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Oral administration of naringenin and a mixture of coconut water and Arabic gum attenuate oxidative stress and lipid peroxidation in gentamicin-induced nephrotoxicity in rats

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Abstract. – OBJECTIVE: This study aimed to investigate the effect of oral administration of naringenin in combination with an aqueous mixture of coconut water (CW) and Arabic gum (AG) on renal function, lipid profile, antioxidant activity, and morphology in gentamicin-induced kidney injury in rats.

MATERIALS AND METHODS: Forty adult male Wistar rats were equally divided into four groups. 1-Negative control group, 2-positive control group (Gentamicin), 3-Naringenin+AG+CW, 4-Gentamicin+Naringenin+AG+CW: groups 2 and 4 were treated with gentamicin. After six weeks, the rats were anesthetized with diethyl ether, and blood was collected by cardiac puncture and dissected to collect the kidneys. Biochemical studies were performed to determine the levels of urea, creatinine, lipids, total antioxidant capacity, and lipid peroxide, antioxidant enzyme activity in the kidney, total phenolic content (TPC), radical-scavenging activity, calcium, magnesium, and potassium in AG, CW, and their mixture. Also, kidney histopathology was performed.

RESULTS: Renal injury manifests as elevated serum urea and creatinine levels. A significant increase in total cholesterol, triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and malondialdehyde (MDA) was also noted. The activities of antioxidant capacity (TAC) and reduced glutathione (GSH) significantly decreased in the serum. There was a reduction in the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) activities in kidney homogenates. Gentamicin administration induces morphological changes in the kidneys. Oral administration of naringenin+AG+CW significantly overturned all of the above-mentioned abnormalities.

CONCLUSIONS: These results show that the naringenin+AG+CW combination exhibited an additive effect against renal dysfunction and structural damage through antioxidant and anti-inflammatory mechanisms, as well as replenishing and balancing intracellular and extracellular electrolytes. Therefore, oral administration of these three ingredients could potentially provide better protection and serve as a unique therapeutic tool against nephrotoxicity caused by gentamicin.

Key Words:
Co-administration, Arabic gum, Naringenin, Coconut water, Nephrotoxicity.

Introduction

The number of patients with chronic kidney disease is increasing rapidly. It is now appearing in one out of seven persons over 20 years of age[1,2]. During the last three decades, a significantly increased number of chronic kidney disease patients has been noted in Saudi Arabia. The ratio of chronic kidney disease patients has reached about 80-120 per million population of the kingdom[3]. According to the Ministry of Health[4-6] in Saudi Arabia, the total cost of kidney dialysis sessions for each patient has reached 46,332 USD (173,784 SR) per year, with a high mortality ratio. The unaffordable prices of conventional medicines in underdeveloped countries and the rising global population have prompted the use of alternative medicines[7]. The nephroprotective activities of various medicinal plants and their extracts (seeds, core, peel, and leaves) have been reported in animal models[8]. Arabic gum (AG) is a water-soluble dietary fiber rich in high-molecular-weight polysaccharides, magnesium, potassium, and calcium salts, which hydrolyze to produce arabinogalactan

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and glycoproteins with different chemical compositions and molecular weight[9-11]. AG contains less than 3% protein and mainly consists (55%) of leucine, hydroxyproline, proline, and serine amino acids[12-14]. The ingested AG remains undigested in the intestine and ferments in the colon to produce beneficial short-chain fatty acids. AG has long been traditionally used to treat patients suffering from hemodialysis and chronic renal failure[15] However, there is a lack of scientific data regarding the impact of AG on already damaged kidneys[15-17].

