Renal and Cardiovascular Damage Induced by Cisplatin in Rats

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Abstract: The anticancer drug cisplatin can cause severe side effects, but to date, the mechanisms of action of these dangerous side effects have not been completely elucidated. The presence of these dangerous side effects prompted research to achieve a better understanding of the biochemical mechanisms underlying the toxicities. In the present study, we demonstrated renal and cardiovascular damage induced by cisplatin as indicated by the assessment of blood pressure, heart rate, biochemical assays and histophathological examination of kidney, heart and aorta in control and cisplatin treated rats. The biochemical assays included the measurements of serum creatinine, urea, high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides (TGs), total cholesterol (TC), albumin, total protein (TPs), nitric oxide (NO), lactate dehydrogenase (LDH), malonaldehyde (MDA), reduced glutathione (GSH) level and cardiac troponin-I (cTn-I) levels in the serum. Moreover, NO, MDA and GSH levels were assayed in kidney and heart tissue homogenates. The biochemical assay showed increased levels of creatinine, urea, HDL, LDL, TGs, TC, cTn-I, NO, LDH, MDA and decreased levels of albumin, TPs and GSH. Various histopathological lesions were observed in the kidneys, heart and aorta from cisplatin administered rats that increased in severity by the day 30. Immunohistochemical staining of vascular endothelial growth factor (VEGF), von Willbrand factor (vWF) and caspase were applied. Caspase-3 expression was detected in the control group on 1st and 4th weeks in the proximal renal tubules, meanwhile, VEGF expressions was increasingly expressed in the kidneys, heart and aorta from cisplatin group only. Meanwhile, vWF was variously expressed in the kidneys, heart and aorta from the control group. A weak expression of vWF was observed in kidneys, heart and aorta from the cisplatin group.
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1-Introduction

Cis-Diaminedichloroplatinum (II) (CDDP), commonly known as cisplatin, has been established as a potent chemotherapeutic agent administered to treat a variety of cancers such as ovarian, bladder, testicular and uterine cervix carcinomas (**Kuhlmann et al., 1997 and Weijl et al., 1997**). Previous studies and data indicated that cisplatin-induced ototoxicity, renal, peripheral sensory and autonomic nervous system toxicity besides potential microvascular damage **(Kohn et al., 2002; Kirchmair et al., 2005** and **Kohn et al., 1997**) in which the stria capillaries **(Kohn et al., 1997**), glomerular capillaries **(Kohn et al., 2002**) and the vasa nervorum **(Kirchmair et al., 2005)** were destructed. However, cisplatin-induced nephrotoxicity is a major complication in the cancer therapy and had a dose-limiting toxicity (**Kuhlmann et al., 1997)**. Patients with chronic nephrotoxicity have focal acute tubular necrosis characterized by cystic dilated tubules lined by a flattened epithelium showing atypical nuclei and atypical mitotic figures with hyaline casts **(Cornelison and Reed, 1993).** Whereas more and more histo-pathological examinations showed that cisplatin injury can cause endothelial cell dysfunction and leukocytes infiltration **(Kelly et al., 1999; Li et al., 2005; Ramesh and Reeves 2002)**. **Lieberthal et al., (1996)** showed morphological characteristics of apoptosis in cisplatin-treated mouse renal proximal tubule cells, but the caspases involved were not studied. Animal experiments and clinical reports have also demonstrated that the vascular effects of cisplatin could contribute to renal dysfunction (**Luke et al., 1992; Winston and Safirstein 1985; Icli et al., 1993)**. The mechanisms of the vascular-injury-associated side-effects of cisplatin might involve the proliferation inhibition on endothelial cells in vitro **(Kirchmair et al., 2005; Yoshikawa et al., 1997; Belotti et al., 1996)** and in vivo **(Kirchmair et al., 2005; Yoshikawa et al., 1997**). The present work was to investigate the deleterious effect of cisplatin on kidneys, heart and aorta in rats by evaluating blood pressure, some biochemical parameters in the serum and tissue homogenate, histopathological changes and immunohistochmical expression of VEGF, vWF, and caspase-3 in kidney, heart and aorta.

2. Materials and methods

2. 1. Animals

Twelve healthy male rats (initially weighing between 200 and 220 g body weight) were used in this

study. The animals purchased from animal house in Helwan, and housed in Department of Physiology, Faculty of Veterinary medicine, Mansoura University Animals were left for one week to acclimatize the place. Rats were kept in cages in a rate of six rats per cage in a controlled environment, maintained under a 12 hours light:dark cycle, 24oC (± 3oC) and 50-70% humidity. Rats were provided with standard diet and water ad-libitum. This experiment was approved by the Animal Care Committee of Mansoura University, Egypt.

2.2. Experimental procedure

For the experiment, rats were randomized into two groups (n = 6). The animals of control group were administered with a single dose of 0.9% saline intraperitoneally and three times of distilled water by oral gavage; animals in the cisplatin group were received a single dose of only cisplatin (4 mg/kg) intraperitoneally besides three times of distilled water by oral gavage.

2.3. Blood pressure and heart rate measurements

Blood pressure and heart rate were determined weekly, in the morning, in conscious, pre-warmed, restrained rats by tail-cuff plethysmography (digital pressure meter, LE 5000, Letica S.A., Barcelona, Spain). The measurements were kindly made in the Faculty of Medicine, Mansoura University. At least three determinations of blood pressure were made in every session and the mean of the three values was taken **(Krege et al., 1995)**.

2.4. Sample collection

Blood was drawn after 12 h fasting at one, two, and four weeks post cisplatin injection; blood samples were collected via retro-orbital bleeding for serum separation. Blood samples were left in plain test tube at room temperature for 1 hour and then centrifuged for 10 min at 3000 rpm to obtain the serum. Serum samples were stored at −80°C for further analysis. Then rats were sacrificed by decapitation. Right and left kidneys, aorta and heart were rapidly excised from all rats. Kidneys were cut into two halves. Heart was promptly sectioned into five slices: 1) basal (included the atria and the base of the ventricles), 2 to 4) middle (included the midportion of the ventricles) and 5) apical (included the ventricular apex). The halves from the right kidneys and slices of the heart were washed three times in ice cold saline and blotted individually on ash-free filter paper, used for preparation of tissue homogenates for estimation of tissue MDA, GSH and NO levels. The crude tissue homogenate was centrifuged at 10,000 rpm, for 15 minutes in cold centrifuge, and the resultant supernatant was used for the different estimations. The remaining halves from the left kidneys, aorta and heart slices were fixed in 10% neutral buffered formalin. Fixed specimens were processed for routine histopathological examination and immunohistochemical staining.

