

# Cyclosporine-A induced nephrotoxicity in male and female rats: Is zinc a suitable protective supplement?

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## ABSTRACT

**Background:** Cyclosporine (CYC) is an immunosuppressant drug used widely in kidney transplant patient. The major side effect of CYC is nephrotoxicity. In this study, three different doses of CYC alone or accompanied with zinc (Zn) supplement were administrated in male and female rats to determine the kidney tissue damages and functions. **Methods:** Male and female rats were treated with 10, 50 or 100 mg/kg/day of CYC alone or accompanied with 10 mg /kg/day of Zn sulfate for 10 days. The parameters related to renal function were determined and the kidney tissues were subjected to histological evaluation. **Results:** All male and female animals were treated with high dose CYC (100 mg/kg/day) alone or accompanied with Zn supplement during the experiment. The data obtained for the serum levels of creatinine (Cr) and blood urea nitrogen/Cr ratio, clearance of Cr, kidney weight (KW), sodium (Na) filtration rate, Na excretion rate and Na excretion fraction (%) in surviving animals suggest a role of gender in the variation of these factors. The kidney tissue damage score (KTDS) was increased as the dosage of CYC was elevated, and the Zn supplement attenuated the KTDS in animals treated with low dose CYC (10 mg/kg/day). **Conclusion:** The CYC-induced nephrotoxicity may be gender-related, and the 10 mg/kg dose of Zn sulphate as a supplement may possibly prevent the induced nephrotoxicity in males due to its antioxidant effects.

**Key words:** Cyclosporine, Gender, Nephrotoxicity, Renal function, Zinc

## INTRODUCTION

Calcineurin inhibitors (CNIs) include cyclosporine (CYC), tacrolimus, voclosporin and pimecrolimus. Patients with organ transplant usually receive CYC and tacrolimus as immunosuppressant agents to avoid organ transplant rejection, but these drugs can cause acute and chronic nephrotoxicity<sup>1</sup>. Among them, the major adverse effects of CYC are nephrotoxicity, hepatotoxicity, hypertension and risk of malignancy, thereby limiting its clinical use. The optimal use dosage of CYC for patient monitoring is one of the major difficulties in the clinic, thus dose adjustment strategy has been suggested for CYC therapy<sup>2</sup>. In addition, many research studies have been conducted to suggest supplemental agents to confer additional protection for the kidney against CYC-induced toxicity. These supplemental agents may include herbal drugs<sup>3-5</sup>, endothelin-1 receptor antagonists<sup>6</sup>, antihypertensive drugs or  $\beta$ -blockers<sup>7-9</sup>, NADPH oxidase activity inhibitor<sup>10</sup>, antioxidants<sup>11</sup>, omega-3 fatty acids<sup>12</sup>, apelin peptide<sup>13</sup>, and/or renin inhibitor<sup>14</sup>.

Gender and sex hormones are also important factors that influence the effects of CYC<sup>15-19</sup>. CYC-mediated side effects may be gender-related in kidney<sup>20</sup> and

heart<sup>21</sup>. El-Bassossy and Eid reported that 21 days of CYC treatment in rats led to sex-related nephrotoxicity due to different responses to inflammatory factors<sup>20</sup>. Until now, the role of gender or sex hormones in kidney toxicity induced by other drugs, such as cisplatin and gentamicin, have been documented<sup>22-33</sup>, although their mechanisms have not been completely understood.

In addition, the antioxidant supplements, such as zinc (Zn), have been widely used in laboratory research to protect the kidney against injury<sup>34-42</sup>. It seems that the administration of this trace element Zn could be considered as a safe protective agent for transplant patients. Therefore, in the current study, we evaluated three different doses of CYC, alone or accompanied with Zn supplement; moreover, kidney tissue damages and functions were investigated.

## METHODS

### Animals

This research was conducted on 110 male (n=55, 268±4 g) and female (n=55, 209±2 g) Wistar rats split among 14 experimental groups. The protocol for our study was approved by the Ethics Committee of Isfahan University of Medical Sciences

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(IR.MUI.REC.1397.2.053).

### Experimental group design

**Group 1 (n=8; 252±11g):** male rats treated with vehicle (sesame oil) as a solvent for CYC.

**Groups 2, 3, and 4 (n=8 per group; 280±11g, 256±15g, and 282±7g, respectively):** male rats treated with 10, 50 and 100 mg/kg/day of CYC dissolved in sesame oil, respectively.

**Groups 5, 6, and 7 (n=8 per group except group 5 (n=7); 266±8g, 272±11g, and 266±13g, respectively):** male rats co-treated with 10, 50 and 100 mg/kg/day of CYC dissolved in sesame oil, respectively, plus 10 mg/kg/day of Zn sulfate as a supplement.

**Groups 8-14 (n=8 per group except group 11 (n=7); 205±5g, 204±6g, 211±6g, 217±10g, 211±4g, 205±6g, and 210±7g, respectively):** female rats were given the same regimen as male rats in Groups 1-7. The treatment duration for each group was 10 days.

### Drugs

CYC was purchased from Zahravi Pharm Co. (Tabriz, Iran). Each capsule of CYC contains 100 mg of CYC. To prepare the desired concentrations, the drug was dissolved in sesame oil (Barij Esans Co, Isfahan, Iran). The Zn sulfate used in this study was from BDH Co. (London, England) with 99% purity.

