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Original Article

Nephroprotective effect of electrolyzed reduced water against cisplatin-induced kidney toxicity and oxidative damage in mice

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Abstract

Background: Cisplatin is a potent chemotherapeutic drug for cancer therapy, but it has serious side effects in clinical treatment, particularly nephrotoxicity. The purpose of this study was to evaluate the protective effect of electrolyzed reduced water (ERW) on renal injury caused by cisplatin.

Methods: Animals were divided into four groups as follows: normal control group, cisplatin control group, ERW control group and ERW + cisplatin group. Each group comprised 10 animals, which were orally treated with normal saline or ERW daily companion by administration of one dose of cisplatin for 28 days. Animals in the cisplatin group received an intraperitoneal single-dose injection of cisplatin (20 mg/kg body weight) as a single i.p. dose on the 25th day of the experiment. We determined the hydration state in urine and the level of serum markers of kidney function, the levels of glutathione (GSH) and thiobarbituric acid-reactive substances (TBARS) levels and the activities of glutathione peroxidase (GPx), glutathione reductase (GR), catalase (CAT) and superoxidase dismutase (SOD) in kidney and histopathological changes.

Results: After administration of ERW, the reduced urinary osmolality was increased and elevated Na^+, K^+, Mg^{2+} and Ca^{2+} levels in urine were significantly decreased in cisplatin-induced renal injury mice. Besides, the results demonstrated that significantly decreased elevated serum levels of creatinine and blood urea nitrogen (BUN) and the levels of TBARS in the kidneys that were induced by cisplatin. Moreover, ERW treatment was also found to markedly increase ($p < 0.05$) the activities of GPx, GR, CAT and SOD, and to increase GSH content in the kidneys. Histopathology showed that ERW protects against cisplatin-induced renal injury to both the proximal and distal tubules.

Conclusion: ERW exhibits potent nephroprotective effects on cisplatin-induced kidney damage in mice, likely due to both the increase in antioxidant-defense system activity and the inhibition of lipid peroxidation.

Keywords: Cisplatin; Electrolyzed reduced water; Kidney; Nephrotoxicity; Oxidative stress

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

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1. Introduction

Cisplatin (*cis*-diamminedichloroplatinum II) is a potent chemotherapeutic drug used in the clinical treatment of several human cancers.[1] Unfortunately, treatment with cisplatin has several side effects, including neurotoxicity, ototoxicity and nephrotoxicity, resulting in dosage limiting in cancer therapy.[2,3] Reactive oxygen species (ROS), including hydroxyl radicals, superoxide anion and hydrogen peroxide (H_2O_2), are known to play critical roles in the pathology and progression of kidney disease,[4] and ROS have been shown to be associated with cisplatin toxicity.[5] In addition, cisplatin induces injury in the renal vasculature that eventually leads to DNA damage, renal antioxidant enzyme inactivity and finally to ischemic tubular cell death, which are strongly associated with the renal toxicity of this compound.[3] In this context, many studies demonstrated that an important mechanism of nephroprotection might be related to the antioxidants' potentiality to scavenge ROS.[4,5]

Electrolyzed reduced water (ERW) is produced by electrolysis of tap water and is characterized by higher dissolved hydrogen, lower dissolved oxygen, lower oxidation-reduction potential and higher pH than any form of tap water.[6] An increasing number of people are using ERW as drinking water in Asia. However, there are limited studies concerning the functional activity of ERW have been published. ERW with ROS scavenging ability might have the potential effect of protecting DNA, RNA and cells against oxidative stress.[7] Several studies have demonstrated that ERW has anticancer effects may be due to inhibition of angiogenesis in A549 cells[8] and suppression of metastasis in melanoma-injected mice.[9] Recently, we reported that ERW has antioxidant-like activity and scavenging activity of hydroxyl radicals, superoxide anion and H_2O_2 detected by chemiluminescence. Moreover, ERW with GSH showed a significantly promoted apoptosis-inducing effect on human promyelocytic leukemia cells and did not affect the normal functioning of the normal cell or the antitumor activity by chemotherapeutic drug.[6]

In various experimental models, ERW protected pancreatic β cells against the diabetogenic agent alloxan-induced damage[10] and increased the release of circulating insulin and ameliorated the sensitivity of insulin in diabetic mice.[11,12] One clinical report showed that hemodialysis with ERW supplementation efficiently reduced oxidative damage to leukocytes and endothelial cells in end-stage renal disease patients on chronic hemodialysis.[13] Recently, we demonstrated that ERW played a protective role in the reduction of oxidative damage and maintain the hepatic antioxidant enzymatic system, including CAT, SOD and GPx.[14] Although the antioxidant activity of ERW is well known, it is still unclear what the protective effect of ERW against cisplatin-induced nephrotoxicity.

