

## Research Article



## Effect of Zinc Sulfate in Protection against Cisplatin-Induced Nephrotoxicity in Cancer Patients

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### ABSTRACT

Cisplatin is a potent antitumor agent that is useful in chemotherapy of various types of cancers. Currently, nephrotoxicity limits its usefulness. The main mechanism responsible for nephrotoxicity is oxidative stress with associated inflammation. This study was to evaluate the protective effect of zinc sulphate on cisplatin-induced nephrotoxicity in cancer patients. Twenty eight patients were participated in the study and were randomized into two groups. Patients in group I (N=14) received six cycles of cisplatin based regimen every 21 days. Patients in group II (N=14) received zinc sulphate in addition to cisplatin based regimen. Serum cystatin C and cystatin C-based GFR were measured at base line and 21 days after 1, 2, 4 and 6 cycles while urinary malondialdehyde and IL-18 were measured at base line and 1 day after 1, 2, 4 and 6 cycles of cisplatin based regimen. Zinc sulfate addition to cisplatin in group II significantly (P<0.05) ameliorated the cisplatin-induced increment in serum cystatin C, urinary malondialdehyde and IL-18 and the cisplatin-induced decline in cystatin C-based GFR that were demonstrated in group I. The significant protective effect of zinc sulfate on cisplatin-induced nephrotoxicity may be through its antioxidant and anti-inflammatory action.

**Keywords:** Cisplatin, nephrotoxicity, zinc sulfate, oxidative stress, inflammation.

### INTRODUCTION

Cisplatin, cis-diamminedichloroplatinum, is an alkylating-like agent with potent antitumor action that is useful in chemotherapy of broad spectrum of human cancers.<sup>1</sup> On the other hand, cisplatin can cause nephrotoxicity as acute kidney injury (AKI) in about 20-30% of patients which can limit the use of cisplatin in cancer patient.<sup>1,2</sup> Cisplatin remains the irreplaceable first line chemotherapeutic agent for various types of cancer even with increased morbidity and mortality due to nephrotoxicity which still to occur despite the use of vigorous hydration with normal saline as a protection measure.<sup>3,4</sup> After Cisplatin uptake by tubular epithelial cells, it activates complex intracellular signalling pathways including DNA damage response pathways, induction of free oxygen radicals along with inactivation of antioxidant systems, leading to cell apoptosis and necrosis.<sup>1</sup> Reactive oxygen species as superoxide anion (O<sub>2</sub><sup>•-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and hydroxyl radical (•OH) are also important in inducing inflammation through the activation of nuclear factor-kB (NF-kB) leading to an intense inflammatory response that further exacerbating tubular cell injury and stimulating fibrogenesis.<sup>1</sup> Activation of NF-kB leads to the renal over expression of TNF-α.<sup>5</sup> TNF-α coordinates the activation of a large network of chemokines and cytokines in the kidney.<sup>5,6</sup> Macrophages- and parenchymal kidney cells-derived IL-18 has an important role in cisplatin-induced nephrotoxicity.<sup>7</sup> Studies in humans that showed urinary IL-18 as an early predictive biomarker of AKI<sup>8</sup> established

the base for the use of urinary IL-18 as an early predictive biomarker of AKI after cisplatin treatment in this study.

Zinc is essential micronutrient that is involved in various aspects of cellular metabolism.<sup>9</sup> It is required for the catalytic activity of many enzymes<sup>10,11</sup> and it has an important role in immune function<sup>11,12</sup>, wound healing<sup>11</sup>, protein and DNA synthesis and cell division<sup>13</sup>. It has a valuable antioxidant and anti-inflammatory effects.<sup>14,15</sup> Zinc sulfate had a protective effect against renal toxicity caused by cisplatin in mice.<sup>16</sup> A preventive effect of organic zinc sources such as zinc gluconate or zinc histidine in animal models with cisplatin induced renal injury was observed.<sup>17-19</sup>

