

## Original Article

# **Angiotensin-Converting Enzyme Inhibitor Captopril: Does it Improve Renal Function in Lipopolysaccharide-induced Inflammation Model in Rats**

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**ABSTRACT.** Renin–angiotensin system as an important regulator of renal function has also a major role in inflammation. In the present study, the effects of captopril on renal dysfunction, renal cytokine levels, and renal tissue oxidative damage were investigated in lipopolysaccharide (LPS)-induced inflammation model in rats. Treatment of five groups of the rats was carried out as follows: (1) saline as a control, (2) LPS 1 mg/kg, and (3–5) 10, 50, or 100 mg/kg captopril 30 min, respectively, before LPS. The treatments were given for 12 days. Finally, the animals were deeply anesthetized, the blood samples were obtained, and the renal tissues were removed and kept for biochemical measurements. Administration of LPS increased serum blood urea nitrogen and creatinine ( $P < 0.001$ ). Pretreatment with all doses of captopril decreased these parameters ( $P < 0.001$ ). LPS also increased interleukin-6 (IL-6), malondialdehyde, and nitric oxide metabolites in the renal tissues ( $P < 0.05 - P < 0.001$ ), which was prevented by captopril ( $P < 0.05 - P < 0.001$ ). The total thiol concentration and superoxide dismutase and catalase activities in the kidney of the LPS group were lower than the control ( $P < 0.001$ ), while they were enhanced when the animals were cotreated by captopril ( $P < 0.01 - P < 0.001$ ). The results of the present study showed that captopril improved renal function and attenuated tissue oxidative stress in LPS-induced inflammation model in rats.

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## **Introduction**

Renal disease or nephropathy may occur as nephritis or inflammatory renal disease and nephrotic, a noninflammatory form.<sup>1</sup> Earlier stages of kidney disease are defined based on the combination of renal damage (albuminuria)

and reduced renal function [glomerular filtration rate (GFR)] estimated from the serum creatinine (Cr) level.<sup>1</sup> Inflammation and oxidative stress are part of the pathophysiology of kidney disease.<sup>2</sup> An increased level of synthesis of inflammatory mediators, for example, interleukin (IL)-1, tumor necrosis factor- (TNF- ), and IL-6, takes part in impairing endothelial and mesangial cell function.<sup>2</sup> It has been reported that both oxidative stress and inflammation have a role in renal failure.<sup>3,4</sup>

Lipopolysaccharide (LPS) is a glycolipid of the Gram-negative bacterial cell wall. LPS increases nitric oxide (NO) production by stimulating the expression of inducible nitric oxide synthase (iNOS) in most organs including the kidney. It also increases reactive oxygen species (ROS) such as superoxide.<sup>5</sup> IL-6 is a predominant cytokine produced in the kidney. LPS induces releasing of inflammatory cytokines, such as IL-1, IL-6, and TNF- . In rodents, LPS has been reported to be able to impair renal function and increases Cr and blood urea nitrogen (BUN) that can be delayed by superoxide dismutase (SOD) or dimethylthiourea.<sup>5</sup> The renal tissue is sensitive to oxidative stress. Oxidative stress in the kidney can cause renal disease by affecting renal hemodynamic actions, by changing glomerular permeability, by inducing apoptosis, and by increasing acute and chronic inflammatory actions.<sup>6</sup>

The renin-angiotensin system (RAS) has been well known as a circulating hormonal system. It has been recently shown that the RAS plays an important role in regulating GFR. On the other hand, a relationship between RAS components and inflammation has been reported. In peripheral tissues, angiotensin (Ang) II has been reported to have a pro-inflammatory role.<sup>7</sup> Ang II binds to two specific receptors: angiotensin receptor type 1 (AT1) and angiotensin receptor type 2. AT1 is responsible for most of the pathophysiological actions of Ang II, by which it promotes cellular proliferation, inflammation, and fibrosis and contributes to renal injury. Furthermore, Ang II contributes to the recruitment of infil-

trating cells into the kidney, causes adhesion of circulating cells to endothelial and mesangial cells, and the migration of inflammatory cells into the kidney. This process is mediated by upregulation of adhesion molecules, cytokines, and chemokines. Angiotensin-converting enzyme (ACE) inhibitors have been shown to diminish inflammatory cell infiltration and inflammatory markers in many animal models of renal injury.<sup>8</sup> In addition, many studies have suggested that ACE activity has a major role in arterial hypertension and nephrotoxicity, and therefore, captopril, an ACE inhibitor, is effectively used as an antihypertensive agent and is suggested to decrease renal oxidative stress. Furthermore, captopril decreases nephrotoxicity induced by cisplatin by decreasing malondialdehyde (MDA) content and significantly increasing glutathione (GSH) content of kidney tissue. In addition, there was a marked decrease in kidney tissue content of NO.<sup>9</sup> In another study, captopril in renal ischemia-reperfusion (IR) injury decreased serum urea and Cr and increased total antioxidant status, total thiol, SOD activity, and GSH peroxidase (GSH-Px) activity in renal tissues. It was shown that captopril pretreatment reduced renal IR injury via anti-inflammatory, antioxidant, and antiapoptotic effects.<sup>10</sup> Considering the evidence that RAS has a role in both inflammation and oxidative stress and regarding the fact that both inflammation and oxidative stress have roles in renal diseases, the present study aimed to further evaluate the effect of captopril on LPS-induced renal dysfunctions, renal inflammation, and oxidative stress in rats.

