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Research Article

Ginkgo biloba Extract Attenuates Hematological Disorders, Oxidative Stress and Nephrotoxicity Induced by Single or Repeated Injection Cycles of Cisplatin in rats: Physiological and Pathological Studies

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Abstract

Background and Objective: Cisplatin (CIS) is known to cause nephrotoxic effect, depression in erythrocytes (anemia), leukocytes (leukopenia) and platelets (thrombocytopenia) in a dose-dependent manners which limits its clinical use. This study was designed to investigate the efficacy of *Ginkgo biloba* (GB) extract against hematological and pathological disorders, oxidative stress and nephrotoxicity induced by single or repeated injection cycles of cisplatin in rats. **Materials and Methods:** Animals were divided into six equal groups (n = 10). Negative and positive control groups (received vehicle, GB 150 mg kg^{-1}, respectively), cisplatin intoxicated groups (received 24 mg kg^{-1} as a single i.p. injection, or as three repeated injection cycles; each cycles consists of 4 daily low dose 2 mg kg^{-1} b.wt., followed by 10 days recovery period) and GB preventive groups (received the same treatment as intoxicated groups in concomitant with daily 150 mg kg^{-1} b.wt., of GB orally, started 5 days before first CIS injection). The possible ameliorative effect of GB was determined on CIS-induce alterations in Relative Kidney Weight (RKW), Blood Urea Nitrogen (BUN), creatinine, blood picture, tissue oxidative parameters, histopathologic and histo-morphometric analysis and quantification of TUNEL positive cells. **Results:** The study revealed significant increase in RKW, serum creatinine and BUN, renal malondialdehyde (MDA), quantitative and semi-quantitative grading of renal pathology and number of TUNEL positive cells/HPF in CIS intoxicated groups. Moreover, significant decrease was reported in renal glutathione (GSH), Hb concentration, RBCs, WBCs and platelets count in CIS intoxicated groups. Significant improvements in some of these parameters were reported with GB prior-treatment. **Conclusion:** Prior-treatment with GB partially attenuated the CIS induced anemia, leukocytopenia, thrombocytopenia and nephrotoxicity through its antioxidant and anti-apoptotic properties.

Key words: Cisplatin-nephrotoxicity, *Ginkgo biloba*, hematological parameters, apoptosis, oxidative stress, morphometric analysis

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cisplatin (CIS) is an effective antineoplastic drug that is commonly used for treatment of varieties solid tumors including head, neck, lung, testicular, ovarian and breast cancers[1]. The possible mechanisms involved in the anticancer activity of CIS may discuss by its ability to binds directly to DNA of tumor cells, forming a cross-link leading to the arrest of DNA synthesis and replication. Treatment with CIS frequently cause nephrotoxicity, acute depression in erythrocytes (anemia), leukocytes (leukopenia) and platelets (thrombocytopenia) in a dose dependent manners in rats[2] and mice[3]. Unfortunately, the previous toxicity of CIS are an inherent adverse effects[4-6], where more than one third of patients develop acute renal injury during CIS treatment. The major nephrotoxic effect of CIS is mainly exerts in the renal proximal tubular cells where it is preferentially accumulated[7,8]. Despite the mechanisms of this renal injury has been the focus of several studies for many years, it still not fully elucidated. However, oxidative stress, in ammation and apoptosis seem to play a crucial role[9-11]. There is no precise treatment for cisplatin-induced nephrotoxicity. Therefore, many investigations have been designed to assess the potential renoprotective effects of several antioxidants and anti-inflammatory agents against the adverse effects of CIS[12-19]. However, the comparative study for the effect of these protective agents against the potential renal damage induced by single injection or repeated injection cycles (a typical clinical dosing regimen) of CIS with an equivalent cumulative dose has not been previously investigated.

Ginkgo biloba (GB) is a common traditional popular medicine which used for treatment of wide range of disorder including cognitive deficiencies, vestibular vertigo, tinnitus and Alzheimer disease[20,21]. It is well known for its anti-inflammatory, anti- apoptotic and antioxidant properties which contributes its ability to scavenge free radicals[22]. Previous investigation reported the crucial prophylactic role of GB in the prevention of several diseases associated with oxidative tissue damage[23,24]. This preventive role may attribute to its active components, namely, avonoglycoside and terpene lactones[22,25]. Therefore, GB may have the ability to protect against CIS-induced nephrotoxicity. This was encouraging to design the current study in order to assess the ameliorative role of GB in rats exposed to single intraperitoneal (i.p.) injection of CIS and was compared to rats receiving an equivalent cumulative dose of CIS in three repeated injection cycles. Moreover, quantification of TUNEL positive cells (as an indicator for apoptosis) and the possible biochemical, hematological and histopathological CIS-induced alteration and mechanisms underlying this alteration were investigated.

MATERIALS AND METHODS

Animals: Sixty adult male albino rats weighing 150-200 g were obtained from Animal Facility, Alexandria University, Egypt. The animals were kept in galvanized metal cages with standard housing facilities (24±1°C and 12 h light/dark cycle). They were supplied with a standard rodent chow diet with *ad libitum* water access and allowed 2 weeks acclimatization period before the experiments. The experimental procedures were carried out in accordance with the animal care guidelines of the National Institute of Health and the experimental protocol was approved by local ethical committee.

Cisplatin and *Gingko biloba*: Cisplatin (CIS) was obtained from Oncotic Pharma Production GmbH, Am Pharmapark, Germany. *Gingko biloba* (GB) dry leaves extract was obtained from Nature's Bounty, Bohemia, USA. The extract is standardized to contain 24% *Ginkgo* flavonoglycosides, 14.4 mg and terpene lactones (6%) which represent the major active contents of GB.

Experimental design: Rats were randomly divided into six equal groups (n = 10, each). Rats in group 1 received distilled water and normal saline (vehicle of GB and CIS) and served as negative control. Rats in group 2 received daily oral GB water extract (150 mg kg^{-1} b.wt., volume 5 mL kg^{-1} b.wt.)[26] and served as positive control. Group 3 received single i.p. injection of CIS (24 mg kg^{-1} 72 h before rat's euthanasia)[27]. Group 4 received three repeated injection cycles of CIS (each cycle consists of four daily low dose treatment 2 mg kg^{-1} b.wt., followed by 10 days recovery period, with cumulative dose 24 mg kg^{-1} b.wt.). Rats in groups 5 and 6 received the same treatment used in groups 3 and 4 in concomitant with daily intra-gastric administration of GB (150 mg kg^{-1} b.wt.). The prior treatment with GB started 5 days before the first injection of CIS and continued up to the end of the experiment. To overcome the possible drug interaction, the treatments with CIS and GB were separated by 60 min. Rats in each group were weekly weighed.

Sample preparation and evaluated parameters: After 72 h of the last CIS administration, rats were anesthetized with light ether; blood samples were collected from the rat's retro-orbital plexus. All rats were weighed before sacrification.

Blood sampling: Two blood samples from each control and treated rats were taken before sacrifying them from the retro-orbital plexus under light ether anesthesia using hematocrit tubes. One sample was taken with EDTA for making blood picture while the other sample was collected on clean dry centrifuge tubes for making sera samples.

Hematological studies: Red blood cells, white blood cells, platelets count, hemoglobin concentration and packed cell volume were measured[28].

Serum analysis: The blood was centrifuged at 3000 rpm for 15 min to obtain clear serum that was stored frozen at -20°C until assayed for BUN urea and creatinine using commercial kits from Diamond Diagnostic Co., Egypt.

