International Immunopharmacology 69 (2019) 19–26

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

S-allyl cysteine protects against lipopolysaccharide-induced acute kidney injury in the C57BL/6 mouse strain: Involvement of oxidative stress and inflammation

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ARTICLE INFO

Keywords:
S-allyl cysteine
Acute kidney injury
Lipopolysaccharide
Apoptosis
Oxidative stress
Inflammation

ABSTRACT

Sepsis is a serious and life-threatening medical condition with a higher rate of patients' morbidity and mortality and with complications such as acute kidney injury (AKI). S-allyl cysteine (SAC) is the active constituent of the medicinal plant garlic (*Allium sativum*) with multiple beneficial effects including anti-inflammatory and anti-oxidant properties. In this research, we tried to determine the protective effect of SAC pretreatment in a mouse model of AKI. To induce AKI, lipopolysaccharide (LPS) was injected once (10 mg/kg, *i.p.*) and SAC was administered at doses of 25, 50, or 100 mg/kg (*p.o.*) 1 h before LPS. Treatment of LPS-challenged C56BL/6 animals with SAC lowered serum level of creatinine and blood urea nitrogen (BUN), partially restored renal oxidative stress-related biomarkers including malondialdehyde (MDA), glutathione (GSH), and activity of superoxide dismutase (SOD) and catalase in addition to improvement of mitochondrial membrane potential (MMP). Furthermore, SAC was capable to bring renal nuclear factor-kappaB (NF-κB), nuclear factor (erythroid-derived 2)-like 2 (Nrf2), toll-like receptor 4 (TLR4), cyclooxygenase-2 (COX2), tumor necrosis factor α (TNFα), interleukin-1β (IL-1β), interleukin-6 (IL-6), Annexin V, and DNA fragmentation partially back to their control levels. Additionally, SAC pretreatment was capable to exert a protective effect, as shown histologically by lower tubular injury and pathologic changes in the kidney. In summary, SAC is capable to alleviate LPS-induced AKI through mitigation of renal oxidative stress, inflammation, and apoptosis in addition to preservation of mitochondrial integrity and its favorable effect exhibits a dose-dependent pattern.

1. Introduction

Acute kidney injury (AKI) is a critical complication in patients hospitalized in critical care units that is usually developed due to a septic condition [1] and finally requires renal replacement therapy [2]. AKI is a major public health concern that adversely affects patients' health and leads to an estimated 1.4 million deaths each year [3]. Renal tubular epithelium is the main site of cell injury and death during AKI and it has even been claimed that tubular epithelial cells play an important role in inflammatory process related to AKI [4]. Several pathogenic factors are responsible for development of AKI. Particularly, inflammation plays a major role in this respect [4,5]. Of related significance, lipopolysaccharide (LPS) is an agent that leads to AKI through activation of TLR4/NF-κB signaling with subsequent production of inflammatory mediators [6]. TLR4 is the main receptor of LPS that plays a key role in the innate immune system [7]. Activation of TLR4 by LPS triggers NF-kB cascade, leading to generation and release of inflammatory cytokines [8] with subsequent kidney damage [9]. In addition, oxidative stress due to increased generation of oxidants including reactive oxygen (ROS) and nitrogen (RNS) species and depletion of endogenous antioxidants such as superoxide dismutase (SOD), catalase, and reduced glutathione (GSH) is also strongly involved in the

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https://doi.org/10.1016/j.intimp.2019.01.026
Received 16 February 2018; Received in revised form 24 December 2018; Accepted 16 January 2019
Available online 18 January 2019

pathogenesis of AKI [10–12]. Besides, sepsis-associated AKI is also associated with acute tubular apoptosis [13]. Particularly, proximal tubule epithelial cells are highly vulnerable to apoptotic process and their injury may contribute to renal failure [14]. On this foundation, regulation of apoptosis and inflammation could protect against development of AKI [15]. Existing evidence also strongly indicate that oxidative stress through its effect on mitochondrial integrity leads to sepsis-induced AKI [16]. It has also been shown that LPS challenge reduces renal mitochondrial membrane potential (MMP) and mitochondrial antioxidants such as superoxide dismutase (SOD), catalase, and glutathione and increases mitochondrial MDA [17]. In clinical settings, except for ordering maintenance of electrolyte and fluid balance, avoidance of exposure to nephrotoxic chemicals and constant clinical monitoring, there is no standardized preventive and/or therapeutic protocols for AKI in severely ill patients [11,18,19].