### PATIENTS AND METHODS

#### Patient

Twenty eight cancer patients were participated in this study for which cisplatin based regimen was indicated as chemotherapy in the oncology unit in al-sadar medical city in Al-Najaf Governorate. All included patients had diagnosed to have malignant tumor by histopathologic and cytologic investigation. Informed consents were obtained from all patients participated in the study. Approval of the study protocol was by the Ethical Committees of Al-Nahrain College of Medicine. Inclusion criteria include no previous administration of radiotherapy or chemotherapy, age of patient's ranges from 18 to 70 years, no serious cardiopulmonary problems. Pregnant, lactating and patients with



metastasis to the central nervous system, psychiatric disorders and hypersensitivity to any platinum derivatives were excluded from the study. Name, sex, age, occupation, education, clinical data and therapeutic data were collected for each participant.

### Study treatment

Patients enrolled in the study were randomized into non zinc sulfate treated group (group I) where Patients (N=14) received cisplatin based regimen every 3 weeks for 6 cycles zinc sulfate treated group(Group II) where Patients (N=14) received zinc sulfate plus cisplatin-based regimen. Cisplatin based regimen was composed of cisplatin in a dose of 75 mg/m<sup>2</sup> and other chemotherapeutic agents such as docetaxel, etoposide, 5-flourouracil and gemcitabine that had no effect on cisplatin-induced nephrotoxicity.<sup>20,21</sup> Zinc sulfate was given as an oral tablet in a dose of 220 mg of zinc sulfate (50 mg of elemental zinc) twice daily for all patients included in group II. It was manufactured by Alhavi CO., Iran. Batch NO. 0100890.

### Collection of blood and urine samples

Peripheral blood and urine samples were collected from patients included in the present study before the 1<sup>st</sup> dose (base line) and after 1<sup>st</sup>, 2<sup>nd</sup>, 4<sup>th</sup> and 6<sup>th</sup> cycles of treatment. Blood samples were collected 21 days after the cycle while urine sample were collected 1 day after the cycle. Each blood sample was left for 15 minutes and centrifuged at 2500 rpm for 15 minutes, and then serum was collected and frozen. Each urine sample was centrifuged at 1500 rpm for 10 minutes and then supernatant was collected and frozen. Serum and urine samples were stored in deep freeze at -80 °C until measurements.

### Assessment of kidney function

Serum cystatin C concentration as a marker of kidney function was measured by enzyme-linked immunosorbent assay using commercially available human cystatin C (Cys C) ELISA kit from Cusabio Biotech Co., LTD. (Catalog Number CSB-E08384h).

The following formula was used to calculate the glomerular Filtration rate from the serum concentration of cystatin C<sup>22</sup>:

$$GFR \left( \frac{ml}{min} \right) = 77.24 \times Cystatin\ C^{-1.2623}$$

### Assessment of kidney oxidative stress

Renal malondialdehyde (MDA), the end product of lipid peroxidation, as a marker for renal oxidative stress was analyzed according to the method of Buege and Aust<sup>23</sup> which is clinically used to measure MDA<sup>24</sup> and is based on formation of a red chromophore that can be detected spectrophotometrically resulted from MDA-thiobarbituric acid (TBA) complex.

### Assessment of acute kidney injury and renal inflammation by urinary IL-18

Urinary IL-18 concentration was measured by enzyme-linked immunosorbent assay using commercially available human IL-18 ELISA kit (Code Number 7620) from MBL International Corporation, Japan.

### Statistical Analysis

Statistical analyses were carried out by SPSS 16.0 for windows. Inc. Data of quantitative variables were expressed as mean ± SEM. Comparison between base line level and other cycle records in same treatment group were done by paired-sample Student's t-test. Comparisons between the two groups variables were done by Chi-Square test or (independent) unpaired-sample Student's t-test as appropriate. In all tests, P<0.05 was considered to be statistically significant unless another level was stated.

## RESULTS

### Patients characteristics

The patients characteristics data are shown in Table 1 and the difference in each characteristic was insignificant (p>0.05) in comparisons between the two randomization groups.