In this study, we reported the nephroprotective effects of ERW against cisplatin-induced acute renal injury *in vivo*. The animals were orally treated with ERW daily companion by administration of one dose of cisplatin for 28 days. The urinary hydration state and the levels of creatinine and BUN in the serum, as well as the levels of GSH and TBARS levels and the activities of GPx, GR, CAT and SOD in kidney, were measured to observe renal damage. The degree of cisplatin-induced renal damage was examined through histopathological examination.

2. Methods

2.1. Chemicals

Cisplatin was purchased from Sigma–Aldrich (St. Louis, MO, USA). All of the other reagents and solvents were of analytical grade.

2.2. Apparatus for producing ERW

The ERW producing system used in this study was as described in detail elsewhere.[6] It consisted of a filtration system for the purification and an electrolyzer for the electrolysis of the water. The electrolysis system maintained the pH between 8.10 and 10.1, the values of ORP between −160 mV and −607 mV, and the water flow rate between 2.0 L/min and 3.4 L/min. The instrument was bound to a water tap. After turning on the equipment, the tap water was first cleansed and then electrolyzed to generate both ERW and electrolyzed oxidized water (EOW). In each experiment, the values of ORP and pH of ERW were maintained at 9.2 ± 0.2 and −360 ± 20 mV, respectively. The characteristics of tap water, distilled-deionized (DD) water, ERW and electrolyzed-oxidized water (EOW) are shown as Table 1. The ERW had been freshly prepared and then used in this study.

2.3. Experimental animals

Eight weeks old male ICR mice were obtained from BLT (BioLASCO Taiwan CO. Ltd.). Mice were quarantined under specific pathogen-free conditions and allowed to acclimate for seven days prior to experimentation. The animals were handled in a humidity- and temperature-controlled laboratory. Water and food were available *ad libitum*. Our Institutional Animal Care and Use Committee approved the procedures for the animal research, and the animals were cared for in accordance with the institutional ethical guidelines.

2.4. Treatment

To establish an optimal animal model of cisplatin-induced renal injury, a dose–response test and a time course experiment of cisplatin's effects on renal function were conducted. For the dose–response study, the mice were divided into four groups of six mice each and given were cisplatin (0, 5, 10 and 20 mg/kg body weight, respectively) intraperitoneally (i.p.). Kidney-functional parameters, serum levels of creatinine and BUN, were evaluated 72 h later. For the time course study, the animals were divided into five groups (0 h, 24 h, 48 h, 72 h and 120 h,

Table 1
Characteristics of ERW, EOW, DD water and tap water.

	pH	ORP (mV)	Cation (mg/L)					Anion (mg/L)			
			Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	NO_3^-	SO_4^{2-}	HCO_3^-
ERW	9.21	−368	7.81	ND	2.32	12.2	17.8	3.52	3.98	64.7	67.2
EOW	5.32	692	4.28	ND	1.11	7.65	10.1	10.5	12.9	147	156
DD water	7.35	402	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tap water	7.53	583	6.32	ND[a]	1.71	11.2	14.4	6.80	7.60	99.4	128

The above properties were determined in this study. The values for ERW and ROW, obtained from an electrolytic water producer made in Taiwan, varied as the electric field strength varied.

[a] ND = no detect.

respectively) of six mice each and were given cisplatin at a dose of 20 mg/kg of body weight. For the ERW protection experiment, the animals were randomly divided into four groups, with each consisting of ten mice. Group I served as the normal control and was orally administered distilled water daily for 28 consecutive days with i.p. administered normal saline (1 mL/kg of body weight) on the 25th day of the experiment. Group II served as the cisplatin control and received distilled water daily for 28 consecutive days in addition to cisplatin (20 mg/kg of body weight) dissolved in normal saline (pH 7.4) as a single i.p. dose on the 25th day of the experiment. Group III served as the ERW control and were received ERW daily for 28 consecutive days with i.p. administered normal saline (1 mL/kg body weight) on the 25th day of the experiment. Group IV received daily ERW for 28 consecutive days in addition to cisplatin (20 mg/kg body weight) as a single i.p. dose on the 25th day of the experiment. Before the start of the experiment, and on the 27th day of the experiment, the mice were individually housed in metabolic cages enabling 24-h urine collections. On the 28th day of the experiment, animals in the ERW protection experiment were sacrificed using CO_2. Blood was collected into heparinized tubes (50 U/mL). Kidney samples were dissected and cleaned immediately with ice-cold saline, and then the left kidney samples were immediately stored at −70 °C until the analysis. The right kidney samples were removed and fixed in a 10% neutral buffered formalin solution for the histopathological examination.