Index weight of kidneys: After blood collection, rats were immediately euthanized; the kidneys were removed, grossly examined and weighed. The Index Weight (IW) for kidney was calculated according to Matousek[29]:

$$IW = \frac{\text{Organ weight}}{\text{Body weight}} \times 100$$

Preparation of renal tissue homogenate: The right kidney was separated from each rat, kept at -80°C and then homogenized in cold potassium phosphate buffer (0.05 M, pH 7.4). The homogenates centrifuged at 5000 rpm for 10 min at 4°C. The resulting supernatant was used for measuring of malondialdehyde (MDA) and reduced glutathione (GSH) levels using colorimetric assay kits according to the manufacturer's instructions (Bio-diagnostic, Egypt).

Estimation of lipid peroxidation in kidney tissue: Lipid peroxides as malondialdehyde (MDA) were measured by a Spectrophotometer (UV-VIS Systronics) after the reaction with thiobarbituric acid reactive substances[30].

Measurement of glutathione in the kidney tissue: Reduced glutathione (GSH) was assayed by spectrophotometric technique[31] using Ellman's reagent. This method is based on the reductive cleavage of 5,5-dithiobis-2-nitrobenzoic acid by sulfhydryl group to yield a yellow color with maximum absorbance at 412 nm.

Histopathological examination and semi-quantitative scoring of renal tissue: The left kidney was collected from each rat then immediately fixed in 10% buffered formalin for 48 h. After fixation, the tissues were processed through the conventional paraffin embedding technique[32]. Sections of 5 µ thicknesses were stained with hemotoxylin and eosin (HE) and then examined by light microscope. The severity of renal damage was scored semi-quantitatively according to previously determined criteria[33]. Tissue deterioration was quantified in 10 microscopic fields from each group using a scale (0: None, 1: Mild, 2: Moderate and 3: Severe) for each criteria with a maximum score of nine (Table 1).

Morphometric analysis (Computer image analysis): Quantitative histo-morphometric analysis was performed on high quality images obtained from light microscopy of HE stained sections using image J software (The program was originally developed by Wayne Rasband (wayne@codon.nih.gov) at the Research Services Branch of the National Institute of Mental Health (Bethesda, MD, USA). Five blindly selected High Power Fields (HPF) from each section were evaluated (10 sections from each experimental group).

Quantification of TUNEL positive cells: Sections of 4 µ thicknesses were prepared from control and treated rat's kidneys. Tissue sections were stained for apoptotic cells using TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling) assay kit according to the recommendations of the manufacturer (Trevigen, TACS® TdT). This assay relies on detection of DNA nicks by terminal deoxynucleotidyl transferase (TdT), an enzyme that will catalyze the addition of dUTPs that are secondarily labeled with a marker as previously described by Gavrieli *et al.*[34]. The TUNEL positive cells was visualize under the light microscope as a dark brown color, other non-reactive cells were stained by hematoxylin to gives a blue shade. Results were expressed as the mean number of TUNEL-positive apoptotic cell/HPF.

Statistical analysis: All values are expressed as Mean±SEM The obtained results were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test using SAS[35]. The p-value was represented as $p \leq 0.01$ and $p \leq 0.05$ to determine the strength of statistically significance.

Table 1: Histopathological criteria used for tissues scoring system

Tissue	Criteria
Kidney (At least 10 microscopic fields were examined from each group)	Deterioration in glomerulus and bowman space
	Deterioration in proximal and distal tubule
	Vascular congestion and inflammatory cell infiltration

RESULTS

Effects of CIS and/or GB on Final Body Weight (FBW) and Relative Kidney Weight (RKW): Final body weight was significantly decreased ($p \leq 0.05$) in rats treated with three repeated cycles of CIS (with or without GB prior-treatment) compared to control rats. The relative kidney weight was significantly increased ($p \leq 0.05$) in all CIS treated rats compared to control rats. While, the pretreatment with GB significantly decreased the RKW compared with the repeated CIS administration alone (Table 2).

Effects of CIS and/or GB on RBCs, WBCs, Hb, PCV and platelets: The single or repeated administration of CIS alone induced a significant decrease in the counts of RBCs, WBCs and platelets, Hb concentration and PCV percent ($p \leq 0.05$) as compared to control groups. While, the rats treated with GB alone showing non-significant difference in the previously mentioned hematological parameters compared to the negative control rats (Table 3). However, pre-treatment of GB significantly increased the counts of WBCs in rats given single or repeated administration of CIS compared with CIS alone. Pre-administration of GB to rats given single doses of CIS insignificantly increased the counts of RBCs and platelets, Hb concentration and PCV percent compared to CIS alone ($p \leq 0.05$). Meanwhile, Pre-administration of GB to rats given repeated doses of CIS significantly increased the counts of RBCs and platelets, Hb concentration and PCV percent compared to CIS alone ($p \leq 0.05$).

Effects of CIS and/or GB on BUN, serum creatinine: Rats received CIS in both administration manners showing significant increase ($p \leq 0.05$) in serum BUN and creatinine levels compared to control rats. However, the pretreatment with GB significantly decreased serum BUN and creatinine levels compared to CIS alone (Table 4).

Effects of CIS and/or GB on renal MDA and GSH: Rats treated with CIS alone showing significant increase ($p \leq 0.05$) in MDA level and significant depletion ($p \leq 0.05$) in GSH level compared to control rats. However, rats pretreated with GB showing significant decrease ($p \leq 0.05$) in MDA level compared to those intoxicated with CIS alone (Table 5). Meawhile, the GSH level was not significantly differed in rats pretreated with GB compared with CIS alone (Table 5).

Table 2: Effects of CIS and/or GB on Final Body Weight (FBW), Relative Kidney Weight (RKW)

	Groups					
Parameters	G1	G2	G3	G4	G5	G6
IBW (g)	176.83±1.74	177.33±1.96	176.50±1.77	180.33±4.14	178.17±2.09	173.50±1.52
FBW (g)	233.17±1.30^{a}	235.83±2.20^{a}	225.50±1.95^{a}	156.83±5.21^{b}	227.33±2.72^{a}	153.83±1.97^{b}
RKW (%)	0.46±0.01^{cd}	0.42±0.02^{d}	0.53±0.02^{c}	0.80±0.03^{a}	0.52±0.02^{c}	0.71±0.02^{b}

Means bearing different letters within the same row are significant at $p \leq 0.05$, initial body weight (IBW), G1: Negative control, received distilled water and normal saline (vehicle of GB and CIS), G2: Positive control, received daily oral GB water extract (150 mg kg^{-1} b.wt.), G3: Received single i.p., injection of CIS (24 mg kg^{-1} b.wt.) 72 h before rat's euthanasia, G4: Received repeated injection cycles of CIS (each cycles consists of four daily low dose treatments 2 mg kg^{-1} b.wt., followed by 10 days recovery period, G5 and 6: Received the same treatment used in groups 3 and 4 in concomitant with daily intra-gastric administration of GB (150 mg kg^{-1} b.wt.)

