

## Antiapoptotic Effect of Captopril in Cisplatin-Induced Kidney Injury in Rats

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### ABSTRACT

**Aim of the work:** captopril protects against cisplatin induced nephrotoxicity; however its potential modulatory effect on hemeoxygenase (HO)-1, antioxidants, as well as inflammatory and apoptotic markers has not yet been verified.

**Materials and methods:** male Sprague-Dawley rats were divided into control (saline), cisplatin (5 mg/kg; i.p), and captopril (60 and 100 mg/kg) given 5 days before and after cisplatin (5 mg/kg; i.p) treatment. **Results:** five-day pre- and post-treatment with captopril (60 and 100 mg/kg; i.p), for a total of 10 days, dose dependently, reduced blood urea nitrogen, as well as serum creatinine and gamma glutamyl transferase, but serum albumin and total protein levels were increased. Captopril restored renal pro-oxidant/antioxidant balance by activating glutathione peroxidase, catalase and superoxide dismutase, and boosting the renal glutathione content. These effects were accompanied by the reduction in serum and/or renal HO-1, tumor necrosis factor- $\alpha$ , monocyte chemoattractant protein-1, nitric oxide, endothelin-1 and caspase-3. Microscopically, captopril especially at 100 mg/kg dose level, prevented cisplatin-induced degenerative changes and inflammatory cell infiltration in the kidney. **Conclusion:** captopril protects against cisplatin nephrotoxicity by its antioxidant, anti-inflammatory and antiapoptotic potentials.

**Keywords:** Captopril; Cisplatin; Renoprotection; Apoptosis; Inflammation.

### INTRODUCTION

Captopril, an angiotensin-converting enzyme inhibitor (ACEI), is widely used for the treatment of hypertension, congestive heart failure, and diabetic nephropathy<sup>[1]</sup>. ACEIs reduce angiotensin (Ang) II and aldosterone that play key roles in their renoprotection<sup>[2]</sup>. In the clinical settings, captopril protects against contrast-induced nephrotoxicity<sup>[3]</sup> and improves the abnormal renal hemodynamics in cyclosporine-treated patients<sup>[4]</sup>. Meanwhile, in the experimental studies captopril ameliorated radiation, doxorubicin, adriamycin, and cisplatin-induced nephropathy, as well as cisplatin cardio- and cadmium testicular toxicity<sup>[5-9]</sup>.

captopril restored kidney functions via an antioxidant character linked to the sulfhydryl (-SH) structural moiety and its ability to conjugate with copper, as well as the preservation of the mitochondria and enhancement of ATP production<sup>[10&11]</sup>. Jones *et al.*<sup>[12]</sup> reported that captopril reduced cisplatin devastating effects on the rat kidney without interfering with its antineoplastic

activity. El-Sayed *et al.*<sup>[13]</sup> showed that captopril renoprotection reside, in part, in its antioxidant potential and endothelin (ET)-1 inhibition.

However, the underlying potential multifactorial actions of captopril remain to be revealed. Therefore, in the present study, the possible modulatory effect of captopril on hemeoxygenase (HO)-1, some antioxidant enzymes and inflammatory mediators, as well as the apoptotic marker caspase (Casp)-3 were evaluated.

### MATERIALS AND METHODS

Adult male Sprague-Dawley rats (150-200g) were obtained from the breeding colony and maintained at the animal house of the National Organization for Drug Control and Research (NODCAR; Giza, Egypt). Animals had free access to food and water *ad libitum*. They were kept under controlled temperature (23±2°C), relative air humidity (60±10%), and 12 hours of light daily. Animals were subjected to one week adaptation period in the animal house before the beginning of the

experiments. All experiments were conducted in accordance with the Animal Research Ethics Committee and were approved by the Research Ethical Committee of Faculty of Pharmacy (Cairo University, Cairo, Egypt; Permit Number: 361) that complies with the Guide for the Care and Use of Laboratory Animals<sup>[14]</sup>.

