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# Protective effects of green tea polyphenol against cisplatin-induced nephrotoxicity in rats

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**Objective**
This study is to compare the effects of green tea polyphenol (GTP) pre-treatment with those of GTP post-treatment on cisplatin (CP)-induced nephrotoxicity in rat.

**Methods**
Male Sprague-Dawley rats were randomly divided into six groups. Animals in the control group received 0.9% saline (intraperitoneal); animals in the GTP group received 0.9% saline and GTP (0.2% GTP as their sole source of drinking water); the CP group received only CP (7 mg/kg, intraperitoneal); the CP+preGTP group received GTP from two days before CP to four days after CP and the CP+postGTP group received GTP for four days after CP. CP-induced renal toxicity was evaluated by plasma creatinine and blood urea nitrogen (BUN) concentrations; kidney tissue γ-glutamyl transpeptidase (GGT) and alkaline phosphatase (AP) activities and histopathological examinations.

**Results**
High serume creatinine and BUN concentrations were observed in CP treated rats. The GGT and AP activites were lower in kidney of CP treated rats compared to control rats. In addition, treatment with CP resulted in development of a marked tubular necrosis, and tubular dilation in kidney of rats. Pretreatment with GTP resulted in markedly reduced elevation of serum creatinine and BUN amounts and changes of GGT and AP activity in kidney induced by CP. CP-induced histopathological changes, including tubular necrosis and dilation, were ameliorated in GTP pre-treated rats, compared to CP alone or GTP post-treated rats

**Conclusion**
These results demonstrate that GTP might have some protective effect against CP-induced nephrotoxicity in rat, and GTP pre-treatment was more effective than GTP post-treatment on reduction of CP-induced renal dysfunction.

**Keywords:** Cisplatin; Green tea polyphenol; Nephrotoxicity

## Introduction

Cisplatin (CP, cis-diaminedichloroplatinum II) was developed as anticancer agent around 1970s and has been used as chemotherapeutic agent in wide range of human cancer treatment [1,2]. Although anticancer chemotherapy is a very effective cancer treatment method since it can be applied regardless of cancer type or by disease stage [3], the side effects of resistant cancer cells and chemotherapeutic agents can be named as a large obstacle of anticancer chemotherapy [4]. By the use of CP, many types of side effects as like nephrotoxicity, acoustic nerve toxicity, neurotoxicity, myelosuppression, nausea and vomiting can appear [5]. Nephrotoxicity is a major toxic side effect that occurs at 20% of the patients who receive the CP treatment, and it may limit the use of CP at the time of performing anticancer treatment [6].

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CP has been known to induce oxidative damages and inflammative responses in cells, which increases lipid peroxidation and causes DNA damages in cells and consequently causes damages on proximal and distal convoluted tubules in kidney [6,7]. Since freed groups are related to the renal damages that are caused by CP, there are studies that reported about the presence of CP toxicity inhibition effects by the use of the following antioxidative materials including vitamin E, vitamin C, carotenoids, selenium and elloagic acids [8-10]. Flavonoids that are contained in a plant have been known to have several physiological activities including anti-inflammative and antioxidative activities [11].

It has been known that green tea (*Camellia sinensis* L.) contains strong antioxidative flavonoids. There is a study that aimed to apply these flavonoids for the prevention and treatment of chronic diseases [12]. Among the flavonoids that are contained in green tea, polyphenol type of tannins and catechins can be acquired. Among these catechins, epicatechin, epicatechin-3-gallate, epigallocatechin and epigallocatechin-3-gallate (EGCG) are the prevalent types that are contained in green tea. EGCG has been reported to have a preventive and treatment effects on several types of chronic diseases along with its anticancer effects [13,14]. As a study that reported about the inhibitory effect of EGCG on the toxicity of CP, El-Mowafy et al. [15] reported about the preventive effect of renal damage by performing Intraperitoneal injection of EGCG for the prevention of renal damages that were caused by CP. Sahin et al. [16] reported about that the injection of EGCG can reduce the oxidative renal damages and inflammative reactions that were caused by CP. Also, Khan et al. [17] reported about the prevention of CP induced renal damage effects that were acquired by green tea extracts. Since the renal damage protective effects that are observed by the administration of green tea extracts or EGCG can reduce the major nephrotoxicity at the time of performing chemotherapy of using CP, it is considered to be a very useful adjuvant when performing the anticancer chemotherapies.