### Treatments

Based on the design of the experimental groups, CYC was administrated daily by subcutaneous injection and for 10 consecutive days. The Zn sulfate was also given daily by intra-peritoneal injection (*i.p.*) for 10 consecutive days based on body weight.

### Measurements

Mortality rate for each group was recorded daily, and the remaining surviving animals (until the 11<sup>th</sup> day) were subjected to placement in metabolic cages for 3 hour urine collection. The volume of urine was determined by scaled micro tube. Finally, the blood samples were obtained and the animals were sacrificed humanely. Then, kidney tissue was fixed in 10% formalin to perform histological evaluation using H&E staining. The tissue damage in the stained tissue (as kidney tissues damage score; KTDS) was scored by a pathologist who was blinded to the study protocol. The score was assigned from 1 to 4 based on intensity of tissue damage while zero was considered as normal. The intensity of tissue damage was considered

based on criteria of vacuolization, dilatation, hyaline cast, debris or degeneration.

The levels of blood urea nitrogen (BUN) and creatinine (Cr) were determined using quantitative diagnostic kits (Pars Azmoon, Iran) by automatic analyzer RA-1000 (Technicon, Ireland). The levels of sodium (Na) in serum and urine were determined by flame photometer assay using Flam Fp20 Model (Seac Co, Italy). The Cr clearance (ClCr) was calculated by the clearance formula:  $ClCr = \text{urine flow (UF)} \times \text{urine Cr level} / \text{serum Cr level}$ .

### Statistical Analysis

Data were reported as mean±SEM. The one way ANOVA and Student's t-test for quantitative data were used to compare the measured parameters between the groups. The Kruskal Wallis H and Mann-Whitney tests were applied to compare the histology findings between the groups. Indeed, p-value <0.05 was considered statistically significant.

## RESULTS

### Animal survival and weight change

The data for animal survival time were tabulated in **Tables 1 and 2**. The entire male and female animals treated with high dose CYC (100 mg/kg/day) expired during the experiment and no animals survived on the last day of experiment (11<sup>th</sup> day- sacrifice day). The survival time of male rats in groups 1-7 were 11.0±0.0, 10.8±0.2, 10.6±0.3, 4.8±0.4, 11.0±0.0, 9.6±0.8 and 3.7±0.5 days (P<0.0001), while the survival time of female rats in groups 8-14 were 11.0±0.0, 11.0±0.0, 10.5±0.3, 7.0±0.7, 11.0±0.0, 9.7±0.8 and 5.1±0.4 days (P<0.0001), respectively (**Tables 1 and 2**).

In male rats, the percentage change of weight of animals in the CYC alone treatment groups were significantly less than vehicle-treated group (vehicle: 11.52±2.3%, CYC 10: 1±4.3%, CYC 50: -1±4.5%, P<0.05). In addition, the percentage change of weight loss in male rats co-treated with CYC (50 mg/kg/day) and Zn (-16.15±1.9 %) was significantly greater than CYC (50 mg/kg) alone treated group (-1±4.50 %) (P<0.05). However, in female rats a greater percentage of weight loss was observed in CYC (50 mg/kg/day), with or without Zn, treatment groups (-14.39±1.89 and -15.50±2.69 %, P<0.001) (**Figure 1**). CYC alone administration increased the kidney weight (KW) normalized to body weight (BW) in male rats significantly when compared with vehicle group (vehicle; 0.67±0.02, CYC 10: 0.72±0.01 g/ 100 g BW, CYC 50: 0.76±0.02 g/ 100 g BW, P<0.05), while addition of Zn supplement did not alter KW towards

