BOLETIN LATINOAMERICANO Y DEL CARIBE DE PLANTAS MEDICINALES Y AROMÁTICAS
© / ISSN 0717 7917 / www.blacpma.ms-editions.cl

Articulo Original / Original Article

Green coffee bean extract attenuates gentamicin induced acute nephrotoxicity in rats

[El extracto de grano de café verde atenúa la nefrotoxicidad aguda inducida por gentamicina en ratas]

Amina Unis[1,2] & Amany Abdelbary[3]

[1]Department of Pharmacology, Faculty of Medicine, University of Alexandria, Egypt
[2]Department of Pharmacology, Faculty of Pharmacy, City University College of Ajman, UAE
[3]Department of Pathology, Faculty of Medicine, University of Alexandria, Egypt

Reviewed by:
Ibrahim Aktas
University of Adiyaman
Turkey

Marcelo Catalán
Universidad Austral
Chile

Correspondence:
Amina **UNIS**
amina@rakmhsu.ac.ae

Section: Biological activity

Received: 28 June 2020
Accepted: 24 January 2021
Accepted corrected: 11 February 2021
Published: 30 March 2022

Citation:
Unis A, Abdelbary A.
Green coffee bean extract attenuates gentamicin induced acute nephrotoxicity in rats
Bol Latinoam Caribe Plant Med Aromat
21 (2): 256 - 267 (2022).
https://doi.org/10.37360/blacpma.22.21.2.16

Abstract: Gentamicin induced acute nephrotoxicity (GIAN) is considered as one of the important causes of acute renal failure. In recent years' great effort has been focused on the introduction of herbal medicine as a novel therapeutic agent for prevention of GIAN. Hence, the current study was designed to investigate the effect of green coffee bean extract (GCBE) on GIAN in rats. Results of the present study showed that rat groups that received oral GCBE for 7 days after induction of GIAN (by a daily intraperitoneal injection of gentamicin for 7days), reported a significant improvement in renal functions tests when compared to the GIAN model groups. Moreover, there was significant amelioration in renal oxidative stress markers (renal malondialdehyde, renal superoxide dismutase) and renal histopathological changes in the GCBE-treated groups when compared to GIAN model group. These results indicate that GCBE has a potential role in ameliorating renal damage involved in GIAN.

Keywords: Green coffee bean extract; Gentamicin induced acute nephrotoxicity; Renal function tests; Malondialdehyde; Superoxide dismutase.

Resumen: La nefrotoxicidad aguda inducida por gentamicina (GIAN) se considera una de las causas importantes de insuficiencia renal aguda. En los últimos años, el gran esfuerzo se ha centrado en la introducción de la medicina herbal como un nuevo agente terapéutico para la prevención de GIAN. Por lo tanto, el estudio actual fue diseñado para investigar el efecto del extracto de grano de café verde (GCBE) sobre la GIAN en ratas. Los resultados del presente estudio mostraron que los grupos de ratas que recibieron GCBE oral durante 7 días después de la inducción de GIAN (mediante una inyección intraperitoneal diaria de gentamicina durante 7 días), informaron una mejora significativa en las pruebas de función renal en comparación con los grupos del modelo GIAN. Además, hubo una mejora significativa en los marcadores de estrés oxidativo renal (malondialdehído renal, superóxido dismutasa renal) y cambios histopatológicos renales en los grupos tratados con GCBE en comparación con el grupo del modelo GIAN. Estos resultados indican que GCBE tiene un papel potencial en la mejora del daño renal involucrado en GIAN.

Palabras clave: Extracto de granos de café verde; Nefrotoxicidad aguda inducida por gentamicina; Pruebas de función renal; Malondialdehído; Superóxido dismutasa

INTRODUCTION

Gentamicin is an aminoglycoside antibacterial that is commonly used worldwide for the treatment of infections produced by gram negative bacteria (Friesen *et al*., 2018). However, its therapeutic use has been associated with high risk rate of nephrotoxicity affecting nearly 10 to 30% of patients (Wargo & Edwards, 2014).

Gentamicin induced acute nephrotoxicity (GIAN) has been widely used as a model to study nephrotoxicity of aminoglycoside drug group, both in experimental animals and humans (Laurent *et al*., 1990). Moreover, researches exploring the mechanisms responsible for aminoglycoside induced nephrotoxicity has been obtained from reviewing the effect gentamicin on animals or cell culture studies (Lopez-Novoa *et al*., 2011).

The pathogenesis of GIAN has been attributed to the accumulation and retention of the drug mainly in the proximal convoluted tubules (Swan, 1997). Such accumulation induces tubular cell death, renal inflammation, oxidative stress and reduction in renal blood flow (Klotman & Yarger, 1983; Başhan *et al*., 2014). Oxidative stress occurs as a result of direct production mitochondrial reactive oxygen species (ROS) due to gentamycin administration. ROS contribute to further impairment of cellular functions, mesangial and vascular contraction and also participate in renal inflammation (Morales *et al*., 2010). Recent studies have demonstrated that therapeutic interventions can ameliorate GIAN (Kandemir *et al*., 2015; Jaikumkao *et al*., 2016). And in order to prevent addition of toxic effects of chemical therapeutic agents, great effort has been focused in recent years upon the introduction of herbal medicine to provide a novel therapeutic agent for GIAN.

Green coffee bean extract (GCBE) derived from unroasted coffee beans has been reported to have multiple beneficial therapeutic effects in both experimental animals and humans. Recent studies demonstrated that consumption of GCBE resulted in reduction of cisplatin-induced renal apoptosis in rats (Nour El-Deen *et al*., 2019), production of antihypertensive effect (Suzuki *et al*., 2002; Kozuma *et al*., 2005), inhibition of fat accumulation (Shimoda *et al*., 2006), and modulation of glucose metabolism (Blum *et al*., 2007). Such effects have been ascribed to polyphenolic antioxidants called chlorogenic acids (CGA) which are present abundantly in GCBE (Farah *et al*., 2008).

To the best of our knowledge, there have been no studies in the literature investigating the effect of GCBE on GIAN. Accordingly, the purpose of this research was to explore the effect of oral administration of GCBE on GIAN in rats.

METHODS AND MATERIALS

Experimental animals

The present study was conducted on 40 adults male Wistar albino rats weighing from 150 to 200 g. The rats were obtained from the Animal House of the Faculty of Medicine, Alexandria University, Alexandria, Egypt.

They were housed under optimal laboratory conditions (relative humidity 85 ± 2%, temperature 22 ± 1 C and 12 h light and 12 h dark cycle). Water and pelleted food were offered ad libitum. All experiments were performed in accordance with national animal care guidelines and were approved by the Faculty of Medicine, Alexandria University Ethics Committee.

Induction of acute nephrotoxicity in rats

Acute nephrotoxicity was induced in rats by a daily intraperitoneal injection of gentamycin (memphis, Egypt) in a dose of (80 mg/kg of body weight) (Abdel-Raheem *et al*., 2009).

Animal grouping

Rats were divided into four equal groups consisting of 10 rats each.