S-allyl cysteine (SAC) is a natural organosulfur agent in aged garlic extract [20]. SAC is simply absorbed through the intestine and its bioavailability is high [20]. SAC has exhibited various beneficial effects in various humankind diseases and there is interestingly no scientific evidence against its adverse effects and it has even been proved that its consumption has multiple pharmacological benefits [21]. SAC has exhibited various beneficial effects [21,22] including alleviation of oxidative stress [23,24] and protection of neurons in ischemic conditions through Nrf2-dependent cascade [25]. In this regard, SAC could attenuate oxidative stress in multiple tissues of streptozotocin-induced diabetic rats [26]. SAC is also capable to exert anti-apoptotic and anti-inflammatory effects [22] and its anti-inflammatory effect in kidney of diabetic mice is ascribed to its inhibition of NF- < kappa > B pathway [27]. Since pathogenic factors including oxidative stress, inflammation, and an apoptotic event are enhanced in LPS-induced AKI [28] and SAC has exhibited anti-oxidant, anti-inflammatory [29–31], and anti-apoptotic [24,32] potential, thus, we designed this study to investigate whether SAC could exert a protective effect in LPS-induced mouse model of AKI with exploration of some related underlying mechanisms.

2. Materials and methods

2.1. Animals

Male C57BL/6 mice (20–24 g) were purchased from Razi Vaccine and Serum Research Institute (Karaj, Iran). All mice were adapted for 1 week (temperature: 23 ± 1 °C, humidity: 45–50%) and kept on a 12-h light/dark cycle. The mice had free access to standard pellet diet and tap water.

2.2. Ethics statement

All animal procedures in this research study were performed according to the Guide for the Care and Use of Laboratory Animals, specified by the National Institutes of Health (NIH) of USA and certified by National Institute for Medical Research Development (NIMAD) of Iran in 2017 (certificate No. 958692) and local Animal Ethics Committee of Shahed University (Tehran, Iran).

2.3. Experimental design

The mice (n = 48) were randomly assigned to six experimental groups (n = 8 per group) including control, SAC 100-pretreated control, LPS, SAC 25-, SAC 50- and SAC 100-pretreated LPS (receiving SAC at doses of 25, 50, or 100 mg/kg; *p.o.*). To produce a general inflammatory response and to induce AKI, LPS from *Escherichia coli* (SigmaAldrich, St Louis, MO, USA; 0111:B4; dissolved in normal saline) injected *i.p.* once at a dose of 10 mg/kg. This dose of LPS was selected from a previous report for development of AKI [28,33]. Mice in treatment groups received SAC (SigmaAldrich, St Louis, USA) dissolved in distilled water, by gavage needle, at doses of 25, 50, or 100 mg/kg, once, 1 h prior to LPS. Control group received only the vehicles (intraperitoneal injection of normal saline and oral administration of distilled water). SAC 100-pretreated control group received SAC *p.o.* at a dose of 100 mg/kg 1 h before *i.p.* injection of normal saline. Selection of doses for SAC was derived from previous researches on its protective and anti-inflammatory effects [23,34]. Also, selection of timetable for administration of SAC and LPS in our model of AKI was according to an earlier report [33]. Twelve hours after LPS, blood samples were taken from retro-orbital plexus under mild anesthesia by diethyl ether and 24 h following LPS injection, mice were euthanized under deep anesthesia with ketamine-HCl (*i.p.* at a dose of 150 mg/kg). Blood was taken from the heart and right kidneys were immediately isolated for next biochemical tests. Since some mice challenged with LPS showed signs of oliguria, urine analysis tests were not done.

2.4. Blood biochemical assessment

Serum samples from mice were prepared by centrifuging at 3000 rpm for 10 min and routine biomarkers of kidney function comprising creatinine and blood urea nitrogen (BUN) were measured by means of specific diagnostic kits from Parsazmun Co., Tehran.

2.5. Determination of renal oxidative stress-associated biomarkers

Left kidney tissue block was homogenized at a w/v ratio of 10% in cold lysis buffer using a rotary homogenizer (IKA Co., Germany). After centrifuging, the supernatant was aliquoted and stored at −70 °C for the following assays.

Malondialdehyde (MDA) content of the supernatant was determined according to earlier studies [35,36]. For determination of MDA concentration or thiobarbituric acid reactive substances (TBARS), supernatant was mixed with a combination of trichloroacetic acid and TBARS in boiling water for 90 min. After cooling, samples were centrifuged at 1000 ×*g* for 10 min and the absorbance was obtained at 532 nm with final results calculated according to tetraethoxypropane standard curve.

Nitrite level was quantitated by Griess procedure [37]. In this assay, supernatant was added to Griess reagent comprising sulfanilamide and *N*-naphthyl ethylenediamine in acidic medium. The absorbance was determined at 540 nm.

Reduced glutathione (GSH) was determined as stated by earlier researches [38–40]. For this aim, the supernatant was centrifuged with 5% trichloroacetic acid. To 0.1 ml of homogenate, 2 ml of phosphate buffer (pH 8.4), 0.5 ml of 5′5 dithiobis (2-nitrobenzoic acid) (DTNB) and 0.4 ml of distilled water was added and the absorbance was read at 412 nm.

Activity of catalase was obtained in accordance to Claiborne's protocol [41]. Shortly, H_2O_2 was added to a mixture of 50 mM potassium phosphate buffer (pH 7.0) and supernatant and H_2O_2 degradation rate was followed at 240 nm.