The present study was performed by using white male Sprague Dawley rats to verify the applicability of green tea polyphenols (GTPs) as the adjuvant of anticancer chemotherapy. To observe its different effects on nephrotoxicity based on the administration time of CP and GTPs, the Sprague Dawley rats were randomly divided into the group that administered with GTPs before injecting CP agent and the other group that administered green tea after treating with CP agent. Biochemical and histopathological investigations were made on their kidney samples to compare the difference of nephrotoxicity between the two groups.

## Materials and methods

### 1. Experimental animals and experimental groups

At the present experiment, the white male Sprague-Dawley rats that were weighed about 180 to 200 g of body weight were used. The mice were purchased from the Samtaco (Daejeon, Korea) and bred under the environment by providing 12 hour light: dark cycle, temperature of 20±2℃ and relative humidity of 60±5%. The CP that was used at the experiment and GTP (polyphenon 60) were purchased from the Sigma Chemical (St Louis, MO, USA). Twenty-five white mice were randomly assigned into the following 5 test groups of the control group, GTP administered group, CP treated group, GTP administered before treating with CP (CP+preGTP), and GTP administered after treating with CP (CP+postGTP). For the control group, tap water was provided as drinking water, For the case of GTP group, GTP was administered after performing the intraperitoneal injection of 0.9% physiological solution, CP group was injected with CP, For the CP+preGTP group, CP was provided at the 2nd days after the administration of GTP was completed and the GTP was then administered again for the next 4 days. For the CP+postGTP group, the GTP was administration was performed for 4 days after the CP administration. The intraperitoneal injection of CP was performed by dissolving CP in a physiological solution (7 mg/kg body weight), and GTPs were provided as drinking water by dissolving in tap water by setting the concentration of 0.2% (w/v) in everyday.

For the control group, tap water was provided as drinking water, For the case of GTP group, GTP was administered after performing the intraperi¬toneal injection of 0.9% physiological solution, CP group was injected with CP, For the CP+preGTP group, CP was provided at the 2nd days after the administration of GTP was completed and the GTP was then administered again for the next 4 days. For the CP+postGTP group, the GTP was administration was performed for 4 days after the CP administration. The intra¬peritoneal injection of CP was performed by dissolving CP in a physiological solution (7 mg/kg body weight), and GTPs were provided as drinking water by dissolving in tap water by setting the concentration of 0.2% (w/v) (Table 1).

**Table 1.** Experimental design

| Experimental groups | Intraperitoneal injection (0.5 mL) | Drinking water |
|---|---|---|
| Control | NS | Tap water |
| GTP | NS | GTP |
| CP | CP | Tap water |
| CP+preGTP | CP | GTP (2 days before and 4 days after CP injection) |
| CP+postGTP | CP | GTP (4 days after CP injection) |

NS, normal saline; GTP, green tea polyphenol (0.2% w/v); CP, cisplatin (7 mg/kg body weight).

### 2. Sampling and biochemical tests

At the 4th day after the injection of CP, experimental animals were anesthetized by using ether. After opening the abdomen, the blood of experimental animals was collected and kidney was collected to be used as the sample to measure enzymatic activities or as the sample to be used for histopathological evaluations. The collected blood was coagulated for the serum separation to be used as the sample for the quantification of blood urea nitrogen (BUN) and creatinine. Both of serum BUN and creatinine quantities were measured by using the automatic hematology analyzer (Hitachi 7180, Hitachi, Tokyo, Japan). For the measurement of kidney enzyme activity, approximately 0.2 g of kidney tissue was homogenized by adding 10 times of 0.1 M phosphate buffer solution (pH 7.4) and used as the sample to measure the activities of alkaline phosphatase (AP) and γ-glutamyl transpeptidase (GGT). The enzymatic activity of GGT was measured by using the method of Tate and Meister [18], which measured the quantity of p-nitroanilide that was produced from γ-glutamyl-nitroanilide, and the AP activity was measured by the method of Tenenhouse et al. [19], which quantified the amount of p-nitrophenol that was produced from p-nitrophenyl phosphate. Protein measurement was made by using the total protein kit that was manufactured by the Sigma-Aldrich (St Louis, MO, USA). The enzyme activity was expressed as μmoles/mg protein/hr.