Group 1: (Normal control group) rats received 1 mL 2% gum acacia as a vehicle orally every day for 7 days simultaneously with a daily intraperitoneal saline injection for 7 days.

Group 2: (GIAN model group) rats received 1 mL 2% gum acacia as a vehicle orally every day for 7 days simultaneously with daily intraperitoneal injection of gentamicin (80 mg/kg) for 7 days to induce acute nephrotoxicity (AN).

Group 3: rats received GCBE orally in a dose of 20 mg/kg/day for 7 days simultaneously with daily intraperitoneal injection of gentamicin (80 mg/kg) for 7 days to induce AN. GCBE was purchased from fair and pure (Germany), a company that follows German pharmaceutical guidelines in production of micronutrients.

Group 4: rats received GCBE orally in a dose of 40 mg/kg/day for 7 days simultaneously with daily intraperitoneal injection of gentamicin (80 mg/kg) for 7 days to induce AN.

At the end of the experiment (24 h after the last saline or gentamicin injection in rat groups), all

rats were anaesthetized with sodium pentobarbital (120 mg/kg intraperitoneally). Blood was withdrawn from the heart for estimation of kidney function tests: serum urea, creatinine and blood urea nitrogen (BUN). Rats were then sacrificed using high dose of sodium pentobarbital and both kidneys from rats were isolated. Then kidneys were divided equally into two longitudinal sections. One half of each kidney was placed in formaldehyde solution for histopathological examination by light microscopy. The other half of the kidney was placed into liquid nitrogen and stored at 20ºC until assayed for renal malonedialdehyde (MAD) and renal superoxide dismutase (GSH).

Estimation of renal functions tests

Determination of serum urea, creatinine and BUN were performed with UREAL kit (serum urea and BUN) and CREJ2 (serum creatinine) using COBAS C311 analyzer (Roche diagnostics, Germany).

Estimation of oxidative stress biomarkers in renal homogenate

One longitudinal half of each kidney was homogenized in phosphate buffer saline (PBS) 50 mM pH (7.4) for estimation of renal MAD and renal SOD. Renal MDA was estimated according to the method of Draper and Hadley (1990), based on the reaction of MDA with thiobarbituric acid (Draper *et al*., 1990). The reaction was performed at 95°C for 15 minutes. The renal homogenate sample was mixed with 2.5 volumes of 10% (w/v) trichloroacetic acid to precipitate the protein. The precipitate was pelleted by centrifugation and an aliquot of the supernatant was allowed to react with an equal volume of thiobarbituric acid 0.67% in a boiling water bath for 15 minutes. After cooling, the absorbance was read at 532 nm. Results were expressed as µg/g/wet tissue. SOD activity was estimated by the method of Sun *et al*. (1988). The measurement of SOD was based on the principle in which xanthine reacts with xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye. The SOD activity is then measured by the degree of inhibition of this reaction. Results were expressed as U/mg.

Renal histopathology

Longitudinal half of each kidney was fixed in a 10% neutral buffered formalin solution, embedded in paraffin, and used for histopathological examination. Five-micrometer thick sections were cut, deparaffinized, and hydrated. For light microscopic purpose, paraffin sections were stained with hematoxylin and eosin (H&E), Periodic acid-Schiff (PAS) and trichrome. For H&E, ten high power fields (40x), were randomly chosen in each proximal tubules and were scored Semi quantitatively (from zero to 4) according to the degree of tubular degeneration, necrosis and tubular interstitial nephritis (Kopple *et al*., 2002; Pai *et al*., 2012; Hur *et al*., 2013).

(a) Scoring of Tubular degeneration (TD): defined as the presence of stained bodies of various sizes and vacuolization containing acidophilus in the cytoplasm of proximal tubule epithelial cells
score 0: Absence of TD
score 1: Mild TD: small and a few focal TD immediately beneath the capsule (0 - 10%)
score 2: Moderate TD: few focal TD along the tubular segment (10 - 25%)
score 3: Severe TD: diffuse and significant TD along the tubular segment (25 - 50%)
score 4: Very severe TD: TD was greater than 50%

(b) Scoring of Tubular necrosis (TN): defined as loss of epithelial cells of the nucleus, dark acidophilic cytoplasm, loss of tubular epithelial cells into tubular lumen, and acellular sections of tubules.
Score 0: Absence of TN
Score1: Mild TN: small and a few focus TN in immediately beneath the capsule (0 - 10%)
Score2: Moderate TN: a few focal TN and along the tubular segment (10 - 25%)
Score3: Severe TD: diffuse and significant TN along the tubular segment (25 - 50%)
Score4: Very severe TN: TN was greater than 50%

(c) Scoring of Tubulo-interstitial nephritis (TIN): defined as infiltration of inflammatory cells in perivascular and interstitial areas.
Score0: Absence of TIN
Score1: Mild TIN: a few pieces of infiltration concentrated on perivascular area (0–10%)
Score2: Moderate TIN: usually infiltrations involved in corticointerstitial and many focal areas (10–25%)
Score3: Severe TIN: diffuse and significant

infiltration areas (25–50%)
Score4: Very severe TIN: TIN was greater than 50%

The three scores for each kidney were added to give the total damage score (maximum 12)

Statistical analysis

Data were expressed as the mean ± standard deviation for continuous variables. Statistical comparisons between all studied groups were performed using a one-way analysis of variance test (ANOVA) while Turkey's Multiple Comparison Test was used as a post-test to detect significance between all groups by comparing group means. For all analyses, a $p<0.05$ was considered statistically significant. Statistical analysis was performed using Graph Pad Prism version 6.0 software (La Jolla, CA).

RESULTS

Effect of green coffee bean extracts on normal kidney functions and morphology

To exclude the possibility that GCBE may affect normal kidney functions, we have performed a pilot study to evaluate the effect of oral administration of 20 and 40 mg/kg/day of GCBE for 7 days on all assessed parameters included in the present study in two rats' groups each consisting of 10 rats. The results of those experiments showed that there was no significant change in kidney functions tests, renal oxidative stress parameters and renal histopathology scoring in GCBE-treated groups when compared to normal control group.

Acute nephrotoxicity induced by gentamicin caused significant changes in kidney functions

Induction of AN by gentamicin injection in rats resulted in statistically significant deterioration in kidney function tests in GIAN model group (group 2) when compared to normal control group (group 1): there was a significant increase in serum urea ($p<0.001$), serum creatinine ($p<0.001$) and serum urea nitrogen ($p<0.001$). (Table No. 1I, Figure No.1).

Green coffee bean extract prevented deterioration of kidney functions in rats with gentamicin induced acute nephrotoxicity

Administration of GCBE for 7 days in a dose of 20 mg/kg/day in group 3 or in a dose of 40 mg/kg/day in group 4 resulted in statistically significant improvement in kidney function tests when compared to group 2 (GIAN model group): there was a significant decrease in serum urea ($p<0.001$), serum creatinine ($p<0.001$) and serum urea nitrogen ($p<0.001$). (Table No. 1, Figure No. 1).