Superoxide dismutase (SOD) activity was according to previous reports [42,43]. In short, supernatant was incubated with xanthine and xanthine oxidase in potassium phosphate buffer (pH 7.8, 37 °C) for 40 min, and nitroblue tetrazolium (NBT) was then added. Thereafter, blue formazan formation was monitored at 550 nm. The amount of protein that inhibited NBT reduction to 50% maximum was regarded as 1 nitrite unit (NU) of SOD activity.

Measurement of protein content was according to Bradford method with bovine serum albumin as its standard [44].

2.6. Measurement of MMP

For determination of mitochondrial membrane potential (MMP) as a reliable index of mitochondrial integrity and cell health, the renal supernatant was re-centrifuged (10,000 rpm for 15 min). The resultant precipitate encompasses mitochondrial fraction from renal tissue. Assessment of changes in MMP was done as reported before [45,46].

Mitochondrial fraction were incubated with 0.2 μmol/l of rhodamine 123 (Sigma-Aldrich, USA) at 37 °C for 5 min, and then the MMP was determined. Fluorescence signals related to mitochondria were excited at 488 nm and emission was followed at 525 nm using a fluorescent microplate reader and fluorescence intensity was finally reported as relative fluorescence unit (RFU).

2.7. Determination of renal NF-κB, Nrf2, TLR4, Cox2, TNFα, IL-1β, and IL-6

The level of these biomarkers in the renal tissue was measured using enzyme-linked immunosorbent assay according to manufacturer's instructions (for Nrf2 and TLR4 from Cloud-Clone Corp. (Houston, Texas, USA), for TNFα from SigmaAldrich (St Louis, MO, USA), and for NF-kB p65, IL-1β and IL-6 from Abcam (Cambridge, MA, USA)). The absorbance of samples was read by Synergy HT microplate reader (BioTek, Winooski, Vermont, USA) and final values were obtained in accordance to plotted standard curves.

2.8. Determination of Annexin V and DNA fragmentation

Annexin V and DNA fragmentation as valid estimators of apoptosis were assessed using Elisa kit (MyBioSource, Inc., USA) and Cell Death Detection ELISA Plus kit (Roche Diagnostics, Germany), respectively and averaged ODs of samples were finally reported.

2.9. Renal histology

Renal tissue (right side) was fixed in 4% formaldehyde, processed, embedded in paraffin, sectioned at a thickness of 5 μm using rotary microtome, and stained with Periodic Acid-Schiff (PAS) and Hematoxylin and Eosin (H&E). Pathologic alterations in PAS staining were blindly graded on a 0–2 scale in accordance to previous reports [47,48]. In this respect, morphologic alterations such as loss of tubular brush border, interstitial edema, casts, dilatation of Bowman's space, and tubular necrosis were evaluated. For H&E staining, a 0–4 scale [49] was used to assess renal tubular damage. In this regard, tubular damage was evaluated by scoring tubular necrosis, dilatation, apoptosis, and cast formation as: 0 = none, 1 = 1–10%, 2 = 11–25%, 3 = 26–45%, and 4 = 46–75%.

2.10. Statistical analysis

All results were shown as means ± SEM. After performing Kolmogorov-Smirnov test for determination of normal distribution of data, one-way ANOVA test was applied and on finding a significant difference, *Tukey* multiple range test was used for pair-wised comparisons. In all calculations, p-value < 0.05 was taken as significant.

3. Results

The beneficial effect of SAC pretreatment on LPS-induced renal function parameters was investigated. As demonstrated in Fig. 1A, serum level of BUN was strikingly and significantly raised 12 and 24 h following LPS challenge ($p < 0.001$) when compared to control group. Furthermore, creatinine level (Fig. 1B) also increased, but to a lower degree versus BUN, 12 ($p < 0.001$) and 24 h ($p < 0.01$) following LPS injection. Conversely, SAC pretreatment of LPS-challenged mice at a dose of 100 mg/kg significantly reduced serum level of BUN ($p < 0.01$) and creatinine ($p < 0.01$).

Following LPS challenge, oxidative stress (due to overproduction of oxygen free radicals and weakened antioxidant system) enhances in kidney tissue [50]. In this regard, there is convincing evidence that oxidative stress damages renal tissue and tubular structures that culminates in AKI [11,51]. For this reason, we assessed the effect of SAC treatment on some renal oxidative stress-related indices. In this respect,