Rats were categorized randomly into four groups (n=12-14 animals, each); thereafter they were subdivided into two experimental subsets. Animals in the control group received saline (CONT). Rats in the second group were injected with a single dose of cisplatin (5 mg/kg, i.p; Sigma-Aldrich, MO, USA<sup>[15]</sup>). Animals in the two other groups were administered captopril in saline (60 and 100 mg/kg/day; i.p; Sigma-Aldrich, MO, USA) for five consecutive days<sup>[13-16]</sup> before and after cisplatin injection for a total of 10 treatments. On the 6<sup>th</sup> day after cisplatin injection, blood was collected and sera were isolated for estimation of the kidney function tests. In the first subset of experiments, both kidneys from animals in each subgroup (n=6-8 rats) were homogenized in 50 mM phosphate buffer (pH 7.4) and samples were stored at -80°C till estimations. In another experimental subset, both kidneys (n=6 rats, each subgroup) were used for the histopathological and the immunohistochemical examinations.

Colorimetric assay kits for the measurement of blood urea nitrogen (BUN) and serum creatinine (Diamond Diagnostics) were used. Stanbio Laboratory Kits (Boerne, TX, USA) were utilized for the determination of the serum albumin and the total protein levels. Moreover,  $\gamma$ -glutamyl transferase (GGT) was estimated using a commercially available kit (Quimica Clinica Aplacada, Tarragona, Spain). All procedures were performed according to the manufacturer's instructions. ET-1 was determined using a commercial ELISA kit (Enzo Life Sciences International, CA, USA). For NO estimation, vanadium trichloride was used to reduce nitrate to nitrite<sup>[17]</sup>. The method of nitrite estimation is based on Griess reaction that was performed using the kit provided by Biodiagnostic (Cairo, Egypt). All procedures were performed according to the manufacturer's instructions. TNF- $\alpha$  and MCP-1 assays were measured according to the manufacturer's instructions using the rat ELISA kits obtained from Assay

Pro Company (Missouri, USA) and Enzo Life Sciences International, respectively.

HO-1 was measured using a commercial ELISA kit provided by Enzo Life Sciences International according to the manufacturer's instruction.

Thiobarbituric acid reactive substances (TBARS), glutathione (GSH), as well as glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) activities were measured using the Biodiagnostic Colorimetric kits according to the manufacturer's instructions.

#### *Renal histopathological examination*

The kidneys of rats in the different groups fixed in 10% neutral buffered formalin were embedded in paraffin and tissue blocks were prepared for sectioning at 4  $\mu$ m thickness. Renal sections were processed according to standard procedures for examination by light microscopy using eosin-hematoxylin stain.

#### *Renal Casp-3 immunohistochemical examination*

Prior to immunohistochemical staining, paraffin sections were properly mounted onto slides, deparaffinized and rehydrated through xylene/alcohol. Antigen retrieval was performed by placing the sections for 20 min in citrate buffer (Thermo Fisher Scientific, Fremont, USA; pH 6.0) at the boiling point then cooled. Active Casp-3 was detected following the incubation with the rabbit polyclonal anti-Casp-3 (CPP32) 1<sup>st</sup> antibody (1:200; Thermo Fisher Scientific) overnight at 4°C. After washing with phosphate buffered saline (PBS), sections were incubated with the biotinylated 2<sup>nd</sup> antibody (37°C, 30 min) then with the Vector Elite ABC kit (Vector Laboratories, CA, USA; 37°C, 30 min). After another wash with PBS, the antibody-biotin-avidin-peroxidase complex was developed using diaminobenzidine tetra hydrochloride (DAB Substrate Kit, Vector Laboratories). Sections were counterstained with hematoxylin, dehydrated and cleared in xylene and cover slipped. The reaction appeared as a brown cytoplasmic reaction.

#### *Statistical analysis*

Statistical significance of differences between means of groups was performed using the SPSS version 16 (Chicago, IL, USA), while the graphs were drawn using a Prism computer program (GraphPad software V5, CA, USA).

One-way analysis of variance (ANOVA) was employed to calculate the statistical significance followed by Tukey or Tukey-Kramer Multiple Comparison Test. A value of  $P < 0.05$  was considered significant.