Table 3: Effects of CIS and/or GB on RBCs, WBCs, Hb, PCV and platelets in male albino rats

	Groups					
Parameters	G1	G2	G3	G4	G5	G6
RBCs×10^{6} C (mm)	10.47±1.64^{a}	11.12±0.41^{a}	5.43±1.43^{c}	2.66±0.02^{d}	7.43±0.30^{bc}	6.87±0.19^{bc}
WBCs×10^{3} C (mm)	12.63±1.97^{a}	11.52±1.04^{ab}	1.10±0.31^{d}	0.80±0.06^{d}	9.45±0.70^{b}	5.15±0.32^{c}
Hb (g dL^{-1})	17.63±3.11^{a}	16.85±0.66^{a}	10.73±2.00^{c}	5.30±0.06^{d}	12.89±0.61^{bc}	12.80±0.72^{bc}
PCV (%)	37.67±6.67^{a}	35.75±1.30^{a}	22.37±5.05^{b}	9.97±0.03^{c}	29.88±2.58^{ab}	28.25±1.49^{ab}
Platelets×10^{3} C (mm)	382.67±24.59^{a}	379.00±22.58^{a}	213.50±13.98^{b}	26.00±1.73^{d}	232.00±32.15^{b}	128.67±9.28^{c}

Means bearing different letters within the same row are significant at $p \leq 0.05$, RBCs: Red blood cells, WBCs: White blood cells, Hb: Hemoglobin, PCV: Packed cell volume

Table 4: Effects of CIS and/or GB on Blood Urea Nitrogen (BUN) and serum creatinine in male albino rats

	Groups					
Parameters	G1	G2	G3	G4	G5	G6
BUN (mg dL^{-1})	33.00±1.53^{d}	27.00±0.86^{d}	158.33±9.46^{b}	219.33±16.42^{a}	85.33±3.52^{c}	154.83±14.45^{b}
Creatinine (mg dL^{-1})	0.60±0.04^{d}	0.52±0.04^{d}	2.88±0.13^{b}	3.57±0.25^{a}	2.30±0.17^{c}	3.22±0.18^{ab}

Means bearing different letters within the same row are significant at $p \leq 0.05$

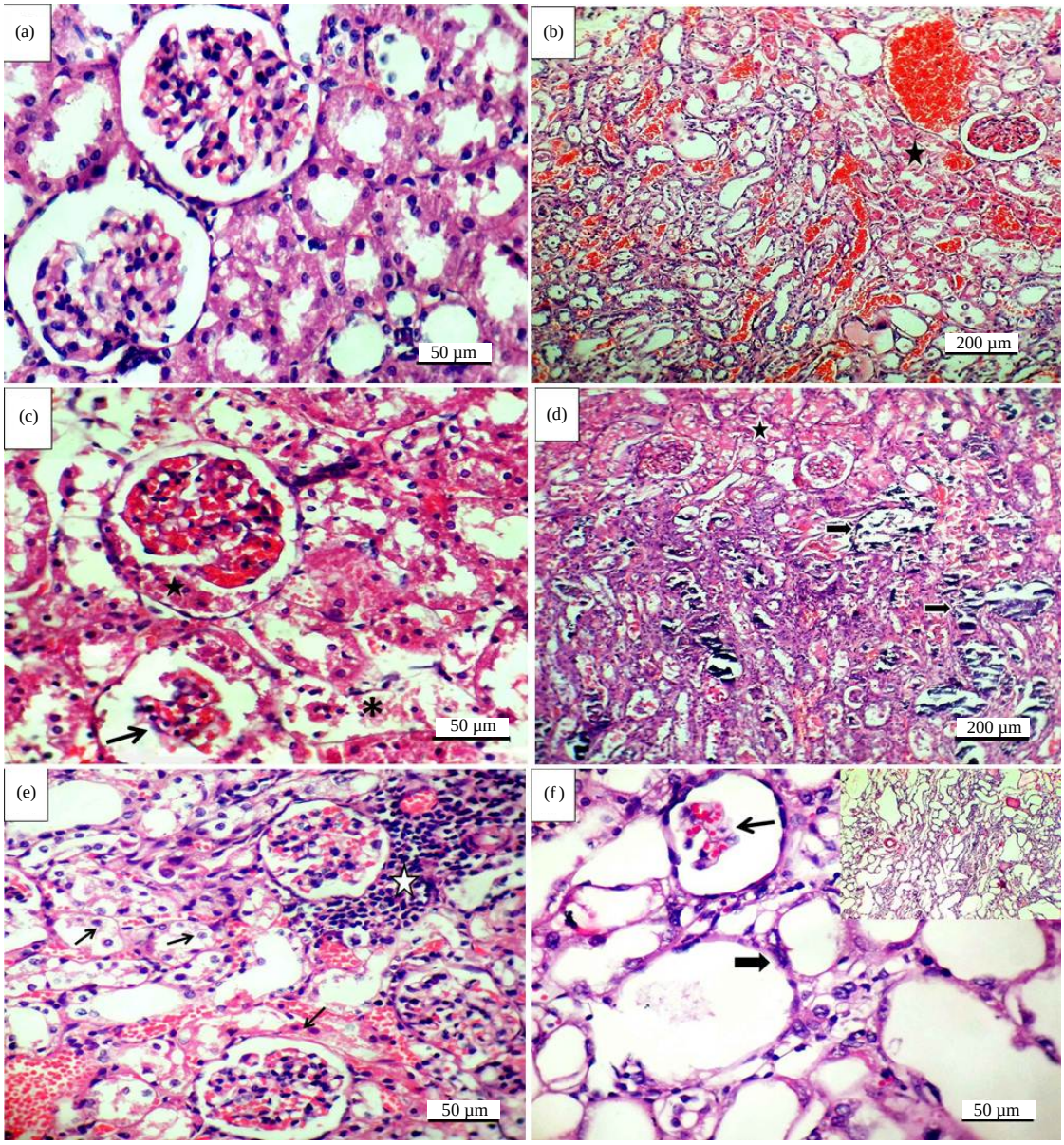

Fig. 1(a-f): Representative photomicrographs of rat kidney (HE) from (a) Control group showing normal renal architecture, (b, c) Rat received single injection of CIS showing, (b) Extensive glomerular and vascular congestion, tubular necrosis (star) and dilated proximal tubules, (c) Atrophied glomerulus (arrow), tubular necrosis (*) and cellular crescent in bowman's space (star) (d-f) Rat received 3 repeated injection cycles of CIS showing, (d) Massive tubular necrosis (star) and mineralization of tubular epithelium (arrows) (e) Interstitial mononuclear cells infiltration (star), tubular necrosis and degeneration (arrows) with glomerular and vascular congestion and (f) Marked dilatation of proximal tubules lined with attenuated or necrotic epithelium (thick arrow), atrophied glomeruli (thin arrow) and mild interstitial edema infiltrated with mononuclear inflammatory cells

Effects of CIS and/or GB on renal pathology and scoring system: The renal tissues from control and GB treated rats showing normal histological morphology (Fig. 1a, 2a). While, the renal tissues from rats intoxicated with single injection of CIS showed extensive glomerular and vascular congestion, vacuolar degeneration, proximal tubular epithelial necrosis and desquamation, intraluminal cast formation, atrophied glomerulus and epithelial cast formation in bowman's space (Fig. 1b, c). On the other hand, rats received single injection of CIS and co-treated with GB showing mildest degrees of previously mentioned lesions reflecting mild ameliorative effect of GB (Fig. 2b). Moreover, the kidney tissues from rats