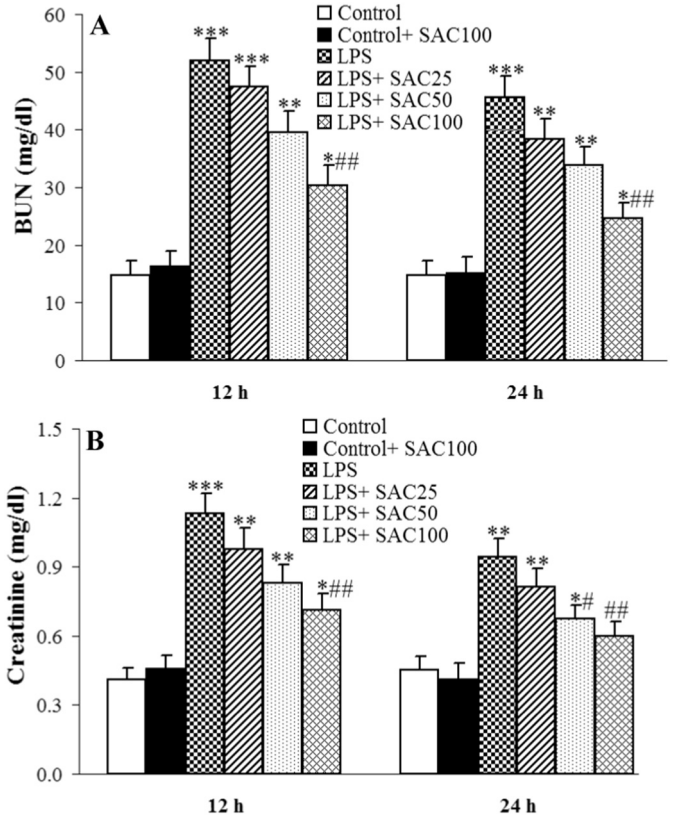

Fig. 1. Serum biomarkers of kidney function comprising BUN (A) and creatinine (B) 12 and 24 h following LPS challenge. LPS was injected once *i.p.* at a dose of 10 mg/kg and s-allyl cysteine (SAC) was administered orally at doses of 25, 50, or 100 mg/kg, 1 h ahead of LPS. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (relative to control), $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ (relative to LPS). n = 7–8 per group.

LPS-challenged mice had a markedly and significantly greater level of renal MDA ($p < 0.01$) (Fig. 2A) and nitrite ($p < 0.001$) (Fig. 2B), lower level of GSH ($p < 0.01$) (Fig. 2C), and lower activity of SOD ($p < 0.01$) (Fig. 2D) and catalase ($p < 0.01$) (Fig. 2E) when compared to control group. Conversely, SAC pretreatment in LPS-challenged mice at a dose of 100 mg/kg significantly lowered MDA ($p < 0.05$), improved SOD activity ($p < 0.05$) and catalase activity ($p < 0.05$), GSH content ($p < 0.05$), and elevated renal level of Nrf2 ($p < 0.05$) (Fig. 2F) with no significant change of nitrite level when compared to LPS-challenged group.

We also measured MMP to evaluate mitochondrial energy metabolism status. In this regard, MMP significantly decreased in LPS-challenged group (Fig. 3A) ($p < 0.01$) relative to control and SAC pretreatment at a dose of 100 mg/kg was capable to significantly raise MMP in LPS group ($p < 0.05$) as compared to LPS-challenged group.

A higher rate of apoptosis has been reported in LPS-induced AKI [28,52]. In this regard, sepsis-associated AKI is associated with acute tubular apoptosis [13]. For assessment of apoptosis severity, we measured renal levels of Annexin V and DNA fragmentation. In this respect, LPS group had a significantly higher level of Annexin V (Fig. 3B) ($p < 0.001$) and DNA fragmentation (Fig. 3C) ($p < 0.001$) versus control group. In contrast, administration of SAC at a dose of 100 mg/kg significantly ameliorated Annexin V ($p < 0.01$) and DNA fragmentation ($p < 0.01$) as compared to LPS group.

LPS exposure is also associated with renal inflammation [53]. Mechanistically, LPS exposure causes AKI through activation of TLR4/NF-κB signaling with subsequent production of inflammatory cytokines such as IL-1β, TNFα, and IL-6 [6]. For this reason, we assessed whether beneficial effect of SAC is mediated through attenuation of

