each): Group 1 (normal control group) received 0.9% NaCl solution by oral gavage. Group 2 (AGE-treated group) received AGE at a dose of 250 mg/kg/day by oral gavage [15]. Group 3 (Gent-treated group) received Gent at a dose of 80 mg/kg/day by intraperitoneal injection [16,17]. Group 4 (AGE & Gent cotreated group) received both AGE and Gent in the same previous doses concurrently within 10 min. All treatments were administered daily for 21 days. Urine samples (in milliliters per 24 h) were collected from each animal using individual metabolic cage to estimate urinary creatinine for

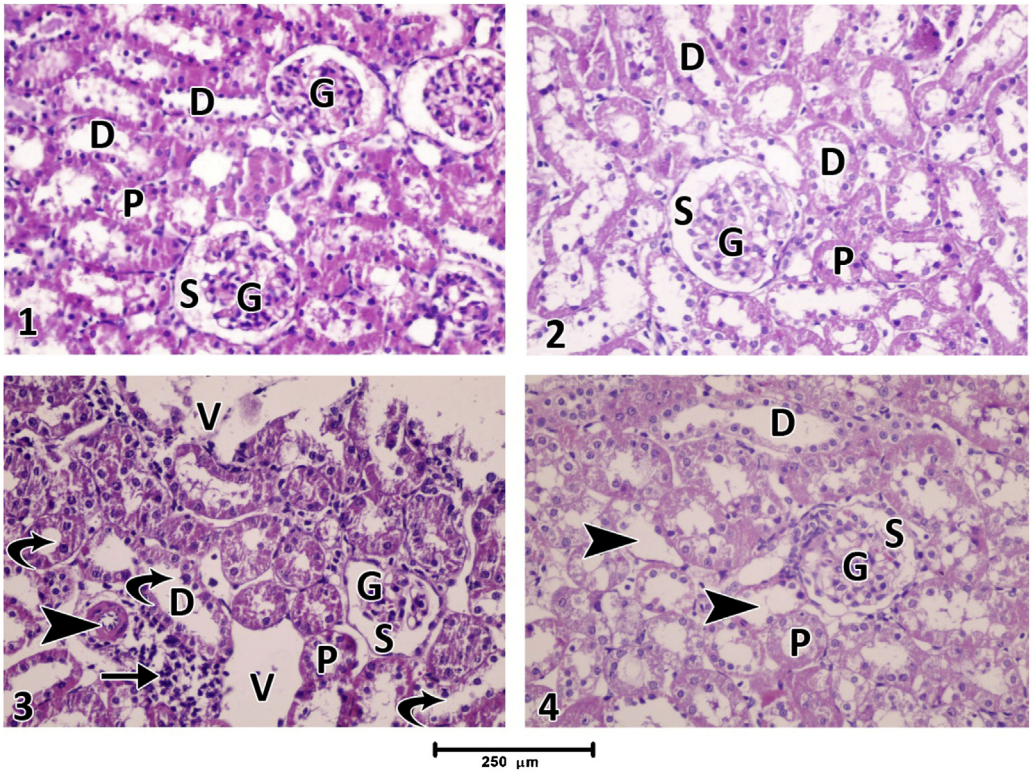

Fig. 2. Haematoxylin & eosin stained sections of the kidney cortex of adult male rat from: 1) the normal control group showing normal glomeruli (G) surrounded by Bowman's capsule with normal renal space (S), and normal proximal (P) and distal (D) convoluted tubules. 2) AGE-treated group showing similar histology as in (1). 3) Gent-treated group revealed fibrous interstitial connective tissue (arrow head), mononuclear inflammatory cellular infiltration (arrow) and vacuolization (V) of the convoluted tubules with tubular dilatation (curved arrows). And 4) Gent & AGE- cotreated group showed almost complete prevention of the histological alterations observed in (3) with only some tubular dilatation (arrow heads) (magnification: x400; scale bars =250 µm).

Table 2
Serum urea and creatinine levels (mg/dL) and glomerular filtration rate (GFR; mL/min) in the different studied groups.

Groups	Urea	Creatinine	GFR
Normal control	30.82 ± 5.22	0.52 ± 0.05	0.0317 ± 0.01
AGE-treated	29.07 ± 5.38	0.47 ± 0.09	0.0531 ± 0.035
Gent-treated	40.41 ± 5.06[a,b]	0.68 ± 0.06[a,b]	0.02 ± 0.01
Gent &AGE cotreated	33.83 ± 3.48[c]	0.52 ± 0.13[c]	0.064 ± 0.05[c]

Data are mean ± SD (n = 8 for each group). [a]P: significant vs. the normal control group. [b]P: significant vs. AGE-treated group; [c]P: significant vs. Gent-treated group ($P \leq 0.05$).

Table 3
Levels of SOD and MDA in kidney tissue homogenate of the different studied groups.

Groups	SOD (u/mg)	MDA (nmol/mg)
Normal control	34.34 ± 7.15	3 ± 0.67
AGE-treated	35.83 ± 5.63	2.55 ± 0.8
Gent-treated	25.2 ± 8.1 [a,b]	4.64 ± 0.67 [a,b]
Gent &AGE cotreated	42.16 ± 4[c]	3.39 ± 1.27[c]

Data are mean ± SD (n = 8 for each group). [a]P: significant vs. the normal control group. [b]P: significant vs. AGE-treated group; [c]P: significant vs. Gent-treated group ($P \leq 0.05$).

3 consecutive days (at 11:30 AM) before scarification of animals to decrease the probability of missing the peaks of urinary creatinine.

Systolic & diastolic blood pressure were measured in conscious rats before the start of the experiment, and at end of the 1st, 2nd, and 3rd week by the indirect tail cuff method using Electrosphygmomanometer Pneumatic Pulse Transducer (Model LE 5001 Pressure Meter, Panlab, Harvard, USA). The animals were pre-warmed in a metal chamber at about 35 °C and allowed to acclimate for 30 min before the recordings were made. Three successive readings were made at the same time of day (8–10 a.m.). Mean SBP & DBP values from the three measurements were calculated.