2.5. Biochemical blood analysis:

2.5.1. Urea and creatinine

Urea and creatinine were measured in the serum by a colorimetric method using commercial kit (Diamon, Egypt) according to the method described by **Tietz (1990).**

2.5.2. *Total protein and albumin :*

Total protein (TPs) and albumin were measured in the serum by UV- calorimetric spectrophotometric method using commercial kits (Vitro Scient, Egypt) according to the method of **Grant et al., (1987).**

2.5.3. *Lipid profile*

Test kits of (StanBio, USA) were used for the determination of plasma lipid profile including total cholesterol (TC), triglycerides (TGs), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) according to **Fossati and Principe (1982).**

2.5.4. *LDH*

Lactate dehydrogenase activity was measured by a kinetic method using commercial kit (Egyptian company for biotechnology) according to **Young (1990)**.

2.5.5. Reduced glutathione (GSH) and Malondialdehyde (MDA) in serum and tissue homogenate

MDA and GSH were measured by a colorimetric spectrophotometric method of **(Ohkawa et al., 1979 and Tietez, 1969, respectively)** using commercial kits (Bio-diagnostic, Egypt).

2.5.6. *NO level in serum and tissue homogenate*

NO was assayed in the serum by a colorimetric method using the diazotization procedure according to **Bartholomew (1984),** while in the heart and kidney homogenates NO was estimated according to **Montgomery and Dymock (1961).**

2.5.7. *Potassium, calcium and phosphorous*

Potassium, calcium and phosphorous were measured in serum by a colorimetric method using commercial kits (Vitro Scient, Egypt) according to the method of **Thomas (1998).**

2.5.8. *cTn-I measurement*

cTn-I was measured in the serum using BioAssay™ ELISA Kit according to the manufacturer procedures. The samples were kindly measured in the Faculty of Medicine, Mansoura University using immuolite 2000 device according to (**Adams et al., 1994 and Wu et al., 1996).**

2.6. Histopathological examinations

Paraffin sections of 5µm thickness were cut and picked up on uncoated slides, dried, deparafinized, rehydrated with graded alcohol, washed and stained with H&E according to **Bancroft et al., (1996).** Histological changes were examined by light

microscopy (binocular, Olympus) in a blinded fashion by one of the authors.

2.7. Immunohistochemical staining

Immunohistochemistry was conducted on paraffin embedded kidneys, heart and aorta of the first and third sacrifices from control and cisplatin rats using the following antibodies caspase-3, VEGF and vWF antibody (Santa Cruz, CA) (1:100). Paraffin sections were cut at 4-μm thick then deparaffinized with xylene, rehydrated in ascending grades of ethyl alcohol, and treated with 3% H2O2 for 10 min. Sections were blocked with 2% animal-free serum for 30 min at 37◦C. Following 1-hour incubation with primary antibodies overnight at 4°C, the sections were washed twice in PBS, and incubated with a peroxidase conjugated secondary IgG antibody for 30 minutes. Bound antibodies were detected using 3, 3'-diaminobenzidine tetrahydrochloride (DAB). The sections were counterstained with Mayer´s hematoxylin. All stained sections were visualized with a light microscopy (binocular, Olympus). Pictures were picked up using Digital camera (Canon 5 mega pixels, 3.2x optical zoom).

2.8. Statistical analysis

Data were analyzed by analysis of variance (ANOVA) using the general liner model procedure of SAS (SAS Institute, 2004). F-test (Fisher least significant difference test) was used to compare between groups and T-test (student T-test) was used to compare between parameters at 1, 2 weeks and 4 weeks of treatments. The mean values were significant at $P<0.05$.

3. Results

3.1. Blood pressure (mmHg) and heart rate (beat/min) in control and cisplatin groups:

Values of diastolic, systolic, mean pressure and heart rate were significantly higher in the cisplatin group compared to the control group and their values gradually increased from the first to the fourth week in the cisplatin group **(Table 1)**.

Table (1): Values of blood pressure (mmHg) and heart rate (beat/min) in control and cisplatin treated rats:

Group	1st Week	2nd Week	4th Week
The value of diastolic pressure (mmHg)			
Control	86.33 ± 0.88^{A}	86.66 ± 0.33^{A}	86.33 ± 0.66^{A}
Cisplatin	97.33 ± 1.45^{Ba}	106.33 ± 1.76^{Bb}	121.33 ± 1.85^{Bc}
The value of systolic pressure (mmHg)			
Control	127.33 ± 0.88^{Aa}	125.00 ± 0.57^{Ab}	123.33 ± 0.33^{Ab}
Cisplatin	134.33 ± 0.66^{Bc}	142.00 ± 0.57^{Bb}	151.00 ± 0.57^{Ab}
The value of mean pressure (mmHg)			
Control	100.00 ± 0.84^{A}	99.44 ± 0.10^{A}	98.66 ± 0.51^{A}
Cisplatin	109.67 ± 1.15^{Ba}	118.22 ± 0.99^{Bb}	131.22 ± 1.39^{Bc}
The value of heart rate (beat/min)			
Control	356.33 ± 3.48^{A}	363.66 ± 2.33^{A}	360.00 ± 2.64^{A}
Cisplatin	378.66 ± 2.73^{Ba}	388.00 ± 3.05^{Bb}	404.66 ± 0.88^{Bc}

- Values are means ±S.E
-Values with the different superscript capital letters in the same column are significantly different at ($P< 0.05$).
- Values with the different superscript small letters in the same raw are significantly different at ($P< 0.05$).