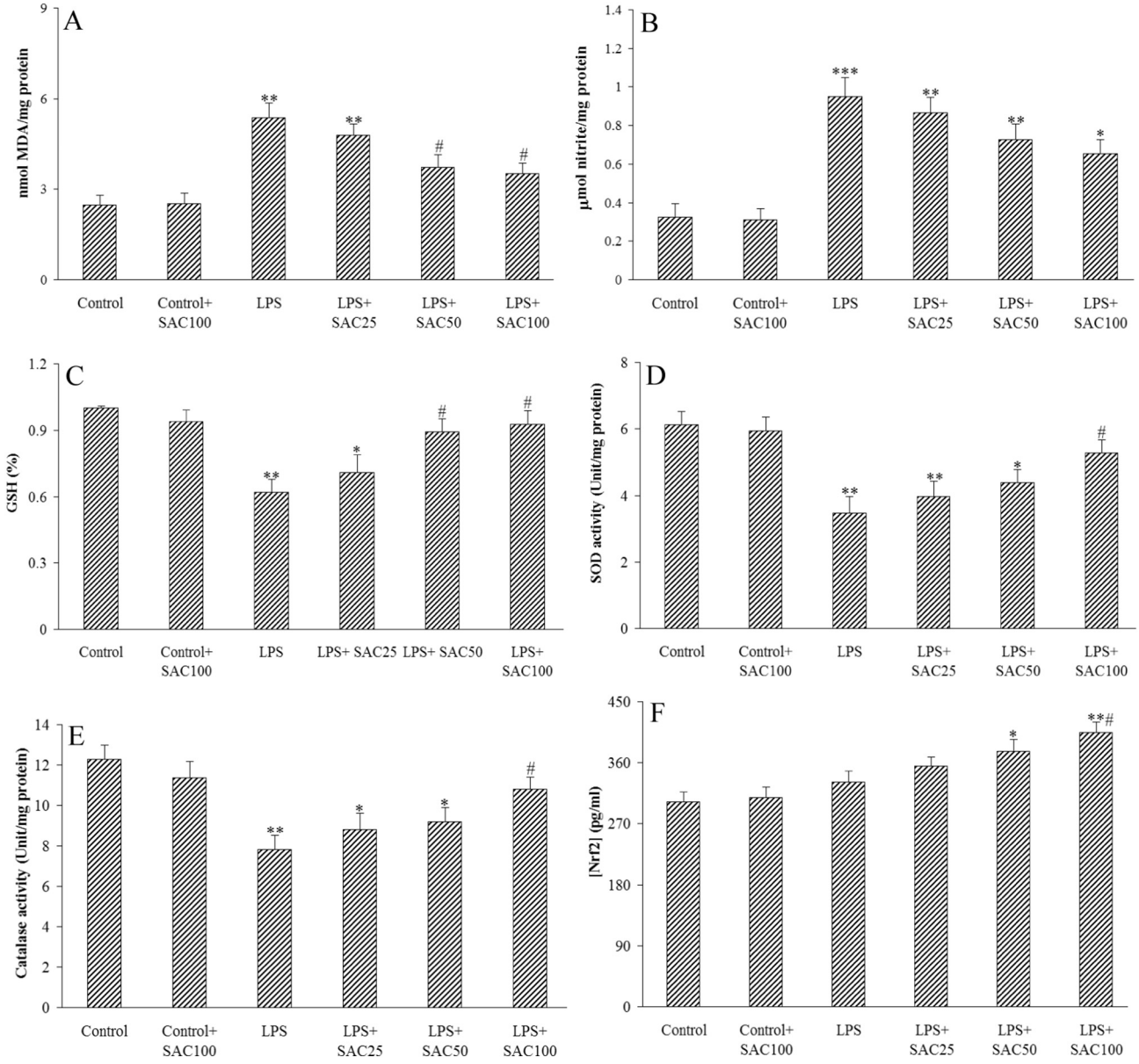

Fig. 2. Renal oxidative stress-related biomarkers in different experimental groups. LPS was injected once *i.p.* at a dose of 10 mg/kg and s-allyl cysteine (SAC) was administered orally at doses of 25, 50, or 100 mg/kg, 1 h ahead of LPS. The figure shows renal MDA concentration (A), nitrite (B), GSH (C), superoxide dismutase (SOD) activity (D), catalase activity (E), and Nrf2 (F). *p < 0.05, **p < 0.01, ***p < 0.001 (relative to control), #p < 0.05 (relative to LPS). n = 7–8 per group.

inflammation and accordingly measured renal level of NF-κB (Fig. 4A), TLR4 (Fig. 4B), and COX2 (Fig. 4C), TNFα (Fig. 5A), IL-1β (Fig. 5B), and IL-6 (Fig. 5C). Our findings showed that renal level of NF-kB ($p < 0.01$), TLR4 ($p < 0.001$), COX2 ($p < 0.001$), TNFα ($p < 0.001$), IL-1β ($p < 0.001$), and IL-6 ($p < 0.001$) markedly and significantly increases in LPS-challenged group when compared to control mice. Conversely, pretreatment of LPS-challenged mice with SAC at a dose of 100 mg/kg was capable to significantly restore NF-kB ($p < 0.01$), TLR4 ($p < 0.01$), COX2 ($p < 0.01$), TNFα ($p < 0.01$), IL-1β ($p < 0.001$), and IL-6 ($p < 0.001$) versus LPS-challenged group.

The effect of SAC administration on renal pathological changes due to LPS was evaluated employing PAS and H&E staining protocols (Fig. 6). In this respect, prominent renal damage including edema of tubular epithelial cells, expansion of Bowman's capsule space, derangement and enlargement of tubular structures, denudation of epithelium, and injury to brush borders was noted 24 h after LPS challenge. In addition, averaged pathologic (Fig. 6A) and renal tubular injury (Fig. 6B) scores were significantly elevated in LPS group versus control ($p < 0.001$). In contrast, SAC administration dose-dependently alleviated LPS-induced pathologic kidney injury ($p < 0.05$ for pathologic scoring in PAS staining and $p < 0.01$ for renal tubular injury in H &E staining).