Blood samples were taken from the retro-orbital vein, centrifuged at 3000 round per minute (rpm) for 15 min and the clear, non haemolysed supernatant sera were removed and kept at -20 C until use. Then, all animals were sacrificed by cervical dislocation under light phenobarbital anesthesia and the kidneys were quickly removed, rinsed with ice-cold saline and divided into 2 halfs, one half was immediately frozen in liquid nitrogen and stored at −80 °C for further analysis and the other was used for the histopathological examination.

2.4. Biochemical measurements

2.4.1. Serum and urinary creatinine measurements

Serum and urinary creatinine levels were measured spectrophotometrically using a kit from Biolabo (REF80107, Maizy, France) following the kit instructions.

2.4.2. Glomerular filtration rate (GFR) estimation

GFR was estimated by measuring creatinine clearance (urinary creatinine concentration (U) (mg/ml) x urine volume (V) (ml/min) / serum creatinine concentration (P) (mg/ml) according to [18].

2.4.3. Serum urea measurements

Serum urea was measured colorimetrically using a kit purchased from Spectrum Diagnostics (REF321 001, Obour City, Egypt) following the instruction of the manufacturer.

2.4.4. Measurement of proinflammatory cytokines (TNF-α, IL-6 INF-γ) in kidney tissue homogenate

1 g of kidney tissue was homogenized with 4 ml 0.9% NaCl at 4000 rpm and then extract were centrifuged at 20,000 rpm for

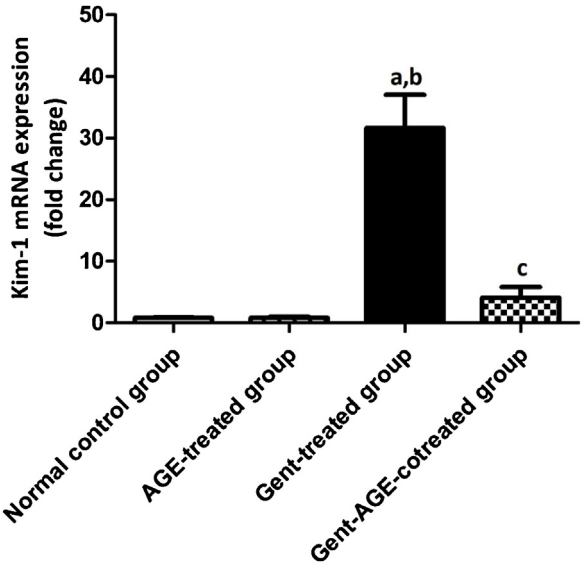

Fig. 3. Changes in mRNA expression of Kim-1 in kidney tissues of the different studied groups measured using real time PCR. Data were expressed mean ± SD. [a]P: significant vs. the normal control group. [b]P: significant vs. AGE-treated group; [c]P: significant vs. Gent-treated group. ($P \leq 0.05$).

20 min, supernatant was collected and stored at −20 °C for subsequent measurement of *IL-6*,TNF-*α*, *INF-γ* [19]. The level of rat TNF-α in the kidney tissue homogenate was measured using ELISA kit from Koma Biotech (Cat. #K0331196, Seoul, Korea) following the manufacturer's instructions [20]. IL-6 was measured in kidney tissue using a rat Interleukin-6 ELISA kit (CUSABIO, Wuhan, China) which employs the quantitative sandwich enzyme immunoassay technique, and expressed as pg/mg. The levels of IFN- γ were measured by a solid phase sandwich ELISA (AbC 606 and AbC 607, respectively; Votrefournisseur AbCysS.A. Paris, France) and expressed as pg/mg.

2.5. Measurement of MDA & SOD

MDA & SOD were measured by colormetric kits supplied by Biodiagnostic co (Biodiagnostic, Giza, Egypt) and expressed as nmol/mg & u/mg respectively.

2.6. Histopathogical study

Half of the kidney was fixed in 10% neutral buffered formalin, dehydrated and embedded in paraffin. 5-μm-thick paraffin sections were made and stained with H&E for histopathological assessment [21].

2.7. Quantification of Kim-1 mRNA in kidney tissue using real time PCR

RNA was isolated from the kidney tissue homogenate using GeneZol™ CT RNA Extraction reagent (Genetix Biotech Asia, Shivaji Marg, New Delhi, India) and further purified using the RNeasy Kit (Takara, Dalian, China) that included DNase treatment. Complimentary DNA (cDNA) was synthesized from RNA using high-capacity cDNA reverse transcription kit (Applied Biosystems Inc, CA, USA). Gene expression was detected by Step One Plus™ Real-Time PCR System (Applied Biosystems Inc, CA, USA) and SYBR Green Real-Time PCR Master Mix (Applied Biosystems Inc, CA, USA). Primer sequences are shown in Table 1. Gene expression changes relative to untreated controls were determined by the $2^{-\Delta\Delta Ct}$ method.

2.8. Statistical analysis

SPSS program version 16 (SPSS Inc., Chicago, USA) was used for analysis of data. Results were expressed as mean ± SD. Statistical analysis was carried out using one-way ANOVA followed by Tukey's post hoc test. Pearson's correlations were assessed. Values of $P \leq 0.05$ were regarded statistically significant.

3. Results

3.1. Effect of Gent and AGE on serum kidney function and GFR

Insignificant differences in the levels of urea, creatinine and GFR were found between normal control and AGE-treated groups. The levels of serum urea and creatinine increased significantly in Gent-treated rats compared with the control **(P = 0.002 and P = 0.01; respectively),** AGE-treated **(P = 0.0001 and P = 0.0001; respectively)** and Gent & AGE-cotreated rats **(P = 0.05 and P = 0.01; respectively).** The cotreatment group showed restoration of the normal serum levels of urea and creatinine with no significant differences from the control and AGE-treated rats. GFR non-significantly decreased in Gent-treated group (0.02 ± 0.01) compared to control & AGE-treated groups. Cotreated rats showed normalized GFR (0.0638 ± 0.04972) reaching a non-significant difference compared with each of the control & AGE-treated groups. However, its level was significantly higher than Gent-treated group (**P = 0.027**) (Table 2).