**Table 1: The mean values of survival time for each group of male and female rats during the experiment**

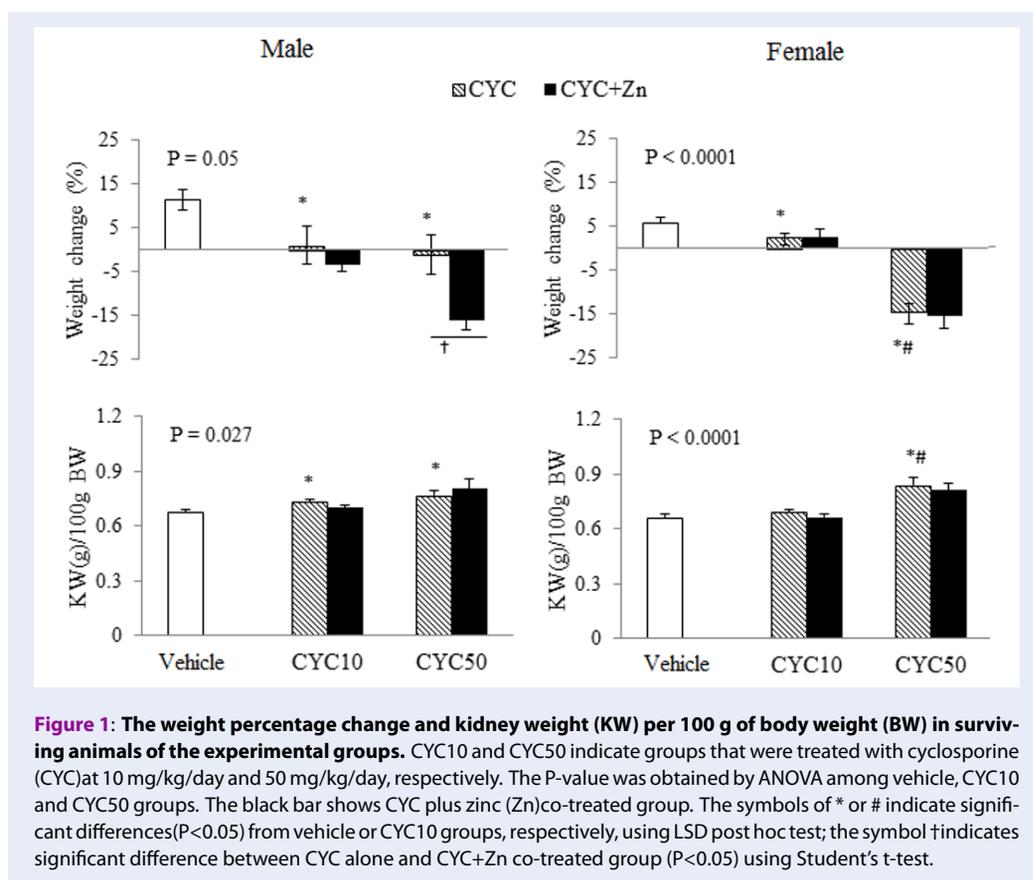
Group	n	Treatment/Day	Number of Death Animals (Male)										Survived	Survival Time (day)	
			1	2	3	4	5	6	7	8	9	10			
1	8	Vehicle	-	-	-	-	-	-	-	-	-	-	-	8	11.0±0.0
2	8	CYC10	-	-	-	-	-	-	-	-	-	1	-	7	10.8±0.2
3	8	CYC50	-	-	-	-	-	-	-	-	1	1	-	6	10.6±0.3
4	8	CYC100	-	-	-	4	3	-	1	-	-	-	-	0	4.8±0.4 <sup>a,b,c</sup>
5	7	CYC10 +Zn	-	-	-	-	-	-	-	-	-	-	-	7	11.0±0.0
6	8	CYC50+Zn	-	-	-	-	1	-	-	1	1	-	-	5	9.6±0.8
7	8	CYC100+Zn	-	1	4	-	2	1	-	-	-	-	-	0	3.7±0.5
P Kruskal-Wallis H (between groups 1-4)														P<0.0001	

CYC10, CYC50 and CYC100 represent the groups treated with 10, 50 and 100 mg/kg/day of cyclosporine respectively. The small alphabetic letters indicate a significant difference from (a) vehicle, (b) CYC10 and (c) CYC50 (P<0.05), as obtained by the Kruskal-Wallis H Test. Each zinc (Zn) + CYC treated group was compared with similar CYC alone treated group, without Zn, using Mann-Whitney test. See the text for the treatment groups.

**Table 2: The mean values of survival time for each group of male and female rats during the experiment (continued)**

Group	n	Treatment/Day	Number of Death Animals (Female)										Survived	Survival Time (day)	P-value between genders) Mann-Whitney Test	
			1	2	3	4	5	6	7	8	9	10				
8	8	Vehicle	-	-	-	-	-	-	-	-	-	-	-	8	11.0±0.0	1.0
9	8	CYC10	-	-	-	-	-	-	-	-	-	-	-	8	11.0±0.0	0.32
10	8	CYC50	-	-	-	-	-	-	-	-	1	2	-	5	10.5±0.3 <sup>a,b</sup>	0.65
11	8	CYC100	-	-	-	-	3	1	-	2	1	1	-	0	7.0±0.7 <sup>a,b,c</sup>	0.06
12	8	CYC10 +Zn	-	-	-	-	-	-	-	-	-	-	-	8	11.0±0.0	1.0
13	8	CYC50+Zn	-	-	-	-	-	2	-	-	-	1	-	5	9.7±0.8	0.70
14	8	CYC100+Zn	-	-	1	1	2	4	-	-	-	-	-	0	5.1±0.4	0.05
P Kruskal-Wallis H (between groups 8-11)														P<0.0001		

CYC10, CYC50 and CYC100 represent the groups treated with 10, 50 and 100 mg/kg/day of cyclosporine respectively. The small alphabetic letters indicate a significant difference from (a) vehicle, (b) CYC10 and (c) CYC50 (P<0.05), as obtained by the Kruskal-Wallis H Test. Each zinc (Zn) + CYC treated group was compared with similar CYC alone treated group, without Zn, using Mann-Whitney test. See the text for the treatment groups.



normal in CYC 10 ( $0.70 \pm 0.01$ g/ 100 g BW) or CYC 50 ( $0.80 \pm 0.05$ g/ 100 g BW) groups. In female rats, the normalized KW in vehicle, CYC10, CYC50, CYC10+Zn, and CYC 50+Zn groups were  $0.66 \pm 0.01$ ,  $0.69 \pm 0.01$ ,  $0.84 \pm 0.04$ ,  $0.66 \pm 0.01$  and  $0.81 \pm 0.03$ g/ 100 g BW, respectively. These findings indicated that the high dose of CYC (50 mg/kg/day) alone increased the normalized KW significantly ( $P < 0.05$ ), and that Zn supplement did not alter it.