2.5. Measurement of the hydration state in urine, and kidney damage in serum

To determine the hydration state in urine, the levels of osmolality, Na^+, K^+, Mg^{2+} and Ca^{2+} were performed by Union Clinical Laboratory (Taichung, Taiwan) using Automated Urine Analyzer (AE-4020). Kidney damage was assessed by the estimation of serum levels of creatinine and BUN using commercially available test kits from Randox Laboratories Ltd. (Antrim, United Kingdom).

2.6. Measurement of GPx, GR, CAT, SOD and GSH in kidneys

The homogenisation procedure was performed under standardised conditions and the supernatant of organ homogenate was used for the GPx, GR, CAT, SOD and GSH assays. All enzyme activities and GSH count were analyzed as stated by the Randox Laboratories Ltd. kit instructions.

2.7. Measurement of lipid peroxidation

Lipid peroxidation was quantified by TBARS assay that is based on the reaction with thiobarbituric acid (TBA), as described by Tsai et al.[14] The homogenates, 10% trichloroacetic acid (TCA) and 0.67% TBA reagent were mixed in a test tube and incubated in a boiling water bath for 30 min. The mixture was then centrifuged (1811× g for 15 min), and the supernatant fluid was determined by measuring the absorbance at 532 nm with a microplate reader (Tecan Group Ltd., Männedorf, Switzerland). The data were expressed as nmol/μg of protein using an extination coefficient for MDA-TBA complex (1.56×10^{-5} M^{-1} cm^{-1}).

2.8. Histopathological evaluation

The kidneys were preserved in a 10% formalin solution and were processed routinely by embedding in paraffin. The tissue sections (4–5 μm) were stained with hematoxylin and eosin and were examined under a light microscope (IX71S8F-2, Olympus, Tokyo, Japan) for histopathological changes. The histological indices of glomerular damage, tubular damage and tubulointerstitial inflammatory infiltrates were quantified based on the method of Uzunoglu et al.[15] with some modifications. The renal damage was graded as following: 0, 0–5% damage; 1, 5–20% damage; 2, 20–40% damage; 3, 40–60% damage; 4, 60–80% damage; and 5, 80–100% damage. The final numerical score of renal damage was calculated by dividing the sum of the numbers per grade of the affected mice by the total number of examined mice.

2.9. Statistical methods

Mean and SD were used to describe the distributions of serum creatinine and BUN, renal GPx, GR, CAT, SOD, GSH and TBARS for study subjects. Comparisons between groups were performed using analysis of variance (ANOVA), followed by the Student-Newman-Keuls test. Data were analyzed using the SPSS statistical package software (SPSS, version 16.0).