## RESULTS

Cisplatin elevated BUN (318%), as well as serum creatinine (465%) and GGT (140%), while decreased serum albumin (70%) and total protein (50%) compared to CONT rats (Table 1). These serological alterations were associated by diffuse acute tubular necrosis, cystic dilatation, cast formation and inflammatory cell infiltration and hemorrhage in the cortex (Fig. 1B). It also enhanced Casp-3 immunoreactivity in the glomerular tuft and proximal tubules to reach almost ten folds compared to normal rats (Figs. 1F & I). Captopril (60 and 100 mg/kg, respectively) reduced BUN (52 and 80%), creatinine (67 and 75%) and GGT (48 and 56%) and increased albumin (214 and 243%) and total protein (66 and 93%) compared to cisplatin rats (Table 1). The kidneys of 60 mg/kg captopril had less degenerative changes and inflammatory cell infiltration, while almost normal renal architecture was noted at the higher dose level (Fig. 1D). The antiapoptotic efficacy of the ACEI was noticed by the reduction in Casp-3-positive cells (60 mg/kg, by 45%; 100 mg/kg, by 55%, as compared to that of cisplatin treated rats (Figs. 1G-I). Serum ET-1 was heightened by 58% by cisplatin as compared to that of the control rats, an effect that was only reduced by the higher dose of captopril by 52%, as compared to that of cisplatin treated rats (Fig. 2). Moreover, cisplatin increased renal TBARS (84%; Fig. 3A) and decreased GSH (64%; Fig. 3B), GPx (79%; Fig. 3C), SOD (55%; Fig. 3D) and CAT (46%; Fig. 3E) compared to the normal animals. On the other hand, captopril (60 and 100 mg/kg, respectively) hampered TBARS (52 and 57%; Fig. 3A) and enhanced the renal GSH (38%; Fig. 3B) above control values only at the higher dose level. Dose dependently, GPx (109 and 180%; Fig. 3C), SOD (58 and 127%; Fig. 3D) and CAT (60 and 86%; Fig. 3E) were elevated by the ACEI compared to renal injured rats. Compared to normal animals, cisplatin increased renal HO-1 level by 448% (Fig. 3F) and captopril decreased it dose dependently (by 35 and 67%; Fig. 3F) compared to cisplatin

administered animals. Serum NO (134%; Fig. 4A) was enhanced, while renal NO (30%; Fig. 4B) was reduced compared to their control counter parts. Administration of captopril (60 and 100 mg/kg) suppressed the serum NO concentration (21 and 43%; Fig. 4A), the ACEI at both dose levels reduced renal NO (by 12 and 25%; Fig. 4B), as compared to cisplatin rats. Cisplatin also increased serum/renal TNF- $\alpha$  (415 and 34%; Fig. 4C & D), as well as serum/renal MCP-1 (86 and 219%; Fig. 4E & F), respectively as compared to CONT rats. Captopril normalized serum TNF- $\alpha$  (76 and 79%; Fig. 4C) and decreased TNF- $\alpha$  renal level (42 and 41%; Fig. 4D) at 60 and 100 mg/kg dose levels, respectively compared to cisplatin rats. The higher captopril dose level suppressed both serum and renal MCP-1 (by 51% and by 62%) as compared to cisplatin animals. The 60 mg/kg dose of the ACEI showed a 40% reduction in renal MCP-1, as compared to cisplatin animals (Figs. 4E-F).

## DISCUSSION

The current study supports the renoprotective effect of captopril [13] and further implies a role for HO-1 and the suppression of TNF- $\alpha$ , MCP-1, ET-1, and Casp 3, especially at the ACEI higher dose level. Captopril also dose-dependently boosted the renal GPx, CAT, SOD, reduced lipid peroxidation and preserved GSH after cisplatin exposure. These positive effects were reflected as improvements of glomerular filtration and renal architecture deteriorated by cisplatin and are in line with previously reported results in the different renal injury models including the present one [12,13,18].

In the current work, captopril was able to decrease Casp-3 immunoreactivity in the kidney evoked by cisplatin, an effect previously documented in murine T cells and diabetic rats [19]. The antiapoptotic efficacy of captopril can be explained by repressing Ang II synthesis by ACE inhibition to reduce the expression of NF $\kappa$ B [20]. Indeed, Ang II directly via ATR1 and indirectly through the synthesis of ET-1 by activating ET<sub>A/B</sub> receptors [21-23] can upregulate NF $\kappa$ B activity. Moreover, Ang II-induced MCP-1 expression is linked to NF $\kappa$ B activation [24]. MCP-1 is a marker for early kidney injury [25] and signifies monocytes and macrophage recruitment that when activated contribute to ROS and TNF- $\alpha$  release. NF $\kappa$ B