Naringenin (2,3-dihydro-5,7-dihydroxy-2-(4-hydroxyphenyl)4H-1-benzopyran-4-one) is a naturally occurring flavonoid of "flavanone flavonoid". It is an important water-insoluble antioxidant. Naringenin-7-rutinoside (narirutin) and naringenin-7-neohesperidoside (naringin) hydrolyze to produce an aglycone known as naringenin. Naringenin is bitter in taste, soluble in dimethyl sulfoxide and alcohol, and relatively insoluble in water[18]. Naringenin is naturally abundant in the peels and seeds of citrus fruits (grapefruit and oranges) and tomato skin[19,20]. Naringenin facilitates a broad range of biological functions. Salehi et al[21] reported that naringenin regulates the immune system and promotes antioxidant defenses and carbohydrate metabolism. It also scavenges reactive oxygen species (ROS) and reduces protein carbonylation and lipid peroxidation. Coconuts have provided food, fiber, drinks, oil, charcoal, and construction materials to humans since ancient times. Therefore, it is considered to be a tree of life[22]. Coconut extract is a beneficial traditional medicine used to cure metabolic disorders[23]. The electrolyte levels in coconut water (CW) are similar to those in human blood, except for minutely different ratios of calcium, phosphorus, sodium, chloride, potassium, and magnesium[24]. Chang and Wu[25] have reported that fresh CW contains (+)-catechin and (−)-epicatechin, which exhibit anticancer, antimicrobial, and antioxidant activity. Fresh CW is rich in cytokinins (a plant growth hormone) that exhibit anti-aging features in the human skin. Nwangwa[26] studied the remedial effects of CW administration in the kidneys of diabetic rats for 30 d. He noticed a significant reduction in sodium (Na), potassium (K), urea, bicarbonate (HCO_3), and creatinine levels, which may prevent renal injury in patients with diabetes who consume CW.

Gentamicin is a potent drug used against Gram-negative bacteria. It is widely used to induce kidney injury and study various parameters. However, nephrotoxicity is a major complication of gentamicin administration as it generates hydroxyl radicals, superoxide anions, hydrogen peroxide, and water. Several studies[27,28] have reported that gentamicin nephrotoxicity is associated with the oxygen metabolites. Gentamicin was used to induce kidney injury in rats.

Materials and Methods

Materials

Acacia Senegal (AG) was obtained from agents that imported AG from Sudan, whereas naringenin was purchased from Sigma (St. Louis, MO, USA). The aminoglycoside antibiotic gentamicin was provided in sulfate form by the inpatient pharmacy of King Fahad Specialist Hospital. They were purchased from Sigma (St Louis, MO, USA), and CW (HARVEST Coconut Water) was purchased from Universal Food Public Company Limited, Soi Bangna-Trad, Khwaeng Bangna Nuea, Khet Bangna, Bangkok, Thailand.

Animals and Diet

Wistar Rats were purchased from the College of Pharmacy, King Saud University, Riyadh, Saudi Arabia, and were used as animal models in this study. The rats were housed in polyacrylate cages inside an air-conditioned room at 25±2°C with standard light and dark cycles (12 h light and 12 h dark). The rats were provided with tap water and standard commercial rat pellets (Number 648; General Organization for Grain Silos and Flour Mills, Riyadh).

Preparation of AG and CW Mixture

AG powder (7.5 g) was dissolved in 30 ml CW and tap water was added to prepare a volume of 100 ml. This solution was used as a substitute for the drinking water.

Preparation of Naringenin

A clear solution of naringenin was prepared by dissolving 2 g naringenin in 200 ml of diluted 40% ethanol.

Experimental Design

Forty adult Wistar male rats weighing 180 g were equally divided into four groups after an adaptation period of one week. The groups were as follows.

A) NC: (negative control): rats in the untreated group were fed standard rat chow and regular tap water *ad libitum*.

B) PC: (positive control): The rats were treated with gentamicin (i/p 80 mg/kg bwt for eight consecutive days) and fed standard rat chow and tap water *ad libitum*.
C) Naringenin+AG+CW: The rats in the control group were orally administered a combination of naringenin (100 mg/kg body weight, using a gavage tube) and an aqueous mixture containing CW (30% drinking water) and AG (7.5 g per 100 ml of drinking water) throughout the experimental period.
D) Gentamicin+Naringenin+AG+CW: The rats in this group were injected with gentamicin for eight days, with *ad libitum* access to standard rat chow and drinking water. Rats in this group were also orally administered a combination of naringenin (100 mg/kg body weight, using a gavage tube) and an aqueous mixture containing CW (30% drinking water) and AG (7.5 g per 100 ml of drinking water) throughout the experimental period.

The experiment continued for six weeks and then rats were fasted overnight, anesthetized with diethyl ether, blood was collected by cardiac puncture, and dissected to collect the kidneys. The blood was left to clot for 60 min at room temperature and then centrifuged for 10 min at 3,000 rpm. The collected sera were labeled and frozen for further analysis. The kidneys were divided into two sections for biochemical and histopathological analysis.