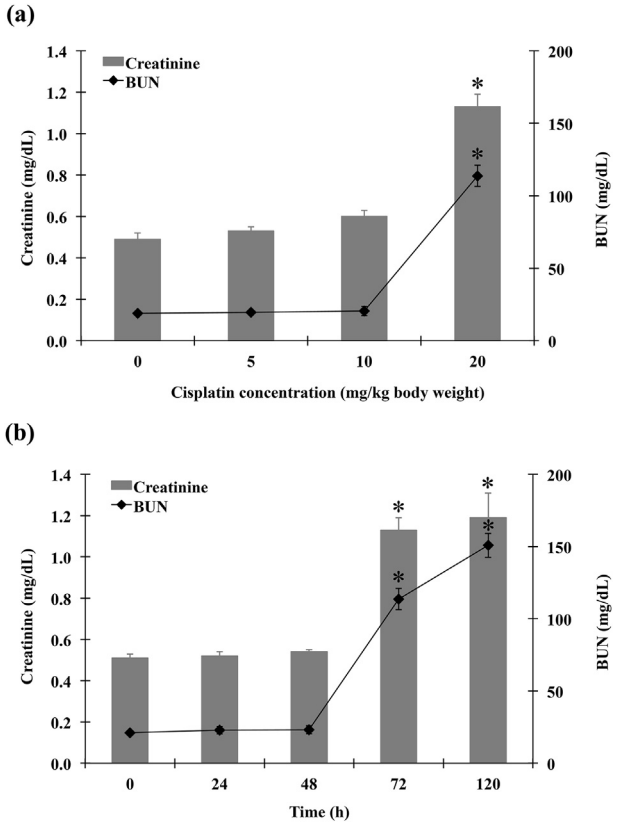

Fig. 1. **Evaluate the nephrotoxicity of cisplatin administration in mice.** Data are mean ± SEM, $n = 6$. *$p < 0.05$ compared with the normal control. (a) Dose–response study. Experimental mice received cisplatin (0, 5, 10, or 20 mg/kg i.p.) and serum BUN and creatinine levels were determined 72 h after cisplatin administration. (b) Time course study. The group of 0 h was given saline. The other groups of experimental mice were given cisplatin with a dose of 20 mg/kg i.p. and serum creatinine and BUN levels were determined at the designated time point. #$n = 5$, because one of the mice in the 120 h group died on 112 h after cisplatin administration.

3. Results

3.1. Dose–response and time course study of cisplatin on nephrotoxicity

Cisplatin treatment induced renal injury as evidenced by the elevation of serum creatinine and BUN. Fig. 1a shows that cisplatin, at the dose of 20 mg/kg, significantly ($p < 0.05$) increased the levels of creatinine and BUN in serum, compared to the control groups. Therefore, the dose of 20 mg/kg of cisplatin was used to induce nephrotoxicity throughout the experiment. Additionally, significant increases in serum creatinine and BUN were observed at 72 h after cisplatin treatment and were maintained at up to 120 h (Fig. 1b). However, one of the mice in the 120-h group died 112 h after cisplatin administration. Therefore, we selected a time point of 72 h after cisplatin administration to measure the potential preventive properties of ERW. As a result, the optimal condition of cisplatin-induced nephrotoxicity in this study was achieved at a dose of 20 mg/kg and an exposure time of 72 h.

3.2. Effect of ERW on urinary hydration state in cisplatin-treated mice

The effects of ERW on urinary osmolality, Na^+, K^+, Mg^{2+} and Ca^{2+} are shown in Table 2. Compared to the normal control group, treatment with cisplatin in mice significantly revealed lower level of osmolality, and higher levels of Na^+, K^+, Mg^{2+} and Ca^{2+} in urine ($p < 0.05$). Nevertheless, ERW in combination with cisplatin increased the reduced osmolality, by 15%, than the level in the cisplatin-treated group. Moreover, the levels of Na^+, K^+, Mg^{2+} and Ca^{2+} were significantly increased in cisplatin-treated mice, compared to the normal control group ($p < 0.05$). In administration of ERW in combination with cisplatin group, the elevated Na^+, K^+, Mg^{2+} and Ca^{2+} levels were significantly reduced by 33%, 34%, 51% and 33%, respectively, compared to the cisplatin-treated group ($p < 0.05$). Additionally, there was no significant difference in the levels of urinary osmolality, Na^+, K^+, Mg^{2+} and Ca^{2+} between the normal control group and the ERW treated group.

3.3. Effect of ERW in cisplatin-induced nephrotoxicity

The effects of ERW on creatinine and BUN are shown in Table 3. Treatment with cisplatin in mice significantly showed higher levels of creatinine and BUN in serum, compared to the normal control group ($p < 0.05$), suggesting the nephrotoxicity in mice was caused by cisplatin. However, ERW in combination with cisplatin significantly reduced the elevated serum levels of creatinine and BUN (31% and 40%, respectively), in comparison with the levels observed in the cisplatin-treated group ($p < 0.05$). Additionally, the levels of creatinine and BUN were not dissimilar between the normal control mice and the ERW-treated mice.

Table 2
Effects of ERW on urinary hydration state in cisplatin intoxicated mice.