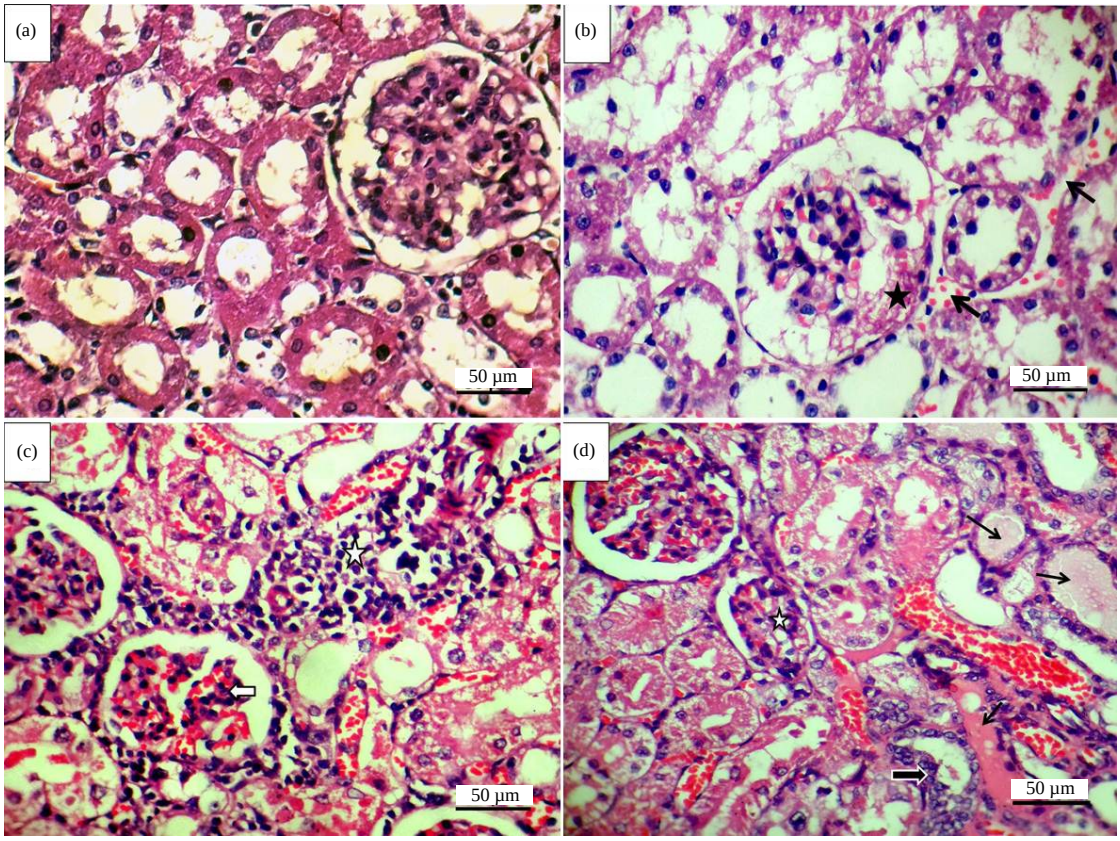

Fig. 2(a-d): Representative photomicrographs of rat kidney (HE) from (a) Rat received GB showing normal renal morphology, (b) Rat received single injection of CIS+GB showing mild tubular injury , cellular cast in bowman's space (star) and mild vascular congestion (arrows), (c and d) Rat received 3 repeated injection cycles of CIS+GB showing, (c) Degenerative and necrotic change of proximal tubules, vascular and glomeruli congestion (arrow), interstitial and perivascular mononuclear cells infiltration (star) and (d) Tubular injury and intraluminal cast formation (thin arrows), glomerular and vascular congestion, atrophied glomerulus (star) and regenerated epithelium (thick arrow)

Table 5: Effects of CIS and/or GB on renal MDA and GSH in male albino rats

	Groups					
Parameters	G1	G2	G3	G4	G5	G6
MDA (nmol g^{-1} tissue)	55.07±2.33^{e}	52.11±1.33^{e}	83.99±1.62^{c}	99.03±1.30^{a}	69.84±0.76^{d}	93.67±2.91^{b}
GSH (nmol g^{-1} tissue)	6.27±0.19^{a}	5.97±0.21^{a}	3.27±0.42^{b}	1.68±0.08^{c}	3.62±0.30^{b}	1.85±0.04^{c}

Means bearing different letters within the same row are significant at $p \leq 0.05$, MDA: Malondialdehyde, GSH: Glutathione

treated with CIS in three repeated injection cycles showed massive tubular necrosis and mineralization, intraluminal cast formation, marked dilatation of cortical tubules lined with necrotic epithelium, interstitial edema infiltrated with mononuclear cells (Fig. 1d-f). While, rats pre-treated with GB and intoxicated with CIS in three injection cycles showed similar pattern of lesions without any noticeable histological improvement associated with using of GB except for mild regeneration of tubular epithelium (Fig. 2c, d). Regarding the semi-quantitative scoring of renal damage, despite the addition of GB to groups 5 and 6 which received CIS in both administration manners, significant increase ($p \leq 0.05$) in kidney damage scores was still recorded in CIS treated and preventive rats compared to control rats. However, significant reduction in kidney damage score ($p \leq 0.01$) was recorded in rats of group 5 (received single injection of CIS and pretreated with GB) compared to rats of group 3 (intoxicated with single dose of CIS alone). Furthermore, significant decrease in renal damage scores ($p \leq 0.05$) was recorded in rats of group 6 (treated with three injection cycles of CIS in concomitant with GB) compared to rats from group 4 (intoxicated with three injection cycles of CIS alone) (Fig. 3).

Effects of CIS and/or GB on quantification of histo-morphometric analysis: By quantitative morphometric analysis, Glomerular Area (GA) and Glomerular Volume (GV) were significantly decreased in acute and chronic CIS intoxicated groups compared to negative and positive control groups. While, prior-treatment with GB improved the situation in rats intoxicated acutely with CIS resulted in non-significant difference in GA and GV compared to control groups. On the other hand, application of GB prior-treatment with chronic CIS administration failed to improve these parameters which show significant decrease compared to control groups (Fig. 4b, d). Regarding the diameter of renal tubules, significant increase was detected only in rats treated with repeated dose of CIS either with or without GB prior-treatment in comparison with control groups (Fig. 4a). Furthermore, the thickness of tubular epithelium showing significant decrease in CIS intoxicated and GB protective groups compared to control groups. While, the rats pretreated with GB before the acute and chronic CIS treatment exhibited significant increase in tubular epithelium thickness compared to rats received CIS alone (Fig. 4c).

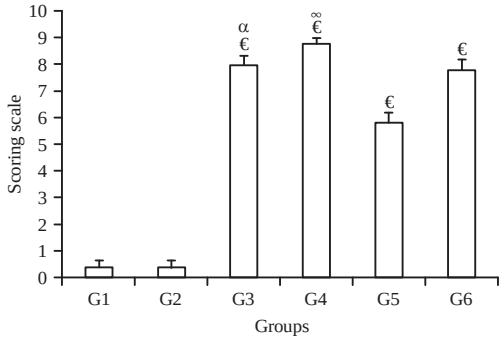

Fig. 3: Severity of kidney tissue damage. €: Significant at p<0.05 compared to control group, α: Significant p<0.01 compared to group 5 received single injection of CIS+GB, ∞: Significant at p<0.05 compared to group 6 received 3 repeated injection cycles of CIS+GB

Effects of CIS and/or GB on quantification of TUNEL positive cells: The number of TUNEL-positive cells was significantly increased in rats treated with CIS in both administration manners as compared to the control and GB treated groups.