When comparing both drug groups, more significant improvement in kidney function tests was observed in the 40 mg/kg/day GCBE treated group ($p<0.001$). (Table No. 1, Figure No. 1).

Gentamicin induced AN caused significant deterioration in renal oxidative stress markers

A significant deterioration in renal oxidative stress markers was detected in group 2 (GIAN model group) when compared to group 1 (normal control): significant decrease in both renal MDA ($p<0.001$) and renal SOD ($p<0.001$). (Table No. 1, Figure No. 2).

Antioxidant effect of green coffee bean extract

Administration of GCBE for 7 days in group 3 (20 mg/kg/day) and in group 4 (40 mg/kg/day) resulted in statistically significant improvement in renal oxidative stress markers when compared to group 2 (GIAN model group): there was significant increase in both renal MDA ($p<0.001$) and renal SOD ($p<0.001$). (Table No. 1, Figure No. 2).

When comparing both drug groups, more significant improvement in both renal MDA and renal SOD was observed in group 4 when compared to group 3 ($p< 0.001$). (Table No. 1, Figure No. 2).

Gentamicin induced AN showed significant renal histopathological changes

Renal sections of rats in the GIAN model group showed significant renal histopathological damage when compared to the normal control group ($p<0.001$). Normal control group showed normal renal tissue with normal tubules and normal tubular interstitial tissue. GIAN model group showing wide spread tubular necrosis, involving more than 50% of tubules. The lining tubular epithelium is either showing cloudy swelling with vacuolated cytoplasm, or totally necrotic lining that are sloughed in the tubular lumina admixed with the eosinophilic tubular casts. The intesrstitial tissue shows dense lymphocytic infiltration (tubular interstitial nephritis) seen mainly within the perivascular zones (Table No. 1, Figure No. 3 to No. 5).

Table No. 1
Comparison between group1 (normal control group), group 2 (GIAN model group), group 3 (GCBE 20 mg/kg/day) and group 4 (GCBE 40 mg/kg/day) regarding kidney function tests, renal MDA renal SOD and renal histopathological changes when assessed 7 days simultaneously with induction of AN by gentamicin injection

Parameter	Group 1 Normal control group	Group 2 AN model group	Group 3 GCBE (20 mg/kg/day) treated group	Group 4 GCBE (40 mg/kg/day) treated group
Serum urea mg/dl	20.84 ± 4.22	158.40 ± 11.50*	96.52 ± 3.36$^{\#}$	80.00 ± 2.09$^{\#\infty}$
Serum creatinine mg/dl	0.88 ± 0.06	7.39 ± 1.04*	4.40 ± 0.28$^{\#}$	3.76 ± 0.08$^{\#\infty}$
Serum blood urea nitrogen (mg/dL)	9.80 ± 1.20	41.96 ± 1.80*	37.84 ± 1.33$^{\#}$	28.57 ± 2.35$^{\#\infty}$
Renal MDA (µg/g wet tissue)	33.29 ± 0.63	61.32 ± 2.72 *	53.75 ± 1.39$^{\#}$	46.71 ± 1.26$^{\#\infty}$
Renal SOD (U/mg)	34.77 ± 1.67	18.01 ± 0.58*	22.92 ± 1.06$^{\#}$	29.61 ± 0.80$^{\#\infty}$
Histopathological renal damage scoring	0 ± 0.00	10.90 ± 0.75*	8.65 ± 0.45$^{\#}$	6.35 ± 0.38$^{\#\infty}$

Data are expressed as Mean ± Standard deviation. The statistical significance between the treated groups, normal control group and GIAN model group, was determined using Tukey's test. *p<0.001 versus normal control group, n$^{\#}$$p$<0.001 versus GIAN model group., $^{\#\infty}$$p$<0.001 versus group 2: GCBE (20 mg/kg/day) treated group. GCBE: green coffee bean extract, GIAN: gentamicin induced nephrotoxicity, MDA: malondialdehyde, SOD: superoxide dismutase

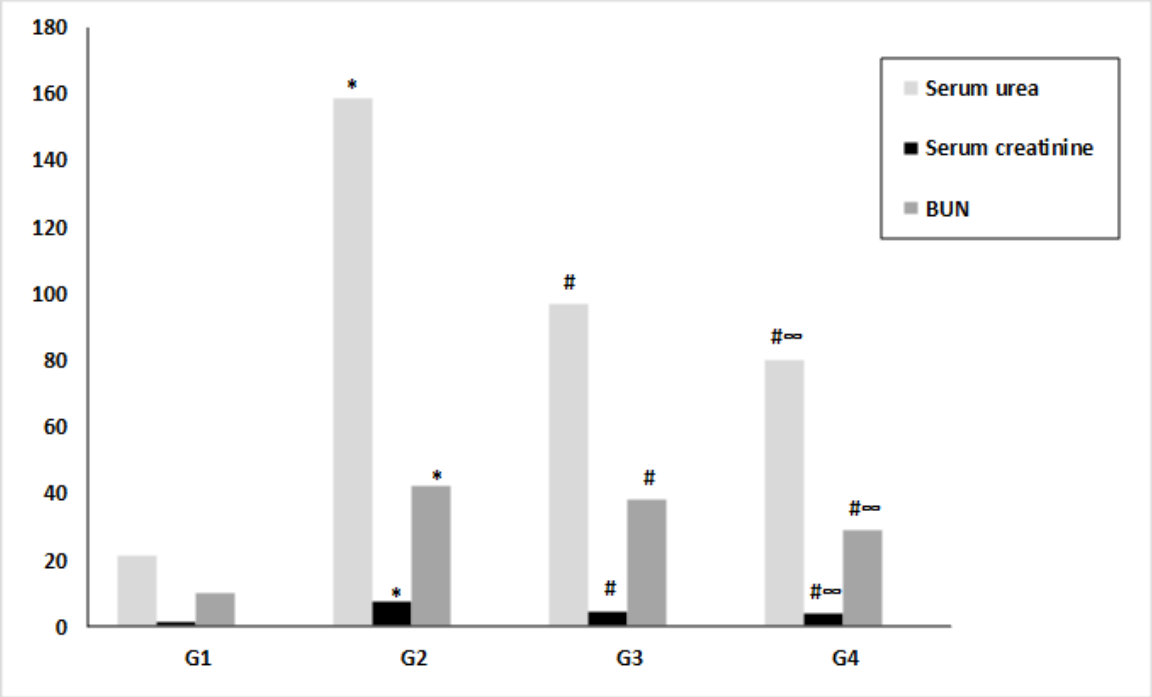


Figure No. 1
Comparison between group G1 (normal control group), group G2 (GIAN model group), group G3 (GCBE 20 mg/kg/day/7day) and group G4 (GCBE 40 mg/kg/day for 7 days) regarding kidney function tests when assessed 24 h after the last saline or gentamicin injection in rat groups. Notes: The statistical significance between the treated groups (G3, G4), G1 normal control group and G2 GIAN model group was determined using Tukey's test.*p<0.001 versus G1 normal control group, #p<0.001 versus G2 GIAN model group, ∞p<0.001 versus G3 (20 mg/kg/day)-treated group. GIAN: gentamicin induced acute nephrotoxicity, GCBE: green coffee bean extract, BUN: blood urea nitrogen