## Materials and Methods

### *Animals and treatments*

Thirty-seven male Wistar rats were used. They were 12 weeks old and  $240 \pm 10$  g in weight. The animals were kept in standard conditions (temperature was  $22^\circ\text{C} \pm 2^\circ\text{C}$  and 12 h light/dark cycle) and had free access to food and water. Working with the animals was carried out in accordance with approved procedures by the Committee on Animal

Research of Mashhad University of Medical Sciences. The animals were divided into five groups ( $n = 7-8$  in each): (1) control group which received 1 mL/kg saline instead of LPS, (2) LPS group which received 1 mg/kg during 12 days, (3-5) LPS-captopril 10 mg/kg (LPS-capto 10), LPS-captopril 50 mg/kg (LPS-capto 50), and LPS-captopril 100 mg/kg (LPS-capto 100) groups which received 10, 50, or 100 mg/kg captopril 30 min before 1 mg/kg LPS.<sup>10-14</sup> LPS was purchased from Sigma (Sigma Chemical Co.). Captopril was kindly provided by Daroupakhsh Company, Tehran, Iran. All drugs were freshly dissolved in saline and were injected intraperitoneally (IP).

#### *Biochemical assessment*

The rats were deeply anesthetized. The blood samples were collected from the retro-orbital sinus and centrifuged to separate the serum. The samples were kept at  $-80^{\circ}\text{C}$  until the use for biochemical assessment. The kidneys were removed, gently washed (twice) in 2-3-volume ice-cold phosphate-buffered solution (PBS), and kept in a refrigerator to use for biochemical measurements.

Serum BUN level was measured using calorimetric method by a commercial kit (Pars Azmoon Company, Tehran, Iran) and using the protocols provided by the kit. Serum Cr was measured by Jaffe's method, using a commercial kit (Pars Azmoon Co., Tehran, Iran). The methods were according to our previous study.<sup>15</sup>

For biochemical measurements, the renal tissues were homogenized in a PBS and the supernatants were separated using a refrigerated centrifuge. The IL-6 content of the renal tissues was determined with a specific rat ELISA kit (eBioscience Co., San Diego, CA, USA) according to the manufacturer instructions. The absorbance was measured using a microplate reader (BioTek, USA), and the concentration of IL-6 was calculated by comparison curve established in the same measurement. Renal tissue NO metabolites ( $\text{NO}_2/\text{NO}_3$ ) were measured according to the Griess reagent method.<sup>11</sup>

Total thiol content, MDA levels, catalase

(CAT), and SOD activities were measured as previously reported.<sup>11</sup> MDA levels, as an index of lipid peroxidation, were measured in renal tissue. MDA reacts with thiobarbituric acid (TBA) as a TBA reactive substance and produces a red-colored complex which has a peak absorbance at 535 nm. Two milliliters TBA/trichloroacetic acid/hydrochloric acid reagent was added to 1 mL homogenate and the solution was incubated in boiling water bath for 40 min. After cooling, the solutions were centrifuged (1000 g for 10 min). The absorbance of supernatant was measured at 535 nm. The MDA concentration was calculated using a formula as previously described.<sup>11</sup>

2,2'-dinitro-5,5'-dithiodibenzoic-acid (DTNB) is a reagent which reacts with the SH groups to produce a yellow complex and was used to measure thiol contents. Briefly, 1 mL Tris-EDTA buffer (pH = 8.6) was added to 50  $\mu\text{L}$  renal tissue homogenate in 1 mL cuvettes, and the absorbance was read at 412 nm against Tris-EDTA buffer (A1). After this, 20  $\mu\text{L}$  DTNB reagent (10 mM in methanol) was added to the mixture, and after 15-min incubation in room temperature, the absorbance was read again (A2). The absorbance of DTNB reagent was also read as a blank (B). Total thiol concentration was calculated using a formula as previously described.<sup>11</sup>

SOD activity was determined using the procedure described by Madesh and Balasubramanian.<sup>16</sup> The method is based on the generation of superoxide by pyrogallol autoxidation and the inhibition of superoxide-dependent reduction of the tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to its formazan, measured at 570 nm. In the presence of SOD in reaction mixture, MTT will produce less amount of colored complex than the control. The reaction was terminated by the addition of dimethyl sulfoxide which also helps to solubilize the formazan formed, and the color evolved was stable for many hours.

One unit of SOD activity was defined as the amount of enzyme causing 50% inhibition in the MTT reduction rate.

CAT activity was measured according to the

method of Aebi.<sup>17</sup> CAT activity was determined by measuring the decrease in absorbance of H<sub>2</sub>O<sub>2</sub> at 240 nm as a result of degradation of H<sub>2</sub>O<sub>2</sub>. The assay buffer contains 50 mM sodium phosphate buffer at pH 7.0 and 10 mM of H<sub>2</sub>O<sub>2</sub>. According to Aebi,<sup>17</sup> one unit is equivalent to 0.01 decrease in absorbance at 240 nm. The activity was calculated as U/L. It was then converted to u/g tissue.