**Table 1:** Characteristics data for all included patients.

Characteristics	Group I	Group II
Number of patients	14	14
Sex (male/female)	10/4	10/5
Age (yr) Mean ± SEM	51.8 ± 6.78	54.15 ± 1.34
Weight (kg) Mean ± SEM	76.21 ± 0.94	78.11 ± 1.24
Height (cm) Mean ± SEM	161.3 ± 3.48	154.4 ± 2.92
Body Surface Area (m <sup>2</sup> ) Mean ± SEM	1.73 ± 2.06	1.72 ± 0.78
no. of Diabetic patients	1	2
no. of Hypertensive patient	2	3
Cisplatin based regimens received by patients:		
Cisplatin + gemcitabine	6	8
Cisplatin + 5-flourouracil + docetaxel	3	3
Cisplatin + 5-flourouracil	2	1
Cisplatin + etoposide	3	2

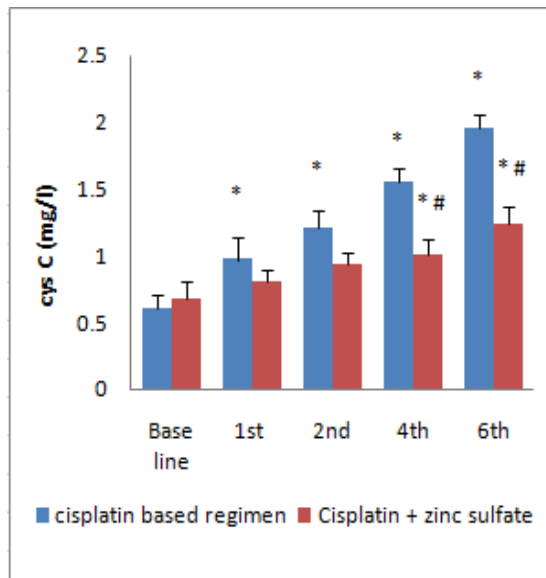
P > 0.05 (unpaired-sample t-test or Chi- Square test were used as appropriate).



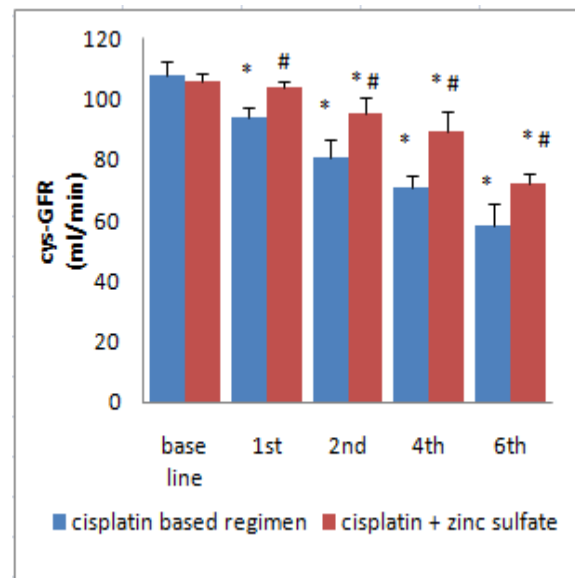
**Changes in kidney function parameters**

After 1, 2, 4 and 6 cycles, cisplatin based regimen caused significant increment ( $P < 0.05$ ) in serum cystatin C (mg/l) level and significant decrement ( $P < 0.05$ ) in cystatin C-GFR (ml/min) level when compared with that of baseline, as shown in Figure 1. In group II, zinc sulfate caused

significant amelioration ( $P < 0.05$ ) of the increased level of serum cystatin C (mg/l) of group I after 4 and 6 cycles of treatment as shown in Figure 1A. In group II, zinc sulfate caused significant amelioration ( $P < 0.05$ ) of the decreased level of cystatin C-GFR (ml/min) of group I after 1, 2, 4 and 6 cycles of treatment as shown in Figure 1B.