Design of treatment	Osmolality (mOsm/kg H_2O)	Na^+ (mmol/L)	K^+ (mmol/L)	Mg^{2+} (mg/dl)	Ca^{2+} (mg/dl)
Normal control	1201 ± 96.64*	77.00 ± 6.53*	77.80 ± 5.69*	1.27 ± 0.27*	4.07 ± 0.64*
Cisplatin (20 mg/kg i.p.)	873 ± 20.22#	116 ± 2.90#	135 ± 15.59#	14.23 ± 0.90#	6.88 ± 0.86#
ERW	1137 ± 95.80*	68.00 ± 2.45*	83.43 ± 14.93*	1.30 ± 0.38*	4.37 ± 0.45*
ERW + cisplatin	1006 ± 114.80	78.00 ± 10.30*	89.10 ± 3.76*	7.03 ± 0.62#,*	4.58 ± 0.90*

Data are mean ± SEM, $n = 10$.
#$p < 0.05$ compared with the normal group.
*$p < 0.05$ compared with the cisplatin group.

Table 3
Effects of ERW on serum creatinine and BUN in cisplatin intoxicated mice.

Design of treatment	Creatinine (mg/dL)	BUN (mg/dL)
Normal control	0.65 ± 0.02*	32.42 ± 1.66*
Cisplatin (20 mg/kg i.p.)	0.91 ± 0.05#	79.61 ± 1.18#
ERW	0.60 ± 0.02*	36.08 ± 1.17*
ERW + cisplatin	0.63 ± 0.03*	48.13 ± 3.78*

Data are mean ± SEM, $n = 10$.
#$p < 0.05$ compared with the normal group.
*$p < 0.05$ compared with the cisplatin group.

3.4. Effect of ERW on kidney antioxidant enzyme activities

Cisplatin-induced nephrotoxicity is associated with the induction of oxidative stress in mice. The effects of ERW on kidney antioxidant enzyme activities are summarized in Table 4. The levels of renal GPx, GR, CAT and SOD in the cisplatin-treated group were markedly reduced by 25%, 63%, 29% and 47%, respectively, compared to the normal control group. On the contrary, administration of ERW in combination with cisplatin had significantly higher levels of GPx, GR, CAT and SOD in kidney than did the cisplatin-treated group. Additionally, there was no significant difference in the activities of renal GPx, GR, CAT and SOD between the normal control group and the ERW treated group.

3.5. Effect of ERW on kidney lipid peroxidation and GSH level

The results of the TBARS in the kidneys are illustrated in Table 5. TBARS levels in the cisplatin-treated group were significantly higher than those in the normal control group ($p < 0.05$). Consistent with the kidney levels of SOD, CAT, GPx and GR, the TBARS levels in the group treated with ERW and cisplatin were significantly lower, by 63%, than the levels in the cisplatin-treated group ($p < 0.05$). Additionally, the levels of GSH in the mouse kidneys are shown in Table 5. Treatment with cisplatin markedly reduced ($p < 0.05$) the GSH levels in the kidneys by 54%, when compared with the normal control group. In contrast, administration of ERW in combination with cisplatin significantly increased GSH levels by 34%, compared to the cisplatin-treated group.

3.6. Histopathological evaluation

The histopathological examination also afforded critical support for the biochemical results. The histological evaluations of renal tubule sections from various treatment groups are shown in Fig. 2. In the normal control animals, the kidney sections showed normal proximal and distal tubular cells, with well-developed cytoplasm and prominent nuclei. The kidneys of the cisplatin-intoxicated mice revealed moderate hydropic changes and severe necrosis in the tubular cells. Compared with the lesions observed in the cisplatin-treated group, the lesions in the group treated with ERW in combination with cisplatin were present to a much milder degree. These animals showed lower levels of edema and only trace levels of necrosis in the tubular cells. In the ERW-treated animals, the kidney sections showed minor or no interstitial edema, as did the normal control group.

The histopathological examinations for glomerular damage, tubular damage and tubulointerstitial inflammatory infiltrates were recorded and scored and are shown in Fig. 3. In this semi-quantitative evaluation, the damage indices of the histopathological assessments in the cisplatin group were significantly higher than those in the normal control group ($p < 0.05$), suggesting that cisplatin caused serious renal cell injury. Administration of ERW in combination with cisplatin significantly reduced the damage indices for renal damage ($p < 0.05$), compared to the cisplatin-treated group.

Table 5
Effects of ERW on renal GSH and MDA in cisplatin intoxicated mice.

Design of treatment	MDA (nmole/mg protein)	GSH (μmole/g wet weight)
Normal control	55.89 ± 3.77*	53.85 ± 3.63*
Cisplatin (20 mg/kg i.p.)	158.04 ± 10.97#	24.48 ± 1.13#
ERW	57.44 ± 3.68*	53.51 ± 1.60*
ERW + cisplatin	100.14 ± 6.74*	32.84 ± 0.81*

Data are mean ± SEM, $n = 10$.
#$p < 0.05$ compared with the normal group.
*$p < 0.05$ compared with the cisplatin group.