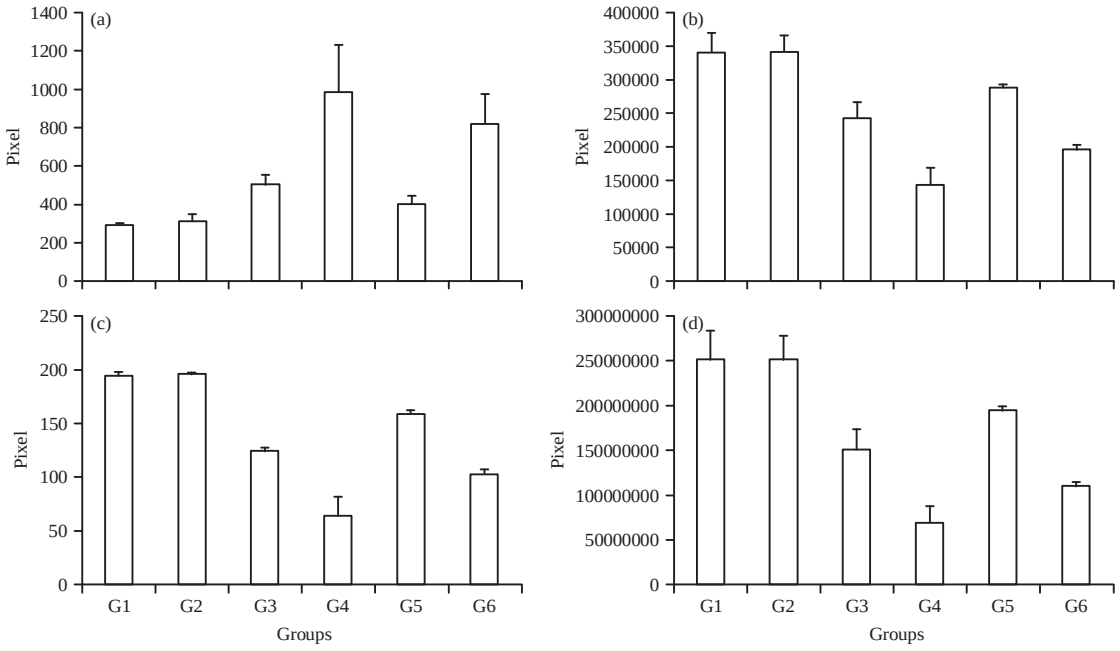

Fig. 4(a-d): Histo-morphometric analysis of renal pathology. Data are presented as the Means±SEM, (a) Tubular diameter estimated by the morphometry of line between the two points on the tubule perimeter, (b) Glomerular area estimated by calculates the glomerular perimeters, (c) Epithelial thickening estimated by calculate the difference between the line morphometry of renal tubules and the line morphometry of its inner lumen and (d) Glomerular volume calculated using spherical approximation formula (GV = 1.2545 (GA) 1.5). G1: Control positive, G2: Control negative, G3: Rats received single CIS injection, G4: Rats received repeated injection cycles of CIS, G5: Rats received single CIS injection+GB, G6: Rats received repeated injection cycles of CIS+GB

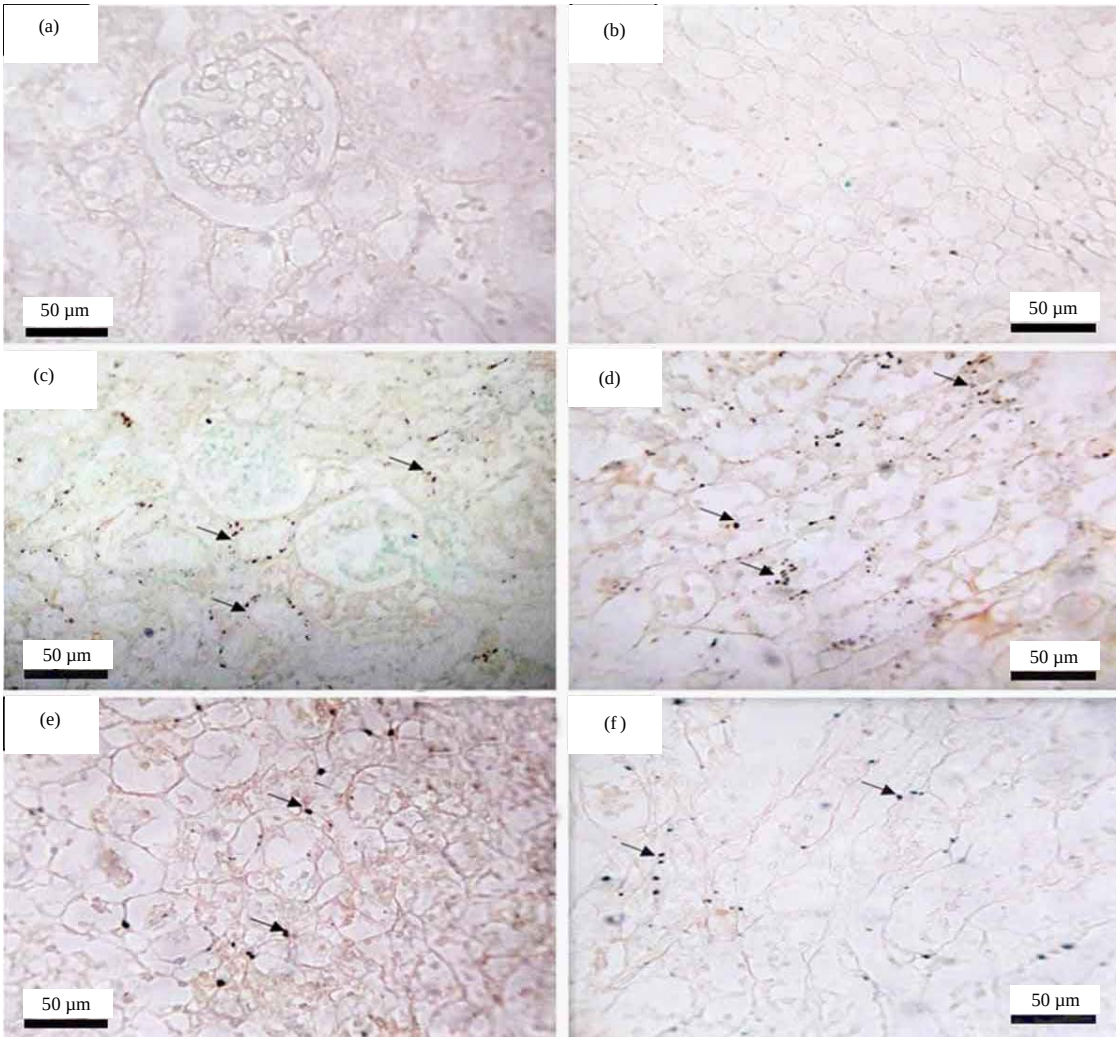

Fig. 5(a-f): Representative photomicrograph of TUNEL assay, TUNEL-positive cells is denoted by arrows, (a) Proximal convoluted tubules (PCT) of control rats, (b) Distal convoluted tubules (DCT) of GB treated rats, (c) PCT from rats treated with repeated injection of CIS, (d) DCT of rats received single dose of CIS, (e) DCT from rats treated with repeated injection of CIS+GB and (f) DCT from rats received single dose of CIS+GB

However, the renal tissue from rats exposed to single dose or three repeated injection cycles of CIS in concomitant with GB prior treatment showing significant reductions in the CIS-induced overexpression of TUNEL positive cells as compared to rats received CIS alone (Fig. 5, 6).