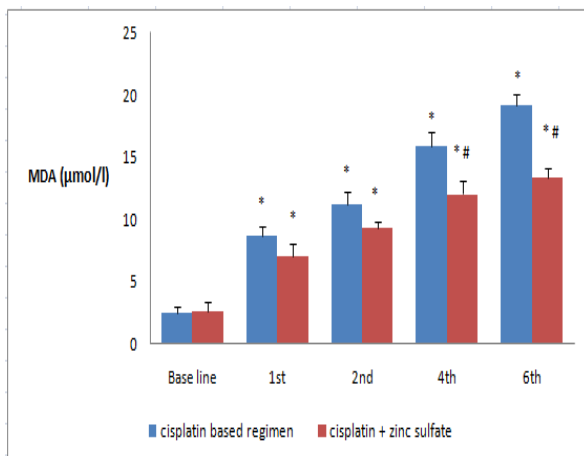
**Figure 1A**



**Figure 1B**

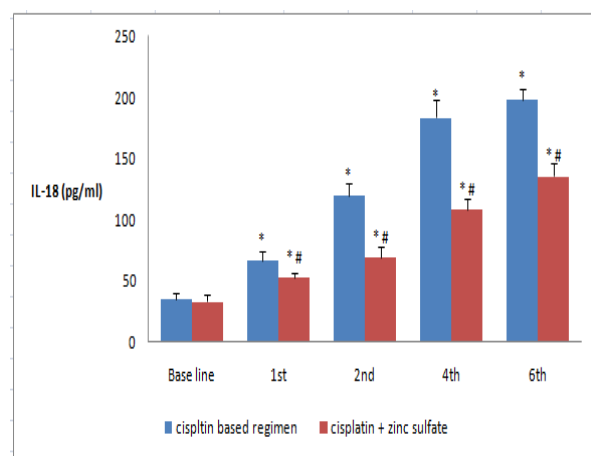
\*  $P < 0.05$  compared to baseline values of the same treatment group.  
#  $p < 0.05$  compared to group I at same cycle of treatment.

**Figure 1:** Mean  $\pm$  SEM values of kidney function parameters (A: serum cystatin C, B: cystatin C-GFR) at baseline and after 1, 2, 4 and 6 cycles in both group I (cisplatin based regimen,  $n=14$ ) and group II (cisplatin + zinc sulfate,  $n= 14$ ).



\*  $P < 0.01$  compared to baseline values of the same treatment group.  
#  $p < 0.05$  compared to group I.

**Figure 2:** Mean  $\pm$  SEM values of urinary MDA ( $\mu\text{mol/l}$ ) at baseline and after 1, 2, 4 and 6 cycles in both group I (cisplatin based regimen,  $n=14$ ) and group II (cisplatin + zinc sulfate,  $n= 14$ ).



\*  $P < 0.01$  compared to baseline values of the same treatment group.  
#  $p < 0.05$  compared to group I.

**Figure 3:** Mean  $\pm$  SEM values of urinary IL-18 (pg/ml) at baseline and after 1, 2, 4 and 6 cycles in both group I (cisplatin based regimen,  $n=14$ ) and group II (cisplatin + zinc sulfate,  $n= 14$ ).

### Changes in urinary MDA level

After 1, 2, 4 and 6 cycles, cisplatin based regimen caused high significant increment ( $p < 0.01$ ) in urinary MDA ( $\mu\text{mol/l}$ ) level when compared with that of baseline, as shown in Figure 2.

In group II, zinc sulfate caused significant amelioration ( $P < 0.05$ ) of the increased level of urinary MDA ( $\mu\text{mol/l}$ ) of group I after 4 and 6 cycles of treatment as shown in Figure 2.

### Changes in urinary IL-18 level

After 1, 2, 4 and 6 cycles, cisplatin based regimen caused high significant increment ( $p < 0.01$ ) in urinary IL-18 ( $\text{pg/ml}$ ) level when compared with that of baseline, as shown in Figure 3. In group II, zinc sulfate caused significant amelioration ( $P < 0.05$ ) of the increased level of urinary IL-18 ( $\text{pg/ml}$ ) of group I after 4 and 6 cycles of treatment as shown in Figure 3.