3.2. Effect of gent and AGE on kidney tissue TNF-α, IL-6 and INF-γ content

The levels of TNF-α, IL-6 and interferon-γ in the kidney tissue homogenate were significantly higher in Gent-treated group in comparison with the control **(P = 0.0001 in all)**, AGE-treated rats **(P = 0.0001 in all).** The administration of AGE concurrently with Gent to adult male rats reduced the levels of these inflammatory markers (TNF- α, IL-6 and interferon-γ) to a significant degree in comparison with rats given Gent alone **(P = 0.0001, 0.01 & 0.0001; respectively)** but their levels remain still significantly higher versus the control **(P = 0.0001, 0.031 & 0.0001; respectively)** and AGE-treated rats **(P = 0.0001, 0.014 & 0.0001; respectively)** (Fig. 1).

3.3. Effect of Gent and AGE on oxidative stress markers (SOD & MDA) in renal tissue homogenate

Intraperitoneal administration of gentamicin to adult male rats reduced the level of natural antioxidant enzyme SOD in renal tissue homogenate to a marked extent as compared to the control (P = 0.038) & AGE-treated groups (P = 0.013). Meanwhile, the level of major lipid peroxidation product MDA significantly elevated in comparison with the control (P = 0.01) & AGE-treated groups (P = 0.0001). Oral intake of garlic concurrently with Gent markedly raised the level of SOD and significantly reduced the level of MDA in comparison with Gent-treated group (P = 0.0001 & 0.042; respectively) but normalized their levels with no significant differences from the control & AGE-treated groups (Table 3).

3.4. Effect of Gent and AGE on systolic (SBP) and diastolic (DBP) blood pressure

The SBP & DBP increased progressively to a significant degree in a time dependent manner after administration of Gent compared to control and AGE-treated groups (**P = 0.0001**). Co-administration of Gent and AGE reduced both SBP & DBP to a significant level in comparison with Gent-treated rats **(P = 0.004 & 0.0001; respectively)** but normalized it to nonsignificant differences compared

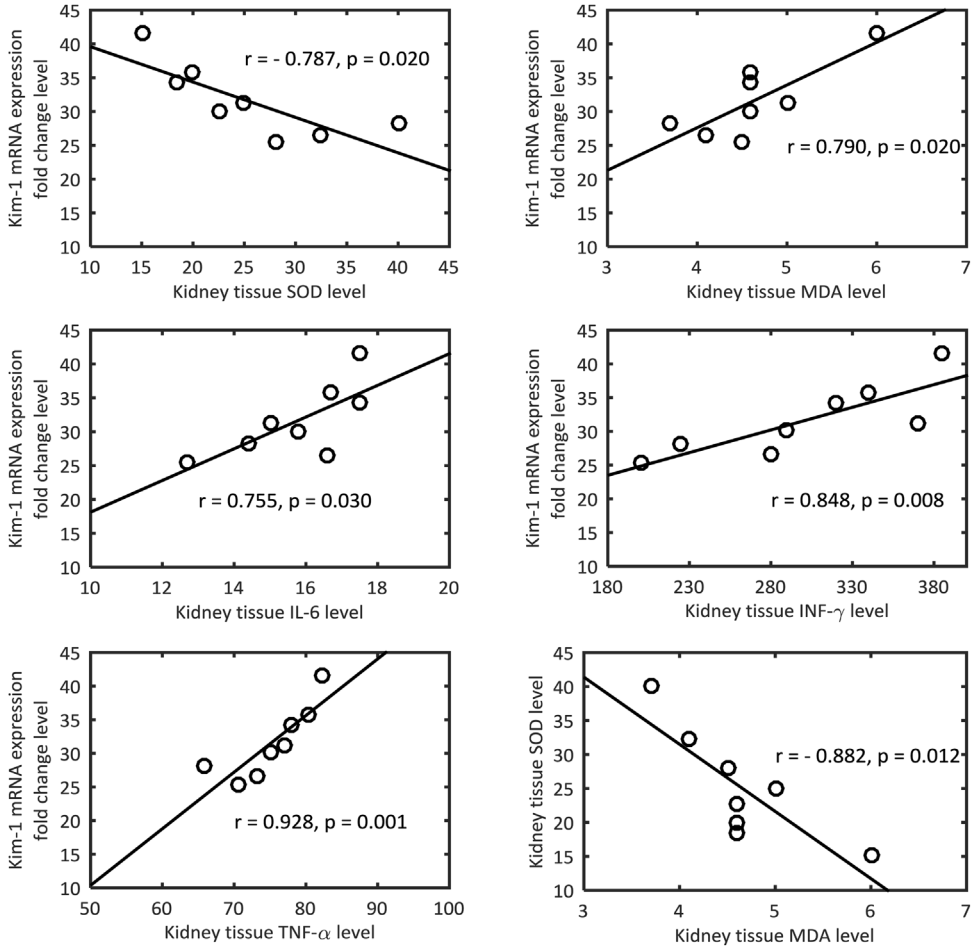

Fig. 4. Correlation between levels of kidney tissue inflammatory, oxidative stress markers and Kim-1 mRNA expression in Gent-treated group.

to controls and AGE-treated rats at the end of the 1st week of treatment. Later on, after 2nd week of experiment, treatment with AGE significantly reduced SBP & DBP compared to Gent-treated rats (**P = 0.0001, P = 0.0001 respectively)** but still markedly higher than control and AGE-treated groups (**P = 0.0001).** Afterthat, at the end of 3rd week, both SBP & DBP were significantly decreased in Gent & AGE cotreated group in comparison with Gent-treated group (**P = 0.0001 P = 0.0001 respectively)** but still markedly elevated than control & AGE-treated groups (**P = 0.0001, 0.0001, 0.001, 0.003; respectively)** (Table 4).

3.5. Renal cortex histopathology

H&E stained paraffin sections of the kidney revealed normal histological architecture in rats of both the control and AGE-treated groups. Renal cortex showed the normal Malpigian renal corpuscles with normal renal space, proximal (PCT) and distal convoluted tubules (DCT). Gent-treated group revealed variable degenerative changes in the glomeruli and renal tubules. The glomeruli showed an apparent increase in the Bowman's spaces. Fibrous connective tissue and mononuclear inflammatory cellular infiltration in the interstitium were noticed. In addition, marked tubular dilatation was noticed. Co-administration of Gent and AGE improved the histological alterations observed in Gent-treated group. The renal cortex had a histological pattern nearly like the control & AGE-treated groups. The renal corpuscles were normal. Renal space was preserved. The proximal and distal convoluted tubules revealed almost normal histological structure. Some tubular dilatations were still noticed (Fig. 2).