4. Discussion

Hydration state is a very important factor in cisplatin-induced kidney injury. Several clinical trials showed firm evidence that the majority of cisplatin-treated patients suffering from hypomagnesaemia, often associated with a reduced glomerular filtration rate, polyuria and electrolyte disturbances such as Na^+, K^+, Mg^{2+} and Ca^{2+} wasting.[16,17] Lower urinary osmolality could also be a major determinant in the increase of cisplatin-induced nephrotoxicity.[18] Our results showed the urinary osmolality was lower and the levels of Na^+, K^+, Mg^{2+}

Table 4
Effects of ERW on renal GPx, GR, CAT and SOD in cisplatin intoxicated mice.

Design of treatment	GSH-Px (nmole NADPH/min/mg protein)	GSH-Rd (nmole NADPH/min/mg protein)	CAT (Units/mg protein)	SOD (Units/mg protein)
Normal control	0.12 ± 0.01*	1.45 ± 0.13*	6.81 ± 0.89*	0.38 ± 0.05*
Cisplatin (20 mg/kg i.p.)	0.09 ± 0.01#	0.53 ± 0.02#	4.81 ± 0.52#	0.20 ± 0.01#
ERW	0.12 ± 0.01*	1.30 ± 0.34*	6.82 ± 0.39*	0.36 ± 0.06*
ERW + cisplatin	0.12 ± 0.01*	1.25 ± 0.09*	6.48 ± 0.30*	0.43 ± 0.01*

Data are mean ± SEM, $n = 10$.
#$p < 0.05$ compared with the normal group.
*$p < 0.05$ compared with the cisplatin group.

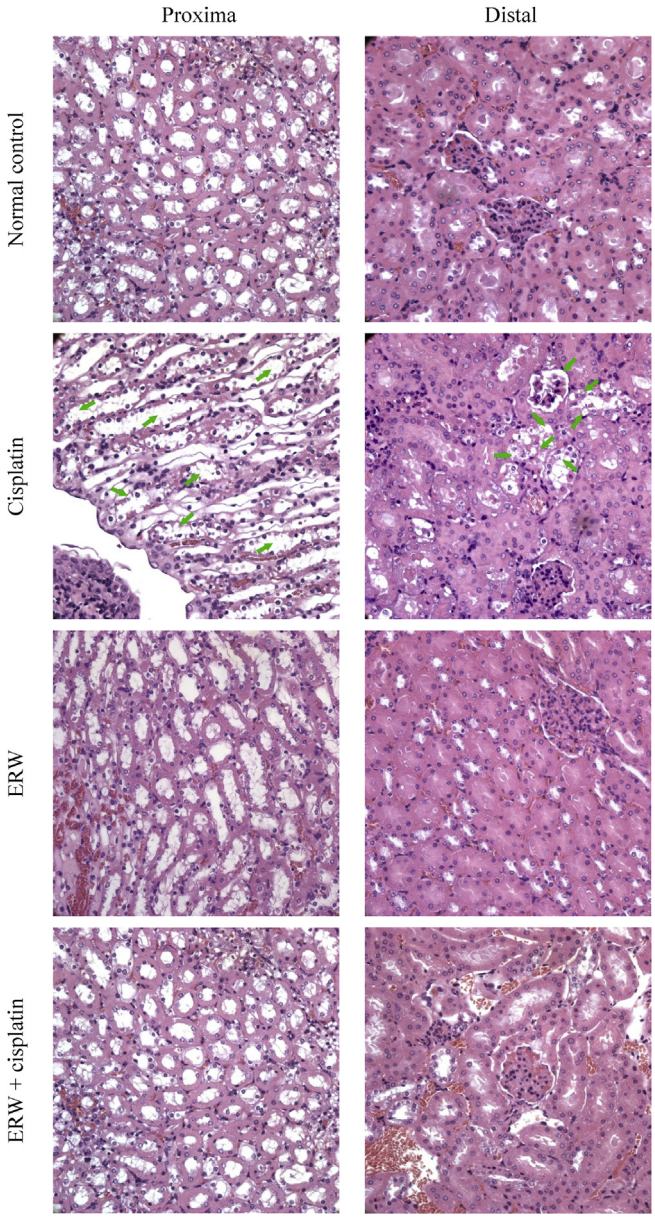

Fig. 2. **Effect of ERW on renal proximal and distal tubules morphological analysis in cisplatin-intoxicated mice.** The sections were stained with hematoxylin–eosin by standard techniques (400×). Normal control group: normal cytoplasm and prominent nucleus; Cisplatin-treated group: areas of tubular necrosis (more serious), show as green arrow; ERW-treated group: minor or no interstitial edema, as normal control group. ERW + cisplatin-treated group: minor edema and trace tubular necrosis.