orchestrates the expression of several key regulatory apoptosis genes that play major roles in acute renal failure [26]. The pro-inflammatory TNF- $\alpha$  synthesized by NF- $\kappa$ B and is capable of activating the extrinsic death receptors to induce programmed cell death [27,28]. In the current work, cisplatin was shown to elevate ET-1, TNF- $\alpha$ , MCP-1, ROS, as well as Casp-3 to support previous study [29]. Therefore, the suppression of both ET-1 and MCP-1 by captopril, seen in our study, as well as the decreased synthesis of AngII was previously reported [30,31] could explain, in part, the present reduction of the proinflammatory cytokine synthesis and hence the consequent apoptosis. Captopril was previously reported to decrease ET-1 in the present model [30], as well as TNF- $\alpha$  and MCP-1 via NF- $\kappa$ B inhibition in other inflammatory models [31-36] to support our results. **Chao *et al.*** [37] reported that captopril reduced MCP-1 is linked to Ang II inhibition. Via NF $\kappa$ B transcriptional activation, cisplatin can increase the generation of superoxide anion and NO by iNOS [38]. The co-existence of both radicals forms peroxynitrite, a potent RNS [39]. Both ROS and RNS are capable to activate the intrinsic apoptotic pathway to ultimately cleave Casp-3 [40].

In the present work, captopril antiapoptotic capacity might be a function of its antioxidative and antinitroactive stress activities evident by the reduction in lipid peroxidation and NO that was associated by the elevation of the antioxidants GSH, GPx, SOD, and CAT, effects that support previous studies in the different models [13,41,42]. The antioxidant effect of captopril is advocated to its -SH structural moiety that directly scavenges ROS by either hydrogen donation or electron-transferring [43], an action that is similar to GSH [44]. Indirectly, captopril activates antioxidant defense enzymes including SOD, CAT, and GPx to inhibit lipid peroxide and NO formation and boost the level of GSH. SOD scavenges superoxide anions, CAT eliminates hydrogen peroxide [45], and GPx reduces hydrogen and lipid peroxides, as well as peroxynitrite [46,48], thus reducing the total free radical pool to prevent apoptosis. The reduction of free radicals by captopril, seen herein, could again be a consequence of Ang II and MCP-1 inhibition, as previously elaborated. Indeed, **Ilieva *et al.*** [31] reported that the ACEI reduced the inflammatory cells infiltration and MCP-1

in an endotoxin induced uveitis in rats. Secondary to the elevated free radical pool, HO-1, a marker of oxidative stress, is enhanced in tubular cells to protect against superoxide anion and hydrogen peroxide generated by Ang II [49]. Cisplatin in the current work and previously [50] was shown to enhance HO-1 that was conversely suppressed by captopril, an effect that is reported for the first time in this study. Such an effect emphasizes the work of **Deng *et al.*** [51] who elucidated that captopril markedly decreased HO-1 in a model of chronic kidney disease. Therefore, captopril ability to inhibit angiotensin converting enzyme (ACE) and reactive oxygen species might be the two factors that might have repressed HO-1.

To this end, via its antioxidative and nitroactive stress, as well as anti-inflammatory potentials associated by the reduction in ET-1, captopril exerts its antiapoptotic effect to guard against cisplatin-induced renal damage.

#### Conflict of interest statement

The authors declare that they have no financial or personal conflicts of interest that influenced or could be perceived to have influenced this work.