3.6. Kim-1 mRNA expression

There was marked upregulation in Kim-1 mRNA level after normalization to GAPDH gene in Gent-treated group compared to the control and AGE-treated group (31.64 ± 5.39 vs 0.79 ± 0.15 & 0.8 ± 0.2; **P = 0.0001 and 0.0001 respectively**). The adult male rats given AGE in conjunction with Gent showed reduced expression of Kim-1 mRNA to a significant degree in comparison with rats given Gent alone (**P = 0.0001**). Gent-AGE-cotreated rats exhibited non-significant differences from the normal control and AGE-treated rats (Fig. 3).

3.7. Correlations

There were significant positive correlations between Kim-1 mRNA expression and IL-6, INF-γ, TNF-α and MDA in Gent-treated group (r = 0.755, P = 0.030, r = 0.848, P = 0.008, r = 0.928, P = 0.001 & r = 0.790, P = 0.020) & AGE-Gent co-treated group (r = 0.749, P = 0.032, r = 0.801, P = 0.017, r = 0.958, P = 0.0001 & r = 0.897, P = 0.003). Significant negative correlations were observed between Kim-1 mRNA expression and Kidney tissue SOD level in both Gent-treated (r = - 0.787, P = 0.020) & Gent-AGE treated groups (r = - 0.871, P = 0.005). In addition, significant positive correlations among TNF-α and both IL-6 and INF-γ were present in Gent-treated (Figs. 4 & 5). While in Gent-AGE treated group, TNF-α was significantly negatively correlated with SOD & positively correlated with MDA, IL-6 & INF-γ. While in normal control & AGE-treated group, no correlation was observed between these parameters (Fig. 6).

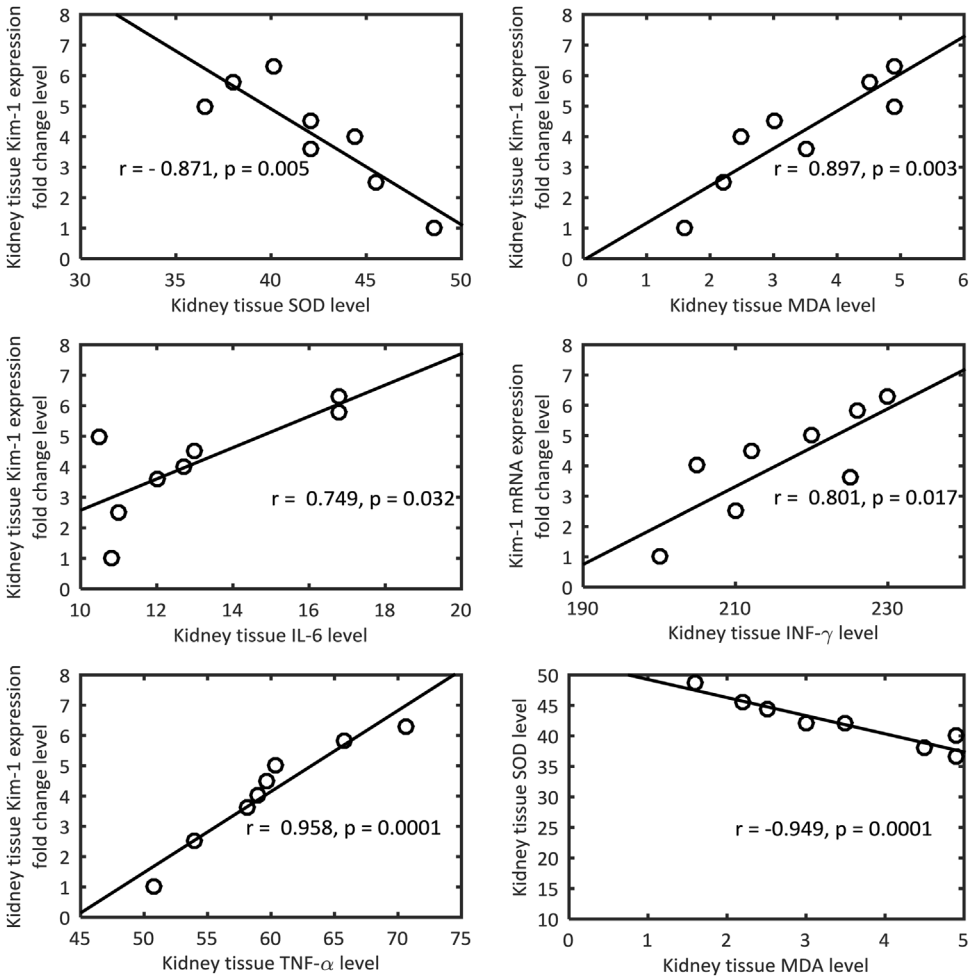

Fig. 5. Correlation between levels of kidney tissue inflammatory, oxidative stress markers and Kim-1 mRNA expression in Gent-AGE-treated group.

4. Discussion

The present study investigated the ability of AGE in halting Gent-induced nephrotoxicity. The model was established and showed marked increases in serum urea and creatinine levels with a decrease in GFR that was confirmed by massive histopathological alterations in the renal tissue. These results reproduced previous observations [22–24]. Gent reduces GFR by increasing renal vascular resistance and decreasing glomerular ultrafiltration coefficient [2]. The increased serum creatinine concentration was due to decrease in GFR [25].