### Statistical Analysis

All data were expressed as means  $\pm$  standard error of the mean. The data were compared by one-way ANOVA, followed by Tukey's *post hoc* comparison test. Differences were considered statistically significant when  $P < 0.05$ .

### Results

#### Renal tissue interleukin-6

The results showed that IL-6 in renal tissues in the LPS group was higher than in the control group ( $P < 0.01$ ). Pretreatment by all doses including 10 ( $P < 0.05$ ), 50 ( $P < 0.01$ ), and 100 ( $P < 0.001$ ) mg/kg of captopril decreased the renal tissue IL-6. However, there

was no significant difference between the three doses (Figure 1).

#### Renal function parameters

The results showed that serum BUN in the LPS group was higher than that of the control group ( $P < 0.001$ ). Pretreatment by all doses including 10 ( $P < 0.01$ ), 50 ( $P < 0.001$ ), and 100 ( $P < 0.001$ ) mg/kg of captopril decreased the serum BUN (Figure 2). There were no significant differences between the three doses of captopril. The results also showed that serum Cr in the LPS group was higher than that of the control group ( $P < 0.05$ ). Pretreatment by 50 ( $P < 0.05$ ) and 100 ( $P < 0.001$ ) mg/kg but not 10 mg/kg of captopril decreased serum Cr (Figure 3). The results also showed that the highest dose of captopril was more effective than both the lowest ( $P < 0.001$ ) and the medium ( $P < 0.01$ ) doses.

#### Malondialdehyde concentrations and thiol contents in renal tissues

Biochemical assessment also showed that the animals of the LPS group had a higher MDA concentration (Figure 4), while thiol content was lower (Figure 5) in their renal tissues compared to the control animals (both  $P < 0.001$ ). Administration of captopril in a dose of 100

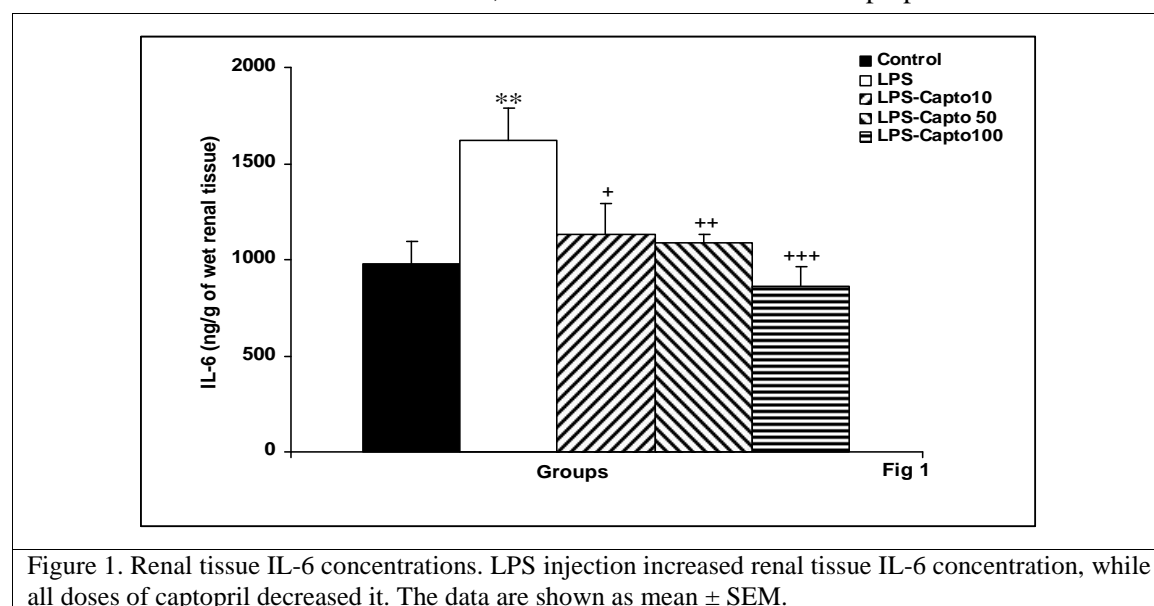


Figure 1. Renal tissue IL-6 concentrations. LPS injection increased renal tissue IL-6 concentration, while all doses of captopril decreased it. The data are shown as mean  $\pm$  SEM.

\*\* $P < 0.01$  compared to the control, + $P < 0.05$ , ++ $P < 0.01$ , and +++ $P < 0.001$  compared to LPS.

IL-6: Interleukin-6, LPS: Lipopolysaccharide, SEM: Standard error of the mean.