3.2. Biochemical blood analysis

The present findings revealed that, serum creatinine and urea levels were significantly increased in cisplatin group when compared with the control group. Their values gradually increased from the first to the fourth week in the cisplatin group **(Table 2)**. Serum TPs and albumin levels were significantly lower in cisplatin group (at $P<0.0059$ and $P<0.0008$, respectively) than in control group **(Table 2)**. TPs levels were nearly constant during the four weeks, meanwhile albumin levels slightly decreased from the first to the fourth week in the cisplatin group. Serum LDL, TGs, TC levels were significantly increased in cisplatin group when compared with the control group at ($P<0.0074$, $P<0.0014$ and $P<0.0053$, respectively) **(Table 2)**; where their values gradually increased from the first to the fourth week. Serum HDL, serum NO, kidney homogenate NO and heart homogenate NO levels were all significantly decreased in cisplatin group compared to control group at ($P<0.03$, $P<0.0009$, $P<0.0001$ and $P<0.0001$, respectively) **(Table 2&3)**. Their values gradually decreased from the first to the fourth week. Serum calcium and potassium levels were significantly ($P<0.004$ and $P<0.007$, respectively) decreased in cisplatin group compared to control group **(Table 4)**. Their values gradually decreased from the first to the fourth week. Meanwhile, levels of serum phosphorus were significantly ($P<0.002$) increased in cisplatin group compared to control group **(Table 4)**, since their values slightly increased from the first to the fourth week. Levels of serum LDH at $P<0.0011$, serum MDA, kidney homogenate' MDA and heart

homogenate' MDA (ANOVA, P<0.000) were significantly increased in cisplatin group when compared with the control group. On reverse, serum GSH, Kidney homogenate' GSH and heart homogenate' GSH levels were significantly decreased in cisplatin group compared to control group **(Table 5)** (P<0.0027, P<0.018 and P<0.002, respectively). Levels of cTn-I in the serum significantly increased in the cisplatin group compared to the control group and their values slightly increased from the first week to the fourth week (P< 0.0002) **(Table 6)**.

Table (2): Effect of cisplatin on the levels of serum creatinine, urea, TPs, albumin and lipid profile in rat

Group	1st Week	2nd Week	4th Week
The level of creatinine (mg/dl)			
Control	0.670 ± 0.37^{A}	0.62 ± 0.04^{A}	0.70 ± 0.01^{A}
Cisplatin	2.91 ± 0.12^{Ba}	3.78 ± 0.09^{Bb}	5.09 ± 0.08^{Bc}
The level of urea (mg/dl)			
Control	32.33 ± 2.33^{A}	27.66 ± 2.02^{A}	24.33 ± 3.84^{A}
Cisplatin	155.66 ± 3.17^{B}	169.33 ± 6.64^{B}	219.66 ± 10.6^{B}
The level of serum protein (mg/dl)			
Control	6.300 ± 0.11^{A}	6.43 ± 0.08^{A}	6.40 ± 0.05^{A}
Cisplatin	4.53 ± 0.03^{Ea}	4.30 ± 0.05^{Bb}	4.13 ± 0.06^{Bb}
The level of serum albumin (mg/dl)			
Control	3.94 ± 0.02^{A}	3.970 ± 0.09^{A}	3.97 ± 0.10^{A}
Cisplatin	2.40 ± 0.01^{Ba}	2.11 ± 0.06^{Bb}	1.94 ± 0.03^{Bc}
The level of HDL (mg/dl)			
Control	40.966 ± 1.10^{Aa}	36.36 ± 1.22^{Bab}	37.86 ± 0.93^{Bb}
Cisplatin	23.900 ± 0.90^{Ba}	22.10 ± 0.60^{Dab}	20.26 ± 0.61^{Eb}
The level of LDL (mg/dl)			
Control	80.36 ± 1.93^{A}	85.83 ± 2.11^{A}	84.93 ± 0.31^{A}
Cisplatin	158.100 ± 3.61^{Aa}	175.90 ± 4.09^{Bb}	180.66 ± 2.09^{Bb}
The level of TGs (mg/dl)			
Control	76.66 ± 1.20^{Aa}	82.33 ± 2.66^{Aab}	84.33 ± 2.18^{Ab}
Cisplatin	213.33 ± 2.02^{Ba}	225.00 ± 2.51^{Bb}	235.33 ± 2.18^{Bc}
The level of TC in rats (mg/dl)			
Control	136.66 ± 1.85^{A}	138.66 ± 3.84^{A}	139.66 ± 1.45^{A}
Cisplatin	224.66 ± 3.18^{Ba}	243.00 ± 4.04^{Bb}	248.00 ± 2.31^{Ba}

- Values are mean ±S.E
- Values with the different superscript capital letters in the same column are significantly different at (P< 0.05).
- Values with the different superscript small letters in the same raw are significantly different at (P< 0.05).

Table (3): Effect of cisplatin on the levels of NO in serum, kidney and heart homogenates of rats

Group	1st Week	2nd Week	4th Week
NO level in serum (µmol/L)			
Control	25.40 ± 0.36^{Aa}	25.10 ± 0.15^{Aa}	25.33 ± 0.68^{Aa}
Cisplatin	15.73 ± 0.54^{Baa}	13.00 ± 0.75^{Bb}	9.90 ± 0.20^{Bc}
NO level in kidney (µmol/g)			
Control	43.86 ± 0.14^{Aa}	43.46 ± 0.31^{Aa}	44.03 ± 0.14^{Aa}
Cisplatin	20.60 ± 0.34^{Ba}	17.20 ± 0.23^{Bb}	13.70 ± 0.26^{Bc}
NO level in heart (µmol/g)			
Control	44.66 ± 0.24^{Aa}	45.00 ± 0.51^{Aa}	5.80 ± 0.11^{Aa}
Cisplatin	31.63 ± 0.34^{Ba}	27.23 ± 1.46^{Eb}	22.46 ± 1.28^{Bc}

- Values are means ±S.E
-Values with the different superscript capital letters in the same column are significantly different at (P< 0.05).
- Values with the different superscript small letters in the same raw are significantly different at (P< 0.05).