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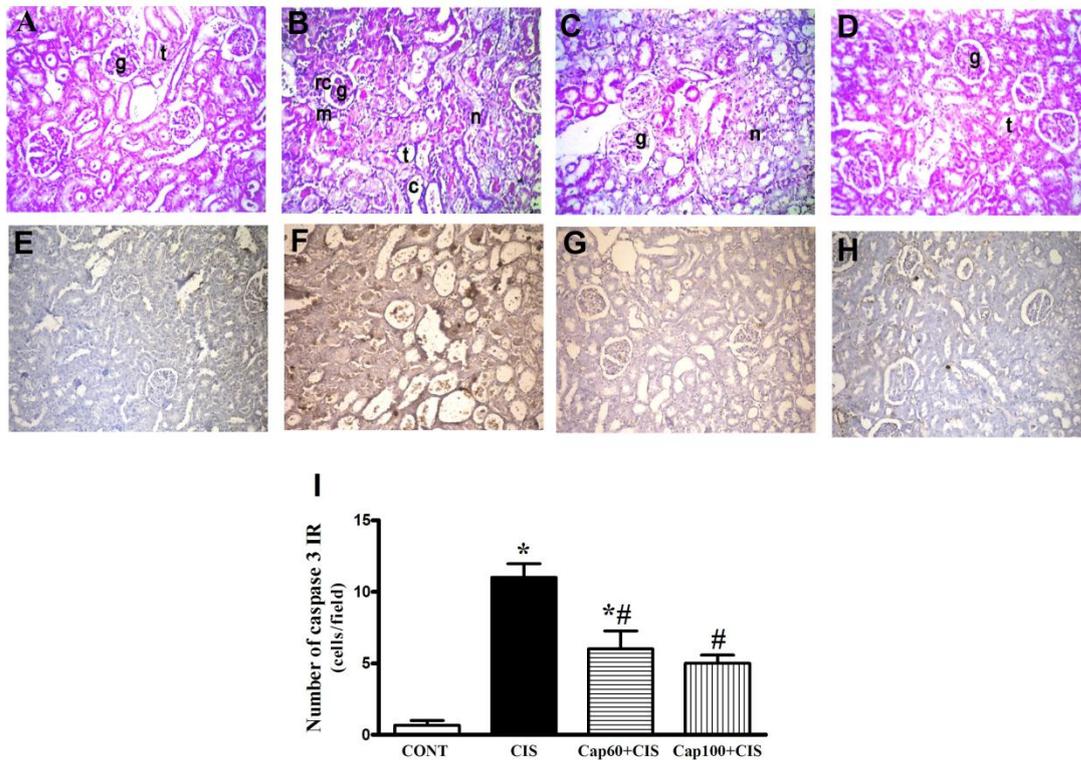
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**Table 1** -Renoprotective effect of captopril (60 mg/kg/day [Cap 60] and 100 mg/kg/day [Cap 100]; i.p.; 10days) on Blood Urea Nitrogen (BUN), and serum Creatinine,  $\gamma$ - Glutamyl Transferase (GGT), Albumin, as well as Total Protein (T protein) in Cisplatin (5 mg/kg [CIS]; i.p.)-induced nephrotoxicity in rats

Groups	BUN (mg/dl)	Serum Creatinine (mg/dl)	Serum GGT (U/l)	Serum Albumin (g/dl)	Serum Total Protein (g/dl)
CONT	47.88±2.67	0.57±0.05	4.88±0.33	5.16±0.14	12.50±0.69
CIS (5 mg/kg)	200.10±3.91	3.25±0.37 *	11.76±0.81 *	1.50±0.04 *	6.13±0.52 *
Cap60 + CIS	15.40±12.58 *	1.05±0.09 #	6.08±0.39 #	4.73±0.07 *#	10.19±0.33 *#
Cap100 + CIS	40.24±7.686 ;	0.80±0.04 #	5.16±0.35 #	5.17±0.14 #	11.87±0.33 #

Data represents mean of 12-14 rats  $\pm$  S.E.M. \*# $P$ < 0.05 compared to the CONT saline, cisplatin (CIS) and Cap 60 + CIS groups, respectively. Statistical analysis was carried out by one way ANOVA followed by Tukey- Kramer Multiple Comparison Test