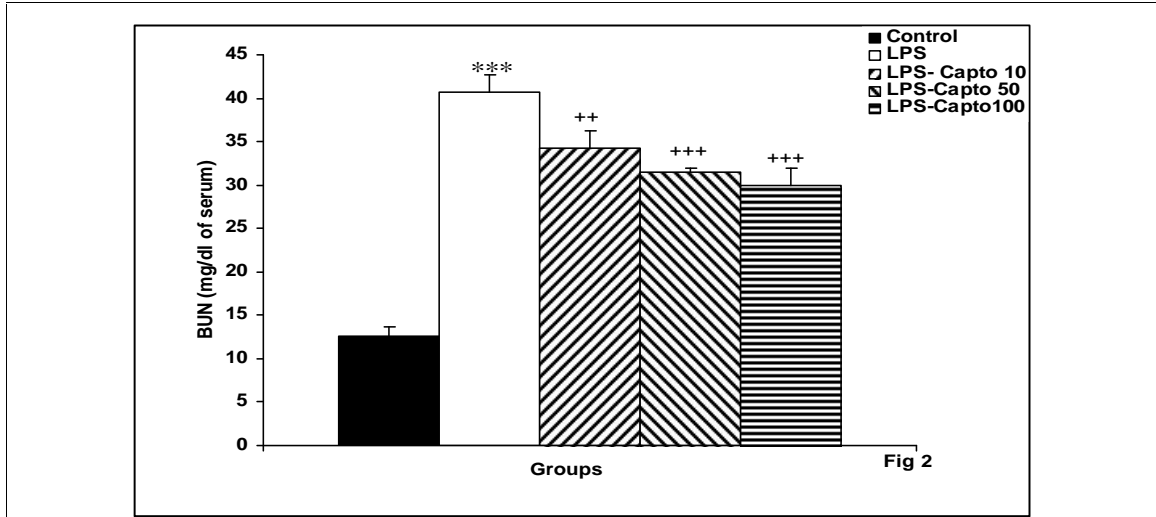


Figure 2. Serum BUN concentrations. LPS injection increased serum BUN concentration, while all doses of captopril decreased it. The data are shown as mean  $\pm$  SEM.

\*\*\* $P$  <0.001 compared to the control, \*\* $P$  <0.01 and +++ $P$  <0.001 compared to LPS. BUN: Blood urea nitrogen, LPS: Lipopolysaccharide, SEM: Standard error of the mean.

mg/kg decreased renal tissue MDA compared to the LPS group ( $P$  <0.001). The results also showed that 100 mg/kg of captopril was more effective than 10 mg/kg captopril on renal tissue MDA ( $P$  <0.01). Neither 10 nor 50 mg/kg of captopril was able to change MDA concentrations in the renal tissues of LPS-

treated rats (Figure 4). In addition, a dose 100 mg/kg of the captopril increased the renal thiol contents compared to the LPS group ( $P$  <0.01; Figure 5), while the two other doses including 10 and 50 mg/kg of captopril were not effective on the renal thiol contents.

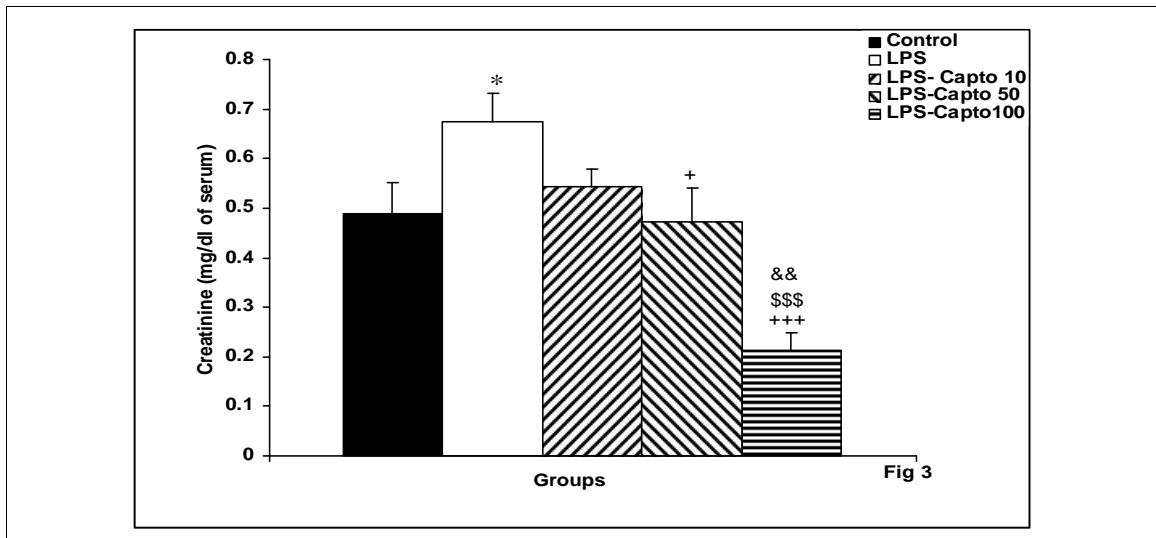


Figure 3. Serum creatinine concentrations. LPS injection increased serum creatinine concentration, while the medium and the highest doses of captopril decreased it. The data are shown as mean  $\pm$  SEM.

\* $P$  <0.05 compared to the control, + $P$  <0.05 and +++ $P$  <0.001 compared to LPS, \$\$\$ $P$  <0.001 compared to 10 mg/kg captopril, && $P$  <0.01 compared to 50 mg/kg captopril. LPS: Lipopolysaccharide, SEM: Standard error of the mean.