Table (4): Effect of cisplatin on the levels of serum mineral ions in rats

Group	1st Week	2nd Week	4th Week
The level of serum calcium (mg/dl)			
Control	12.15 ± 0.01^{Aab}	12.50 ± 0.17^{Aa}	11.71 ± 0.23^{Ab}
Cisplatin	8.23 ± 0.10^{Ba}	7.19 ± 0.17^{Bb}	6.25 ± 0.17^{Bc}
The level of serum phosphorus (mg/dl)			
Control	2.54 ± 0.21^{A}	2.43 ± 0.13^{A}	2.60 ± 0.01^{A}
Cisplatin	3.77 ± 0.01^{Ba}	3.87 ± 0.05^{Ba}	4.08 ± 0.03^{Bb}
The level of serum potassium (mmol/L)			
Control	5.83 ± 0.07^{Aa}	5.97 ± 0.06^{Aa}	5.95 ± 0.04^{Aa}
Cisplatin	4.29 ± 0.02^{Ba}	4.15 ± 0.03^{Bb}	4.06 ± 0.03^{Bb}

- Values are means ±S.E

-Values with the different superscript capital letters in the same column are significantly different at ($P< 0.05$).

- Values with the different superscript small letters in the same raw are significantly different at ($P< 0.05$).

Table (5): Effect of cisplatin on the levels of LDH, MDA and GSH in serum and tissue homogenates in rats

Group	1st Week	2nd Week	4th Week
The level of LDH in serum (U/L)			
Control	62.33 ± 6.74^{Aa}	82.66 ± 3.93^{Ab}	70.66 ± 4.26^{Aab}
Cisplatin	396.33 ± 3.7^{Ba}	438.66 ± 11.2^{Bb}	470.66 ± 4.26^{Bc}
The level of MDA in serum (nmol/ml)			
Control	19.766 ± 0.38^{A}	20.50 ± 0.40^{A}	21.06 ± 0.08^{A}
Cisplatin	44.53 ± 0.40^{Ba}	50.10 ± 0.60^{Bb}	57.46 ± 1.26^{Bc}
The level of MDA in kidney homogenate (ng/ml)			
Control	18.30 ± 0.36^{A}	19.26 ± 0.43^{A}	19.266 ± 0.24^{A}
Cisplatin	40.03 ± 0.20^{Ba}	44.10 ± 0.20^{Bb}	51.100 ± 1.21^{Bc}
The level of MDA in heart homogenate (ng/ml)			
Control	29.73 ± 0.34^{A}	30.53 ± 0.52^{A}	29.333 ± 0.33^{A}
Cisplatin	47.76 ± 0.17^{Ba}	58.50 ± 0.36^{Bb}	65.10 ± 0.63^{Bc}
The level of GSH in serum (mmol/L)			
Control	1.05 ± 0.03^{Aa}	1.09 ± 0.05^{Aa}	1.07 ± 0.02^{Aa}
Cisplatin	0.30 ± 0.006^{Ba}	0.27 ± 0.01^{Ba}	0.20 ± 8.78^{Bb}
The level of GSH in kidney homogenate (mg/g)			
Control	2.033 ± 0.03^{Aa}	2.02 ± 0.06^{Aa}	1.97 ± 0.05^{Aa}
Cisplatin	0.90 ± 0.03^{Ba}	0.85 ± 0.03^{Ba}	0.72 ± 0.01^{Bb}
The level of GSH in heart homogenate (mg/g)			
Control	3.53 ± 0.03^{Aa}	3.48 ± 0.04^{Aa}	3.59 ± 0.03^{Aa}
Cisplatin	1.25 ± 0.03^{Ba}	1.106 ± 0.06^{Ba}	0.85 ± 0.02^{Bb}

- Values are mean ±S.E

-Values with the different superscript capital letters in the same column are significantly different at ($P< 0.05$).

- Values with the different superscript small letters in the same raw are significantly different at ($P< 0.05$).

Table (6): Effect of Cisplatin on the level of cTn- I in rats (ng/ml):

Group	1st Week	2nd Week	4th Week
Control	0.057 ± 0.003^{A}	0.047 ± 0.003^{A}	0.053 ± 0.003^{A}
Cisplatin	0.210 ± 0.006^{Ba}	0.233 ± 0.009^{Ba}	0.317 ± 0.009^{Bb}

- Values are mean ±S.E

-Values with the different superscript letters in the same column are significantly different at ($P< 0.05$).

- Superscript capital letter means significantly different between groups and superscript small letters means significantly different between times.

3.3. Histopathological evaluation

3.3.1. Kidneys

In the control group, normal histological picture was seen in rat kidneys **(Fig.1A)**. Meanwhile, in the cisplatin group after 1st week, the basement membranes of the affected tubules appeared to be irregularly arranged or injured. Glomerular damage (including atrophy, shrinkage, collapse and sclerosis), tubular damage (including tubular casts, epithelial cells degeneration, tubular epithelial cell detachment from the basement membrane and desquamation into tubular lumina, tubular dilation at the corticomedullary junction) were characteristic. Macrophages were seen inside lumen of the damaged renal tubules. Lipofuscin pigment was clearly visible inside the renal epithelium **(Fig.1B-D)**. Mild congestion, perivascular edema and interstitial edema could be seen. At 2nd week, epithelial cells degeneration increased, besides, marked congestion, perivascular edema, interstitial edema and hemorrhage **(Fig.1E)**. Mononuclears were detected accompanying perivascular edema. Additionally, fibrotic areas consisting of spindle-shaped fibroblastic cells and mononuclear cells began to be clearly developed around the affected tubules in the corticomedullary junction **(Fig. 1F)**. Some renal tubules were lined by epithelium with basophilic cytoplasm **(Fig.1G)**. The extent of the glomerular damage increased **(Fig.1H)**. Polymorphnuclears leukocytes were rarely seen. Dilated pelvis was observed in one animal. Arteriolar hyalinization was observed in the renal tissues where some renal arterioles had thickened wall and narrowed lumen. **(Fig.1I)**. After the 4th week, severe tubular necrosis and calcification were seen at the corticomedullary junction. Adjacent to the areas of necrosis, dilated renal tubules were lined by regenerating, flattened epithelial cells with basophilic cytoplasm **(Fig.2 A&B).** The fibrotic areas became more evident especially around the variously dilated tubular lumina in the corticomedullary junction indicating progressive fibrosis. Moreover, the extent of glomerular atrophy became severely increased by the day 30.