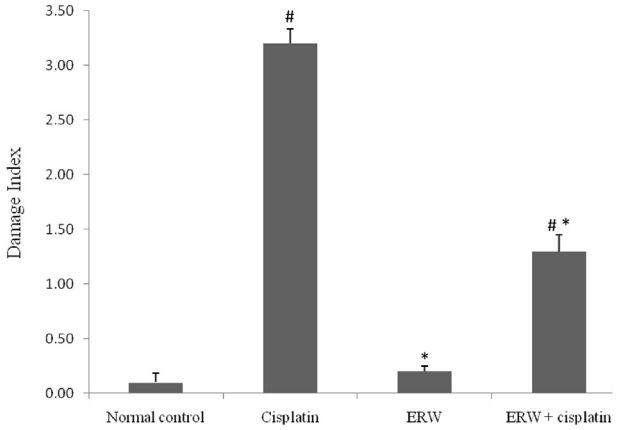

Fig. 3. **Effects of ERW on histopathological renal damage index in mice treated with cisplatin.** The damage indices of renal histopathological examinations were quantified based on Uzunoglu et al.[15] method. $^{\#}p < 0.05$ compared with the normal group. $^{*}p < 0.05$ compared with the cisplatin group.

and Ca^{2+} in urine were higher comparing to normal control group that confirmed the kidney failure in the cisplatin-induced mice. Since ROS is closely related to the renal failure induced by cisplatin, many antioxidants have been studied as nephroprotective agents such as rosmarinic acid, crocin and fisetin that prevent oxidative damage by exposure to cisplatin.[19–21] In this study, we demonstrated that administration of ERW not only caused the level of osmolality closer to the baseline but ameliorated the electrolyte disturbances in cisplatin-induced mice. ERW containing high levels of hydrogen molecules improves various diseases and provides protection by multiple pathways. Several studies reported that ERW has the ability to extinguish ROS and excited sensitizer molecules.[7,22] Moreover, hydrogen molecules protect cells and tissues against strong oxidative stress by scavenging hydroxyl radicals.[23] Hydrogen rich water also has been found to decrease cisplatin-induced nephrotoxicity as assessed by serum creatinine and BUN levels.[24] Recently, we demonstrated that ERW prevents liver damage induced by carbon tetrachloride[14] and attenuates amyloid β-induced cytotoxicity in human neuroblastoma SK-N-MC cells.[25] In the case of carbon tetrachloride-induced hepatotoxicity, ERW protects the liver against carbon tetrachloride challenge, with plasma alanine aminotransferase and aspartate aminotransferase levels being reduced to normal control levels, oxidative stress being suppressed, and GPx, CAT and SOD activities also being restored. Several studies have demonstrated that antioxidant enzymes, such as GPx, GR, CAT and SOD, protect against oxidative tissue damage.[14,26,27] Davis et al.[26] showed that overexpression of MnSOD protected against cisplatin-induced renal epithelial cell injury. Ma et al.[27] showed that catalase and its derivatives not only extenuate cisplatin-evoked nephrotoxicity but also enhance the efficiency of cisplatin in cancer therapy. Therefore, free radical-scavenging enzymes are important to protect against the cell oxidative stress caused by cisplatin. However, these antioxidant enzymes are easily inactivated by reactive oxygen species, which result in decreasing in the activities of these enzymes in cisplatin toxicity.[28] In the present study, our results point that the activities of GPx, GR, CAT and SOD were significantly upgraded by the treatment of ERW to cisplatin-intoxicated mice, implying that ERW has the capacity to maintain/restore the level of enzymatic antioxidants in cisplatin-injured kidneys.