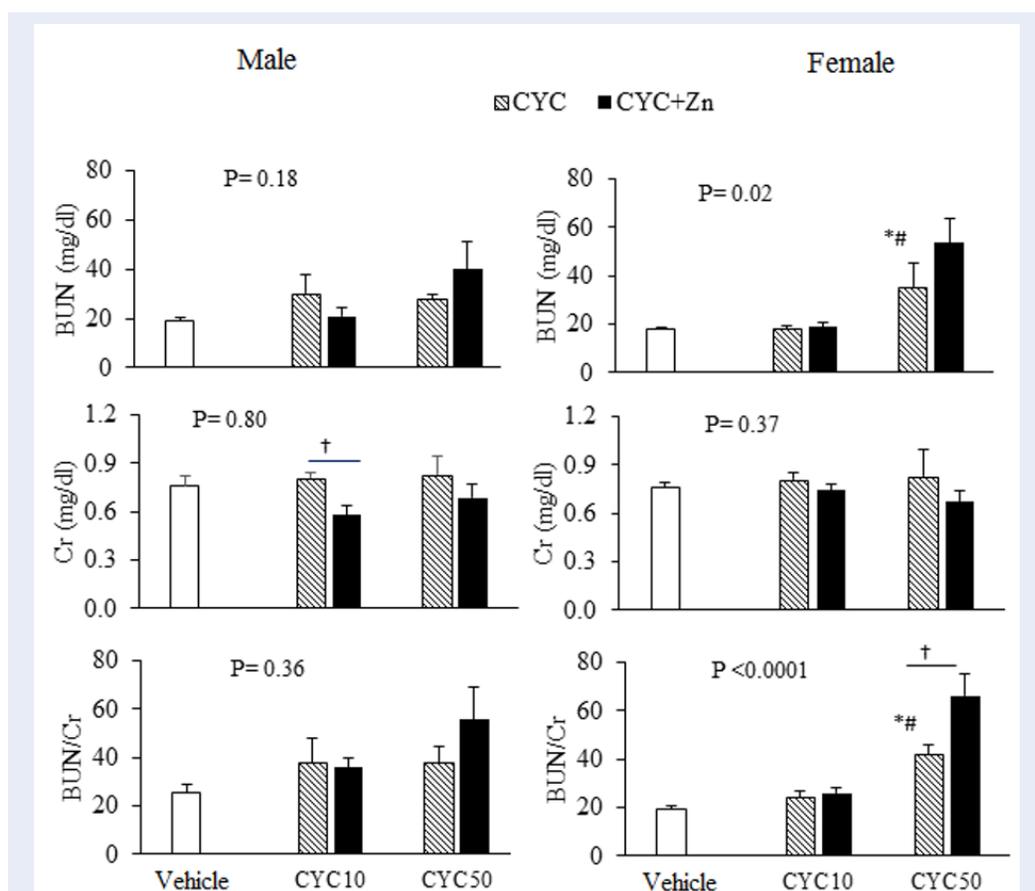
### The serum levels of blood urea nitrogen (BUN) and Creatinine (Cr)

In male rats, the levels of BUN in vehicle, CYC10, CYC50, CYC10+Zn and CYC50+Zn groups were  $18.69 \pm 1.42$ ,  $30.14 \pm 7.62$ ,  $27.95 \pm 2.02$ ,  $20.82 \pm 3.43$  and  $39.81 \pm 11.31$  mg/dL, respectively, while the serum levels of Cr in these groups were  $0.76 \pm 0.06$ ,  $0.81 \pm 0.03$ ,  $0.82 \pm 0.11$ ,  $0.58 \pm 0.06$  and  $0.69 \pm 0.08$  mg/dl. The serum levels of BUN/Cr ratio were also evaluated in male rats groups of vehicle; CYC10, CYC50, CYC10+Zn and CYC50+Zn were  $25.88 \pm 3.04$ ,  $37.94 \pm 9.84$ ,  $37.92 \pm 6.79$ ,  $36.01 \pm 4.07$  and  $55.83 \pm 13.07$ , respectively. The result indicated that

the serum level of BUN, Cr and BUN/Cr ratio increased insignificantly in male rats treated with CYC, and that Zn supplement decreased the serum levels of BUN insignificantly and Cr significantly ( $P < 0.05$ ) in low dose of CYC-treated rats (Figure 2, left panel). In female rats, the levels of BUN in vehicle, CYC10 and CYC 50, respectively, were  $17.84 \pm 1.18$ ,  $17.96 \pm 1.16$  and  $35.37 \pm 9.83$  mg/dL. However, the Zn supplement did not attenuate these markers toward normal levels. The serum level of BUN in CYC 10+Zn and CYC50+Zn groups were  $18.86 \pm 1.78$  and  $53.73 \pm 10.11$ mg/dL, respectively (Figure 2, right panel). These results indicated that the CYC (50 mg/kg/day) increased the serum levels of BUN and BUN/Cr ratio significantly ( $P < 0.05$ ) in females.