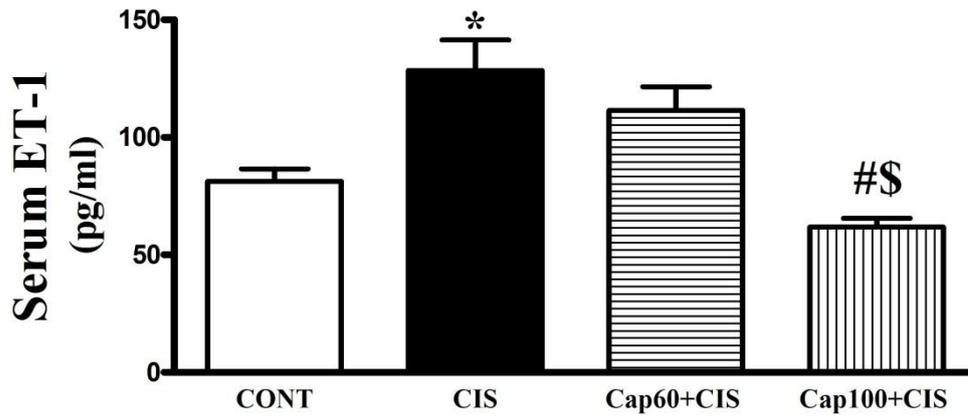
**FIGURE 1**



**FIGURE 1**

Renoprotective effect of daily administration of captopril (Cap; 60, 100 mg/kg, 10 days; i.p) on the histological appearance (40x), activated caspase (Casp)-3 immunoreactive (+IR) cells (200x), and immunoreactivity count in the kidney tissue in the control and cisplatin (CIS; single dose of 5 mg/kg; i.p) rats. In control (CONT) normal kidney architecture was noted [A]; CIS (single dose of 5 mg/kg; i.p)-treated rats showed degenerative changes, coagulative necrosis (n), tubular casts (rc), cystic dilatation (c) and inflammatory cells infiltration (m) [B]; Cap60+CIS showed apparently fewer coagulative necrosis (n) in the tubules [C]; Cap100+CIS improved histological appearance of the kidney [D]; CONT did not show any positive Casp-3 expression in the kidney [E]. Animals received CIS show positive Casp-3 expression [F] that is markedly reduced by Cap60 [G] and Cap100 [H] treatments in a dose dependent manner. Panel [I] represents positive Casp-3 cells mean (n=6 rats, per group)  $\pm$  SEM. Cells were counted in a fixed field size 10 000  $\mu\text{m}^2$ .  $P < 0.05$  compared to CONT, CIS, and Cap60+CIS groups, respectively. Statistical analysis was carried out by one way ANOVA followed by Tukey Multiple Comparison Test.

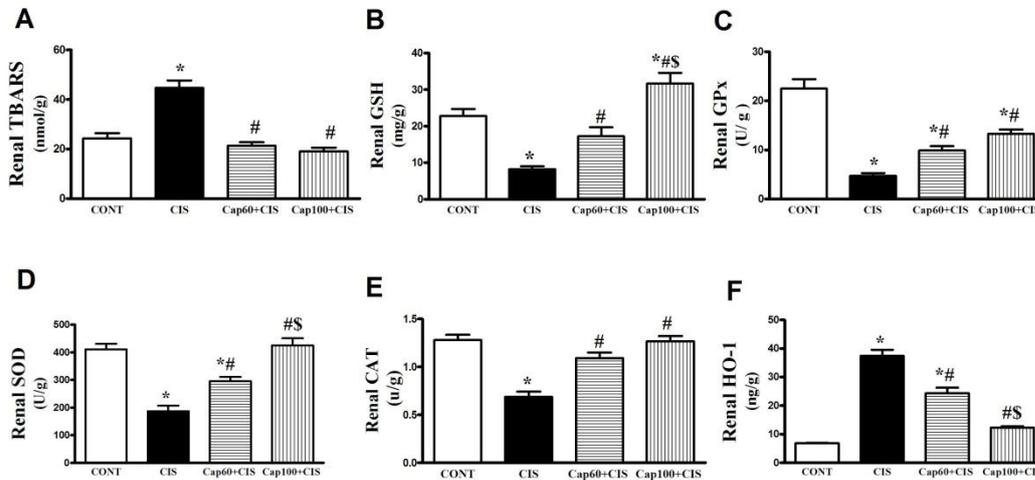
**FIGURE 2**



**FIGURE 2**

Renoprotective effect of daily administration of captopril (Cap; 60, 100 mg/kg, 10 days; i.p) on serum endothelin (ET)-1 in cisplatin (CIS; single dose of 5 mg/kg; i.p)-treated rats. Data represent mean  $\pm$  SEM (n=6-8 rats, per group). <sup>##</sup> $P < 0.05$  compared to control (CONT), CIS, and Cap60+CIS groups, respectively. Statistical analysis was carried out by one way ANOVA followed by Tukey-Kramer Multiple Comparison Test.