DISCUSSION

The clinical use of anti-neoplastic drug cisplatin (CIS) is limited by its severe nephrotoxic, anemia, leukopenia and thrombocytopenia effects[5,36]. About 20-30% of CIS treated patients suffered acute renal failure[37,38]. The pathophysiological mechanism underlying CIS nephrotoxicity is still unclear, it includes some complicated factors such as DNA damage, oxidative stress, inflammatory response, activation of apoptotic pathways and accumulation of CIS where it converts into nephrotoxins[39]. Therefore, cisplatin associated nephrotoxicity increases according to dose, frequency of administration and cumulative dose[40]. Hence, the present study was assigned to induce nephrotoxicity in two different model of rats using an equivalent cumulative dose of CIS and different frequency of administration (as a single injection or three repeated injection cycles), structural and functional alterations in experimental rats kidney was evaluated by hematologic, biochemical, pathologic and histo-morphometric analysis to investigate the potential

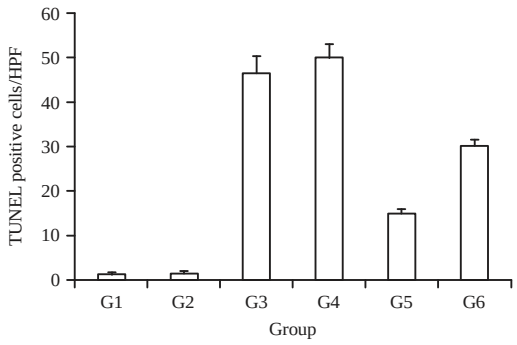

Fig. 6: Quantification of apoptotic cells in kidney sections, identified by TUNEL assay. Data are presented as the Means±SEM. Groups treated with CIS alone or with GB prior-treatment (G3, G4, G5 and G6) had significant increase ($p<0.05$) in TUNEL positive cells compared to control groups (G1 And G2). Groups treated with CIS+GB (G5 and G6) had significant decrease ($p<0.05$) in TUNEL positive cells versus groups treated with CIS alone (G3 and G4)

beneficial effects of *Ginkgo biloba* (GB) on cisplatin-induced nephrotoxicity. The GB is a natural herbal antioxidant; the ameliorative effect of GB contributed to its anti-inflammatory and antioxidant properties and its ability to scavenge free radicals, that could be attributed to its active components, namely, avonoglycoside and terpene lactones[22,25].

An important indicator in the toxicological investigations is organ weight[41] in this study, significant increase in kidneys weight was recorded in all CIS treated groups which may contributed to congestion of intertubular and glomerular blood capillaries and interstitial inflammatory cells infiltrations. This result was in agreement with other earlier investigators[42]. Anemia, leukopenia and thrombocytopenia are major and frequent complications for treatment with anti-neoplastic drugs[43]. Hence, different hematological parameters should be evaluated during the chemotherapy[44]. In this study, significant decrease in the counts of RBCs, WBCs and platelets, Hb concentration and PCV percent in rats received CIS were reported. These results were agreed with the previous investigator who concluded a pronounced depression in erythrocytes (anemia), leukocytes (leukopenia) and platelets (thrombocytopenia) in a dose dependent manners after cisplatin treatment in rats[2] and mice[3]. These findings may attributed to the suppressive effect of antineoplastic drugs on hematopoiesis of the host[44]. Additionally, the pretreatment with GB ameliorated the previously mentioned hematological disorders although it not identical to control rats, these results revealed a beneficial effect of GB on the hemopoietic system. These findings are in accordance with those reported by Aruna and Naidu[45], Abdel-Baieth[46] and Zhou *et al.*[47] confirming the beneficial effects of GB on the blood picture.

Moreover, several markers can be used in diagnosis of Acute Renal Failure (ARF), among these markers are Blood Urea Nitrogen (BUN) and creatinine[48]. In the current study, significant increase in serum BUN and creatinine levels was reported in CIS treated rats compared to control rats. While, rats treated with CIS and GB exhibited significant reduction in these parameters compared to CIS treated rats reflecting the ameliorative effect of GB, this finding was in harmony with the previous investigator[33,49,50] who concluded the renoprotective effect of GB against CIS-induced nephrotoxicity and with Sener *et al.*[24] who reported the ameliorative effect of GB against renal damage induced by ischemic reperfusion. Furthermore, lipid peroxidation (LPO) is an indicator of the oxidative stress, which plays a major role in the toxicity of many drugs including CIS. Most of the previous investigations concluded that CIS administrations resulted in severe oxidative stress and increased formation of free radicals[51,52]. The MDA is a known stable end product of LPO which used to measure the cumulative LPO indirectly. Therefore, MDA and GSH in renal tissues were evaluated as a marker of LPO and oxidative enzymes. Levels of MDA were significantly increased and level of GSH was significantly reduced in CIS treated rats compared to the control rats probably due to impairment of cellular oxidant defense system. However, renal MDA was significantly reduced in rats pretreated with GB and intoxicated with CIS compared with rats received CIS alone, this significant attenuation in MDA level probably because of ability of GB to scavenge oxygen free radicals in the renal tissues of rats. Similar results were previously reported by other earlier researcher[24,33,49,50].

In commitment with biochemical and oxidative analysis of serum and renal tissues, the histopathological examination of renal tissues from CIS treated rats revealed acute tubular necrosis indicate irreversible renal injury, marked dilatation of proximal convoluted tubules, necrosis and desquamation of tubular epithelium, intraluminal cast formation, severe glomerular atrophy and epithelial cast formation in bowman's space, interstitial edema and mononuclear cells infiltration. These changes may be explained by the ability of CIS to induced LPO, DNA damages, inflammatory responses and activation of apoptotic pathways. The obtained lesions run parallel with the previous investigation conducted by Okuyan *et al.*[33], Song *et al.*[50] and Pan *et al.*[53]. On the other hand, similar pattern of lesions was observed in rats received CIS after GB prior treatment but in low distribution manner. Semi-quantitative scoring confirmed the ameliorative role of

GB in reduction of severity of the CIS induced renal damage compared to CIS intoxicated groups albeit it was not identical to control tissues, these findings are in agreement with earlier investigations[24,33,50], who concluded the renoprotective role of GB against CIS. While, the quantitative reno-morphometry revealed significant increase in diameter of renal tubules (indicating severe dilatation of proximal tubules), which only reported in rats treated chronically with CIS alone or with GB prior-treatment. Moreover, significant decrease in glomerular area and volumes (indicating glomerular atrophy) was reported in CIS treated groups as well as in rats received repeated doses of CIS with GB prior-treatment, while non-significant atrophy was reported in rats treated with single injection of CIS after GB treatment which reflect the ability of GB to ameliorate the nephrotoxic effect of acute injection of CIS but not in repeated injection cycles. On the other hand, the morphometric analysis of tubular epithelial thickening showing significant decrease (due to necrosis and desquamation of tubular epithelium) in all CIS intoxicated and protective groups compared to control rats , while it showed significant difference between the CIS intoxicated and GB protective rats which may attributed to the renoprotective effect of GB on renal epithelium. Cisplatin is DNA damaging anti-neoplastic drug, it reacts with DNA of tumor cells forming different types of functional adducts resulting in the induction of apoptosis[54]. In our study, apoptosis was confirmed with TUNEL assay, TUNEL positive cells were significantly increased in CIS treated and preventive groups compared to positive and negative control groups, where as it is significantly decreased in kidney tissues from rats pre-treated with GB and intoxicated with CIS compared to those received CIS alone, which implied that the apoptotic cascade might play a key role in GB nephroprotective effect. This result was in consistent with other investigator[50,55,56] who reported significant increase in TUNEL positive cells after CIS treatment in rats.