### 3. Histopathological tests

Portions of the kidney that was collected from experimental animals were immediately used for the sufficient fixation at 10% formalin solution and paraffin formatted after dehydrating the samples, and then the tissue was sliced as the sections with 4 μm thickness and stained with hematoxylin and eosin (H&E) for the 200 folded optical microscopic observation. The following categories of renal tubules including tubular necrosis, tubular

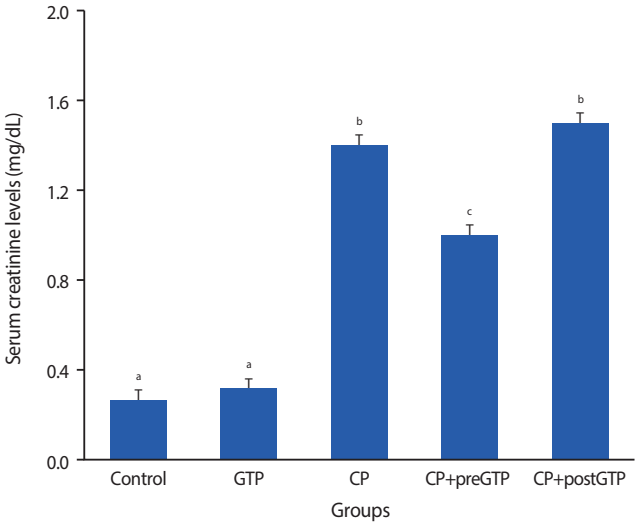


**Fig. 1.** The effects of dietary green tea polyphenol (GTP) on creatinine levels in cisplatin (CP) treated rats. Rats were administered normal saline (control, intraperitoneal injection), GTP (0.2% GTP drinking water) alone, CP alone (7 mg/kg body weight, intraperitoneal injection), CP+preGTP (GTP received from 2 days before CP to 4 days after CP) and CP+postGTP (GTP received 4 days after CP); creatinine levels were determined 4 days after these treatments. Values are mean±standard deviation (n=5). [a,b,c] Values with different superscripts are significantly different at the $P<0.01$ by Duncan's multiple range test.

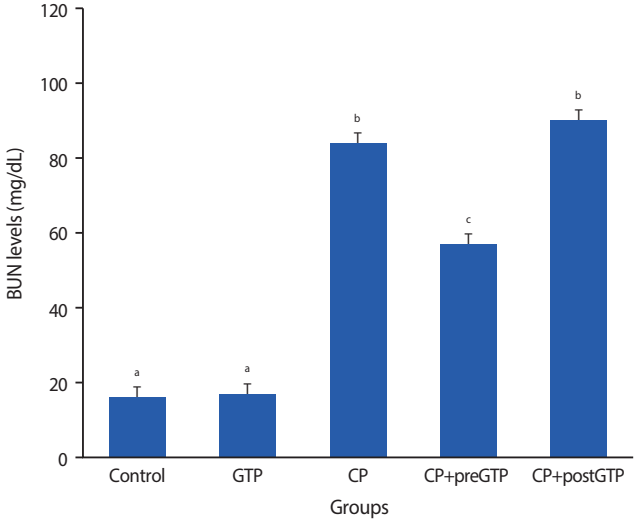


**Fig. 2.** The effects of dietary green tea polyphenol (GTP) on blood urea nitrogen (BUN) amounts in cisplatin (CP) treated rats. Rats were administered normal saline (control, intraperitoneal injection), GTP (0.2% GTP drinking water) alone, CP alone (7 mg/kg body weight, intraperitoneal injection), CP+preGTP (GTP received from 2 days before CP to 4 days after CP) and CP+postGTP (GTP recieved 4 days after CP); BUN amounts were determined 4 days after these treatments. Values are mean±standard deviation (n=5). [a,b,c] Values with different superscripts are significantly different at the $P<0.01$ by Duncan's multiple range test.

**Table 2.** The effects of dietary GTP on enzyme activities of kidney in cisplatin treated rats

| Groups | GGT | AP |
|---|---|---|
| Control | 45.3±3.3[a] | 15.3±1.0[a] |
| GTP | 42.7±3.2[a] | 14.5±2.0[a] |
| CP | 22.8±5.0[b] | 8.7±0.6[b] |
| CP+preGTP | 33.7±5.2[c] | 11.2±1.5[c] |
| CP+postGTP | 24.1±7.4[b] | 9.3±1.8[b] |

Enzyme activities are expressed moles/mg protein/hr. Values are mean±standard deviation (n=5). [a,b,c] Different superscripts within the same column are significantly different at the $P<0.01$ by Duncan's multiple range test.
GTP, green tea polyphenol; GGT, γ-glutamyl transpeptidase; AP, alkaline phosphates; CP, cisplatin.