One of the possible mechanisms of Gent-induced nephrotoxicity is through increasing the production of the proinflammatory cytokines TNF-α, IL-6 & INF-γ as seen in our study. This result is similar to a previous report that reasoned it to increases in the production of ROS from mitochondria [26]. The latter stimulates the expression of inducible nitric oxide synthase and nitric oxide (NO) in glomeruli and mesangial cells [27]. NO reacts with superoxide anion to form peroxynitrite. This creates a state of nitrosative stress in the kidney with the increases in locally secreted proinflammatory cytokines [28]. Also, these findings are similar to a former study done by [29]. Excessive production of hydrogen peroxides and superoxide anions as a result of reduced activity of SOD demonstrated in our study activates NF-κB that augments the expression of pro-inflammatory cytokines: TNF-α, IL-6 and INF-γ [30]. The normalization of inflammatory cytokines after oral intake of garlic extract could be explained by the its allicin content that exhibited anti-inflammatory effect by reducing the expression of intercellular adhesion molecule-1 & inflammatory cytokines (TNF-α, IL-1α, IL-6, IL-8, T-cell, interferon-γ and IL-2) while stimulating IL-10 production [31].

The decreased SOD activity and increased MDA level in renal tissue of gent-treated rats confirmed a state of oxidative stress in the kidney. This is in partial agreement to studies conducted by [4,29,32]. A previous study proved that gentamicin directly increased generation of ROS via inhibiting NADH dehydrogenase & cytochrome C oxidase enzyme activities of the mitochondrial respiratory chain [4]. Restoration of normal balance between oxidants & antioxidants could be due to the potent antioxidant activity of AGE [3]. The excessive generation of hydrogen sulfide (H_2S), the main end product of diallyl trisulfide present in AGE enhances the expression of the nuclear factor E2-related factor-2 (Nrf-2) with consequent increased its downstream antioxidant enzymes [33]. Nrf2 is cytoplasmic transcription factor, translocated in to the nucleus and subsequently attached to the antioxidant responsive element existed in the promoter area of several genes which encrypt the antioxidant enzymes [34].

The significant progressive increase in arterial blood pressure in animals injected with Gent might be due to the associated structural alterations in renal tissue that decreased GFR and urine output. The consequent increase in plasma volume and cardiac output triggered the increased ABP [35]. The damage of glomerular and tubular cells and glomerular congestion induced by Gent reduced renal blood flow, decreased amount of sodium that reached macula densa and distal tubules. All these factors would induce the release of renin from the juxtaglomerular apparatus which in turn

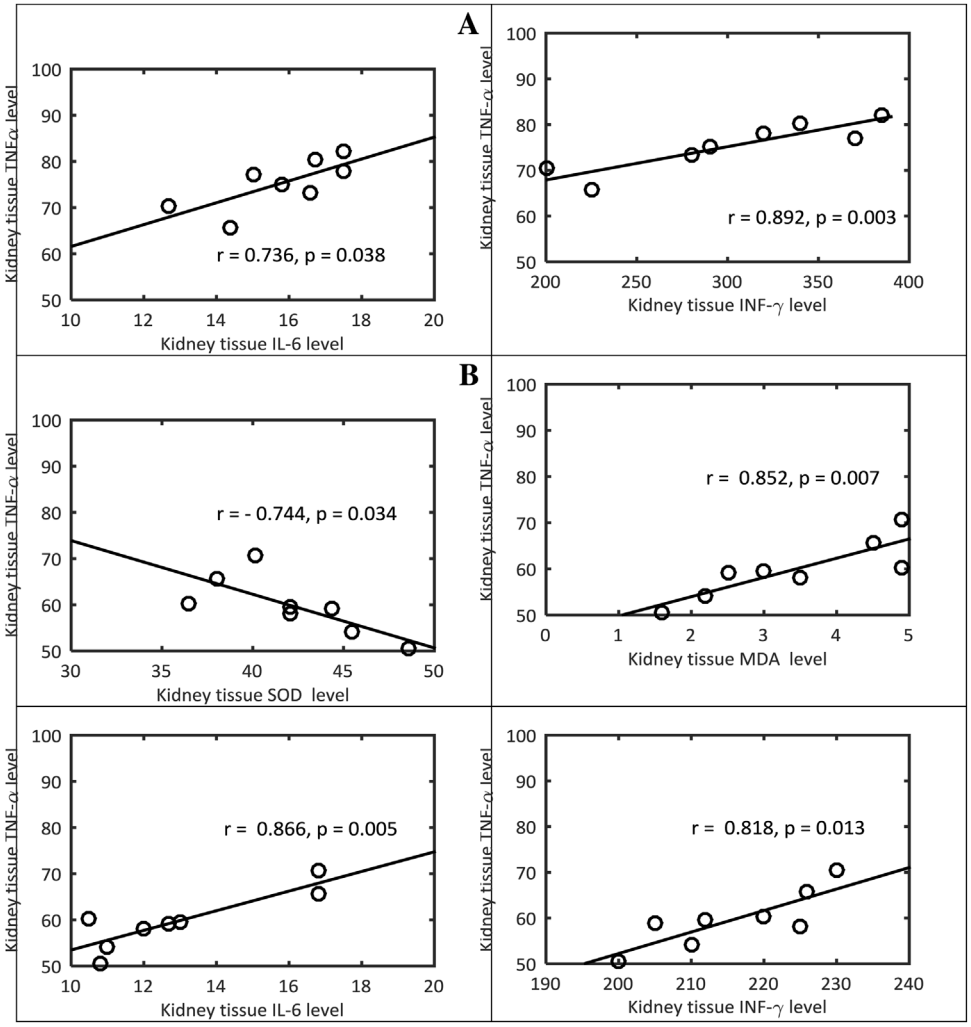

Fig. 6. Correlation between levels of kidney tissue inflammatory, oxidative stress markers in: A. Gent-treated group. B. Gent-AGE treated group.

Table 4
Changes in systolic & diastolic blood pressure (SBP; DBP; mmHg) at different weeks in various studied groups.