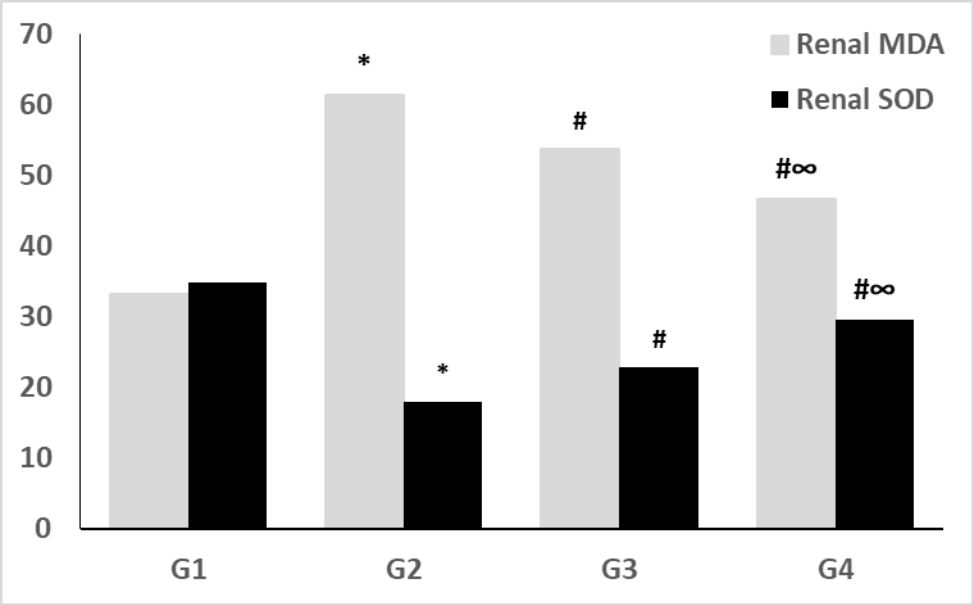


Figure No. 2

Comparison between group G1 (normal control group), group G2 (GIAN model group), group G3 (GCBE 20 mg/kg/day/7day) and group G4 (GCBE 40 mg/kg/day for 7 days) regarding renal MDA and renal SOD when assessed 24 h after the last saline or gentamicin injection in rat groups. Notes: The statistical significance between the treated groups (G3, G4), G1 normal control group and G2 GIAN model group was determined using Tukey's test.*p<0.001 versus G1 normal control group, #p<0.001 versus G2 GIAN model group, ∞p<0.001 versus G3 (20 mg/kg/day)-treated group. GIAN: gentamicin induced acute nephrotoxicity, GCBE: green coffee bean extract, MDA: malondialdehyde, SOD: superoxide dismutase

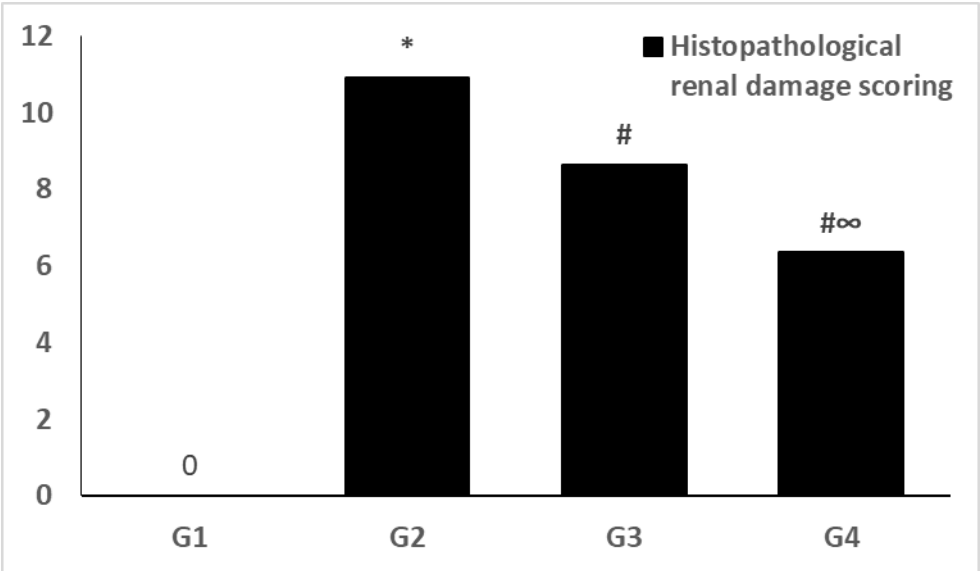


Figure No. 3

Comparison between group G1 (normal control group), group G2 (GIAN model group), group G3 (GCBE 20 mg/kg/day/7day) and group G4 (GCBE 40 mg/kg/day for 7 days) regarding renal histopathological changes scoring when assessed 24 h after the last saline or gentamicin injection in rat groups. Notes: The statistical significance between the treated groups (G3, G4), G1 normal control group and G2 GIAN model group was determined using *Tukey's test*. *p<0.001 versus G1 normal control group, $^{\#}p$<0.001 versus G2 GIAN model group, $^{\infty}p$<0.001 versus G3 (20 mg/kg/day)-treated group. GIAN: gentamicin induced acute nephrotoxicity, GCBE: green coffee bean extract