**Table 3.** The score of tubular injury

| Groups | GGT | AP |
|---|---|---|
| Control | 0.1±0.3[a] | 0.1±0.3[a] |
| GTP | 0.1±0.3[a] | 0.1±0.3[a] |
| CP | 3.6±0.5[b] | 4.3±0.6[b] |
| CP+preGTP | 2.4±0.5[c] | 3.1±0.6[c] |
| CP+postGTP | 3.4±0.5[b] | 4.1±0.5[b] |

Values are mean±standard deviation (n=5). Tubular injury was scored as follows: 0, no damage; 1, up to 10% of the tubular area showing tubular injury; 2, 10% to 25% of the tubular area showing tubular injury; 3, 25% to 50% of tubular area showing tubular injury; 4, 50% to 75% of tubular area showing tubular injury; 5, more than 75% of the tubular area showing tubular injury. [a,b,c] Different superscripts within the same column are significantly different at the $P<0.01$ by Duncan's multiple range test. GTP, green tea polyphenol; CP, cisplatin.

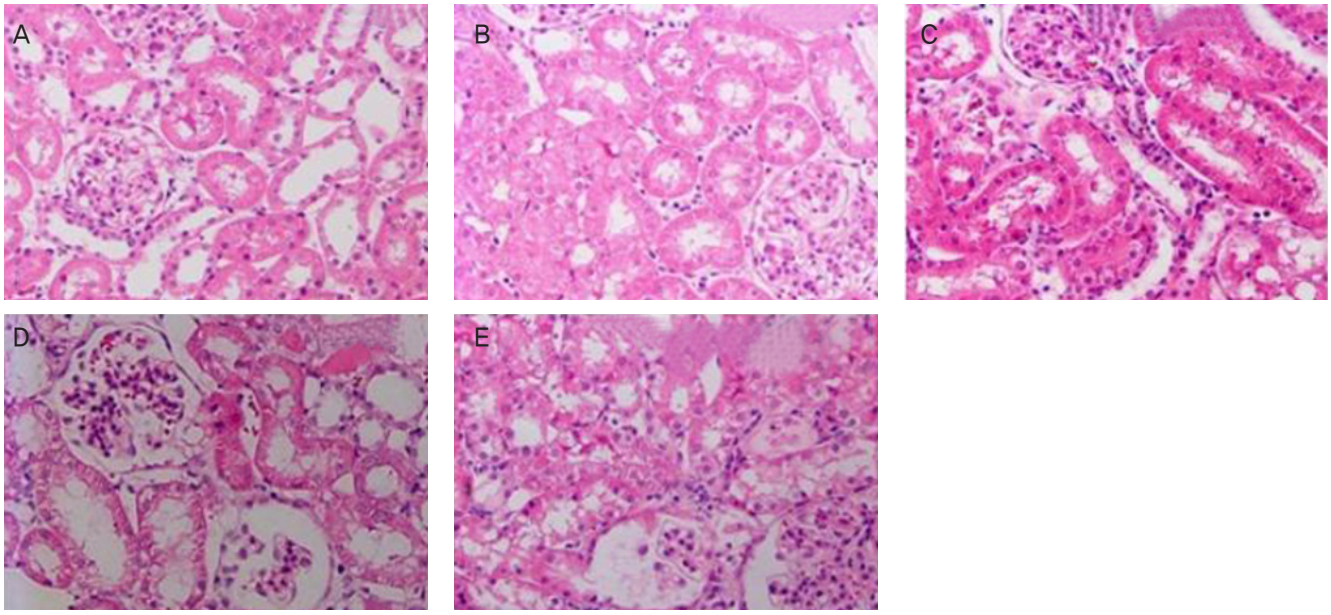


**Fig. 3.** Histopathological analysis in renal cortex in the groups of rats studied: (A) control, (B) green tea extract (GTP), (C) CP+GTP pre-treatment (CP+preGTP), (D) cisplatin (CP), and (E) CP+GTP post-treatment (CP+postGTP). Rats were studied 4 days after CP injection (7 mg/kg). GTP was given in the drinking water (2 g/L) 2 days before and/or 4 days after CP injection (haematoxylin and eosin staining, ×200).

degeneration and tubular dilation were evaluated by using the following criteria: no damage, 0; less than 10% of damages, 1; 10% to 25% of damages, 2; 25% to 50% of damages, 3; 50% to 75% of damages, 4; more than 75% of damages, 5.