Groups	Weeks			
	0 SBP/DBP	1st SBP/DBP	2nd SBP/DBP	3rd SBP3/DBP
Normal control	124.4 ± 5.73 /71.8 ± 5.15	117.3 ± 8.5 /73.3 ± 7.48	121 ± 13.82 /71.8 ± 7.87	125.6 ± 10.18 /72.5 ± 8.16
AGE-treated	119.4 ± 9.27 /72.5 ± 4.23	121 ± 8.77 /69.2 ± 5.03	122.6 ± 9.58 /70.8 ± 4.17	117.6 ± 10.9 /72.5 ± 8.16
Gent-treated	120.6 ± 8.19 /73.3 ± 8.48	166 ± 34.56a,b /97.5 ± 5.92	212.3 ± 11.85a,b /108.5 ± 7.62	226.3 ± 16.2a,b /117 ± 15.45
Gent & AGE-cotreated	119.6 ± 9.71 /76.6 ± 5.31	130.1 ± 11.44c /76.9 ± 4.49	175.3 ± 24.22a,b,c / 89.5 ± 5.01	181.8 ± 20.93a,b,c /93.8 ± 6.25

SBP & DBP were measured before the start of experiment (SBP0, DBP0), after one week (SBP1, DBP1), two weeks (SBP2, DBP2) and 3 weeks (SPB3, DBP3). Data are mean ± SD (n = 8 for each group). [a]P: significant vs. the normal control group. [b]P: significant vs. AGE-treated group; [c]P: significant vs. Gent-treated group ($P \leq 0.05$).

activated the renin-angiotensin-aldosterone that played a role in renovascular hypertension [36].

Aqueous garlic extract lowers the blood pressure possibly through reducing the activity of angiotensin converting enzyme (ACE) and inhibition of the renin-angiotensin system [37]. A study conducted by [38] reported that in the two-kidney-one-clip hypertensive rat model, a marked reduction in SBP was noted in the garlic-treated rats (given a single daily oral dose of 50 mg of AGE for 4 weeks). This decrease correlates positively with a reduction in ACE activity in the serum, aorta, heart, lung and kidney. In agreement with our results, a recent study found a marked reduction in ABP in rats given concurrently Gent & AGE for 21 days [39]. It was found that patients with renal impairment had a state of oxidative stress as a result of increased production of free radicals and reduced antioxidant capacity and this was increased in a graded manner with increasing renal dysfunction [40]. These free radicals were implicated to be a mediator of hypertension [41]. The organic sulfur compounds and polyphenols of AGE prevent cellular injury & reduce ABP through scavenging free radicals [42]. Also, the diallyl trisulfide present in AGE yields hydrogen sulfide (H2S), which

is a vasodilator that opposes hypertension [43]. Besides, H2S also antagonizes the renin-angiotensin system [44].

The histological renal alterations observed in our rats given Gent reproduced previously reported observations [22,45,46]. The proximal renal tubular cells are the primary target site where Gent accumulates with the help of specific transporters. This causes severe tubular necrosis with sloughing of tubular cells into the lumen resulting in slowing of fluid flow that aggravates drug accumulation. This culminates into deterioration of function and renal failure [47]. Gent accumulation in the lysosomes causes their rupture with the release of acid hydrolases that damage cytoplasmic organelles contributing to apoptosis and necrosis of proximal tubular and mesangial cells [45]. These necrotic changes induced the release of pro-inflammatory cytokines which exacerbate the leukocytes migration to the injured site [48]. This was observed in our study by the presence of mononuclear cellular infiltration in renal tissue of Gent-treated rats and the high levels of TNF-α, IL-6 & INF-γ in their kidney tissues.

Our results showed a significantly increased expression of Kim-1 mRNA in kidney tissue of rats injected with Gent. This was similar to the results obtained by other researchers [4,8]. The increased Kim-1 mRNA expression in renal tissue observed in our study might be mediated by TNF-α that induced the expression of matrix metalloproteinase (MMP)-3, specific enzyme present in proximal renal tubular cells, that in turn, increased Kim-1 expression. The positive correlation between TNF-α & Kim-1 mRNA expression could be a potential explanation of this result. In addition, the induced proliferation of renal cells in response to Gent overdose might be another reason for the higher Kim-1 mRNA expression [4]. Furthermore, The increased ROS as a result of Gent nephrotoxicity might cause increased expression of MMP-3 and consequent increased Kim-1 mRNA expression [49]. This is proved by positive correlation between MDA & Kim-1 mRNA expression and negative correlation between Kim-1 mRNA expression & SOD.

Our study found that oral supply of AGE in addition to Gent for 21 days ameliorated the renal stress induced by Gent. This was evidenced by normalization of the increases in inflammatory cytokines, MDA and Kim-1 mRNA expression in kidney tissue. Such nephroprotection was reflected as significant reductions in serum urea and creatinine levels, improvement in GFR, reduction of SBP & DBP & prevention of histological structural alterations in renal tissue. These results are in concordance with a previous study conducted by Abd El Fadil et al. 2016. Furthermore, Ahmed et al 2015 described similar histopathological renal alterations in rats given a single i.p. injection of methotrexate, (20 mg/kg) either alone or with oral garlic extract (1 mL/100 g. b.w.) for 7 days before and after methotrexate administration. The organic sulfur compounds present in AGE inhibits neutrophil infiltration, release of inflammatory mediators including TNF-α and IL-1β, oxidative stress and subsequent cytotoxicity, renal tissue apoptosis and necrosis [41]. This might be verified by the positive correlation between TNF-α, IL-6, INF-γ, MDA & Kim-1 mRNA expression in kidney tissue of AGE-Gent cotreated rats.

5. Conclusion

The present study showed that AGE given orally attenuated nephrotoxicity induced by Gent injection in rats. This effect was mediated by reducing level of inflammatory markers (TNF-α, IL-6 & INF-γ) in renal tissue homogenate, reversing the state of oxidative stress in the kidney, decreasing expression of Kim-1 mRNA in renal tissue as well as preventing/repairing the histological alterations induced by Gent. Such observations stands for the potential role of garlic supplementation as an adjuvant in the therapeutic strategies aimed at delaying chronic renal disease progression and its complications particularly during long courses of Gent therapy. However, further experiments aiming at identification of other molecular mechanisms are planned for.