Biochemical Analysis

Biochemical studies were performed to determine the levels of urea, creatinine, triglycerides, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), reduced glutathione, and protein. All the chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). Commercial Kits were purchased from the Bio-Merieux Laboratory Reagents and Products (France).

Total Phenolic Content (TPC)

The TPC was determined according to the method described by Singleton and Rossi[29]. A gallic acid standard curve was constructed to generate an equation for calculating the results. The results are expressed as milligrams of gallic acid equivalents per 100 g of dry sample (mg GAE/100 g DW).

Radical-Scavenging Activity (DDPH)

DPPH radical scavenging activity was determined according to the method described by Musa et al[30]. A Trolox standard curve was constructed to generate an equation to calculate the results. The results are expressed as milligrams of Trolox equivalents per 100 g of dry sample (mg TE/100 g DW).

Radical-Scavenging Activity (ABTS)

The method of Re et al[31] was followed to determine ABTS activity. A Trolox standard curve was constructed to generate an equation to calculate the results. The results are expressed as milligrams of Trolox equivalents per 100 g of dry sample (mg TE/100 g DW).

Ferric Reducing/ Antioxidant Power (FRAP)

The FRAP was determined according to the procedure described by Benzie and Strain[32]. A Trolox standard curve was constructed to generate an equation to calculate the results. The results are expressed as milligrams of Trolox equivalents per 100 g of dry sample (mg TE/100 g DW).

Calcium, Magnesium, and Potassium in AG, CW, and Their Mixture

The method of Waziri et al[33] was adopted to assess the minerals, and the samples were weighed in a clean porcelain crucible and dried at 45°C in an oven. The porcelain crucible was then placed in a muffle furnace for 5 h at 550°C for dry-ashing. The ash was dissolved in hydrochloric acid (6N) and heated to boiling temperature. An atomic absorption spectrophotometer (Shimadzu Atomic absorption spectrophotometer flame model NO. AA-7000F, International Equipment Trading Ltd, 955 Campus Drive Mundelein, IL, USA) was used to measure calcium (Ca) and magnesium (Mg) in the digested ash, and potassium (K) was measured using a Jenway PFP7 Keison Products flame photometer (Chelmsford, Essex, England).

Serum Urea and Creatinine

Commercial kits (Sigma, St. Louis, MO, USA) were used to determine serum urea and creatinine levels.

Serum Lipid Profile

Commercial kits (bio-Merieux Laboratory Reagents and Products, France and kits Kostner et from NubencoInterprises INC. Paramus, NJ, USA) were used to determine Cholesterol[34], HDL[35] and triglycerides[36] all assays were performed using an automatic analyzer (Hitachi, 777, Tokyo, Japan).

The following formula of Friedwald et al[35] was used to calculate LDL concentration

$$LDL = (\text{Total Cholesterol}) - (HDL) - 0.16 \times (\text{Triglycerides})$$

Malondialdehyde (MDA)

Lipid peroxides were calculated as malondialdehyde (MDA) according to Namıduru et al[37]

Total Antioxidant Capacity

The total antioxidant capacity of the serum was determined at 505 nm according to Koracevic et al[38].

Antioxidant Enzyme Activity in the Kidney

Commercial reagents were purchased from Nanjing Jiancheng Bioengineering Company (Nanjing, China) for measuring superoxide dismutase (SOD), glutathione peroxidase (GSHPx), catalase (CAT), and reduced glutathione (GSH). Kidney tissue (1 g) was cut into small pieces and homogenized in ice-cold saline buffer (0.85%, pH 7.4) (1:9, w/v) using an Ultra-Turrax homogenizer (T8, IKA-labortechnik Staufen, Germany). Kidney homogenates were centrifuged at 1,000×g at 4°C for 15 min, and the supernatants were collected. Supernatants were used in the SOD, GSHPx, CAT, and GSH assays to estimate the antioxidative profile of the kidney. The activities of SOD, CAT, and GSH were measured according to Minami and Yoshikawa[39], and Paglia and Valentine[40] respectively. The procedure described by Sedlak and Lindsay[41] was used to photometrically determine the GSH concentration in the homogenates of the renal cortex. All assays were conducted using clinical chemistry assay kits according to the recommended protocols.