### 4. Statistical analysis

The experimental results of the present study were expressed as mean±standard deviation, one-way ANOVA tests were performed for the experimental results by using a SPSS ver. 12.0 (SPSS Inc., Chicago, IL, USA). Significance between samples was determined by using the Duncan's multiple range tests at the probability less than 0.05.

## Results

### 1. Change of serum creatinine and BUN amounts in CP and GTP administered Sprague Dawley rats

Serum creatinine and BUN amount in the GTP and CP administered Sprague Dawley rats could be expressed as in Figs. 1 and 2, respectively. The serum creatinine and BUN amount in the GTP administered group did not show any significant differences, but the CP administered group resulted to show 3.6 fold increase of serum creatinine level by showing 1.45±0.13 mg/dL, and the BUN amount resulted to show 2.6 fold increase by showing 88.26±8.69 mg/dL. Therefore, it was possible to see that the CP administration was responsible for inducing the kidney damages. In addition, the creatinine and BUN amount in the preGTP administered CP administered group respectively ranged about 69% and 63% compared to the CP administered group, which indicated smaller kidney damages than the CP administered group. Contrary to that, the creatinine and BUN amount in the postGTP administered CP administered group respectively ranged about 107% and 109% by showing no significant difference by the post GTP administration compared to the CP administered group. Therefore, the result indicated to show that there is no significant difference in the degree of kidney damages between the postGTP administered CP administered group and the CP administered group.

### 2. Changes of renal GGT and AP activities in CP and GTP administered Sprague Dawley rats

The serum GGT and AP activities that were measured in GTP and CP administered Sprague Dawley rats could be listed as in Table 2. No significant difference of renal GGT and AP activities was observed in the GTP administered group. The renal GGT activity in CP administered group was found to show 49% reduction compared to that of the control group by showing 22.80±5.02 μmoles/mg protein/hr, and the AP activity was found to show 43% reduction compared to that of the control group by showing 8.73±0.65 μmoles/mg protein/hr, which resulted to show occurrences of renal cell damages by the reduction of renal GGT and AP enzyme activities. Contrarily, the GGT and AP activities of the preGTP administered CP administered group respectively resulted to show 47% and 28% increment compared to the CP administered group by showing higher GGT and AP activities compared to the CP administered group by showing less kidney damages in preGTP administered CP administered group compared to the CP administered group. But, the GGT and AP activities of postGTP administered CP administered group did not show any significant difference compared to the results of the CP administered group.

### 3. Changes of renal tissue in CP and GTP administered Sprague Dawley rats

The optical observation results of the H&E stained kidney tissue sections of CP and GTP administered Sprague Dawley rats were provided in Fig. 3 and Table 3. At the CP administered group, tubular dilation was observed more than 75% of the samples by showing the occurrences of renal tubular damages by the administration of CP administration. In the preGTP administered CP administered group, the occurrences of renal tubular expansion was resulted to show 50% to 75% reduction compared to the results of the CP administered group by showing smaller renal tubule damages in preGTP administered CP administered group. Contrary to that, postGTP administered CP group did not show any significant differences in the degree of tubular dilation compare to the results of the CP administered group. Renal tubular necrosis was observed about 50% to 75% at the CP administered group, but it was observed about 25% to 50% at the preGTP administered CP administered group by showing the reduction of renal tubular necrosis at the preGTP administered CP administered group. However, the degree of renal tubular necrosis in the postGTP administered CP administered group did not show any significant differences compare to the CP administered group.

## Discussion

As an extensive anticancer chemotherapeutic agent, CP has been widely used in the treatment of many solid cancers. But, its applicability can be limited if its toxic side effects are once occurred. Nephrotoxicity is a major side effect that limits the CP anticancer therapies, which has been reported to be inhibited by the administration of green tea extracts or catechins. The present experiment observed the effects of GTP and CP according to their administration time and verified the applicability of GTPs as an agent in reducing the CP nephrotoxicity. When the effect of GTP administration on the nephrotoxicity of CP was evaluated by measuring the serum creatinine and BUN amounts after administering green tea extracts or after the intraperitoneal injection of CP, no significant difference of serum creatinine and BUN amounts was observed between the control group and green tea extract administered group.