References

[1] B.H. Ali, Agents ameliorating or augmenting experimental gentamicin nephrotoxicity: some recent research, Food Chem. Toxicol. 41 (11) (2003) 1447–1452.
[2] C. Martinez-Salgado, F.J. Lopez-Hernandez, J.M. Lopez-Novoa, Glomerular nephrotoxicity of aminoglycosides, Toxicol. Appl. Pharmacol. 223 (1) (2007) 86–98.
[3] I. Seckiner, O. Bayrak, M. Can, A.G. Mungan, N.A. Mungan, Garlic supplemented diet attenuates gentamicin nephrotoxicity in rats, Int. Braz. J. Urol. 40 (4) (2014) 562–567.
[4] M. Adil, A.D. Kandhare, G. Dalvi, et al., Ameliorative effect of berberine against gentamicin-induced nephrotoxicity in rats via attenuation of oxidative stress, inflammation, apoptosis and mitochondrial dysfunction, Ren. Fail. 38 (6) (2016) 996–1006.
[5] B.D. Sahu, M. Kuncha, G.J. Sindhura, R. Sistla, Hesperidin attenuates cisplatin-induced acute renal injury by decreasing oxidative stress, inflammation and DNA damage, Phytomedicine 20 (5) (2013) 453–460.
[6] V. Sinha, L.M. Vence, A.K. Salahudeen, Urinary tubular protein-based biomarkers in the rodent model of cisplatin nephrotoxicity: a comparative analysis of serum creatinine, renal histology, and urinary KIM-1, NGAL, and NAG in the initiation, maintenance, and recovery phases of acute kidney injury, J. Investig. Med. 61 (3) (2013) 564–568.
[7] L. Guo, T. Takino, Y. Endo, T. Domoto, H. Sato, Shedding of kidney injury molecule-1 by membrane-type 1 matrix metalloproteinase, J. Biochem. 152 (5) (2012) 425–432.
[8] Y. Zhou, V.S. Vaidya, R.P. Brown, et al., Comparison of kidney injury molecule-1 and other nephrotoxicity biomarkers in urine and kidney following acute exposure to gentamicin, mercury, and chromium, Toxicol. Sci. 101 (1) (2008) 159–170.
[9] V.S. Vaidya, J.S. Ozer, F. Dieterle, et al., Kidney injury molecule-1 outperforms traditional biomarkers of kidney injury in preclinical biomarker qualification studies, Nat. Biotechnol. 28 (5) (2010) 478–485.
[10] V. Lanzotti, The analysis of onion and garlic, J. Chromatogr. A 1112 (1–2) (2006) 3–22.
[11] S.K. Banerjee, S.K. Maulik, Effect of garlic on cardiovascular disorders: a review, Nutr. J. 1 (4) (2002) 1–14.
[12] R. Asadpour, M. Azari, M. Hejazi, H. Tayefi, N. Zaboli, Protective effects of garlic aquous extract (Allium sativum), vitamin E, and N-acetylcysteine on reproductive quality of male rats exposed to lead, Vet. Res. Forum 4 (4) (2013) 251–257.
[13] F. Khanum, K.R. Anilakumar, K.R. Viswanathan, Anticarcinogenic properties of garlic: a review, Crit. Rev. Food Sci. Nutr. 44 (6) (2004) 479–488.
[14] C.R. Nwokocha, R.I. Ozolua, D.U. Owu, M.I. Nwokocha, A.C. Ugwu, Antihypertensive properties of Allium sativum (garlic) on normotensive and two kidney one clip hypertensive rats, Niger. J. Physiol. Sci. 26 (2) (2011) 213–218.
[15] S.L. Becerra-Torresa, C. Soria-Fregozoa, F. Jaramillo-Juárezb, J.L. Moreno-Hernández-Duquec, Allium sativum aqueous extract prevents potassium dichromate-induced nephrotoxicity and lipid oxidation in rats, J. Pharm. Pharmacogn. Res. 2 (2) (2014) 45–52.
[16] M.T. Boroushaki, E. Asadpour, H.R. Sadeghnia, K. Dolati, Effect of pomegranate seed oil against gentamicin -induced nephrotoxicity in rat, J. Food Sci. Technol. 51 (11) (2014) 3510–3514.
[17] R. Reimschuessel, D. Williams, Development of new nephrons in adult kidneys following gentamicin-induced nephrotoxicity, Ren. Fail. 17 (2) (1995) 101–106.
[18] J.C. Joshi, N. Machhan, S. Sharma, R.D. Budhiraja, Possible role of sodium cromoglycate, a mast cell stabilizer in halting gentamicin nephrotoxicity in rats, Asian J. Pharm. Res. Dev. 5 (1) (2017) 1–9.
[19] R.D. Marsoul, R.M. Abboud, M.T. Abbas, Effect of garlic oil on cyclosporine induced renal toxicity in rats, Int. J. Pharm. Pharm. Res. Hum. 5 (2) (2016) 209–221.
[20] M.I. Yousef, H.M. Hussien, Cisplatin-induced renal toxicity via tumor necrosis factor-α, interleukin 6, tumor suppressor P53, DNA damage, xanthine oxidase, histological changes, oxidative stress and nitric oxide in rats: protective effect of ginseng, Food Chem. Toxicol. 78 (2015) 17–25.
[21] J.D. Bancroft, M. Gamble, Theory and Prac¬tice of Histological Techniques, 6th edition, Churchill Livingstone, Philadelphia, PA, 2008.
[22] E. Hur, A. Garip, A. Camyar, et al., The effects of vitamin d on gentamicin-induced acute kidney injury in experimental rat model, Int. J. Endocrinol. 2013 (313528) (2013) 1–7.
[23] A. Sardana, S. Kalra, D. Khanna, P. Balakumar, Nephroprotective effect of catechin on gentamicin-induced experimental nephrotoxicity, Clin. Exp. Nephrol. 19 (2) (2015) 178–184.
[24] Z.M. Yarijani, H. Najafi, S. Hamid Madani, Protective effect of crocin on gentamicin-induced nephrotoxicity in rats, Iran. J. Basic Med. Sci. 19 (3) (2016) 337–343.
[25] R.A. Star, Treatment of acute renal failure, Kidney Int. 54 (6) (1998) 1817–1831.