CONCLUSION

This study provides evidence that CIS adversely induce anemia, leucocytopenia, thrombocytopenia, renal structure and function through increasing the oxidative damage and activation of apoptotic pathways. However, the severity of damage was more pronounced in repeated injection cycles of CIS than in case of single injection, which could explained by the cumulative effect of CIS and its conversion to toxic product. Moreover, the prior-treatment with GB attenuated the damaging effect of CIS-treated rats-albeit it not identical to control rats.

SIGNIFICANCE STATEMENTS

- Cisplatin (Cis) cause nephrotoxicity, acute depression in erythrocytes, leukocytes and platelets
- *Ginkgo biloba* (GB) is a common traditional popular medicine
- Intoxication with cisplatin increased relative kidney weight, serum creatinine and blood urea nitrogen, renal malondialdehyde (MDA), quantitative and semi-quantitative grading of renal pathology and number of TUNEL positive cells/HPF
- Intoxication with cisplatin decreased renal glutathione (GSH), Hb concentration, RBCs, WBCs and platelets count
- Prior-treatment with GB partially attenuated the CIS induced anemia, leukocytopenia, thrombocytopenia and nephrotoxicity through its antioxidant and anti-apoptotic properties

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REFERENCES

1. Delord, J.P., C. Puozzo, F. Lefresne and R. Bugat, 2009. Combination chemotherapy of vinorelbine and cisplatin: A phase I pharmacokinetic study in patients with metastatic solid tumors. Anticancer Res., 29: 553-560.
2. Ohno, S., F.R. Strebel, L.C. Stephens, Z.H. Siddik and H. Baba *et al.*, 1993. Haematological toxicity of carboplatin and cisplatin combined with whole body hyperthermia in rats. Br. J. Cancer, 68: 469-474.
3. Khynriam, D. and S.B. Prasad, 2001. Hematotoxicity and blood glutathione levels after cisplatin treatment of tumor-bearing mice. Cell Biol. Toxicol., 17: 357-370.
4. Ali, B.H. and M.S. Al Moundhri, 2006. Agents ameliorating or augmenting the nephrotoxicity of cisplatin and other platinum compounds: A review of some recent research. Food Chem. Toxicol., 44: 1173-1183.
5. Pabla, N. and Z. Dong, 2008. Cisplatin nephrotoxicity: Mechanisms and renoprotective strategies. Kidney Int., 73: 994-1007.
6. Rodrigues, M.A.C., N.A.G. dos Santos, M.C.D.S. Faria, J.L. Rodrigues and A. Kinoshita *et al.*, 2016. Carvedilol protects the kidneys of tumor-bearing mice without impairing the biodistribution or the genotoxicity of cisplatin. Chem. Biol. Interact., 245: 59-65.

7. Ramesh, G. and W.B. Reeves, 2005. p38 MAP kinase inhibition ameliorates cisplatin nephrotoxicity in mice. Am. J. Physiol. Renal Physiol., 289: F166-F174.
8. Yousef, M.I. and H.M. Hussien, 2015. 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: 17-25.
9. Schrier, R.W., W. Wang, B. Poole and A. Mitra, 2004. Acute renal failure: Definitions, diagnosis, pathogenesis and therapy. J. Clin. Invest., 114: 5-14.
10. Behling, E.B., M.C. Sendao, H.D. Francescato, L.M. Antunes, R.S. Costa and L. Bianchi-Mde, 2006. Comparative study of multiple dosage of quercetin against cisplatin-induced nephrotoxicity and oxidative stress in rat kidneys. Pharmacol. Rep., 58: 526-532.
11. Yano, T., Y. Itoh, M. Matsuo, T. Kawashiri, N. Egashira and R. Oishi, 2007. Involvement of both tumor necrosis factor-α-induced necrosis and p53-mediated caspase-dependent apoptosis in nephrotoxicity of cisplatin. Apoptosis, 12: 1901-1909.
12. Do Amaral, C.L., H.D.C. Francescato, T.M. Coimbra, R.S. Costa, J.D.A.C. Darin, L.M.G. Antunes and M.D.L.P. Bianchi, 2008. Resveratrol attenuates cisplatin-induced nephrotoxicity in rats. Arch. Toxicol., 82: 363-370.
13. Kang, K.P., D.H. Kim, Y.J. Jung, A.S. Lee and S. Lee *et al*., 2009. Alpha-lipoic acid attenuates cisplatin-induced acute kidney injury in mice by suppressing renal inflammation. Nephrol. Dial. Transplant., 24: 3012-3020.
14. Khan, S.A., S. Priyamvada, W. Khan, S. Khan, N. Farooq and A.N.K. Yusufi, 2009. Studies on the protective effect of green tea against cisplatin induced nephrotoxicity. Pharmacol. Res., 60: 382-391.
15. Kim, E.S., J.S. Lee, M. Akram, K.A. Kim, Y.J. Shin, J.H. Yu and O.N. Bae, 2015. Protective activity of *Dendropanax morbifera* against cisplatin-induced acute kidney injury. Kidney Blood Pressure Res., 40: 1-12.
16. Lee, S., K. Jung, D. Lee, S.R. Lee, K.R. Lee, K.S. Kang and K.H. Kim, 2015. Protective effect and mechanism of action of lupane triterpenes from *Cornus walteri* in cisplatin-induced nephrotoxicity. Bioorgan. Med. Chem. Lett., 25: 5613-5618.
17. Park, J.Y., D. Lee, H.J. Jang, D.S. Jang and H.C. Kwon *et al*., 2015. Protective effect of *Artemisia asiatica* extract and its active compound eupatilin against cisplatin-induced renal damage. Evid. Based Complement. Altern. Med., Vol. 2015. 10.1155/2015/483980.
18. Dugbartey, G.J., H.R. Bouma, I. Lobb and A. Sener, 2016. Hydrogen sulfide: A novel nephroprotectant against cisplatin-induced renal toxicity. Nitric Oxide, 57: 15-20.
19. Karwasra, R., P. Kalra, Y.K. Gupta, D. Saini, A. Kumar and S. Singh, 2016. Antioxidant and anti-inflammatory potential of pomegranate rind extract to ameliorate cisplatin-induced acute kidney injury. Food Funct., 7: 3091-3101.
20. Kwon, Y.S., H.S. Ann, T. Nabeshima, E.J. Shin and W.K. Kim *et al*., 2004. Selegiline potentiates the effects of EGb 761 in response to ischemic brain injury. Neurochem. Int., 45: 157-170.
21. Tan, M.S., J.T. Yu, C.C. Tan, H.F. Wang and X.F. Meng *et al*., 2015. Efficacy and adverse effects of *Ginkgo biloba* for cognitive impairment and dementia: A systematic review and meta-analysis. J. Alzheimer's Dis., 43: 589-603.
22. Naik, S.R., V.W. Pilgaonkar and V.S. Panda, 2006. Neuropharmacological evaluation of *Ginkgo biloba* phytosomes in rodents. Phytother. Res., 20: 901-905.
23. Oken, B.S., D.M. Storzbach and J.A. Kaye, 1998. The efficacy of ginkgo biloba on cognitive function in alzheimer disease. Arch. Neurol., 55: 1409-1415.
24. Sener, G., E. Sener, O. Sehirli, A.V. Ogunc, S. Cetinel, N. Gedik and A. Sakarcan, 2005. *Ginkgo biloba* extract ameliorates ischemia reperfusion-induced renal injury in rats. Pharmacol. Res., 52: 216-222.
25. Naik, S.R. and V.S. Panda, 2007. Antioxidant and hepatoprotective effects of *Ginkgo biloba* phytosomes in carbon tetrachloride-induced liver injury in rodents. Liver Int., 27: 393-399.
26. Gong, Q.H., Q. Wu, X.N. Huang, A.S. Sun, J. Nie and J.S. Shi, 2006. Protective effect of *Ginkgo biloba* leaf extract on learning and memory deficit induced by aluminum in model rats. Chin. J. Integrative Med., 12: 37-41.
27. De Freitas, M.R., A.A. Figueiredo, G.A. de Castro Brito, R.F. de Carvalho Leitao, J.V. de Carvalho Junior, R.M. Gomes Junior and R. de Albuquerque Ribeiro, 2009. The role of apoptosis in cisplatin-induced ototoxicity in rats. Braz. J. Otorhinolaryngol., 75: 745-752.
28. Schalm, O.W., N.C. Jain and E.J. Carrot, 1975. Veterinary Haematology. 3rd Edn., Lea and Febiger, Philadelphia, pp: 498-512.
29. Matousek, J., 1969. Effects on spermatogenesis in guinea-pigs, rabbits and sheep after their immunization with sexual organ fluids of bulls. J. Reprod. Fertil., 19: 63-72.
30. Placer, Z.A., L.L. Cushman and B.C. Johnson, 1966. Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. Anal. Biochem., 16: 359-364.
31. Sedlak, J. and R.H. Lindsay, 1968. Estimation of total, protein-bound and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal. Biochem., 25: 192-205.
32. Culling, C.F., 1983. Handbook of Histological and Histochemical Techniques. 3rd Edn., Butterworth, London, Boston.