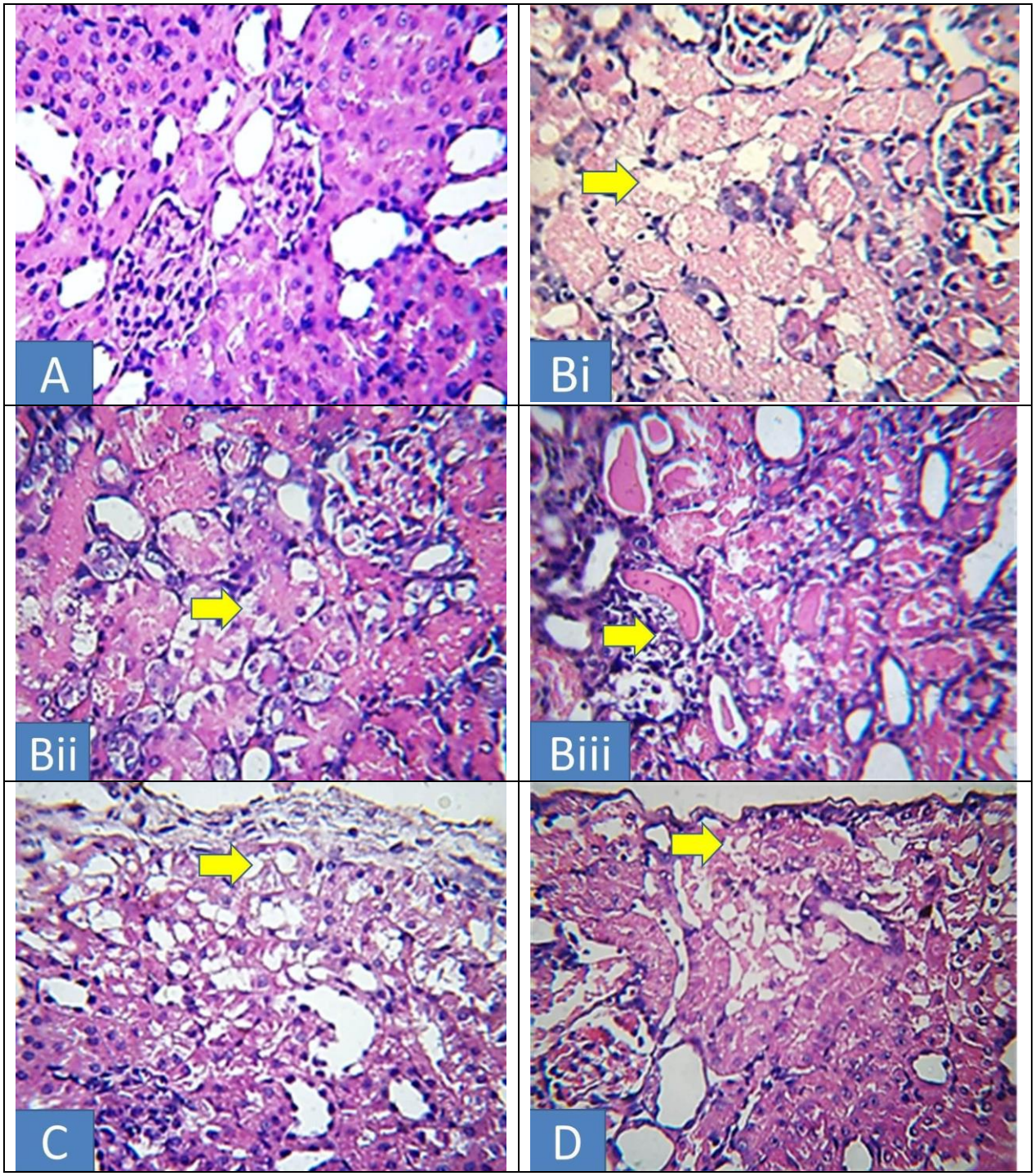
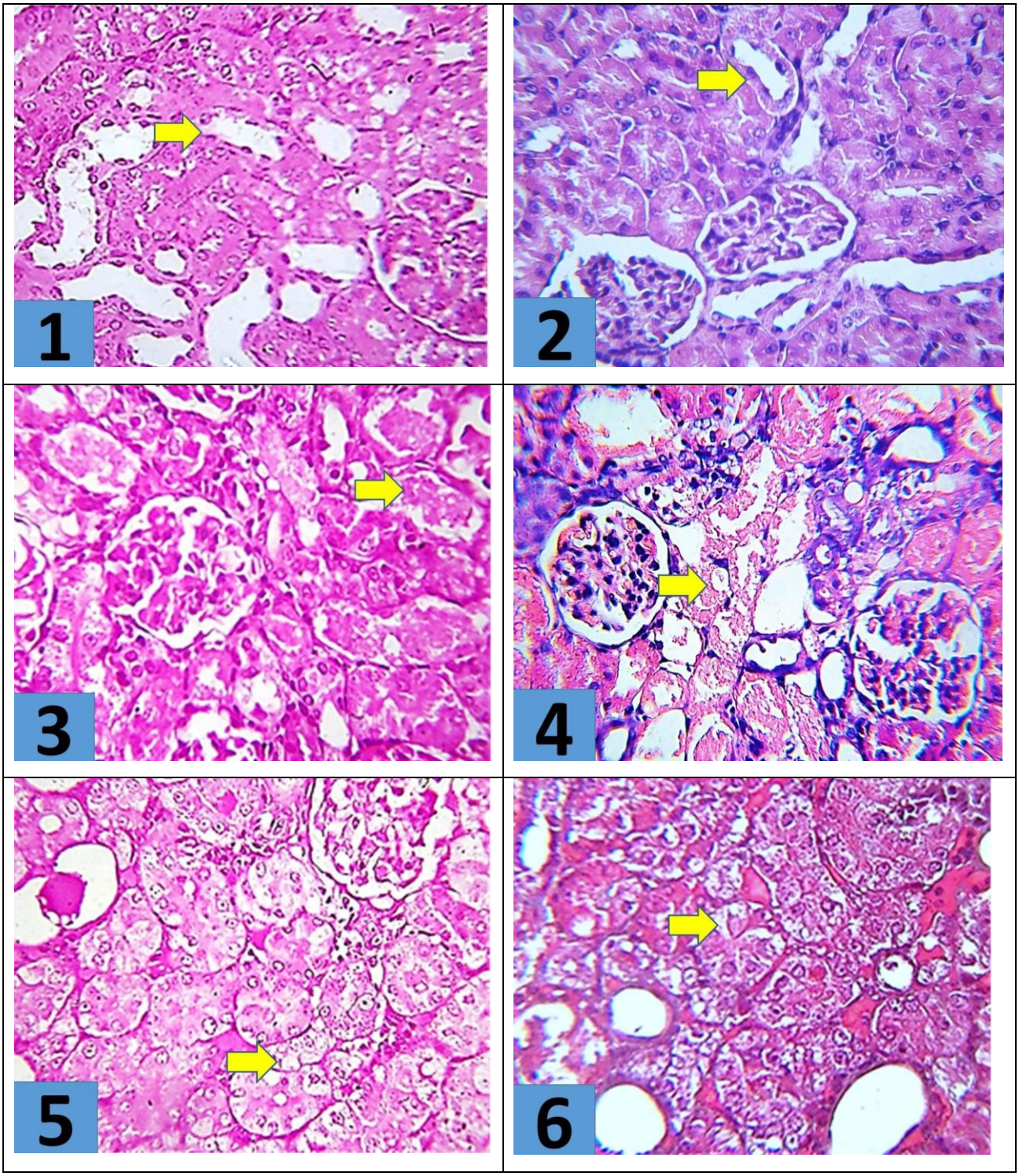


Figure No. 4
Histopathological changes in the study groups (H&E x100). (A) G1: Normal control group showing a section of normal kidney. (B) G2 GIAN model group showing Bi: very severe tubular necrosis, acellular sections of the tubules involving more than 50% of tubules (arrow) .Bii: Cytoplasmic vacuolization of the tubular epithelial lining (arrow). Biii: perivascular and interstitial lymphocytes lymphocytic infiltration (arrow). (C) G3 GCBE (20 mg/kg/day)-treated group showing Mild TD, focal cytoplasmic vacuolization (arrow), mild TIN, no TN. (D) G4 GCBE (40 mg/kg/day)-treated group showing Mild TD, cytoplasmic vacuolization immediately beneath the capsule (arrow), no TIN or TN. GIAN: gentamicin induced acute nephrotoxicity, GCBE: green coffee bean extract.TD: tubular degeneration, TN: tubular necrosis, TIN: tubule interstitial nephritis