3.3.2. Heart

In cisplatin group on 1st and 2nd weeks, congestion **(Fig.2C)**, perivascular edema in the small intramuscual arterioles and focal muscular hyalinization were consistently described in the heart **(Fig.2D&E)**. Coronary arterioles and intramuscular arterioles showed increased medial muscle thickness, protruded arteriolar endothelium, irregular intimal layer, hyalinization with decreased the luminal area **(Fig.2F)**. Perivascular few inflammatory cells infiltration **(Fig.2G)** was detected on histological examination of the myocardium of the rats. On 4th week, congestion in intramuscular arterioles, edema and hemorrhage became obvious among muscle fibers. Furthermore, the cardiac myofibers in this group were markedly found to be in disarrayed pattern **(Fig.2H)**. Controversially, the heart of the control rats showed normal histological picture. Cardiomyocytes showed single, oval and centrally located nuclei with regularly arranged cardiac myofibres. Normal small intramyocardial arteries were seen with single-cell layer endothelium **(Fig. 2I)**.

3.3.3. Aorta

The tunica intima, media and adventitia of the control group rat specimens showed normal histology. In cisplatin group at 1st week, the histopathological examination of the aorta, there was moderate increase in the thickness tunica media layers **(Fig.3A&B)**. This was due to the proliferation of smooth muscle cells in the aortic wall. At 2nd and 4th weeks, marked increase in the thickening of the tunica media and narrowing in the aortal lumen were seen in cisplatin treated rat **(Fig.3C)**. In some animals, irregular luminal layer of the endothelial cell lining was demonstrated **(Fig.3D)**.

3.4. Immunohistochemical results

Table (7) summarized the results of immunohistochemical staining of caspase-3, VEGF and vWF.

3.4.1. Expression of Caspase in the Kidneys, Heart and Aorta.

In the control group, caspase-3 expression was detected from 1st week in the proximal renal tubules **(Fig.4A)**, while no expression was detected in the kidney from cisplatin group. In cisplatin group only, caspase-3 was stained in the heart under the endothelial lining the endocardium **(Fig. 4B)**. Positive caspase-3 expression was also noticed throughout the aorta and stained the endothelial lining **(Fig.4C)**. On 4th week, a dramatic expression was found in the heart and aorta from cisplatin group.

3.4.2. Expression of VEGF in the Kidneys, Heart and Aorta.

We examined the effect of cisplatin on the expression of VEGF which contributed to the vascular inflammation. We qualitatively measured the expression of VEGF by the immunohistochemistry in the kidneys, heart and aorta. VEGF was increasingly expressed in the kidneys, heart and aorta from cisplatin group by the 4th week. It was stained in large arteries, medium and small sized arterioles besides afferent and efferent arterioles of kidneys **(Fig.4D)**, in intramuscular arterioles and capillaries of the heart **(Fig.4E)** and intensely throughout the aorta **(Fig. 4F)**.

3.4.3. Expression of vWF in the Kidneys, Heart and Aorta.

vWF was variously expressed in the kidneys, heart and aorta from the control group. In the kidney strong expression was detected in large arteries, medium and small sized arterioles **(Fig. 4G)**. The fenestrated endothelium of the glomeruli was only focally positive or completely negative for vWF. All

endothelial cells lining the endocardium and coronary arteries in the heart and aorta were strongly positive **(Fig.4H&I)**. Meanwhile, weak positive staining was recorded in the capillary endothelial cells **(Fig.4H)**. Controversially, a weak expression of vWF was observed in the kidneys, heart and aorta from the cisplatin group.

Table (7): Summarized the results of immunohistochemistry

	Control group			Cis group		
	Caspase	VEGF	vWF	Caspase	VEGF	vWF
After 1st week						
kidney	+	-	+, ++	-	++	+
Heart	-	-	+, ++	+	++	+
Aorta	-	-	+, ++	+	++	+
After 4th week						
kidney	+	-	+, ++	-	+++	+
Heart	-	-	+, ++	++	+++	+
Aorta	-	-	+, ++	++	+++	+