4. Discussion

Obtained data in this research study demonstrated that SAC pretreatment of LPS-challenged group dose-dependently ameliorates serum BUN and creatinine, restores most renal biomarkers of oxidative stress and inflammation, preserves mitochondrial integrity, and mitigates apoptosis.

In our study, LPS-challenged mice showed kidney dysfunction, as demonstrated by higher serum BUN and creatinine that was in agreement with earlier reports [33,54,55]. In spite of these confirmatory reports, however some investigators had not reached to such findings with regard to serum creatinine and even refuted that serum creatinine be considered as a consistent and reliable biomarker of kidney function

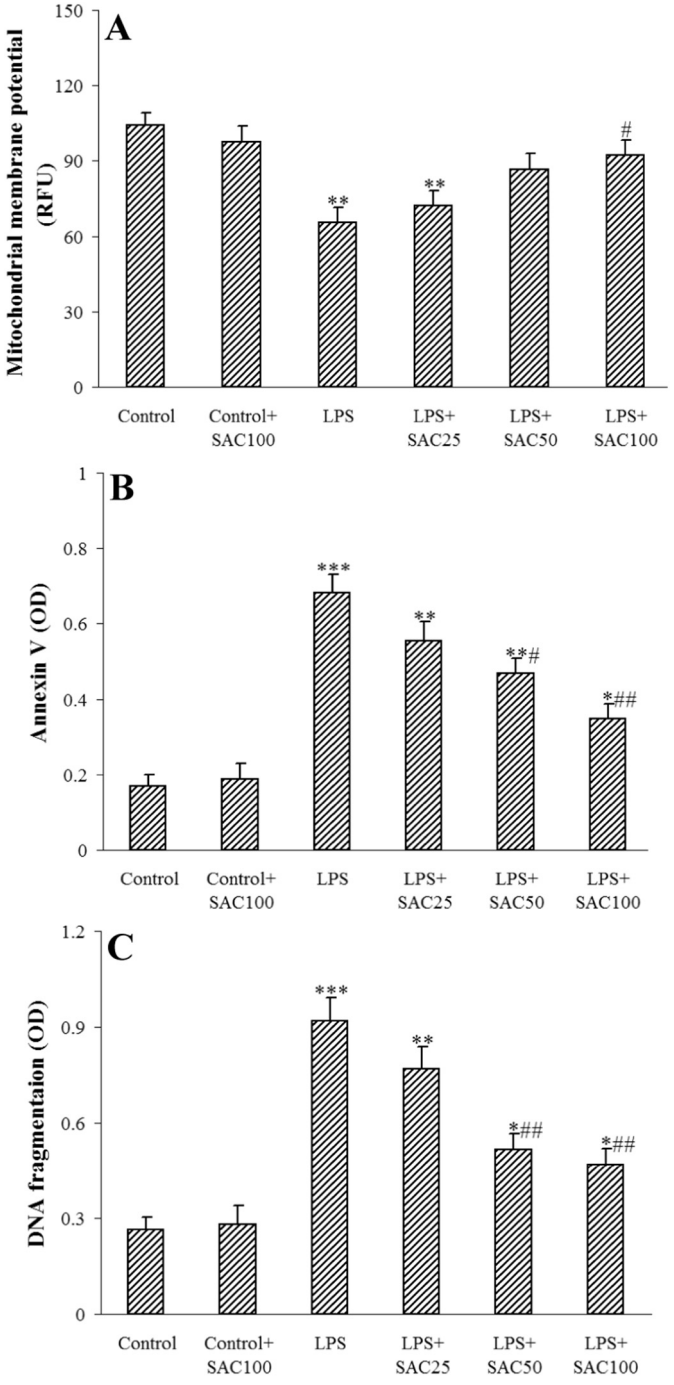

Fig. 3. Renal mitochondrial membrane potential as shown by relative fluorescence unit (RFU) (A) and apoptotic biomarkers including Annexin V (B) and DNA fragmentation (C). LPS was injected once *i.p.* at a dose of 10 mg/kg and s-allyl cysteine (SAC) was administered orally at doses of 25, 50, or 100 mg/kg, 1 h ahead of LPS. *p < 0.05, **p < 0.01, ***p < 0.001 (relative to control), #p < 0.05, ##p < 0.01 (relative to LPS). n = 6 per group.