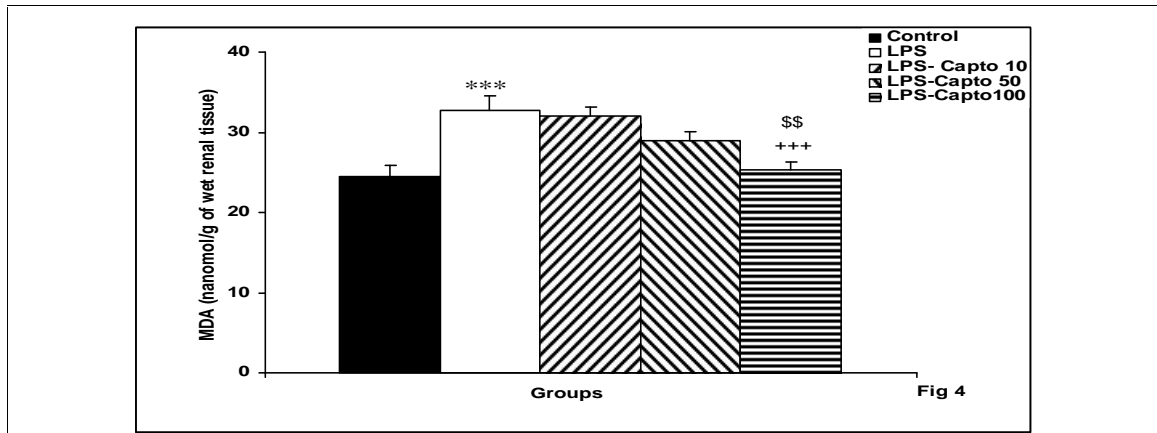


Figure 4. Renal tissue MDA concentrations. LPS injection increased renal tissue MDA concentration, while the highest dose of captopril decreased it. The data are shown as mean ± SEM.

\*\*\*  $P < 0.001$  compared to the control, +++  $P < 0.001$  compared to LPS, \$\$  $P < 0.01$  compared to 10 mg/kg captopril. MDA: Malondialdehyde, LPS: Lipopolysaccharide, SEM: Standard error of the mean.

*Superoxide dismutase and catalase in renal tissues*

The results also showed that LPS administration decreased renal SOD ( $P < 0.001$ ) which was prevented by the highest dose of the captopril ( $P < 0.001$ ; Figure 6). The lowest and the medium doses were not able to affect renal tissue SOD. In addition, the highest dose of captopril was more effective than the lowest ( $P < 0.001$ ) and the medium ( $P < 0.01$ ) doses. In addition, the medium dose was more effective

than the lowest dose ( $P < 0.05$ ). The results also showed that treatment by LPS decreased renal CAT ( $P < 0.001$ ). Pretreatment by 50 ( $P < 0.05$ ) and 100 ( $P < 0.001$ ) mg/kg but not 10 mg/kg of captopril improved CAT in the renal tissues compared to the LPS group ( $P < 0.05 - P < 0.001$ ; Figure 7). In addition, the highest dose of captopril was more effective than the medium ( $P < 0.05$ ) and the lowest ( $P < 0.001$ ) doses of captopril on the CAT activity in renal tissues of LPS-treated rats.

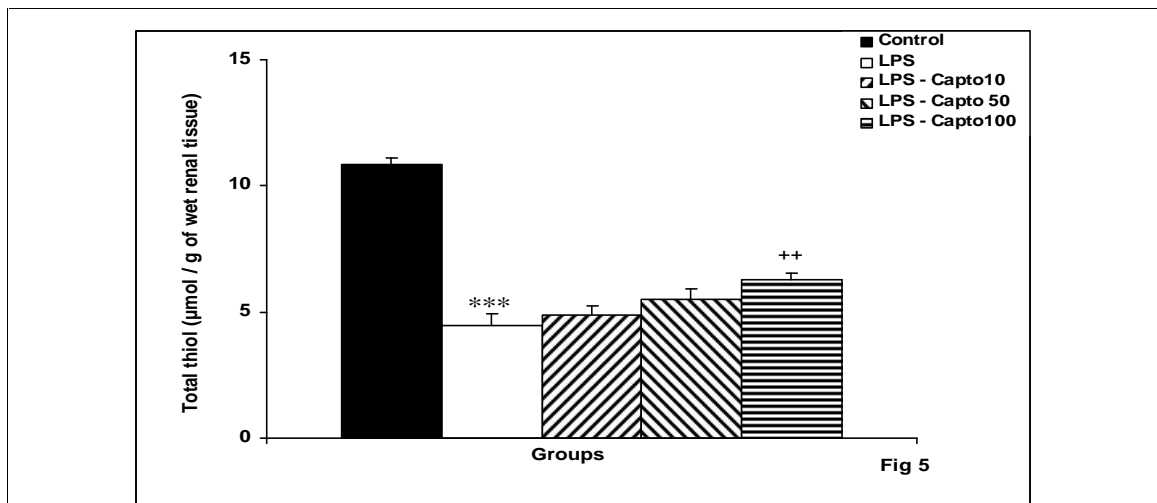


Figure 5. Renal tissues thiol contents. LPS injection decreased renal tissue thiol concentration, while the highest dose of captopril increased it. The data are shown as mean ± SEM.