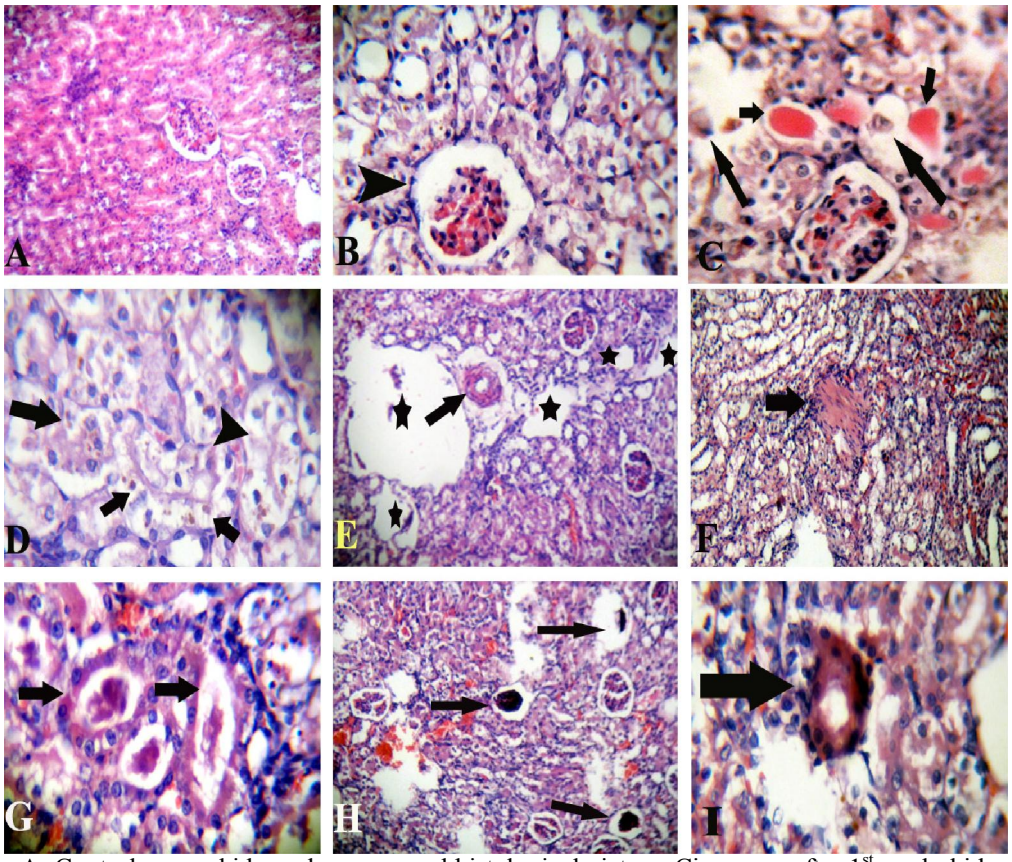

Fig. 1(A-I): A: Control group, kidney shows normal histological picture. Cis group, after 1st week, kidney shows **B:** glomerular shrinkage (arrowhead) and epithelial cells degeneration, tubular epithelial cell detachment from the basement membrane and mild tubular dilation**, C:** tubular hyaline casts (short arrows) and macrophages were seen inside lumen of the damaged renal tubules (long arrows)**, D:** epithelial vacuolation (arrowhead), desquamation into tubular lumina (long arrow) with lipofuscin pigment inside the renal epithelium (short arrows)**.** Cis group, after 2nd week, kidney shows **E:** marked perivascular edema (arrow) and interstitial edema (astrisks)**, F:** interstitial fibrotic area (arrow)**, G:** dilated renal tubules containing tubular casts are lined by epithelium with basophilic cytoplasm (arrows), **H:** several atrophied glomeruli (arrows) and **I:** arteriolar hyalinization with thickened wall and narrowed lumen (arrow) **(H&E, A. x 100, B. x 200, C. x 200, D. x 200, E. x 100, F. x100, G. x200, H. x100 and I. x 200).**

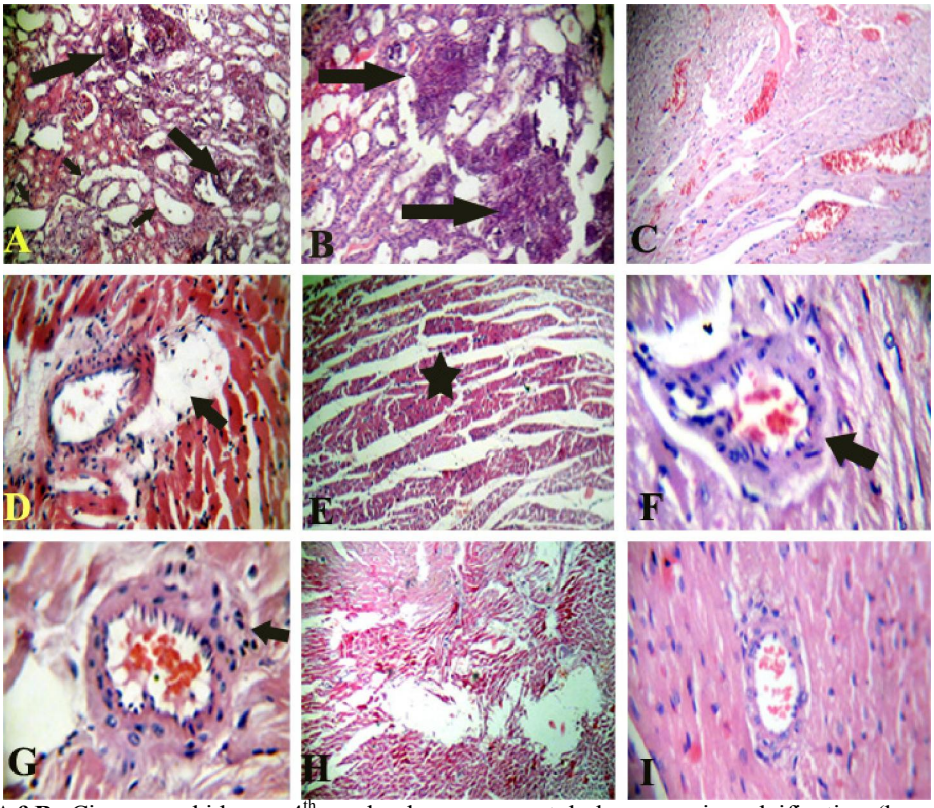

Fig. 2 (A-I): A&B: Cis group, kidneys, 4th week, shows severe tubular necrosis, calcification (long arrows) at the corticomedullary junction and dilated renal tubules lined by regenerating, flattened epithelial cells with basophilic cytoplasm (short arrows)**.** Cis group, heart 1st week, shows **C:** congestion in the small intramuscual arterioles, **D:** perivascular edema (arrow), **E**: focal muscular hyalinization (asterisk), **F.** coronary arteriole with narrowed lumen (arrow) and **G.** perivascular few inflammatory cells infiltration (arrow) with protruded endothelium. **H**. Cis group, heart 4thweek, cardiomyocytes are arranged in a disarrayed pattern. **I.** Control group, heart shows regularly arranged cardiomyocytes with single, oval and centrally located nuclei. Normal small intramyocardial artery is seen with single-cell layer endothelium **(H&E, A. x 100, B. x200, C. x 100, D. x 100, E. x 100, F. x 200, G. x 200, H. x 100 and I. x 200).**

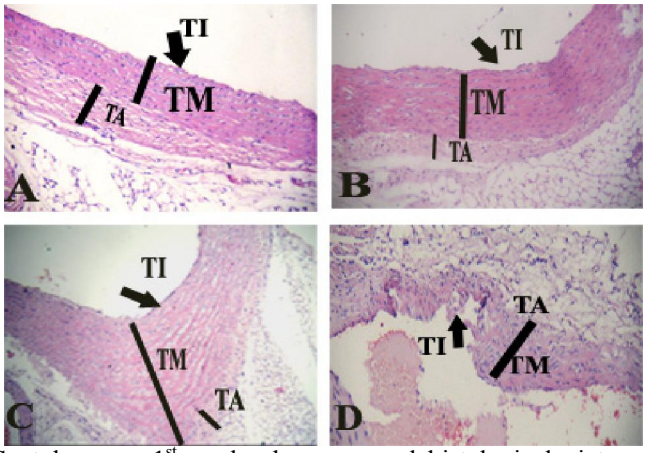

Fig. 3 (A-D): Aorta. **A:** Contol group, 1st week, shows normal histological picture consisting of tunica intima (arrow, TI), tunica media (TM), and tunica adventitia (TA). **B:** Cis group, 1st week, shows moderate increase in the thickness of TM compared to the control group due to the proliferation of smooth muscle cells. **C:** Cis group, 4th week shows marked increase in the thickening of the TM. **D.** Cis group, 4th week, reveals irregular luminal layer of the endothelial cell lining **(H&E, A. x 100, B. x100, C. x 100, D. x 100).**