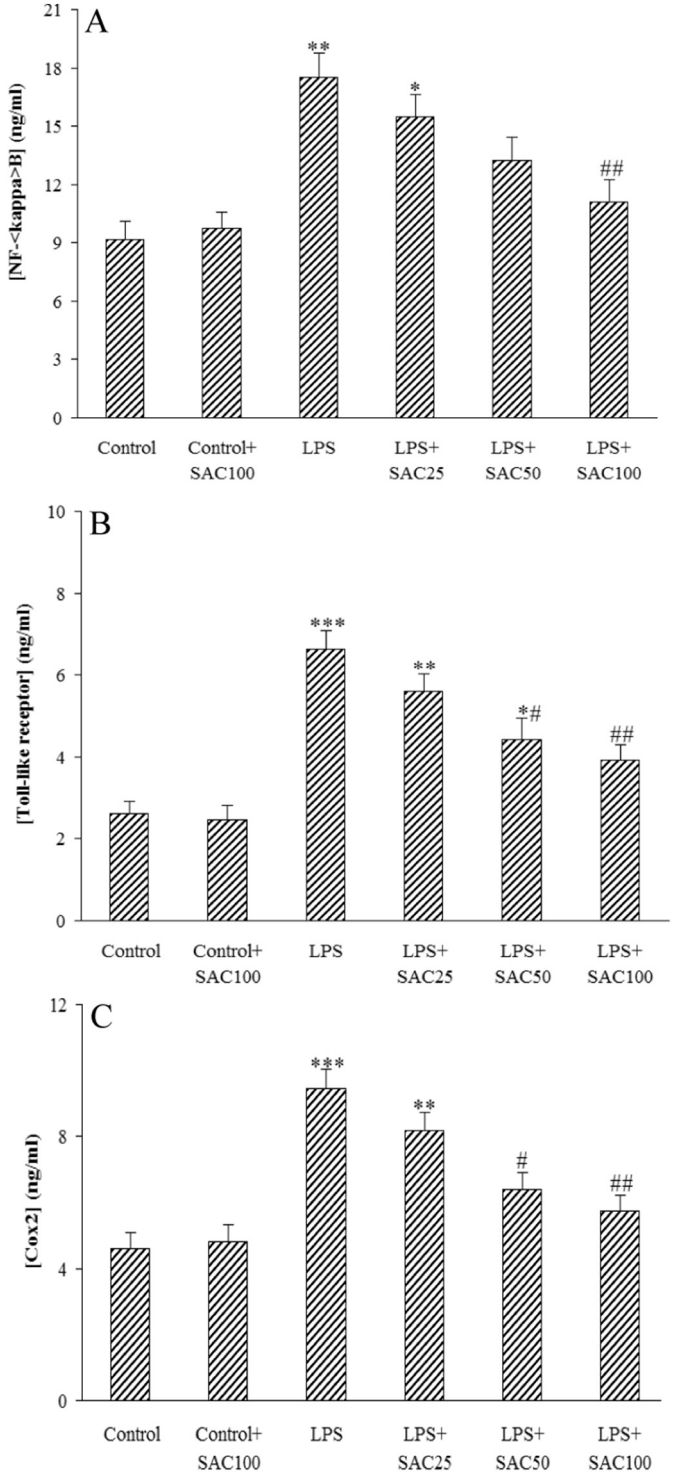

Fig. 4. Renal inflammation-related biomarkers in different experimental groups. LPS was injected once *i.p.* at a dose of 10 mg/kg and s-allyl cysteine (SAC) was administered orally at doses of 25, 50, or 100 mg/kg, 1 h ahead of LPS. The figure shows renal level of NF-kB (A), TLR4 (B), and Cox2 (C). *p < 0.05, **p < 0.01, ***p < 0.001 (relative to control), #p < 0.05, ##p < 0.01 (relative to LPS). n = 6 per group.

in AKI and related conditions [56,57]. In interpretation of this controversy, according to Hu et al., it has been demonstrated that following LPS challenge (at a dose of 10 mg/kg) in mice, at least a period of 24 h is required for serum creatinine to be increased at an appropriate degree [55]. SAC pretreatment in this study successfully reduced serum creatinine and BUN in LPS model of AKI. In support of these findings, Mong et al. in 2012 reported that SAC due to its anti-inflammatory and anti-oxidative effects could protect the kidneys against deleterious effects of diabetes in mice [27].

Oxidative stress as a result of overproduction of reactive oxygen species (ROS) not sufficiently counteracted by endogenous enzymatic and non-enzymatic antioxidants is elevated in LPS-induced model of AKI [58]. Oxidative stress is believed to play a key role in the development of AKI [59]. According to literature, LPS challenge rapidly