1
2
3
4
5
6

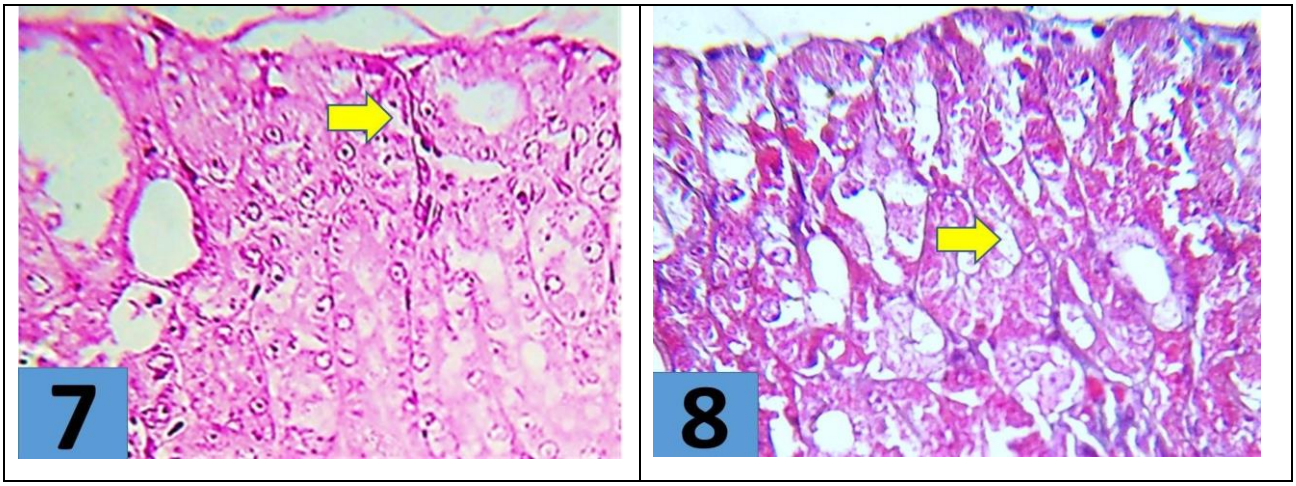


Figure No. 5
Histopathological changes in different groups using PAS & Trichrome stains (x200):

- **Group 1 normal control; (1) PAS, (2) trichrome: showing tubules with no pathological changes (arrow)**
- **GIAN Model group; (3) PAS (4) trichrome: showing tubular necrosis, arrows point to the necrotic acellular tubules containing tubular casts**
- **Drug treated group GCBE 20 mg/day; (5) PAS and (6) trichrome showing vacuolar degeneration of the tubular epithelial lining (arrows)**
- **Drug treated group GCBE 40 mg/day; (7) PAS showing subcapsular vacuolar degeneration of the tubules. (arrow) and (8) trichrome showing subcapsular vacuolar degeneration of the tubules with focal tubular necrosis (arrow)**

GCBE administration caused regression of renal histopathological changes

Significant regression of histopathological changes was recorded in both GCBE treated groups when compared to the model group (p<0.001). (Table No. 1, Figure No. 3).

GCBE (20 mg/kg/day)-treated group showed cloudy swelling of the lining epithelium with focal cytoplasmic vacuolization. Mild interstitial perivascular lymphocytic infiltration (tubular interstitial nephritis) is noted. No tubular necrosis is detected. (Figure No. 4).

GCBE (40 mg/kg/day)-treated group showing Mild cloudy swelling of the tubular epithelial lining, some tubules are showing evident cytoplasmic vacuolization that is seen immediately beneath the capsule, however no tubular necrosis or interstitial lymphocytic infiltration (tubular interstitial nephritis) could be detected in this group

When comparing both drug groups, more significant improvement in renal histopathological changes was observed in group 4 when compared to group 3 (p<0.001). (Table No. 1, Figure No. 3 to No. 5).

DISCUSSION

In the present study, oral administration of GCBE resulted in improvement of kidney function tests, renal oxidative stress markers as well as renal pathological changes in rats with GIAN.

Morphological studies demonstrated that Gentamicin induced AN in rats is characterized by the presence of epithelial edema, proximal tubule epithelial desquamation, tubular necrosis, and glomerular hypertrophy (Stafford *et al*., 2005). Those changes are responsible of deterioration of renal functions and hence failure of the kidneys to excrete waste products and to maintain fluid and electrolyte homeostasis (Balakumar *et al*., 2010; Nasri, 2012). This is in accordance with the present study which showed significant decline in the renal functions (increase in serum urea, serum creatinine and BUN) in GIAN model group when compared to the normal control groups. Moreover, reported similar results (Ali, 2003; Singh *et al*., 2012).

Many therapeutic agents have been recently used as an attempt to prevent gentamicin induced renal damage, e.g. captopril (Chowdhury *et al*., 2018) and sitagliptin (Al Suleimani *et al*., 2018). However, due to associated adverse effects of such therapeutic agents, natural products like herbs have been recently introduced to prevent GIAN, e.g. fish oil (El-Ashmawy *et al*., 2018), *Punica granatum* (Mestry *et al*., 2018) and *Rumex vesicarius* (Subramaniyan *et*

al., 2018).

Green coffee beans extract (GCBE), has proven to contain numerous phytochemicals that possess antioxidant, anti-inflammatory, neuroprotective and anticancerous properties (Mohamed *et al*., 2018).

In the literature, this is the first study to explore the effect of GCBE on GIAN in rats.

The present study demonstrated that oral administration of GCBE protected against gentamicin -induced impairment of renal functions through producing significant decrease in serum urea, serum creatinine and BUN when compared to the GIAN model group.

The current study hypothesizes that the possible explanation for improvement in renal function following administration of GCBE may be due to its proven role in reduction of oxidative stress and renal histopathological changes.

Oxidative stress plays an important role in the pathogenesis of GIAN. This could be explained in the context that the kidney is known to be an organ with high susceptibility to damage by reactive oxygen species, probably due to the abundance of long chain polyunsaturated fatty acids in the composition of renal lipids (Fishman *et al*., 2012). Similarly, the present study revealed deterioration of oxidative stress markers (renal MDA and renal SOD) in the GIAN model group when compared to the normal control group. Similar results were reported (Ojano-Dirain *et al*., 2014; Kandemir *et al*., 2015).

To explore the antioxidant effect of GCBE in the present study, we examined its effect on two important antioxidant enzymes; renal MDA and renal SOD. Results of the current study revealed that oral administration of GCBE caused significant amelioration in both renal MDA and renal SOD when compared to their levels in GIAN model group. In addition, more significant improvement was demonstrated in the group that received GCBE in a dose of 40 mg/kg/day. The proven antioxidant effect of GCBE on other injuries of the kidneys was previously reported. Noureldeen et al demonstrated improvement in renal oxidative stress markers after oral administration of GCBE in rats with cisplation induced renal apoptosis (Nour El-Deen *et al*., 2019). Also the antioxidant effect of GCBE has been demonstrated in other organs like liver, brain and skin (Nogaim *et al*., 2020; Pergolizzi *et al*., 2020).