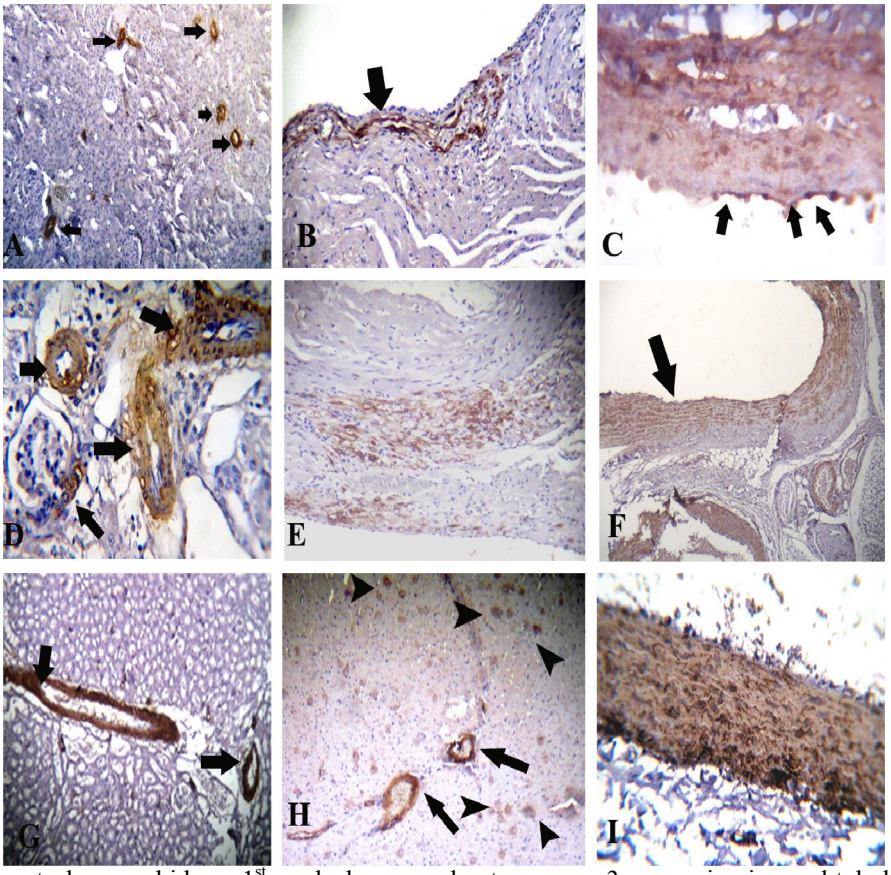

Fig. 4 (A-I): A. control group, kidney, 1st week shows moderate caspase-3 expression in renal tubules**. B.** Cis group, heart 1st week, shows moderate caspase-3 expression under the endothelial lining the endocardium**. C**. Cis group, aorta 4th week, shows caspase-3 expression throughout the aorta and stained the endothelial lining. **D**. Cis group, kidney 1st week, shows VEGF in arterioles besides afferent and efferent arterioles**. E.** Cis group, heart 1st week, shows positive staining for VEGF in capillaries**. F.** Cis group, aorta 1st week, shows intense positive staining for VEGF throughout the wall of the aorta. **G.** Control group, kidney 1st week, shows strong expression of vWF was detected in large and small sized arterioles. **H.** Control group, heart 1st week, shows strong positive staining of vWF in coronary arteries and weak positive staining in the capillaries. **I.** Control group, aorta 4th week, shows strong positive staining of vWF throughout the wall of the aorta **(IHC counterstained with Mayer´s hematoxylin, A. x 100, B. x100, C. x200, D. x 200, E. x100, F. x100, G. x100, H. x100 and I. x200).**

4. Discussion

Cisplatin is one of the potent anticancer drugs that is not only cause nephrotoxicity but also induces vascular endothelial dysfunction leading to cardiovascular diseases **(Demkow and Stelmaszczyk-Emmel, 2013; Santos et al., 2012; Sekijima et al., 2011)**. Induction of endothelial dysfunction mediated by inflammation and oxidative stress leading to imbalance in endothelial cells adhesion molecules and endothelial growth factors **(Duyndam et al., 2007;Nuver et al., 2010;Yu et al., 2008**). Therefore, cisplatin is considered an important model for cytoxicity studies **(Casares et al., 2012)**. The in vivo mechanisms of cisplatin nephrotoxicity are complex. These events cause tubular damage and tubular dysfunction with sodium, potassium, and magnesium wasting. In this study, serum calcium and potassium levels significantly decreased in the cisplatin group compared to the control group. Meanwhile, levels of serum phosphorus significantly increased. These data may be resulted from the decrease in glomerular filtration that was found in most treated patients with cisplatin as reported by **Yao et al., (2007**). High concentrations of cisplatin induced necrosis in proximal tubule cells, whereas lower concentrations induced apoptosis through a caspase-9–dependent pathway **(Lieberthal et al., 1996)**. Our microscopical findings revealed tubular damage in the kidneys of cisplatin exposed rats including tubular casts, epithelial cells degeneration, tubular epithelial cell detachment from the basement membrane and desquamation into tubular lumina besides tubular dilation at the corticomedullary junction. In animal models cisplatin damages the proximal tubules, specifically the S3

segment of the outer medullary stripe. **Cornelison and Reed (1993), Meyer and Madias (1994) and Vickers et al., (2004)** mentioned that, the site of injury in kidneys involves either (the distal tubules and collecting ducts or the proximal and distal tubules but the glomerulus has no obvious morphologic changes**.** However in this study, the glomerular damage (including atrophy, shrinkage, collapse and sclerosis) was clearly obvious in rat kidneys from cisplatin group. The sites affected probably depend on differences in dose and timing of biopsy specimens. Long-term cisplatin treatment and injury may cause cyst formation and interstitial fibrosis **(Cornelison and Reed 1993**). In our findings, fibrotic areas were demonstrated with mononuclear cells infiltration in the interstitial tissue from 2nd week. It was shown that cisplatin nephrotoxicity primarily causes tubulointerstitial lesions and the severity of necrosis is dose-, concentration-, and time dependent.