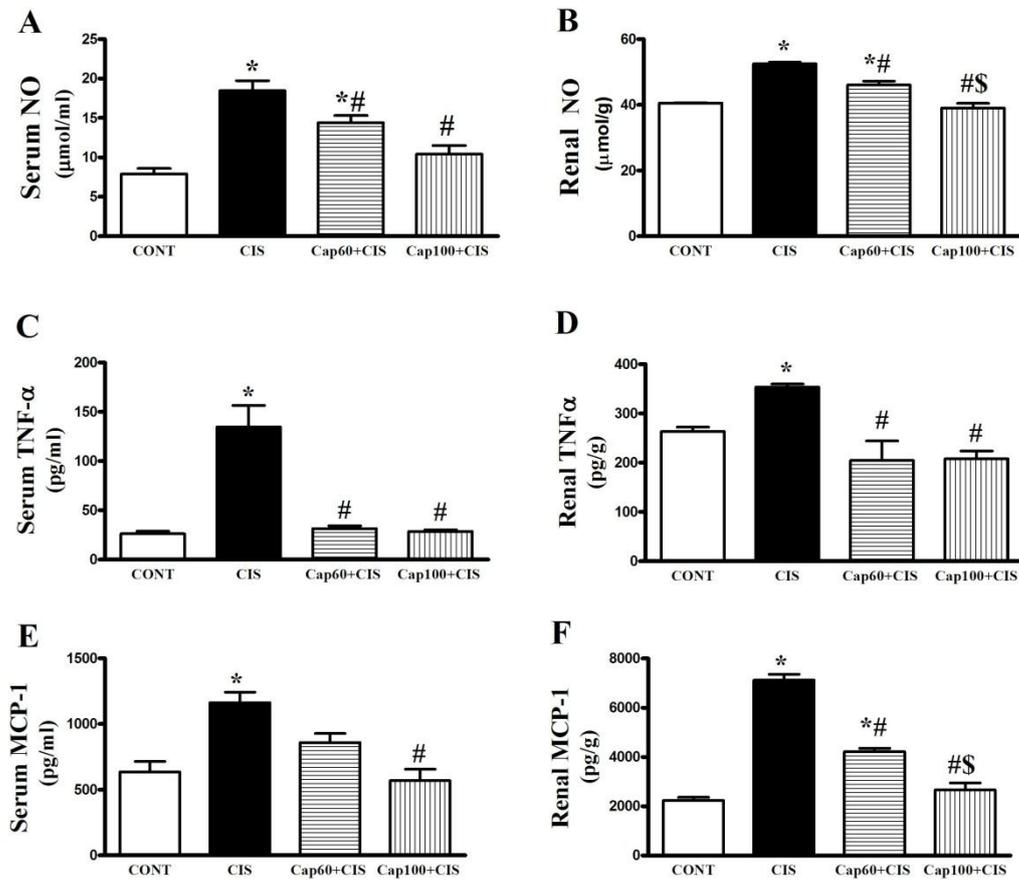
**FIGURE 3**



**FIGURE 3**

Renoprotective effect of daily administration of captopril (Cap; 60, 100 mg/kg, 10 days; i.p) on renal thiobarbituric acid reactive substance (TBARS) [A], glutathione (GSH) [B], glutathione peroxidase (GPx) [C], superoxide dismutase (SOD) [D], catalase [E], and hemeoxygenase (HO)-1 [F] in cisplatin (CIS; single dose of 5 mg/kg; i.p)-treated rats. Data represent mean  $\pm$  SEM (n=6-8 rats, per group). <sup>##</sup> $P < 0.05$  compared to control (CONT), CIS, and Cap60+CIS groups, respectively. Statistical analysis was carried out by one way ANOVA followed by Tukey-Kramer Multiple Comparison Test.

**Figure 4**



**FIGURE 4**

Renoprotective effect of daily administration of captopril (Cap; 60, 100 mg/kg, 10 days; i.p) on serum/renal nitric oxide (NO) [A and B, respectively], tumor necrosis factor (TNF)-α [C and D, respectively], and monocyte chemoattractant protein (MCP)-1 [E and F, respectively] in cisplatin (CIS; single dose of 5 mg/kg; i.p)-treated rats. Data represent mean ± SEM (n=6-8, each group. \*# $P < 0.05$  compared to control (CONT), CIS, and Cap60+CIS groups, respectively. Statistical analysis was carried out by one way ANOVA followed by Tukey-Kramer Multiple Comparison Test.