## DISCUSSION

### Effects of cisplatin based regimen on kidney function parameters

In the current study, cisplatin based regimen caused significant increment ( $P < 0.05$ ) in serum cystatin C after the first cycle and highly significant increment ( $P < 0.01$ ) after 2, 4 and 6 cycles in comparison to base line level which agrees with that results demonstrated by Zhang and Zhou<sup>25</sup>. Serum cystatin C had been selected in this study as a more sensitive clinical marker than serum creatinine for the early evaluation of cisplatin-induced disturbance in GFR because it is, unlike serum creatinine, not affected by factors that are not correlated to renal function that affect creatinine production or elimination such as body mass, nutrition or sex.<sup>26</sup> Benohr and colleagues<sup>27</sup> demonstrated that serum cystatin C level correlated well to GFR measured by insulin clearance. In the present study, GFR based on serum cystatin C was significantly decreased ( $p < 0.05$ ) after 1 and 2 cycles and more over highly significant decrement ( $p < 0.01$ ) was recorded after 4 and 6 cycles of cisplatin treatment. These results are in accordance with that revealed by Boelke<sup>28</sup> where they further recommended cystatin C for GFR estimation in patients receiving cisplatin as alternative method to the estimated creatinine clearance in clinical practice. The significant reduction in GFR after cisplatin is might be due to the decrease in renal blood flow because of the increased renal vascular resistance secondary to tubular-glomerular feedback in response to the increased sodium chloride delivery to the macula densa resulted from cisplatin-induced inhibition of renal reabsorption.<sup>29</sup>

### Effects of cisplatin based regimen on renal oxidative stress parameter

In the present study, there is highly significant increment ( $p < 0.01$ ) in urinary MDA ( $\mu\text{mol/l}$ ) level after 1, 2, 4 and 6 cycles in cisplatin based regimen treated group in

comparison to base line level. These results are in consistence with that revealed by Zhou<sup>30</sup> where they recorded an increase in urinary MDA excretion 24 h after cisplatin treatment in 8 cancer patients. In the presence of cisplatin, ROS are produced through the xanthine-xanthine oxidase system, mitochondria, and NADPH oxidase in cells<sup>31</sup> and are implicated in the pathogenesis of AKI due to cisplatin.<sup>32</sup> In addition, formation of GSH-cisplatin adduct decreases GSH intracellular level decreasing the scavenging activity, inhibition of antioxidant enzymes as superoxide dismutase, glutathione peroxidase, and catalase.<sup>33,34</sup> and increasing calcium concentration that leads to over production of ROS.<sup>32</sup> The main damage to cells results from the ROS-induced alteration of macromolecules such as polyunsaturated fatty acids in membrane lipids, essential protein, DNA and ultimately cell death.<sup>35</sup>

### Effects of cisplatin based regimen on renal inflammatory response parameter

In the present study, cisplatin based regimen caused highly significant increment ( $p < 0.01$ ) in urinary IL-18 ( $\text{pg/ml}$ ) level after 1, 2, 4 and 6 cycles in comparison to base line level. These data indicated the presence of AKI after the first cycle of cisplatin treatment. Cisplatin administration significantly up regulated several cytokines and chemokines and the most important of them are TNF- $\alpha$ , monocyte chemo attractant protein-1 and intercellular adhesion molecule.<sup>6</sup> This leads to the recruitment and accumulation of inflammatory cells. IL-18 is produced in the kidney predominantly by recruited inflammatory cells, probably macrophages but also by activated injured renal parenchymal cells.<sup>36</sup> Up to our knowledge, there is no similar study that measured urinary or even serum level of IL-18 in patient receiving cisplatin. On the other hand, there are some reports of measuring urinary IL-18 in patients with AKI. A case-control study on patients with the Acute Respiratory Distress Syndrome was performed and revealed that urine IL-18 values predicted development of AKI (defined as a 50% increase in serum creatinine) 24 and 48 hours later.<sup>37</sup>

### Effects of cisplatin based regimen + zinc sulfate on kidney function parameters

In the present study, zinc sulfate caused significant ( $P < 0.05$ ) lowering of serum cystatin C level of zinc sulfate treated group in comparison to that of cisplatin based regimen treated group after 2, 4 and 6 cycles of treatment. Zinc sulfate in the present study showed more prominent ameliorative effect on cystatin C based GFR where cystatin C-GFR of zinc sulfate treated group was significantly higher ( $P < 0.05$ ) than that of cisplatin only treated group after 1, 2, 4 and 6 cycles of treatment. Up to our knowledge, there is no similar study on cancer patients or any experimental models that studied the effect of zinc sulfate on serum cystatin C and cystatin C-GFR as renal function test in cisplatin nephrotoxicity





models but results shown by Parham<sup>38</sup> may agree with our work where they concluded that Zinc supplementation reduced albumin excretion in microalbuminuric type 2 diabetic patients and improved kidney function.