### Renal function parameters

The findings related to renal function parameters were normalized to KW. The normalized UF in male rats for vehicle, CYC10, CYC50, CYC10+Zn and CYC50+Zn groups were  $6.91 \pm 1.81$ ,  $2.84 \pm 1.35$ ,  $3.42 \pm 1.21$ ,  $2.87 \pm 0.48$  and  $4.74 \pm 0.62$   $\mu$ L/min/g tissue, while the normalized ClCr in these groups were  $465.90 \pm 120.29$ ,  $249.19 \pm 54.09$ ,  $305.27 \pm 90.80$ ,



**Figure 2: Serum levels of blood urea nitrogen (BUN), creatinine (Cr) and BUN/Cr ratio in surviving animals of the experimental groups.** CYC10 and CYC50 represent the groups treated with cyclosporine (CYC) at a dose of 10 mg/kg/day or 50 mg/kg/day, respectively. The P-value was obtained by ANOVA among vehicle, CYC10 and CYC50 groups. The black bar shows CYC plus zinc (Zn) co-treated group. The symbols of \* or # indicate significant differences ( $P < 0.05$ ) from vehicle or CYC10 groups, respectively, using LSD post hoc test. The symbol † indicates a significant difference between CYC alone and CYC+Zn co-treated group ( $P < 0.05$ ) using Student's t-test.

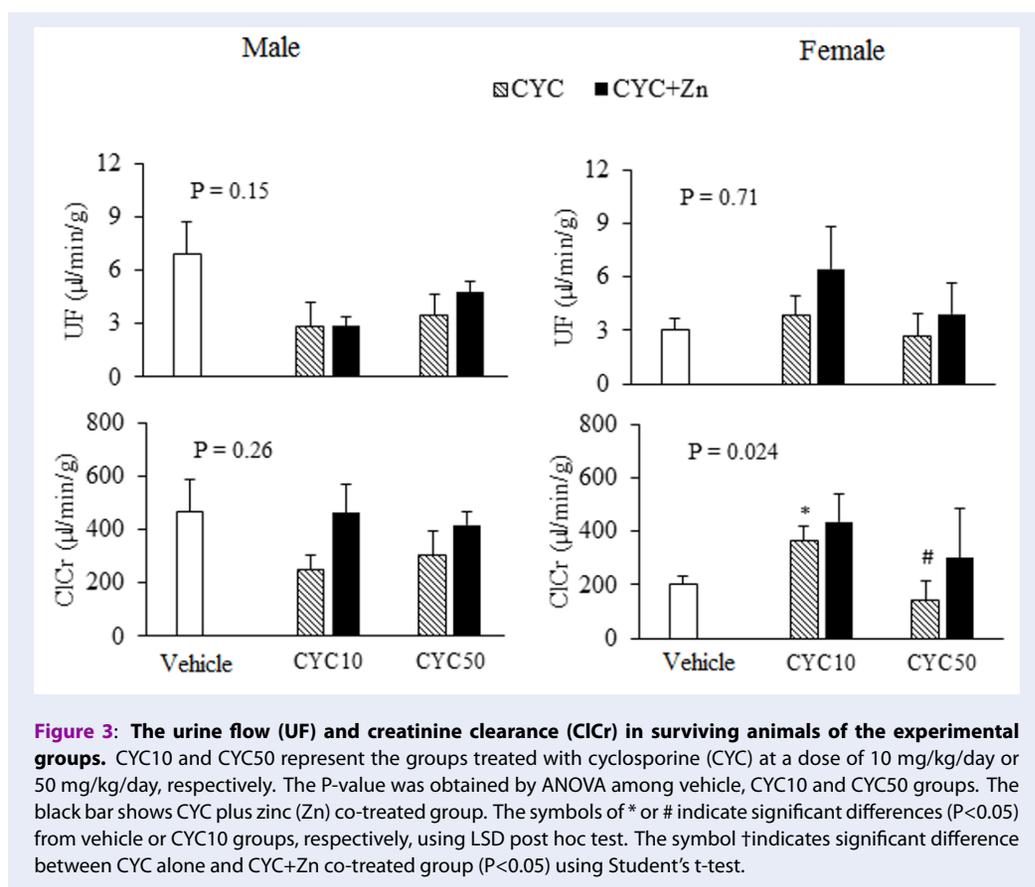
460.82±107.20 and 417.58±51.54  $\mu\text{L}/\text{min}/\text{g}$  tissue, respectively. The results revealed that CYC decreased UF and  $\text{ClCr}$  in male rats insignificantly.

The normalized UF in female rats for vehicle, CYC10, CYC50, CYC10+Zn and CYC50+Zn groups were 3.05±0.60, 3.83±1.04, 2.67±1.26, 6.43±2.36 and 3.89±1.78  $\mu\text{L}/\text{min}/\text{g}$  tissue, and the normalized  $\text{ClCr}$  in the mentioned groups were 201.01±33.10, 366.03±50.18, 138.63±74.82, 435.75±100.60 and 300.53±186.55  $\mu\text{L}/\text{min}/\text{g}$  tissue, respectively. These findings showed that CYC (at dose of 10 mg/kg/day) alone increased  $\text{ClCr}$  significantly in female rats when compared with vehicle group ( $P < 0.05$ ) (Figure 3). However, Zn supplement did not provide the protective effect in either males or females.