Immunohistochemically, caspase-3 expression was detected from the 1st week in the proximal renal tubules in the kidneys from the control group and in the heart from the cisplatin group under the endothelial lining the endocardium. Positive caspase-3 expression was also noticed throughout the aorta and stained the endothelial lining. An extensive positive expression was found in the heart on the 4th week. Similarly, **Lau (1999)** found that cisplatin treatment increased caspase-3 expression in the heart. The absence of caspase-3 expression from the kidneys of cisplatin group may be because the increase of necrosis instead of apoptosis

Cisplatin may inhibit proliferation of endothelial cells in vitro and that inhibition may also enhance the antitumour effects of the agent **(Kohn et al., 1997)**. Cisplatin is thought also to trigger a degenerative process of medium-thickness vessel walls, thus causing occlusive vascular disease in the long term **(Morlese et al., 2007)**. The increase in the hypertension is as an indicator for chemotherapy-induced vascular toxicity **(Daher and Yeh, 2008)**. In the same context, the increased systolic and diastolic blood pressure in our results indicating hypertension. In addition, our histopathological findings showed various lesions in the vascular elements of the heart and aorta including congestion, perivascular edema and hemorrhage in the small intramuscual arterioles, increased medial muscle thickness, protruded arteriolar endothelium, irregular intimal layer and hyalinization with decreased the luminal area in the coronary arterioles and intramuscular arterioles. The vascular endothelium has many functions and, accordingly, endothelial dysfunction is responsible for numerous health problems including atherosclerosis, high blood pressure, sepsis, thrombosis, vasculitis, and bleeding, among others. One of the most important functions of the endothelium is to secrete NO, a relatively unstable diatomic free radical, which can be synthesized by a broad range of organisms. This molecule has a role as a messenger in many biological processes including involvement in the regulation of neuron communication, antimicrobial activity, ventilation, hormone secretion, inflammation and immune responses as well as vascular tone **(Higashi and Chayama, 2002).** NO is a potent vasodilator and levels are often reduced when endothelial function is impaired, making it a vascular risk factor **(Luksha et al., 2012).** In this work, the assay of NO levels in serum, kidney and heart tissue homogenate were significantly lower in the cisplatin group compared to the normal group indicating the endothelial damage. Moreover, an elevation in the concentrations of serum creatinine, urea, TC, TGs, LDL, HDL and MDA and reduction of serum albumin, TPs, and GSH levels were recorded. Higher LDH and MDA and lower GSH in the serum indicated the elevated lipid peroxidation and exhausted antioxidant activities in cisplatin treated rats. Several studies suggested that renal injury following cisplatin treatment is correlated with oxidative damage (**Chang et al., 2002; Ueda et al., 2000**). The oxidative stress mainly results from formation of cisplatin–GSH conjugation (**Hanigan and Devarajan, 2003**).The conjugation contributes to GSH depletion and alteration of redox state in kidney, and consequently leads to an increase in generation of superoxide and other oxygen radicals (**Santos et al., 2007**). It has been also reported that cisplatin caused a decrease in the activities of antioxidant enzymes, such as superoxide dismutase, catalase, glutathione peroxidase in kidney tissues (**Antunes et al., 2000**). **Masuda et al., (1994**) mentioned that cisplatin was able to generate superoxide anion in cell-free system. In addition, the increased lipid profile may constitute hazardous effect on cardiovascular system.

vWF is a glycoprotein involved in blood coagulation, that is required for normal hemostasis. vWF is produced by endothelial cells and megakaryocytes throughout the body **(Sadler, 2013)**. The highest levels of vWf in blood plasma or tissue samples are indicative of damaged endothelium **(Teitel, 1991).** In this study, vWF was variously expressed in the kidneys, heart and aorta from the control group. In the kidney, large arteries, medium and small sized arterioles were strongly positive and the fenestrated endothelium of the glomeruli was only focally positive or completely negative for vWF. In the heart, all the endothelial cells lining the endocardium and coronary arteries in the heart and aorta were strongly positive. Meanwhile, weak positive staining was recorded in the capillary endothelial cells. A weak expression of vWF was observed in the kidneys, heart and aorta from the cisplatin group. These results were unexplainable and needed further research to be

understood. Although vWF is widely used as a specific endothelial cell marker, little is known about the mechanisms that regulate its expression *in vivo*. The distribution of vWF in vascular endothelium is heterogeneous and associated with regional variations in mRNA levels **(Page et al., 1991; Smith et al., 1996)**. vWF is expressed at higher in human tissues, in the endothelium of larger vessels and in the adult endocardium (**Page et al., 1991**). These differences in vWF distribution among vessels are maintained when endothelial cells are grown in culture **(Smith et al., 1996)**. In *en face* preparations of rat aorta, immunoreactivity to vWF antibody appears to vary from one endothelial cell to another (**Tomlinson et al., 1991**). Expression levels of vWF mRNA and protein differ significantly among the endothelial cells of different murine tissues and in the venous and arterial endothelial cells of the same tissue (**Yamamoto et al., 1998**). The distribution of vWF protein in the endothelium is regulated by such factors as blood flow and platelet number. In addition, thrombin generation may recruit non-expressing endothelial cells to produce vWF. These findings suggest that vWF synthesis is controlled at the transcriptional level and that the extracellular environment may determine cell-to-cell variations in expression levels (**Zanetta et al., 2000**).

Furthermore, the disarrayed pattern of the cardiac myofibers in cisplatin group was probably due to the degeneration of the structural protein in mitochondria of the cytoplasm that occurred in protein degradation. The levels of serum cTn-I significantly increased in the cisplatin group compared to the normal group. Cardiac troponin is the current standard method of serologic detection of myocardial injury in patients with acute coronary syndromes **(Wu and Ford, 1999)**. In addition, increases in cTn-I were associated with increased mortality in critically ill patients **(Mann, 1999)** and even slight increases in cTn-I (≥0.04 ng/ml) were associated with increased mortality rates in patients with advanced chronic heart failure **(Olivetti et al., 1997)**.

On conclusion, according to biochemical, histopathological and immunohistochemical evaluations, cisplatin has deleterious effects on kidneys, heart and aorta of rats.

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