33. Okuyan, B., F.V. Izzettin, O. Bingol-Ozakpinar, P. Turan and Z.N. Ozdemir *et al*., 2012. The effects of *Ginkgo biloba* on nephrotoxicity induced by cisplatin-based chemotherapy protocols in rats. IUFS J. Biol., 71: 103-111.
34. Gavrieli, Y., Y. Sherman and S.A. Ben-Sasson, 1992. Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J. Cell Biol., 119: 493-501.
35. SAS., 2004. JMP SAS Institute Statistical Analysis System: Users Guide. SAS Institute Inc., Cary, NC., USA., Pages: 1290.
36. Mansour, M.A., A.M. Mostafa, M.N. Nagi, M.M. Khattab and O.A. Al-Shabanah, 2002. Protective effect of aminoguanidine against nephrotoxicity induced by cisplatin in normal rats. Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol., 132: 123-128.
37. Hanigan, M.H. and P. Devarajan, 2003. Cisplatin nephrotoxicity: Molecular mechanisms. Cancer Ther., 1: 47-61.
38. Miller, R.P., R.K. Tadagavadi, G. Ramesh and W.B. Reeves, 2010. Mechanisms of cisplatin nephrotoxicity. Toxins, 2: 2490-2518.
39. Peres, L.A.B. and A.D.D. Cunha Jr, 2013. Acute nephrotoxicity of cisplatin: Molecular mechanisms. Journal Brasileiro de Nefrologia, 35: 332-340.
40. Madias, N.E. and J.T. Harrington, 1978. Platinum nephrotoxicity. Am. J. Med., 65: 307-314.
41. Yavasoglu, A., M.A. Karaaslan, Y. Uyanikgil, F. Sayim, U. Ates and N.U.K. Yavasoglu, 2008. Toxic effects of anatoxin-a on testes and sperm counts of male mice. Exp. Toxicol. Pathol., 60: 391-396.
42. Fouad, A.A., A.I. Al-Sultan, S.M. Refaie and M.T. Yacoubi, 2010. Coenzyme Q10 treatment ameliorates acute cisplatin nephrotoxicity in mice. Toxicology, 274: 49-56.
43. Hoagland, H.C., 1982. Hematologic complications of cancer chemotherapy. Semin. Oncol., 9: 95-102.
44. Doll, D.C. and R.B. Weiss, 1983. Chemotherapeutic agents and the erythron. Cancer Treat. Rev., 10: 185-200.
45. Aruna, D. and M.U.R. Naidu, 2007. Pharmacodynamic interaction studies of *Ginkgo biloba* with cilostazol and clopidogrel in healthy human subjects. Br. J. Clin. Pharmacol., 63: 333-338.
46. Abdel Baieth, H.A., 2009. Evaluation of *Ginkgo biloba* extract on hematological changes affected with hazards of electromagnetic field exposure. Int. J. Biomed. Sci., 5: 229-236.
47. Zhou, H., C. Wang, J. Ye, H. Chen and R. Tao, 2015. Effects of dietary supplementation of fermented *Ginkgo biloba* L. residues on growth performance, nutrient digestibility, serum biochemical parameters and immune function in weaned piglets. Anim. Sci. J., 86: 790-799.
48. Thadhani, R., M. Pascual and J.V. Bonventre, 1996. Acute renal failure. N. Engl. J. Med., 334: 1448-1460.
49. Gulec, M., M. Iraz, H.R. Yilmaz, H. Ozyurt and I. Temel, 2006. The effects of ginkgo biloba extract on tissue adenosine deaminase, xanthine oxidase, myeloperoxidase, malondialdehyde and nitric oxide in cisplatin-induced nephrotoxicity. Toxicol. Ind. Health, 22: 125-130.
50. Song, J., D. Liu, L. Feng, Z. Zhang, X. Jia and W. Xiao, 2013. Protective effect of standardized extract of *Ginkgo biloba* against cisplatin-induced nephrotoxicity. Evidence-Based Compl. Alternat. Med., Vol. 2013. 10.1155/2013/846126
51. Antunes, L.M.G., J.D.C. Darin and M.L.P. Bianchi, 2000. Protective effects of vitamin C against cisplatin-induced nephrotoxicity and lipid peroxidation in adult rats: A dose-dependent study. Pharmacol. Res., 41: 405-411.
52. Antunes, L.M.G., J.D.C. Darin and M.L.P. Bianchi, 2001. Effects of the antioxidants curcumin or selenium on cisplatin-induced nephrotoxicity and lipid peroxidation in rats. Pharmacol. Res., 43: 145-150.
53. Pan, H., K. Shen, X. Wang, H. Meng, C. Wang and B. Jin, 2014. Protective effect of metalloporphyrins against cisplatin-induced kidney injury in mice. PLoS One, Vol. 9. 10.1371/journal.pone.0086057
54. Muggia, F.M., 2004. Recent updates in the clinical use of platinum compounds for the treatment of gynecologic cancers. Seminars Oncol., 31: 17-24.
55. Taniguchi, T., N. Yuasa, M. Maeda and T. Horiuchi, 1982. Chronological observations on hemato-pathological changes in chicks inoculated with chicken anemia agent. Nat. Instit. Anim. Health Quart., 23: 1-12.
56. Qi, S.H. and D.C. Wu, 2013. Bone marrow-derived mesenchymal stem cells protect against cisplatin-induced acute kidney injury in rats by inhibiting cell apoptosis. Int. J. Mol. Med., 32: 1262-1272.