Studies have been previously conducted to uncover the antioxidant contents of GCBE which include cholinergic acids (CGA) and related compounds (Bothiraj & Vanitha, 2020). The main groups of CGA found in green coffee beans include caffeoylquinic acids, dicaffeoylquinic acids, feruloylquinic acids, p-coumaroylquinic acids and mixed diesters of caffeic and ferulic acids with quinic acid. Those CGA constitute the main components of the phenolic fraction of GCBE (Farah & Donangelo, 2006). The overall antioxidant mechanism of action of phenols is thought to be through reducing reactive oxygen species production by neutralizing them or by chelating metal ions (Afanas *et al*., 1989).

Cellular damage of the proximal convoluted tubules together with loss of integrity of their brush border leading to necrotic changes are important landmarks in the pathogenesis of GIAN. It has been postulated that gentamicin injection leads to the development of acute tubular necrosis due to its accumulation in the proximal convoluted tubules leading to activation of inflammatory processes, leukocyte infiltration, contraction of mesangial cells and decrease in renal blood flow (Martinez-Salgado *et al*., 2007; Lopez-Novoa *et al*., 2011). The present study revealed significant histopathological changes in kidney sections obtained from rats in GIAN model group when compared to normal control group. These changes were mostly in the form of tubular degeneration, necrosis and tubular interstitial nephritis. Similar findings were also reported by other investigators (Promsan *et al*., 2016).

The present study showed that oral GCBE administration caused significant regression of renal histopathologic changes when compared to GIAN model group. In accordance with our results Nour El-Deen *et al*. (2019), reported attenuation of renal apoptosis after administration of GCBE to rats. Such improvement was attributed according to the researchers to the significant proven antioxidant effect of GCBE.

CONCLUSIONS

GCBE was found to significantly attenuate GIAN in rats through improving renal functions and reducing renal oxidative stress. In the literature, this is the first study highlighting this novel finding. Further studies are needed to elucidate the exact cellular mechanism of its renoprotective effect.

REFERENCES

Abdel-Raheem IT, Abdel-Ghany AA, Mohamed GA. 2009. Protective effect of quercetin against gentamicin-

induced nephrotoxicity in rats. **Biol Pharm Bull** 32: 61 - 67. **https://doi.org/10.1248/bpb.32.61**

Farah A, Donangelo CM. 2006. Phenolic compounds in coffee. **Braz J Plant Physiol** 18: 1590 - 1677. **https://doi.org/10.1590/S1677-04202006000100003**

Afanas IB, Dorozhko AI, Brodskii AV, Kostyuk VA, Potapovitch AI. 1989. Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. **Biochem Pharmacol** 38: 1763 - 1769. **https://doi.org/10.1016/0006-2952(89)90410-3**

Al Suleimani YM, Abdelrahman AM, Karaca T, Manoj P, Ashique M, Nemmar A, Ali BH. 2018. The effect of the dipeptidyl peptidase-4 inhibitor sitagliptin on gentamicin nephrotoxicity in mice. **Biomed Pharmacother** 97: 1102 - 1108. **https://doi.org/10.1016/j.biopha.2017.10.107**

Ali BH. 2003. Agents ameliorating or augmenting experimental gentamicin nephrotoxicity: some recent research. **Food Chem Toxicol** 41: 1447 - 1452. **https://doi.org/10.1016/s0278-6915(03)00186-8**

Balakumar P, Rohilla A, Thangathirupathi A. 2010. Gentamicin-induced nephrotoxicity: do we have a promising therapeutic approach to blunt it? **Pharmacol Res** 62: 179 - 186. **https://doi.org/10.1016/j.phrs.2010.04.004**

Bashan İ, Bashan P, Secilmis MA, Singirik E. 2014. Protective effect of L-arginine on gentamicin-induced nephrotoxicity in rats. **Indian J Pharmacol** 46: 608 - 612. **https://doi.org/10.4103/0253-7613.144915**

Blum J, Lemaire B, Lafay S. 2007. Effect of a green decaffeinated coffee extract on glycaemia. A pilot prospective clinical study. **NutraFoods** 6: 13 - 17.

Bothiraj KV, Vanitha V. 2020. Green coffee bean seed and their role in antioxidant–A review. **Int J Res Pharmaceut Sci** 11: 233 - 240. **https://doi.org/10.26452/ijrps.v11i1.1812**

Chowdhury MS, Saha SK, Eva EO, Sarker MM. 2018. A study on the effect of captopril on gentamicin induced nephrotoxicity in rats. **Mymensingh Med J** 27: 57 - 67.

Draper HH, Hadley M. 1990. Malondialdehyde determination as index of lipid peroxidation. **Methods Enzymol** 86: 421 - 431.

El-Ashmawy NE, Khedr NF, El-Bahrawy HA, Helal SA. 2018. Upregulation of PPAR-γ mediates the renoprotective effect of omega-3 PUFA and ferulic acid in gentamicin-intoxicated rats. **Biomed Pharmacother** 99: 504 - 510. **https://doi.org/10.1016/j.biopha.2018.01.036**

Farah A, Monteiro M, Donangelo CM, Lafay S. 2008. Chlorogenic acids from green coffee extract are highly bioavailable in human. **J Nutr** 138: 2309 - 2315. **https://doi.org/10.3945/jn.108.095554**

Fishman AI, Alexander B, Eshghi M, Choudhury M, Konno S. 2012. Nephrotoxin-induced renal cell injury involving biochemical alterations and its prevention with antioxidant. **J Clin Med Res** 4: 95 - 101. **https://doi.org/10.4021/jocmr833w**

Friesen WJ, Johnson B, Sierra J, Zhuo J, Vazirani P, Xue X, Tomizawa Y, Baiazitov R, Morrill C, Ren H, Babu S, Moon YC, Branstrom A, Mollin A, Hedrick J, Sheedy J, Elfring G, Weetall M, Colacino JM, Welch EM. 2018. The minor gentamicin complex component, X2, is a potent premature stop codon read through molecule with therapeutic potential. **PLoS One** 13: e0206158. **https://doi.org/10.1371/journal.pone.0206158**

Hur E, Garip A, Camyar A, Ilgun S, Ozisik M, Tuna S, Olukman M, Ozdemir ZN, Sozmen EY, Sen S, Akcicek F, Duman S. 2013. The effects of vitamin D on gentamicin-induced acute kidney injury in experimental rat model. **Int J Endocrinol** 2013: 313528. **https://doi.org/10.1155/2013/313528**

Jaikumkao K, Pongchaidecha A, Thongnak L, Wanchai K, Arjinajarn P, Chatsudthipong V, Chattipakorn N, Lungkaphin A. 2016. Amelioration of renal inflammation, endoplasmic reticulum stress and apoptosis underlies the protective effect of low dosage of atorvastatin in gentamicin-induced nephrotoxicity. **PLoS One** 11: e0164528. **https://doi.org/10.1371/journal.pone.0164528**