#### Effects of cisplatin based regimen + zinc sulfate on renal oxidative stress parameter

In the present study, urinary MDA level of zinc sulfate treated group was significantly lower ( $P < 0.05$ ) than that of cisplatin only treated group after 4 and 6 cycles of treatment. These data suggest that zinc sulfate might inhibit oxidative status in the kidney. This effect may be due to the antioxidant and anti-inflammatory effects of zinc.<sup>14,15</sup> To the best of our knowledge, no similar study was reported in cancer patients but there are some experimental studies that agree with results demonstrated by the present study. Tuzcu<sup>39</sup> showed that the administration of zinc picolinate decreased MDA and 8-isoprostane production (as parameters of oxidative stress) in kidney of cisplatin-treated rats. Anderson<sup>40</sup> reported that people with type 2 diabetes mellitus supplemented with dietary zinc had a significant reduction in MDA levels in serum and kidney.

The antioxidant effect of zinc might be through many mechanisms. Zinc plays a key role in suppression of free radicals as it acts as a cofactor of the main antioxidative enzyme CuZnSOD, inhibits the NADPH-dependent lipid peroxidation<sup>41</sup> and prevents lipid peroxidation via inhibiting glutathione depletion.<sup>42</sup> Zinc competes with the transition metals to bind to the cell membrane and decreases the production of free radicals; thus, it may exert a direct antioxidant effect because of the ability of zinc to replace iron and copper from binding sites.<sup>41,43</sup> Zinc may also induce the production of metallothionein, which is an effective scavenger for hydroxyl radical. It has been suggested that Zn-metallothionein complexes provide protection against immune-mediated free-radical damage.<sup>41,44</sup>

#### Effects of cisplatin based regimen + zinc sulfate on renal inflammatory response parameter

In the present study, urinary IL-18 level of zinc sulfate treated group was significantly lower ( $P < 0.05$ ) than that of cisplatin only treated group after 1, 2, 4 and 6 cycles of treatment. Up to our knowledge, there is no similar study on cancer patient or even on experimental model that assess the effect of zinc sulfate on renal IL-18 as marker for inflammation but rather many reports studied the effects of zinc sulfate on inflammation. Prasad<sup>14</sup> reported that zinc supplementation to normal healthy subjects inhibited the induction of TNF- $\alpha$  and IL-1 by blood mononuclear cells, and exhibited a protective effect against TNF- $\alpha$ -induced NF- $\kappa$ B activation in isolated mononuclear cells. Prasad<sup>42</sup> also reported that after zinc gluconate supplementation, generation of TNF- $\alpha$  was significantly lower in the zinc-supplemented group than in

the placebo group in healthy elderly subjects (55 to 87 years). On experimental level, Tuzcu<sup>39</sup> showed that level of renal TNF- $\alpha$ , which is a potent proinflammatory cytokine, increased significantly after cisplatin treatment, and zinc picolinate prevents the rise of TNF- $\alpha$  in the kidney of cisplatin-treated rat.

According to the results from these studies, zinc sulfate in the present study might inhibit cisplatin-induced upregulation of TNF- $\alpha$ <sup>6</sup>, and so inhibition of the recruitment and accumulation of inflammatory cells that are responsible for renal IL-18 production.<sup>36</sup>

#### CONCLUSION

The results from the present study revealed that there is significant amelioration on kidney injury caused by addition of zinc sulfate to cisplatin based regimen in patients of group II that is evidenced by the observed improvement in kidney function. This ameliorative effect may be through the observed antioxidant and anti-inflammatory action of zinc sulfate.

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