In male rats, the normalized Na filtration rate in the vehicle, CYC10, CYC50, CYC10+Zn, and CYC50+Zn

groups were 75.52±18.98, 42.69±9.38, 51.39±15.71, 80.66±20.69, 93.73±9.12  $\mu\text{mol}/\text{min}/\text{g}$  tissue, respectively, while the normalized Na excretion rate in these groups were 1.19±0.18, 0.38±0.16, 0.36±0.11, 0.60±0.11, 0.39±0.16  $\mu\text{mole}/\text{min}/\text{g}$  tissue and the percentage of Na excretion fraction were 1.96±0.29, 0.72±0.19, 0.72±0.16, 0.88±0.21 and 0.40±0.15%, respectively. The results analyses indicated that the CYC alone decreased Na filtration rate (insignificantly), Na excretion rate, and percentage of Na excretion fraction (significantly,  $P < 0.05$ ) in male rats (Figure 4, left panel).

Similar observation for the normalized Na excretion rate and percentage of Na excretion fraction were also observed in female rats (Figure 4, right panel). The normalized Na filtration rate for the vehicle, CYC10, CYC50, CYC10+Zn, CYC50+Zn female groups were



**Figure 3: The urine flow (UF) and creatinine clearance (ClCr) in surviving animals of the experimental groups.** CYC10 and CYC50 represent the groups treated with cyclosporine (CYC) at a dose of 10 mg/kg/day or 50 mg/kg/day, respectively. The P-value was obtained by ANOVA among vehicle, CYC10 and CYC50 groups. The black bar shows CYC plus zinc (Zn) co-treated group. The symbols of \* or # indicate significant differences ( $P < 0.05$ ) from vehicle or CYC10 groups, respectively, using LSD post hoc test. The symbol † indicates significant difference between CYC alone and CYC+Zn co-treated group ( $P < 0.05$ ) using Student's t-test.

34.19 $\pm$ 5.27, 67.31 $\pm$ 9.82, 24.72 $\pm$ 13.44, 77.43 $\pm$ 18.55, 51.20 $\pm$ 31.60  $\mu\text{mol}/\text{min}/\text{g}$  tissue, while the Na excretion rate in these groups were 0.59 $\pm$ 0.09, 0.30 $\pm$ 0.05, 0.06 $\pm$ 0.05, 0.59 $\pm$ 0.13, 0.47 $\pm$ 0.23  $\mu\text{mol}/\text{min}/\text{g}$  tissue, respectively. In the similar female rat groups, the percentage of Na excretion fractions were 1.81 $\pm$ 0.28, 0.46 $\pm$ 0.07, 0.25 $\pm$ 0.08, 0.85 $\pm$ 0.11, and 1.05 $\pm$ 0.19%, respectively. As the data show, the Zn supplement increased the Na excretion rate and the percentage of Na excretion fraction, only in female rats.