Cisplatin-induced nephrotoxicity is manifested as increases in the serum levels of creatinine and BUN.[1,29] A major mechanism of cisplatin's nephrotoxic effects might be related to oxidative stress.[30] Many studies have demonstrated cisplatin-induced free radical generation in the microsomes via the cytochrome *P*450 system.[30,31] The ability of an

antioxidant to remove ROS has been attested as a critical role that contributes to nephroprotective efficacy. In fact, multiple lines of evidence from animal model studies have indicated that antioxidant agents, such as manganese superoxide dismutase,[26] sesame oil[2] and sulforaphane,[5] reduced cisplatin-induced nephrotoxicity by the inhibition of peroxidation. The results of the present study demonstrated that treatment with ERW significantly decrease cisplatin-evoked renal damage, as evidenced by reduced creatinine and BUN serum levels (Table 3), suggesting that ERW has the ability to restore renal damage caused by cisplatin-induced nephrotoxicity. Our results are in agreement with previous studies that molecular hydrogen has ability to alleviate cisplatin-induced nephrotoxicity as shown by decreased levels of creatinine and BUN in serum and reduced apoptosis in the kidneys.[24] A clinical study also indicated that in chronic hemodialysis patients, treatment with ERW was beneficial for palliating chronic hemodialysis-induced oxidative stress, as evidenced by decreased blood ROS production, RBC lipid peroxidation and hemolysis.[32]

The initiation of oxidative stress has been related to various acute and chronic diseases, which are commonly supposed to be important events cause by underlying increases in lipid peroxidation.[33] Lipid peroxidation by cisplatin has been attested as a critical mechanism of cisplatin-induced kidney damage.[30] TBARS is revealed during the peroxidation of biological membranes and polyunsaturated fatty acids.[14,30] The elevation of TBARS levels in the kidneys implies that enhancing lipid peroxidation leads to tissue damage and to the breakdown of the antioxidant defense mechanisms, preventing the formation of superabundant free radicals.[14] The results of this study reported that cisplatin-induced nephrotoxicity caused an elevation in TBARS levels in kidney. In contrast to the cisplatin-induced toxicity group, administration of ERW significantly reduced the levels of TBARS, implying that ERW could protect mice from cisplatin-evoked lipid peroxidation.

In antagonism to the toxic effects of cisplatin-induced ROS formation through the cytochrome *P*450 pathway, the detoxification pathway is also involved in the conjugation of cisplatin with thiol-containing molecules, including GSH.[34] GSH is an endogenous non-enzymatic antioxidant along the pathway of detoxification that downgrades the accumulation of endogenous ROS and oxidative stress caused by cisplatin.[29] The cisplatin-induced ROS accumulation leading to renal injury could be proved in the CYP2E1-null mouse model.[31] Previous studies on the mechanism of cisplatin-induced nephrotoxicity have shown that neutralization of cisplatin was depleted of GSH and other thiol-containing molecules, causing change in cellular redox status and the accumulation of endogenous ROS.[34] Therefore, GSH conjugation is extremely indispensable for reducing the toxic effects of cisplatin. The results of this study found that the renal levels of GSH were significantly reduced in cisplatin-toxified mice then did the control mice. Nevertheless, administration of ERW had significantly higher levels of GSH in cisplatin-toxified mice, suggesting that ERW can protect against cisplatin-induced depletion of renal GSH.

There are some limitations in our study. Because the cisplatin-induced renal damage model is based on animals, further research is necessary if we want to test the effect of ERW in clinical patients. There are three limitations that were listed below. First, the mechanisms associated with pharmacodynamic study and pharmacokinetic study should be fully investigated and understood. Second, the cytotoxicity and functionality of ERW should be further evaluated in various organs. Third, ERW is rich in hydrogen molecules, but the dissolved hydrogen in ERW decreased rapidly to zero at 8 h. Therefore, the ERW we used in our experiments was fresh prepared and treated. We replaced fresh prepared ERW for mice every 6 h to confirm the condition of our experiment was standardized.

In the present study, the ERW is successful in the preclusion of cisplatin-evoked renal damage. To our knowledge, this is the first study to report a significant attenuation cisplatin-induced renal injury by ERW *in vivo*. The results of this study demonstrated that nephrotoxicity induced by cisplatin was distinguished decreased by the treatment of ERW, as evidence by reduced the serum kidney-functional indices. Furthermore, the histological evaluations clearly indicate that the large hydropic and necrosis in the tubular induced by cisplatin were remarkably decreased by the administration of ERW. This nephroprotection appears to be associated with the elevation renal antioxidant defense system and suppression renal lipid peroxidation. Overall, dietary ERW may be helpful for nephroprotection against cisplatin-induced nephrotoxicity *in vivo*.

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