\*\*\*  $P < 0.001$  compared to the control, ++  $P < 0.01$  compared to LPS. LPS: Lipopolysaccharide. SEM: Standard error of the mean.

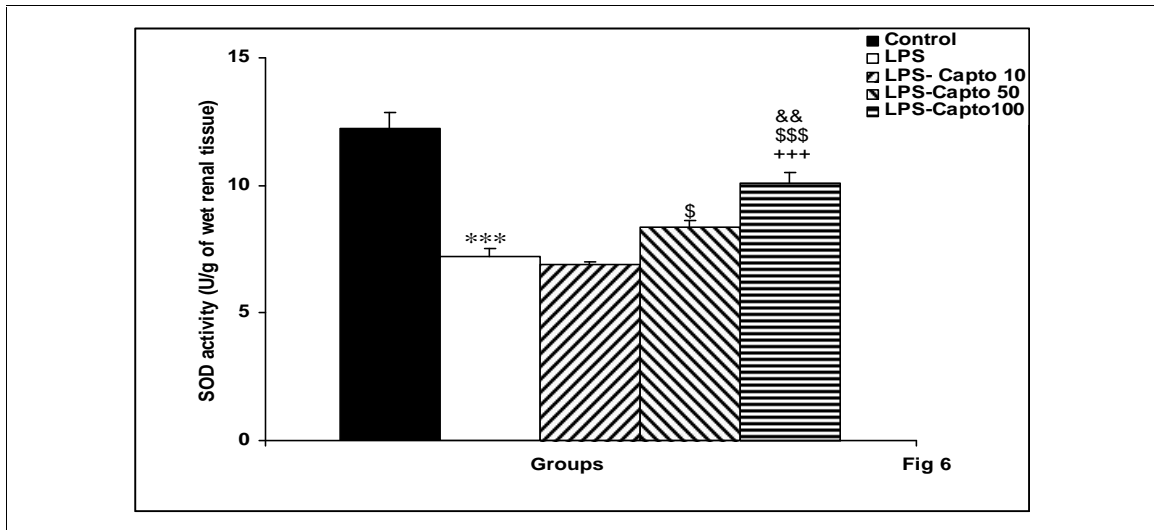


Figure 6. Renal tissue SOD activity. LPS injection decreased renal tissue SOD activity, while the highest dose of captopril increased it. The data are shown as mean  $\pm$  SEM.

\*\*\*  $P < 0.001$  compared to the control, +++  $P < 0.001$  compared to LPS, \$  $P < 0.05$  and \$\$\$  $P < 0.001$  compared to 10 mg/kg captopril, &&  $P < 0.01$  compared to 50 mg/kg captopril. SOD: Superoxide dismutase, LPS: Lipopolysaccharide, SEM: Standard error of the mean.

*Renal tissues NO metabolites*

LPS administration increased the NO metabolite concentrations in the renal tissues of the LPS group compared to the control group ( $P < 0.001$ ). Pretreatment by all doses including 10, 50, and 100 mg/kg captopril decreased the renal tissue NO metabolites ( $P < 0.001$  for all;

Figure 8). The results also showed that the highest dose of captopril was more effective than the lowest dose ( $P < 0.05$ ). There was no significant difference between the highest dose and the medium dose of captopril. There was also no significant difference between the effect of the medium and the lowest dose of

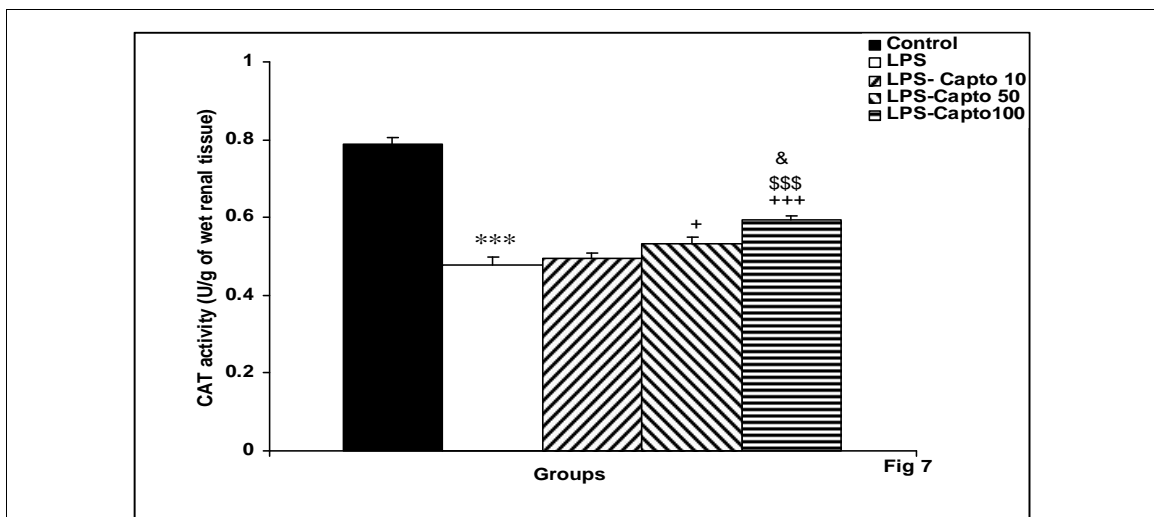


Figure 7. Renal tissue CAT activity. LPS injection decreased renal tissue CAT activity, while the medium and the highest doses of captopril increased it. The data are shown as mean  $\pm$  SEM.