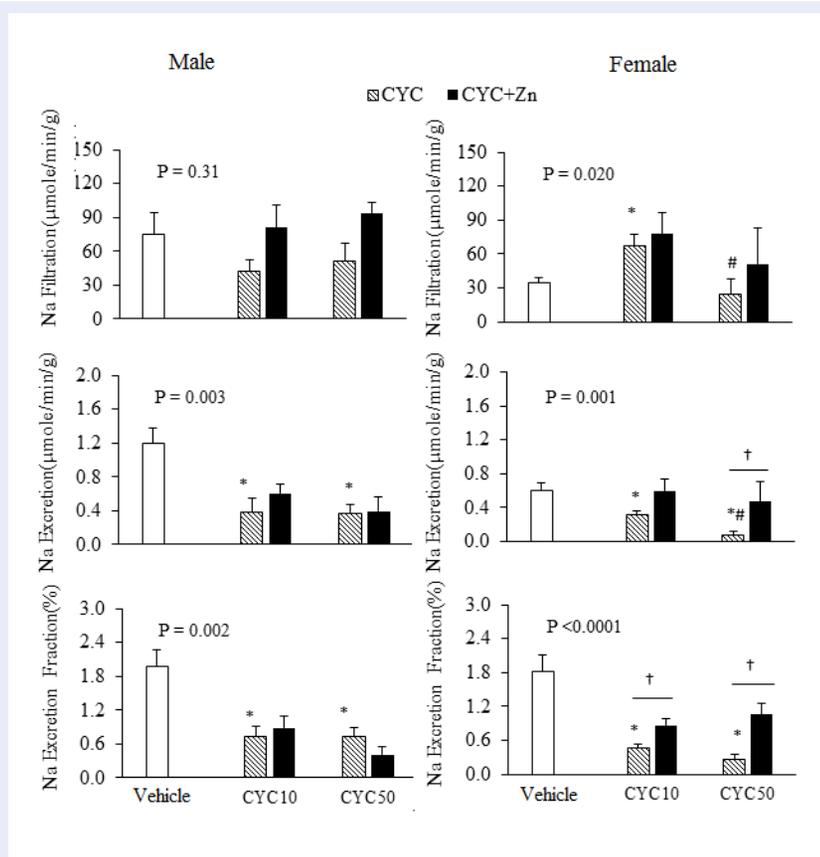
### The kidney histology findings

In the male rats, the mean value of KTDS for vehicle, CYC10, CYC50, CYC100, CYC10+Zn and CYC50+Zn, CYC100+Zn groups were 0.5 $\pm$ 0.18, 1.37 $\pm$ 0.18, 1.75 $\pm$ 0.16, 2.42 $\pm$ 0.20, 0.66 $\pm$ 0.21, 2 $\pm$ 0.18, 2.7 $\pm$ 0.18, while in the female gender of above groups these findings were 0.5 $\pm$ 0.18, 1.62 $\pm$ 0.26, 2 $\pm$ 0.20, 2.71 $\pm$ 0.18, 1 $\pm$ 0.18, 2.25 $\pm$ 0.26, 2.83 $\pm$ 0.16, respectively. The histology data indicated that KTDS was increased by CYC dose dependently of both male and female rats (Figure 5). Hence, Zn supplement decreased the KTDS in male and female rats treated

with CYC (10 mg/kg/day) significantly ( $P < 0.05$ ). The samples of images from the animals in each group of experiment are shown in Figure 6.

## DISCUSSION

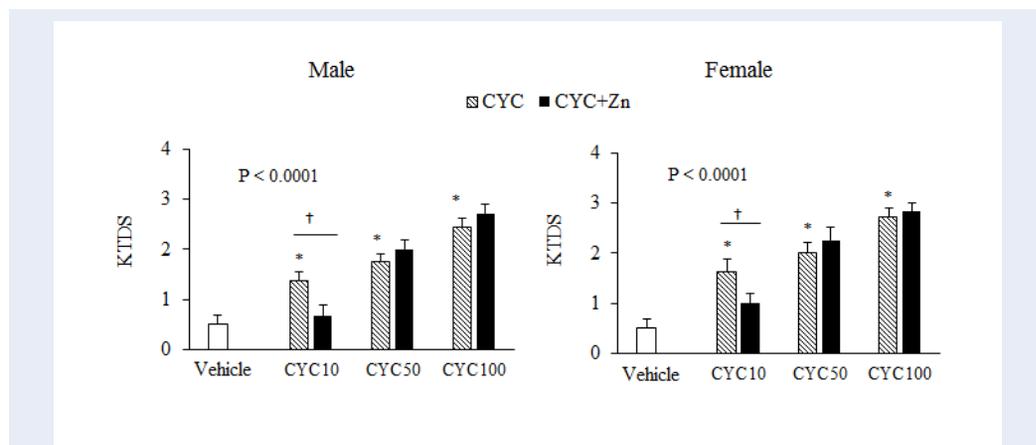
The major findings of this study indicated that CYC-induced nephrotoxicity was dose-related in both male and female rats. In addition, Zn supplementation accompanied with low dose of CYC (10 mg/kg/day) attenuated CYC and induced tissue damage. The protective role of Zn in male rats treated with low dose of CYC (10 mg/kg/day) also was observed by attenuation of serum level of Cr. In addition, when UF, ClCr, Na filtration rate, Na excretion rate and Na excretion fraction are considered, the data indicated that low dose of CYC accompanied with Zn increased all the mentioned markers insignificantly. On the contrary, when KW and body weight change were targeted, Zn supplementation with low dose of CYC did not alter weight loss and KW. Finally, if pathological findings are looked as ultimate findings, the interpretation of the data will be easier, and we can assumed that Zn could be a protectant agent against CYC (10 mg/kg/day) induced nephrotoxicity. CYC



**Figure 4: The sodium (Na) filtration rate, Na excretion rate and Na excretion fraction (%) in surviving animals of the experimental groups.** CYC10 and CYC50 represent the groups treated with cyclosporine (CYC) at 10 mg/kg/day or 50 mg/kg/day dose, respectively. The P-value was obtained by ANOVA among vehicle, CYC10 and CYC50 groups. The black bar shows CYC plus zinc (Zn) co-treated group. The symbols of \* or # indicate significant differences ( $P < 0.05$ ) from vehicle or CYC10 groups, respectively, using LSD post hoc test. The symbol † indicates a significant difference between CYC alone and CYC+Zn co-treated group ( $P < 0.05$ ) using Student's t-test.

reduced the body weight percentage change and increased the normalized KW. The weight gain by CYC was reported before<sup>43</sup>. Under conditions of induced nephrotoxicity, both body weight loss and KW gain occurred<sup>25-27</sup>. Together with BUN, Cr and BUN/Cr ratio data, it seems that weight loss and KW gain are related to CYC induced nephrotoxicity, which are confirmed by pathology findings. The weight loss and KW gain by CYC (50 mg/kg) was different in female rats; this difference may be related to body water content. Male and female rats have different distribution of body fluid compartment, and this fact may affect the alteration of markers, such as body weight loss, KW gain and the serum level of BUN. In addition, Zn supplementation decreases the serum levels of BUN and Cr in male rats treated with low dose of CYC (10 mg/kg/day) when compared with CYC alone treated rats (Figure 2). Such observation was not detected in