Protein Assay

Bovine serum albumin was used as a standard to measure protein concentration in the kidney homogenate, as described by Lowry et al[42]

Histopathology Analysis

Paraffin embedding (dehydration in ethanol, clearing in xylene, and impregnation in melted wax) was used to process the kidney specimens for histopathological analysis. Five µm thick sections were prepared, stained with hematoxylin and eosin (H&E), and examined for pathological findings.

Statistical Analysis

The results are presented as mean±SE of ten replicates. One-way analysis of variance (ANOVA) was performed, and the means were compared using Bonferroni multiple comparisons with a computer-based fitting program (Prism, GraphPad, La Jolla, CA, USA). The level of statistical significance was set at $p<0.05$.

Results

The results depict the non-toxicity of oral administration of AG, naringenin, and CW to rats (Naringenin+AG+CW group). Therefore, this formula can be considered safe in mammals.

Total Phenolic Compounds (TPC) and Antioxidant Activities

Table I presents the TPC and antioxidant activities of the studied samples. Antioxidant activity is shown based on radical scavenging activity and reducing power. Radical scavenging activity was determined using DPPH and ABTS free radicals. The mixture of CW and AG exhibited higher scavenging activity than CW alone. An FRAP assay was conducted to measure the reducing activities of the samples. CW and AG exhibited higher reducing activity and TPC. The activity of naringenin was significantly higher than that of other samples.

Calcium, Magnesium, and Potassium Concentration in CW and AG

The levels of calcium, magnesium, and potassium in CW, AG, and their mixtures are listed in Table II. AG contained higher levels of all three elements, especially potassium, than CW. However, the mixture of CW and AG showed decreased concentrations of all three elements compared with the individual concentrations.

Effect of Experimental Treatments on Serum Urea and Creatinine of Rats

The effects of the experimental treatments on serum urea and creatinine levels in the rats are shown in Table III. Gentamicin (80 mg/kg/day for 8 days) produced a typical pattern of nephrotoxicity by significantly increasing the concentrations of creatinine and urea in rat serum. The level of urea significantly increased from 21.2±0.81 to 92.7±0.47 mg/dl. However, administration of Naringenin+AG+CW significantly reduced the level of urea to 25.2±1.51 mg/dl. Gentamicin administration significantly increased the level of creatinine from 0.66±0.01 mg/dl in normal rat serum to 1.51±0.02 mg/dl indicating toxic effects in kidneys. Administration of the treatment protocol decreased the level of serum creatinine to 0.64±0.01 mg/dl.

Table I. Total phenolic compounds (TPC) and antioxidant activities of CW, AG, and Naringenin.

Samples	TPC*	DPPH**	ABTS**	FRAP**
CW	31.3±4.8^{c}	48.9±6.2^{c}	70.2±14.4^{c}	128.1±6.6^{c}
CW+AG	57.3±9.6^{b}	219.3±102.8^{b}	177.1±23.5^{b}	147.8±28.2^{b}
Naringenin (20 mg/mL)	3,597±435^{a}	8,494±238^{a}	8,109±521^{a}	10,513±166^{a}

*mg Gallic Acid Equivalent /100 gm.
**mg Trolox Equivalent/100 gm.
Mean sin the same column having different superscripts are significantly different at (p<0.05).

Table II. Calcium, magnesium, and potassium concentration in CW and AG.

Samples	Calcium (ppm)	Magnesium (ppm)	Potassium (ppm)
AG	3,561	183	7,447
CW	156	53	3,232
CW+AG	1,025	94	2,424

The mineral content of naringenin was not determined, as it is a pure flavonoid.

Table III. Effect of experimental treatments on serum urea and creatinine of rats.

Groups	Urea (mg/dl)	Creatinine (mg/dl)
NC	21.2±0.81^{b}	0.66±0.01^{b}
PC	92.7±0.47^{a}	1.5±0.02^{a}
Naringenin+AG+CW	27.6±0.53^{b}	0.70±0.01^{b}
Gentamicin+Naringenin+AG+CW	25.2±1.51^{b}	0.64±0.01^{b}

NC: normal control. PC: positive control. Naringenin+AG+CW: oral administration of naringenin (100 mg/kg bwt, using a gavage tube), and an aqueous mixture of CW (30% of drinking water) and AG (7.5 g per 100 ml of drinking water) without gentamicin treatment. Gentamicin+Naringenin+AG+CW: The same as (Naringenin+AG+CW), but with gentamicin treatment.
The results are expressed as the Mean±SE of 10 rats.
The values with different superscript letters in a column are significantly different (p<0.05).