\*\*\*  $P < 0.001$  compared to the control, +  $P < 0.05$  and +++  $P < 0.001$  compared to LPS, \$\$\$  $P < 0.001$  compared to 10 mg/kg captopril, &  $P < 0.05$  compared to 50 mg/kg captopril. CAT: Catalase, LPS: Lipopolysaccharide, SEM: Standard error of the mean.

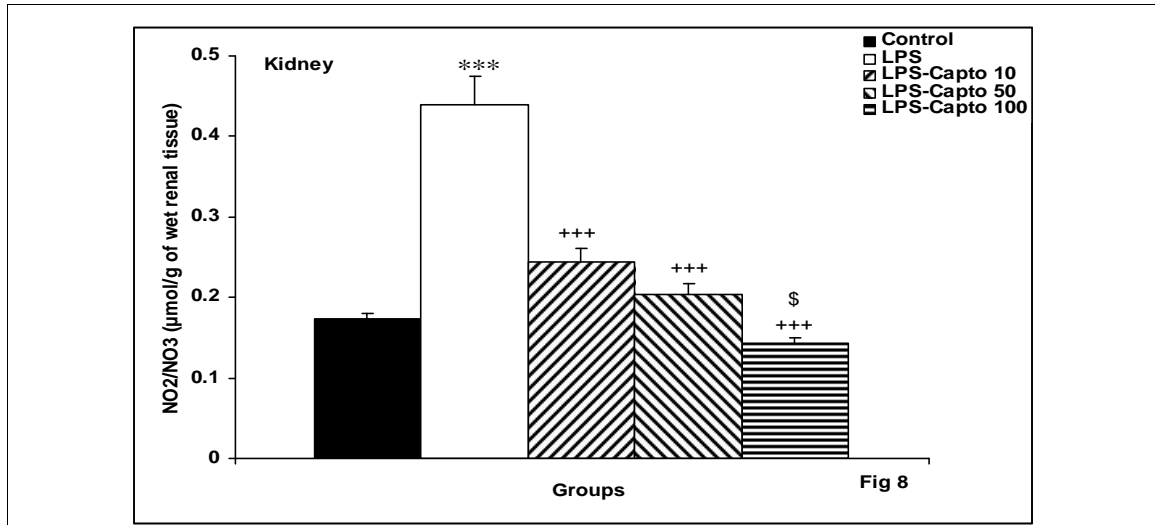


Figure 8. Renal tissue NO metabolites. LPS injection decreased renal tissue NO metabolites, while all doses of captopril increased it. The data are shown as mean  $\pm$  SEM.

\*\*\*  $P < 0.001$  compared to the control, +++  $P < 0.001$  compared to LPS, \$  $P < 0.05$  compared to 10 mg/kg captopril. NO: Nitric oxide, LPS: Lipopolysaccharide, SEM: Standard error of the mean.

captopril on renal tissue NO metabolites (Figure 8).

### Discussion

In the current study, LPS administration induced an inflammation status in the renal tissues. The results also showed that the inflammation was accompanied with a high level of serum BUN and Cr, implying a kidney dysfunction status. The higher doses, for example, of 10 mg/kg of LPS as a single injection, have been used to induce acute renal injury and sepsis in animal model.<sup>18</sup> In addition, lower doses of LPS have also been reported to be able to induce renal injury and inflammation.<sup>13,14</sup> In the present study, a lower dose (1 mg/kg) of LPS was administered daily during 12 consecutive days and renal dysfunction was manifested by an increased level of serum BUN and Cr. An increased level of renal tissue IL-6, which was seen in the present study, confirmed that a lower dose LPS was able to induce renal tissue inflammation and injury in rats. We also showed previously that LPS in a dose used in the present study was able to induce renal fibrosis in rats accompanied with a high level of

biochemical criteria of renal dysfunctions.<sup>15</sup> Other researchers also showed that even a single injection of 1 mg/kg of LPS was followed by inflammation in the tissues.<sup>19</sup>