female. The protective effect of Zn in kidney injury may be dose and gender related<sup>34,39</sup>, and actually the involved mechanisms is not well clear. Some of the renal function markers also were different between genders. Collectively, this difference possibly is related to sex hormones. CYC affects sex hormones<sup>44,45</sup>, and *in-vitro* study demonstrated that the therapeutic dose of CYC may alter ovarian function<sup>45</sup>. The Zn also may protect the kidney gender dependently<sup>39</sup>. Therefore, the different responses from the measured markers to CYC and Zn in male and female rats may be expected, but the exact mechanisms need to be defined. There is one limitation in our study related to dose of Zn. In the current study, we only used 10 mg/kg/day of Zn sulphate, and possibly this dose of Zn supplement may be appropriate for a lower dose of CYC. A higher dose of CYC (50 mg/kg/day) needs higher doses of Zn sulphate.



**Figure 5: The kidney tissue damage score (KTDS) for the entire experimental groups.** CYC10, CYC50 and CYC100 indicate the groups treated with cyclosporine (CYC) at a dose of 10 mg/kg/day, 50 mg/kg/day, or 100 mg/kg/day, respectively. The P-value was obtained by Kruskal-Wallis H Test. The co-treatment of zinc (Zn) and CYC groups were compared to that of CYC alone with or without the treated group, using Mann Whitney Test. The black bar shows CYC+Zn treated group. The symbols of \* or # indicate significant differences ( $P < 0.05$ ) from vehicle or CYC10 groups respectively using Mann Whitney Test, and the symbol † indicates significant difference between CYC alone and CYC+Zn treated group ( $P < 0.05$ ).

## CONCLUSIONS

The high dose of CYC (100 mg/kg) demonstrated the highly toxic effect. No animals survived on the last day of experiment, and the other dose of CYC induced nephrotoxicity gender dependently. However, the 10 mg/kg of Zn sulphate as a supplement may prevent induced nephrotoxicity in males, possibly due to its antioxidant effects.

## COMPETING INTERESTS

The authors have declared that no conflict of interest exists

## AUTHORS' CONTRIBUTIONS

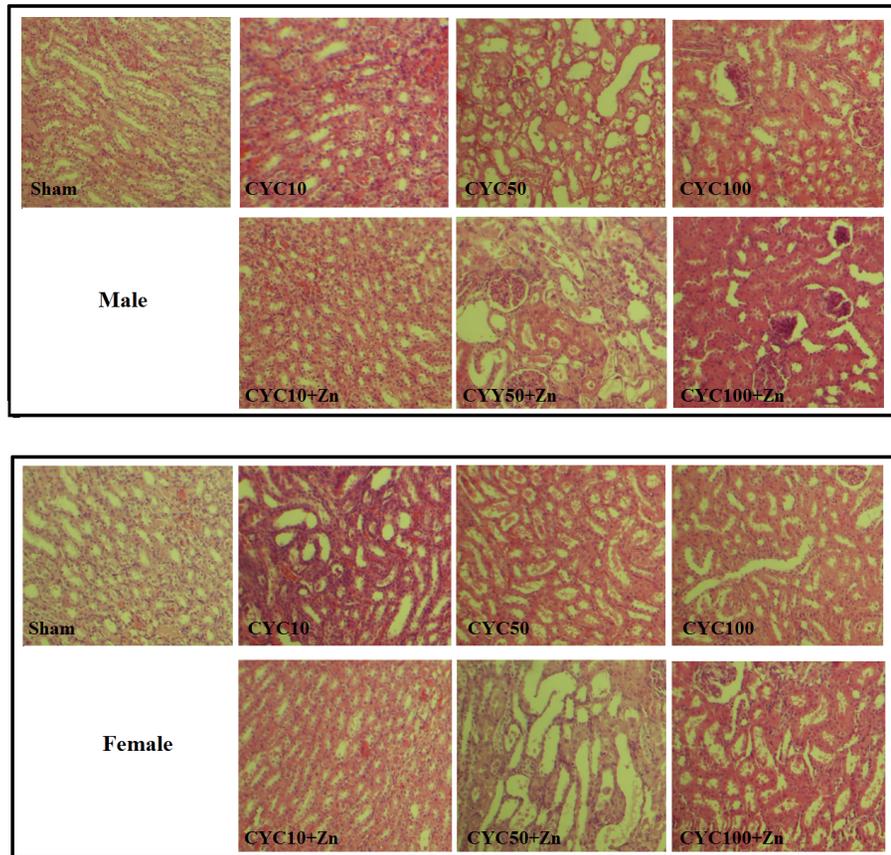
SC, SK and AT conducted the experimental procedures and data analysis, MM, YG, and MM conducted study design, and finalized the article. MN conducted study design, experimental procedures, data analysis and finalized the article.

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**Figure 6: The kidney tissue images with magnification of 100X.** The CYC10, CYC50 and CYC100 represent the groups treated with cyclosporine (10 mg/kg/day, 50 mg/kg/day and 100 mg/kg/day, respectively). The CYC10+Zn, CYC50+Zn and CYC100+Zn indicate the groups treated with cyclosporine (CYC: 10 mg/kg, 50 mg/kg and 100 mg/kg plus 10 mg/kg) and of zinc (Zn) sulfate, respectively.

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