The serum creatinine and BUN amounts in CP administered group respectively resulted to show 3.6 fold increase

and 2.6 fold increase compared to the results of the control group. Serum BUN and creatinine amounts have been reported as the evaluation items of showing a renal function. Fatima et al. [20] reported that intraperitoneal injection of CP can increase the serum creatinine and BUN amounts. At the present experiment, a similar result was acquired by indicating the presence of kidney damages after the intraperitoneal injection of CP. As the effects of green tea extracts by its administration time, the serum creatinine and BUN amount in the preGTP administered CP administered group respectively resulted to show 69% and 63% reduction compared to the CP administered group by showing a better renal function, but the postGTP administered CP administered group respectively recorded about 107% and 109% by showing no significant differences of renal function compared to the CP administered group.

Khan et al. [17] reported an increment of serum creatinine and BUN amount by the administration of CP, but reported a significant decrement of creatinine and BUN amount by the combined administration of CP and green tea extracts and stated the presence of inhibitory effects of renal damages by green tea extracts. The results were gathered when they evaluated the renal damages occurred by performing 4 times of CP administration with 5 day interval (3 mg/kg body weight) during the 25 days when they are administered with 3% green tea extracts. The results of the preGTP administered CP administered group at the present study were very similar to their results.

In addition, Sahin et al. [16] reported the increase of creatinine and BUN amounts at the 10th day after the injection of CP onto Sprague Dawley rats, but the injection of CP 2 days after the injection of EGCG resulted to show significant decrease of creatinine and BUN amounts and stated that the administration of EGCG can prevent the kidney damages that may be caused by the administration of CP, which was similarly concluded as the results of the present experiment. In the experiment that was performed by Khan et al. [17], oral administration of green tea extracts was progressed 5 days before administering the CP. The study result of Sahin et al. [16] was acquired by performing the intraperitoneal injection of either of green tea extracts or EGCG 2 days before injecting the CP, which could be considered as the corresponding results that were gathered at the preGTP administered CP administered group at the present experiment. But since the postGTP administered CP administered group didn't show any significant differences in the amount of creatinine and BUN, it is considered that postGTP administration has no effect on preventing the kidney damages or on stimulating renal functions. Both of GGT and AP are the prevalent enzymes that present in the brush border membrane of the microtubules of a kidney, which have been reported to be reduced at the event when microtubules were damaged. Khan et al. [17] reported the reduction of GGT and AP activities by the administration of CP, but reported no reduction of enzymatic activities by the combined administration of CP and green tea extracts. Fatima et al. [20] reported the reduction of renal GGT and AP activities at the time of injecting CP, which was prevented by the administration of vitamin C, and stated the preventive effect of vitamin C microtubule damages in kidney. At the present experiment, the preGTP administered CP administered group resulted to show higher GGT and AP activities compared to the CP administered group, which enabled to consider plausible microtubule damage protective effects of GTPs. Contrary to that, the GGT activity of the postGTP administered CP administered group did not show any significant difference compared to the result of the CP administered group, which may indicate no microtubule damage protective effect when it was administered after the administration of CP.

Under the circumstance of tubular dilation or necrosis, the parameter that enables to estimate the presence of histological damages in microtubules, Atessahin et al. [10] reported that the administration of CP stimulated the tubular dilation and increased the renal necrosis, which was inhibited by the administration of ellagic acid and concluded the protective effect of ellagic acid in kidney damage. At the present experiment, the preGTP administered CP administered group revealed the reduction of microtubular dilation and necrosis compared to the CP administered group, but no significant difference observed in the postGTP administered group, which suggests that GTP treatment should be advanced to have the preventive renal damages effects that were caused by CP injection. Considering the results that were gathered from this experiment, green tea polypheonols can reduce the nephrotoxicity when it was administered before the CP injection, but its administration after the CP injection did not have such reduction of nephrotoxicity. Therefore, the preventive effect of green tea extracts in reducing the CP induced nephrotoxicity may be acquired when patients are

pre-treated with GTPs. Such effects of GTPs can be considered as an experiment result that showed the possible use of it as the adjuvant that reduce the toxic side effects during CP treatment. To verify the effects and safety of such approaches, it is considered to have more experiments and clinical studies.

## Conflict of interest

No potential conflict of interest relevant to this article was reported.

## Acknowledgments

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