Kandemir FM, Ozkaraca M, Yildirim BA, Hanedan B, Kirbas A, Kilic K, Aktas E, Benzer F. 2015. Rutin attenuates gentamicin-induced renal damage by reducing oxidative stress, inflammation, apoptosis, and autophagy in rats. **Renal Fail** 23: 1 - 8. **https://doi.org/10.3109/0886022x.2015.1006100**

Klotman PE, Yarger WE. 1983. Reduction of renal blood flow and proximal bicarbonate reabsorption in rats by gentamicin. **Kidney Int** 24: 638 - 643. **https://doi.org/10.1038/ki.1983.205**

Kopple JD, Ding H, Letoha A, David B, Qing PY, Dux L, Wang HY, Sonkodi S. 2002. L-carnitine ameliorates gentamicin-induced renal injury in rats. **Nephrol Dial Transplant** 17: 2122 - 2131. **https://doi.org/10.1093/ndt/17.12.2122**

Kozuma K, Tsuchiya S, Kohori J, Hase T, Tokimitsu I. 2005. Antihypertensive effect of green coffee bean extract on mildly hypertensive subjects. **Hypertens Res** 28: 711 - 718. **https://doi.org/10.1291/hypres.28.711**

Laurent G, Kishore BK, Tulkens PM. 1990. Aminoglycoside induced renal phospholipidosis and nephrotoxicity. **Biochem Pharmacol** 40: 2383 - 2392. **https://doi.org/10.1016/0006-2952(90)90078-y**

Lopez-Novoa JM, Quiros Y, Vicente L, Morales AI, Lopez-Hernandez FJ. 2011. New insights into the mechanism of aminoglycoside nephrotoxicity. **Kidney Int** 79: 33 - 45. **https://doi.org/10.1038/ki.2010.337**

Martinez-Salgado C, López-Hernández FJ, Lopez-Novoa JM. 2007. Glomerular nephrotoxicity of aminoglycosides. **Toxicol Appl Pharmacol** 223: 86 - 98. **https://doi.org/10.1016/j.taap.2007.05.004**

Mestry SN, Gawali NB, Pai SA, Gursahani MS, Dhodi JB, Munshi R, Juvekar AR. 2018. *Punica granatum* improves renal function in gentamicin-induced nephropathy in rats via attenuation of oxidative stress. **J Ayurveda Integr Med** 9476: 30323 - 30326. **https://doi.org/10.1016/j.jaim.2017.09.006**

Mohamed HE, Asker ME, Younis NN, Shaheen MA, Eissa RG. 2018. Modulation of brain insulin signaling in Alzheimer's disease: new insight on the protective role of green coffee bean extract. **Nutr Neurosci** 1: 1 - 10. **https://doi.org/10.1080/1028415x.2018.1468535**

Morales AI, Detaille D, Prieto M, Puente A, Briones E, Arévalo M, Leverve X, López-Novoa JM, El Mir M. 2010. Metformin prevents experimental gentamicin-induced nephropathy by a mitochondria-dependent pathway. **Kidney Int** 77: 861 - 869. **https://doi.org/10.1038/ki.2010.11**

Nasri H. 2012. Acute kidney injury and beyond. **J Ren Inj Prev** 1: 1 - 2. **https://doi.org/10.12861/jrip.2012.01**

Nogaim QA, Bugata LSA, Prabhakar PV, Reddy UA, Kumari I, Mahboob M. 2020. Protective effect of Yemeni green coffee powder against the oxidative stress induced by Ochratoxin A. **Toxicol Rep** 7: 142 - 148. **https://doi.org/10.1016/j.toxrep.2019.11.015**

Nour El-Deen A, Mansour AM, Taha A, Fahmy E. 2019. Effect of green coffee on cisplatin induced renal apoptosis in adult male albino rats. **Food Nutr Sci** 10: 358 - 368. **https://doi.org/10.4236/fns.2019.104028**

Ojano-Dirain CP, Antonelli PJ, Le Prell CG. 2014. Mitochondria-targeted antioxidant MitoQ reduces gentamicin-induced ototoxicity. **Otol Neurotol** 35: 533 - 539. **https://doi.org/10.1097/mao.0000000000000192**

Pai PG, Nawarathna SC, Kulkarni A, Habeeba U, Reddy CS, Teerthanath S, Shenoy JP. 2012. Nephroprotective effect of ursolic acid in a murine model of gentamicin-induced renal damage. **ISRN Pharmacol** 2012: 410902. **https://doi.org/10.5402/2012/410902**

Pergolizzi S, D'Angelo V, Aragona M, Dugo P, Cacciola F, Capillo G, Dugo G, Lauriano ER. 2020. Evaluation of antioxidant and anti-inflammatory activity of green coffee beans methanolic extract in rat skin. **Nat Prod Res** 34: 1535 - 1541. **https://doi.org/10.1080/14786419.2018.1523161**

Promsan S, Jaikumkao K, Pongchaidecha A, Chattipakorn N, Chatsudthipong V, Arjinajarn P, Pompimon W, Lungkaphin A. 2016. Pinocembrin attenuates gentamicin-induced nephrotoxicity in rats. **Can J Physiol Pharmacol** 94: 808 - 818. **https://doi.org/10.1139/cjpp-2015-0468**

Shimoda H, Seki E, Aitani M. 2006. Inhibitory effect of green coffee bean extract on fat accumulation and body weight gain in mice. **BMC Complement Altern Med** 6: 1 - 9. **https://doi.org/10.1186/1472-6882-6-9**

Singh AP, Junemann A, Muthuraman A, Singh N, Grover K, Dhawan R. 2012. Animal models of acute renal failure. **Pharmacol Rep** 64: 31 - 44. **https://doi.org/10.1016/s1734-1140(12)70728-4**

Stafford GI, Jäger AK, Van Staden J. 2005. Effect of storage on the chemical composition and biological activity of several popular South African medicinal plants. **J Ethnopharmacol** 97: 107 - 115. **https://doi.org/10.1016/j.jep.2004.10.021**

Subramaniyan V, Shaik S, Bag A, Manavalan G, Chandiran S. 2018. Potential action of *Rumex vesicarius* (L.) against potassium dichromate and gentamicin inducednephrotoxicity in experimental rats. **Pak J Pharm Sci** 31: 509 - 516.

Sun Y, Oberley LW, Li Y. 1988. A simple method for clinical assay of superoxide dismutase. **Clin Chem** 34: 497 - 500.

Suzuki A, Kagawa D, Ochiai R, Tokimitsu I, Saito I. 2002. Green coffee bean extract and its metabolites have a hypotensive effect in spontaneously hypertensive rats. **Hypertens Res** 25: 99 - 107. **https://doi.org/10.1291/hypres.25.99**

Swan SK. 1997. Aminoglycoside nephrotoxicity. **Semin Nephrol** 17: 27 - 33.

Wargo KA, Edwards JD. 2014. Aminoglycoside-induced nephrotoxicity. **J Pharm Pract** 27: 573 - 577.