It has also been reported that LPS injection induces an oxidative stress status in many organs, including the heart, brain, and renal tissue.<sup>11</sup> It has been previously reported that a single injection of LPS in a dose similar to the dose which was used in the present study was followed by oxidative stress status in the renal tissue.<sup>19</sup> In the present study, LPS administration was accompanied by an increased level of MDA and NO metabolites, while thiol content and SOD and CAT activities were decreased in the renal tissue. Oxidative changes in the thiol groups and lipid peroxidation can serve as important *in vivo* biomarkers of oxidative stress. In recent studies, it has been suggested that ROS contributes in the pathogenesis of LPS-induced renal oxidative damage.<sup>20</sup> The free radicals can initiate and progress the inflammation since the activation of phospholipase A2 by inflammatory stimuli leads to release of inflammatory mediators such as IL-6.<sup>21</sup> IL-6 is a multifunctional cytokine and has been considered as an inflammation-promoting



one.<sup>22</sup> IL-6 has been reported to be involved in glomerulonephritis and inflammatory renal disease.<sup>23</sup> In this study, IL-6 was increased by LPS and showed renal inflammation. Furthermore, in another study, LPS increased cytokines such as IL-6 and TNF- $\alpha$  in the kidney.<sup>24</sup> Considering these facts, it seems that both oxidative stress and renal inflammation were accompanied with an increased level of serum BUN and Cr which was seen in the present study. Consistently, both pro-oxidant and pro-inflammatory compounds have been reported to be markedly increased in patients with renal failure<sup>25</sup> and are among the factors that may initiate inflammation.<sup>26</sup> In addition, many of the therapies currently used in patients with renal dysfunction or at risk for worsening renal function exert anti-inflammatory effects.<sup>2</sup>

ACE inhibitors such as captopril are widely used for cardiac disorders.<sup>27</sup> These drugs have been frequently used in the prevention and treatment of progressive renal failure. For example, captopril has been reported to be positively effective on renal function and reduces albuminuria in hypertensive insulin-dependent diabetics with nephropathy.<sup>28</sup> In the present study, captopril improved renal dysfunction induced by LPS which was presented by a decrease in BUN and Cr.

In accordance with our results, it was recently reported that renal tissue Ang II was increased after injection of LPS.<sup>14</sup> Captopril has been able to reduce Cr level in the serum of the patients with acute renal failure.<sup>29</sup> In another clinical study, captopril ameliorated nephropathy which was accompanied with a decrease in Cr and BUN.<sup>30</sup> It has also been reported that a rise in serum Cr was decreased by captopril in diabetic rats.<sup>28</sup> In the recent study, there was no significant difference between the different doses of captopril on BUN. The highest dose of captopril was more effective in attenuating serum Cr than the medium and the lowest doses. The highest dose also reduced the serum Cr to a lower level of the control group. This effect of the highest dose of captopril was not evaluated in the present study, and it needs to be investigated in future studies. However, it has been previously reported that ACE

activity is positively correlated with BUN and Cr levels.<sup>31</sup>

Captopril has a sulfhydryl (-SH) group and can also react rapidly with hydroxyl radicals and hypochlorous acid at micromolar concentrations.<sup>27</sup> In addition, captopril was found to improve the enzymatic activity of SOD and selenium-dependent GSH-Px.<sup>9</sup> Captopril was a free radical scavenger and improved the nephrotoxicity induced by doxorubicin.<sup>32</sup> In the present study, captopril decreased NO metabolites and MDA concentration and increased total thiol concentration and SOD and CAT activity in the renal tissues of LPS-treated rats. Considering the results of the present study, it seems that the effects of captopril on oxidative stress criteria were dose dependent and the highest dose was more effective than the medium and the lowest doses. Captopril in doses similar to the doses which was used in the present study showed nephron-protective effects against diazinon-induced nephrotoxicity accompanied with a decrease in BUN and Cr and improving effects on renal tissue oxidative damage.<sup>33</sup> Captopril in 5-fluorouracil-induced nephrotoxicity increased GSH and decreased MDA level of renal tissues.<sup>34</sup>

It is suggested that there is an association between markers of inflammation and arterial hypertension and cardiovascular diseases, and interventional studies confirmed that treatment of hypertension with antihypertensive drugs attenuates the markers of inflammation.<sup>35</sup> It has also been reported that ACE inhibitors and angiotensin receptor blockers attenuate mortality rate of patients suffering from pneumonia.<sup>36</sup>

Besides the anti-oxidative stress, some of the beneficial effects of ACE inhibitors including captopril have been attributed to their anti-inflammatory properties.<sup>37</sup> In the present study, captopril attenuated IL-6 concentration in the renal tissue of LPS-treated rats.

Recently, the effect of ACE inhibitors and captopril on inflammation has been reported to be complicated, and they possess several modes of action, including inhibition of bradykinin and cytokines. Some studies showed that captopril at low doses (up to 5

mg/kg, single dose) induced inflammation by causing stimuli resulting in rise in the bradykinin level.<sup>38</sup> However, at higher doses (up to 400 mg/kg, single dose), captopril was able to inhibit inflammation.<sup>39</sup> Furthermore, in another study, captopril decreased IL-6 in an arthritis model in rats.<sup>40</sup> Moreover, captopril exerted a significant protection against radiation injury in rats since it reduced inflammation and fibrosis.<sup>41</sup> It has been assumed that the immunosuppressive action of captopril might be partly responsible for reduction of chronic inflammation.<sup>42</sup>

### Conclusion

In this study, captopril as a well-known ACE inhibitor improved renal function which was accompanied by attenuating IL-6 as a marker of inflammation. Protection against oxidative damage of renal tissues may also be considered as another explanation for beneficial effects of captopril in LPS-induced inflammation model in rats which was seen in the present study.

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**Conflict of interest:** None declared.

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