Effect of Experimental Treatments on Serum Lipid Profile

The effects of the experimental treatment on the levels of serum cholesterol, triglycerides, LDL, and HDL are presented in Table IV. The levels of cholesterol, triglycerides, and LDL in the NC group remained at 55.4±3.67, 38.3±0.75, and 17.6±1.14 mg/dl, respectively. Gentamicin administration increased the level of these metabolites to 74.7±3.55, 40.2±1.59, and 24.2±0.89 mg/dl, respectively. The combined administration of AG+Naringenin+CW to rats injected with gen-

Table IV. Effect of experimental treatments on serum lipid profile.

Groups	Cholesterol (mg/dl)	Triglyceride (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
NC	55.4±3.67^{d}	38.3±0.75^{b}	17.6±1.14^{c}	34.1±3.07^{d}
PC	74.7±3.55^{a}	40.2±1.59^{a}	24.2±0.89^{a}	32.9±2.73^{c}
Naringenin+AG+CW	62.9±5.204^{c}	29.0±1.77^{c}	20.5±1.296^{b}	44.7±2.77^{a}
Gentamicin+Naringenin+AG+CW	68.7±1.48^{b}	47.7±9.48^{a}	22.4±3.01^{a}	40.2±1.05^{b}

NC: normal control. PC: positive control. Naringenin+AG+CW: oral administration of naringenin (100 mg/kg bwt, using a gavage tube), and an aqueous mixture of CW (30% of drinking water) and AG (7.5 g per 100 ml of drinking water) without gentamicin treatment. Gentamicin+Naringenin+AG+CW: The same as (naringenin+AG+CW), but with gentamicin treatment. The results are expressed as the Mean±SE of 10 rats. The values with different superscript letters in a column are significantly different (p<0.05).

tamicin improved these metabolites, except for triglycerides. The level of HDL 34.1±3.07 mg/dl in the NC group was reduced in the PC group to 32.9±2.73 mg/dl. A significant increase in HDL levels was observed after administration of AG+Naringenin+CW.

Effect of Experimental Treatments on Serum Antioxidant and Oxidative Stress Parameters in Rats

The effects of experimental treatment on MDA, total antioxidant capacity, and reduced glutathione are indicated in Table V. Serum malondialdehyde (MDA) significantly increased from 1.24±0.02 µmol/L in the NC group to 5.55±0.87 µmol/L in the PC group. AG+Naringenin+CW treatment reduced the level of MDA to 3.00±1.63 µmol/L. The level of total antioxidant capacity in the NC group was observed as 0.36±0.02 mM/L that was significantly decreased to 0.11±0.04 mM/L in the PC rats. AG+Naringenin+CW treatment significantly increased in total antioxidant capacity (TAC) level to 0.32±0.02 mM/L. The level of reduced glutathione was noted as 2.76±0.36 µmol/L in the NC group that was significantly decreased in the PC group to 1.15±0.56 µmol/L. However, AG+Naringenin+CW treatment significantly increased the level to 2.94±0.16 µmol/L.

Effect of Experimental Treatment on Antioxidant Enzyme Activities in Kidney Homogenate

SOD, CAT, and GSH-px activities in the kidney homogenates of all groups are presented in Table VI. The level of SOD was found to be 9.92±0.24 (mU/mg protein) in the NC group which was decreased to 5.06±0.52 (mU/mg protein) in the PC group. A higher level of SOD 8.04±0.82 (mU/mg protein) activity was noted in the Naringenin+AG+CW group. Similar trends were observed for the CAT and GSH-Px levels.

Histopathology

Histological changes were not observed during microscopic examination of the NC kidneys (Figure 1). The normal appearance of the renal tubular epithelial cells and glomeruli was clear under a microscope (H&E at a magnification of 100X). The PC group (Figure 1, PC) exhibited an altered architecture with extensive destruction of glomeruli and tubular structures. Hypercellularity was observed in the glomeruli, which was associated

Table V. Effect of experimental treatments on serum antioxidant and oxidative stress parameters in rats.