[26] A.I. Morales, D. Detaille, M. Prieto, et al., Metformin prevents experimental gentamicin-induced nephropathy by a mitochondria-dependent pathway, Kidney Int. 77 (10) (2010) 861–869.
[27] J.C. Leung, T. Marphis, R.D. Craver, D.M. Silverstein, Altered NMDA receptor expression in renal toxicity: protection with a receptor antagonist, Kidney Int. 66 (1) (2004) 167–176.
[28] J. Pedraza-Chaverri, D. Barrera, P.D. Maldonado, et al., S-allylmercaptocysteine scavenges hydroxyl radical and singlet oxygen in vitro and attenuates gentamicin-induced oxidative and nitrosative stress and renal damage in vivo, BMC Clin. Pharmacol. 4 (5) (2004) 1–13.
[29] H. Erjaee, F. Azma, S. Nazifi, Effect of caraway on gentamicin-induced oxidative stress, inflammation and nephrotoxicity in rats, Vet. Sci. Dev. 5 (2) (2015) 90–94.
[30] B. Sahu, S. Tatireddy, M. Koneru, et al., Naringin ameliorates gentamicin-induced nephrotoxicity and associated mitochondrial dysfunction, apoptosis and inflammation in rats: possible mechanism of nephroprotection, Toxicol. Appl. Pharmacol. 277 (1) (2014) 8–20, http://dx.doi.org/10.1016/j.taap.2014.02.022.
[31] D.H. El-Kashef, A.E. El-Kenawi, G.M. Suddek, H.A. Salem, Protective effect of allicin against gentamicin-induced nephrotoxicity in rats, Int. Immunopharmacol. 29 (2) (2015) 679–686, http://dx.doi.org/10.1016/j.intimp.2015.09.010.
[32] K. Manshare, A. Anand, S. Mahajan, et al., Evaluation of nephroprotective activity of gallic acid in gentamicin-induced rat model of nephrotoxicity, Int. J. Green Pharm. (2018) 48–52.
[33] C.Y. Tsai, S.Y. Wen, M.A. Shibu, et al., Diallyl trisulfide protects against high glucose-induced cardiac apoptosis by stimulating the production of cystathionine gamma-lyase-derived hydrogen sulfide, Int. J. Cardiol. 195 (2015) 300–310, http://dx.doi.org/10.1016/j.ijcard.2015.05.111.
[34] S. Sajadimajd, M. Khazaei, Oxidative stress and cancer: the role of Nrf2, Curr. Cancer Drug Targets 18 (6) (2018) 538–557, http://dx.doi.org/10.2174/1568009617666171002144228.
[35] Marc E. de Broe, George A. Porter, Clinical Nephrotoxins: Renal Injury From Drugs and Chemicals, 3rd ed., New York:Springer Science+Business Media, 2008, pp. 42–54.
[36] J.T. Dipiro, R.L. Talbert, G.C. Yee, G.R. Matzke, B.G. Wells, L.M. Posey, Pharmacotherapy A: Pathophysiologic Approach, 7th ed., Mc Graw Hill, New York, 2008, pp. 705–759.
[37] M. Hosseini, S.M. Shafiee, T. Baluchnejadmojarad, Garlic extract reduces serum angiotensin converting enzyme (ACE) activity in nondiabetic and streptozotocin-diabetic rats, Pathophysiology 14 (2) (2007) 109–112.
[38] A.M. Sharifi, R. Darabi, N. Akbarloo, Investigation of antihypertensive mechanism of garlic in 2K1C hypertensive rat, J. Ethnopharmacol. 86 (2–3) (2003) 219–224.
[39] A.M.S. Gomaa, A.T. Abdelhafez, H.A. Aamer, Garlic (Allium sativum) exhibits a cardioprotective effect in experimental chronic renal failure rat model by reducing oxidative stress and controlling cardiac Na+/K+-ATPase activity and Ca2+ levels, Cell Stress Chaperones (2018) 1–8.
[40] M. Tepel, M. Echelmeyer, N.N. Orie, W. Zidek, Increased intracellular reactive oxygen species in patients with end-stage renal failure: effect of hemodialysis, Kidney Int. 58 (2) (2000) 867–872.
[41] M. Deniz, G. Sener, F. Ercan, B.C. Yegen, Garlic extract ameliorates renal and cardiopulmonary injury in the rats with chronic renal failure, Ren. Fail. 33 (7) (2011) 718–725.
[42] M. Ouarda, C. Abdennour, Evaluation of the therapeutic efficiency of raw garlic on reproduction of domestic rabbits under lead induced toxicity, Ann. Biol. Res. 2 (3) (2011) 389–393.
[43] J.L. Greaney, J.L. Kutz, S.W. Shank, S.S. Jandu, L.M. L. Alexander, Impaired hydrogen sulfide-mediated vasodilation contributes to microvascular endothelial dysfunction in hypertensive adults, Hypertension 69 (2017) 902–909.
[44] A. Mohamadi, S.T. Jarrell, S.J. Shi, et al., Effects of wild versus cultivated garlic on blood pressure and other parameters in hypertensive rats, Heart Dis. (Hagerstown, Md) 2 (2000) 3–9.
[45] V.B. De Souza, R.F.L. De Oliveira, H.F. De Lucena, et al., Gentamicin induces renal morphopathology in wistar rats, Int. J. Morphol. 27 (1) (2009) 59–63.
[46] N. Suliska, E.Y. Sukandar, The effectivity of captopril, losartan and amilodipine on hypertension in rat model of gentamicin-induced renal failure, Int. J. Pharm. Pharm. Sci. 6 (6) (2014) 146–151.
[47] B.C. Widemann, P.C. Adamson, Understanding and managing methotrexate nephrotoxicity, Oncologist 11 (6) (2006) 694–703.
[48] T.J. Geleilete, G.C. Melo, R.S. Costa, R.A. Volpini, T.J. Soares, T.M. Coimbra, Role of myofibroblasts, macrophages, transforming growth factor-beta endothelin, angiotensin-II, and fibronectin in the progression of tubulointerstitial nephritis induced by gentamicin, J. Nephrol. 15 (6) (2002) 633–642.
[49] C. Punsawad, P. Viriyavejakul, Increased expression of kidney injury molecule-1 and matrix metalloproteinase-3 in severe Plasmodium falciparum malaria with acute kidney injury, Int. J. Clin. Exp. Pathol. 10 (7) (2017) 7856–7864.