Treatments	MDA (µmol/L)	TAC (mM/L)	GSH (µmole/L)
NC	1.24±0.02^{c}	0.36±0.02^{a}	2.76±0.36^{a}
PC	5.50±0.87^{a}	0.11±0.04^{d}	1.15±0.56^{c}
Naringenin+AG+CW	3.18±1.33^{b}	0.19±0.02^{c}	1.92±0.38^{b}
Gentamicin+Naringenin+AG+CW	3.00±1.63^{b}	0.32±0.02^{b}	2.94±0.16^{a}

NC: normal control. PC: positive control. Naringenin+AG+CW: oral administration of naringenin (100 mg/kg bwt, using a gavage tube), and an aqueous mixture of CW (30% of drinking water) and AG (7.5 g per 100 ml of drinking water) without gentamicin treatment. Gentamicin+Naringenin+AG+CW: The same as (naringenin+AG+CW), but with gentamicin treatment. The results are expressed as the Mean ± SE of 10 rats. The values with different superscript letters in a column are significantly different (p<0.05).

Table VI. Effect of experimental treatment on antioxidant enzyme activities (U/mg protein) of kidney homogenate.

Treatments	SOD (mU/mg protein)	CAT (mU/g protein)	GSH-px (mU/mg protein)
NC	9.92±0.24^{a}	65.14±3.22^{a}	**8.82±0.62^{a}**
PC	5.06±0.52^{d}	36.8±3.68^{d}	**5.15±0.82^{c}**
Naringenin+AG+CW	7.04±0.62^{c}	46.53±1.88^{c}	**6.74±0.44^{b}**
Gentamicin+Naringenin+AG+CW	8.04±0.82^{b}	54.94±1.92^{b}	**8.22±1.8^{a}**

NC: normal control. PC: positive control. Naringenin+AG+CW: oral administration of naringenin (100 mg/kg bwt, using a gavage tube), and an aqueous mixture of CW (30% of drinking water) and AG (7.5 g per 100 ml of drinking water) without gentamicin treatment. Gentamicin+Naringenin+AG+CW: The same as (Naringenin+AG+CW), but with gentamicin treatment. The values with different superscript letters in a column are significantly different (p<0.05).

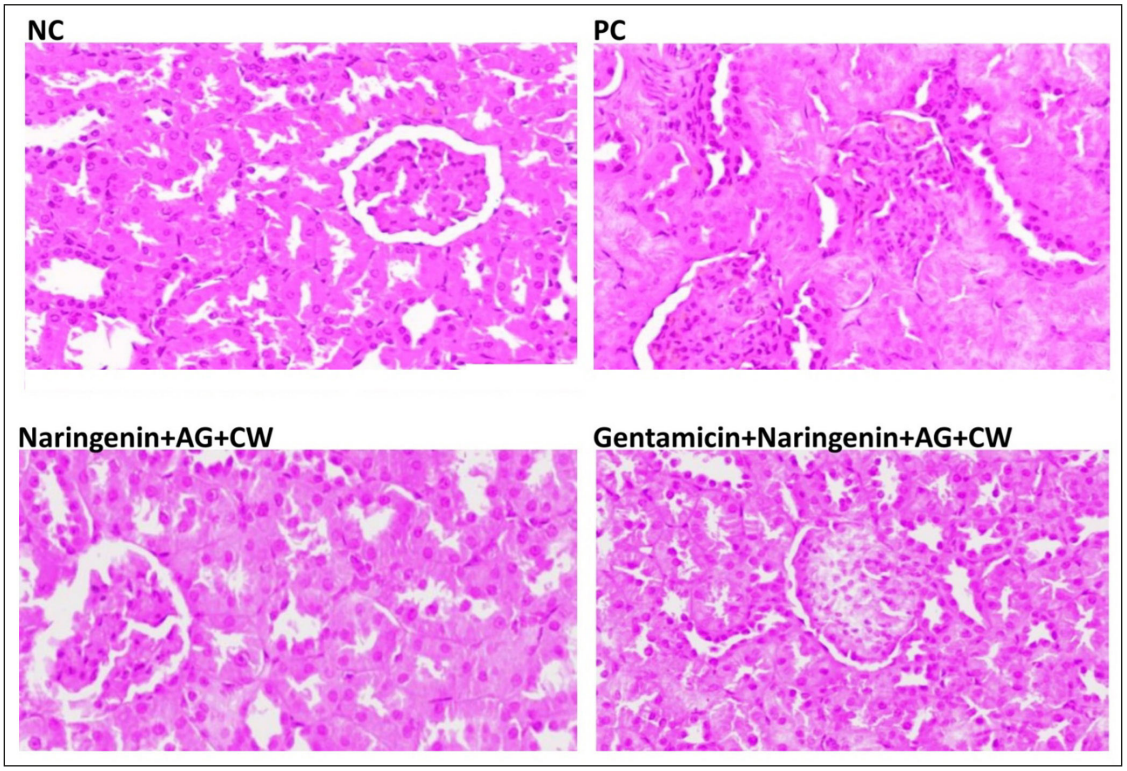

Figure 1. Displays Histopathological examinations of dissected kidney samples from different groups of rats. The samples were stained with H&E at a magnification of 100X. The labels used in the figure are as follows:
- NC: Normal Control
- PC: Positive Control
- Naringenin+AG+CW: Oral administration of naringenin (100 mg/kg bwt, using a gavage tube), along with an aqueous mixture of CW (30% of drinking water) and AG (7.5 g per 100 ml of drinking water), without gentamicin treatment.
- Gentamicin+Naringenin+AG+CW: Similar to the Naringenin+AG+CW group, but with gentamicin treatment.

with narrowing of the urinary and necrotic areas of the renal tubules. Figure 1 depicts an improvement in the kidney structure after AG+Naringenin+CW administration, which is obvious from minor glomerular and tubular damage. Figure 1 (Naringenin+AG+CW) shows reduced lesions and which appear almost normal.

Discussion

Kidney disease (KD) is a serious global health concern worldwide. According to the Saudi Center for Organ Transplantation (SCOT)[3], the rate of dialysis has reached 136 new cases per million population (PMP) in Saudi Arabia. A steady increase in KD has been observed in Saudi Arabia over the last few years. The expensive treatment of KD has encouraged patients from different countries to consult traditional medicine. Phospholipids[43], fish oil[44], and antioxidants such as selenium, vitamin E, and dimethyl sulfoxide[45,46], have been tested against gentamicin nephrotoxicity, but none have been completely successful.

In this study, three agents (AG, Naringenin, and CW) were tested against gentamicin-induced nephrotoxicity. The TPC and antioxidant activities of these compounds were analyzed before their application in gentamicin-induced nephrotoxicity in rats.

Radical scavenging activity was measured using DPPH and ABTS free radicals. The mixture of CW and AG exhibited higher scavenging activity than CW alone. The FRAP assay-based reducing activity revealed that CW and AG had higher reducing activity. A similar trend was noted for the TPC. AG significantly contributed to the antioxidant activity of the mixture, whereas the naringenin activity was much higher than that of the other compounds. High TPC and antioxidant activity of AG have been previously reported[47]. The results of the DPPH assay revealed the

high antioxidant activity of CW with hydrodistillation and petroleum ether extraction[48] or without extraction[49]. CW contains antioxidant compounds including caffeic acid and ascorbic acid[50]. Mahayothee et al[50] has reported ABTS, DPPH, and TPC values of coconuts at various maturity stages.

Naringenin+AG+AG+CW was beneficial in rats with gentamicin-induced nephrotoxicity. Gentamicin administration damages the kidneys by increasing serum urea and creatinine levels and disturbing lipid profiles and antioxidants in the body. Naringenin, AG, and CW were co-administered in this study as they exhibited different modes of action to produce beneficial effects. AG is a water-soluble dietary fiber. It remains undigested in the gastrointestinal tract and is fermented in the large intestine to produce beneficial short-chain fatty acids[51,52]. The anti-inflammatory properties of AG may ameliorate gentamicin-related renal damage[53,54]. The curing effect of AG may help normalize the physiological function of kidneys damaged by gentamicin. Cindoruk et al[55] has reported that AG does not cure fenofibrate-induced hepatocellular damage in rats with cholestasis. Ali et al[56] elaborated on the protective effect of AG against kidney failure owing to its fibrous nature, which acts as an entero-sorbent agent to transfer excessive blood urea and creatinine from the body. However, the antioxidant properties and protective roles of AG have not yet been studied. Therefore, AG was co-administered with naringenin, a strong antioxidant (naringenin). The mixture of AG and CW was rich in polyphenols (Table I), which might have acted as an antioxidant to produce higher scavenging activity in this treatment model. AG also contains fermentable carbohydrates that reduce serum urea and creatinine levels by facilitating the transfer of urea nitrogen from the blood to the cecal lumen, where it is trapped in feces and excreted[57]. Propionate (a product of bacterial fermentation of AG) is a glucogenic substance that is rapidly utilized as an energy source by the liver to prevent ammonia formation and deamination of amino acids[57-59]. A few animal and epidemiological studies[60-62] have demonstrated increased oxidative damage and decreased tissue antioxidant levels after nephrotoxicity and renal ischemia.

The generation of free radicals is partially responsible for the nephrotoxicity of gentamicin. Therefore, enhanced lipid peroxidation and external antioxidants may be helpful for addressing this issue. Naringenin is a potent antioxidant. Naringenin antioxidant activity decreased serum cholesterol and LDL levels and increased HDL and GSH levels. Its antioxidative activity restored the activities of antioxidant enzymes (SOD, CAT, and GSH-Px) and may also play a role in scavenging kidney-damaging lipid peroxides. Naringenin has been found[63] to effectively attenuates cisplatin-induced nephrotoxicity in rats. Middleton et al[64] reported that the possible therapeutic role of flavonoids in free radical-mediated diseases has attracted substantial interest. The nephrotoxicity-reducing properties of the CW were also analyzed in this study. Table II presents the concentrations of Mg, K, and Ca in AG and CW. It appears that CW function in the present study was related to replenishing and balancing intracellular and extracellular electrolyte levels during kidney injury. Matsuda et al[65] demonstrated that gentamicin administration in rats led to a shift in the concentration ratio of electrolytes, particularly reduced potassium and increased sodium and chloride levels in the proximal tubular cells. Similarly, Nwangwa[26] administered CW in diabetic rats and reported a significant reduction in the renal function parameters including creatinine, urea, sodium, potassium, and bicarbonate. Omary et al[66] reported that electrolyte supplementation decreased the risk of nephrotoxicity after cisplatin chemotherapy. CW is also known to treat kidney stones and reduce creatinine levels to improve kidney functions[67]. Thus, the presence of higher amounts of potassium in CW makes it an ideal drink for treating kidney injury[68]. Pere et al[69] found that dietary magnesium and potassium were beneficial against nephrotoxicity.

The co-administration of the three ingredients restored the kidney structure damaged by gentamicin administration. This indicated that these three compounds can cooperate to cure the damaging effects of gentamicin. Histopathological examination of the kidneys further confirmed that the combination of naringenin+AG+CW attenuated gentamicin-induced damage, including degeneration of glomeruli, necrosis, hypercellularity, glomerular congestion, and epithelial desquamation in the renal tubules. Ali et al[56] have also observed reduced necrosis in AG-treated animal groups.

Conclusions

To our knowledge, the present study is the first to investigate the effect of oral co-administration of AG, naringenin, and CW in a model

of acute kidney injury induced by the antibiotic gentamicin. This combination produced noticeable improvements in serum urea and creatinine levels, lipid profiles, oxidative stress, and endogenous antioxidant levels. Co-administration of naringenin, AG, and CW also protected against necrosis and degeneration of glomeruli, glomerular congestion, and epithelial desquamation in the renal tubules caused by gentamicin. These results indicate that naringenin, AG, and CW exert additive effects through antioxidant and anti-inflammatory mechanisms, and possibly replenish and balance intracellular and extracellular electrolyte levels during kidney injury. The results also suggest that the co-administration of these three ingredients could provide better protection against nephrotoxicity.

Authors' Contributions

Conceptualization, H.M., and N.S. Methodology, H.A., K.H., E.M., S.A.A. and S.A.; Formal analysis, H.A.A., and H.A.; Investigation, H.A.A., and N.S.; Resources, H.M., and N.S.; Data Curation, H.A.A. Writing the original draft preparation, H.A.A.; writing the review and editing, H.M. and N.S., Supervision: H.M. and N.S. All the authors have read and agreed to the published version of the manuscript.

Data Availability

The data presented in this study are available upon request from the corresponding author.

Conflicts of Interest

The authors declare no conflict of interest.

Ethics Approval

This study was conducted in accordance with the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the National Committee of Bioethics NCBE at King Abdul-Aziz City for Science and Technology, KACST, KSA. Review Board Number: 10026645. Approval is valid for three years, starting on November 4, 2021.

Informed Consent

Not applicable.

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