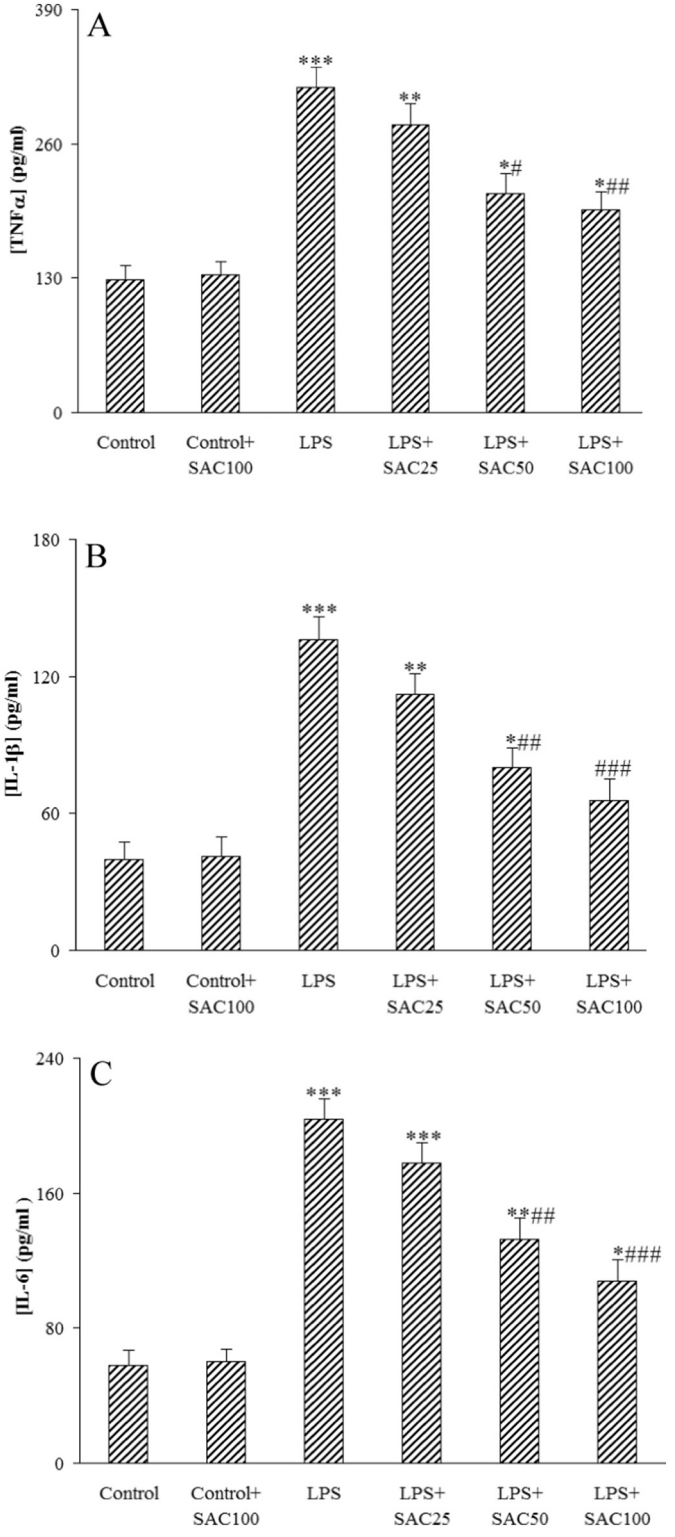

Fig. 5. Renal inflammation-related biomarkers in different experimental groups. LPS was injected once *i.p.* at a dose of 10 mg/kg and s-allyl cysteine (SAC) was administered orally at doses of 25, 50, or 100 mg/kg, 1 h ahead of LPS. The figure shows renal level of TNFα (A), IL-1β (B), and IL-6 (C). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (relative to control), $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ (relative to LPS). n = 6 per group.

evokes extra generation of ROS that leads to complications of sepsis [59]. In this study, renal content of MDA as a reliable index of lipid peroxidation elevated and renal activity of self-protective enzymes including catalase and SOD and also GSH content decreased after LPS injection, noticeably showing the occurrence of oxidative stress phenomenon in the kidney tissue. In support of our obtained data, Liu et al. in 2015 have also found out enhanced oxidative stress burden comprising an increase of nitric oxide and MDA and a decrease of GSH and lower activity of SOD and catalase in kidney tissue from male C57BL/6 mice following a challenge of LPS [17]. Conversely, SAC alleviated renal oxidative stress burden due to LPS challenge in a dose-dependent pattern. Consistent with our findings, Escribano et al. in 2017 have shown that SAC could alleviate oxidative damage in an experimental model of multiple sclerosis through normalization of lipid peroxidation, carbonylation of proteins and improving endogenous antioxidants and its effects was according to a dose-dependent manner [60]. Similar findings have also been reported by Zarezadeh et al. in 2017 showing that SAC could attenuate LPS-induced behavioral deficits through alleviation of oxidative stress and inflammation [34].

It has also been reported that inflammatory cytokines play pivotal roles in the pathogenesis of LPS-induced AKI [61]. LPS-induced AKI is coupled to higher levels of inflammatory cytokines including IL-1β, IL-6, and TNF-α [62] and greater expression level of inflammation cascade elements such as NF-*k*B [62] and Cox2 and TLR4 [28,63]. Since these factors are somehow involved in the pathogenesis of LPS-induced AKI, their suppression could ameliorate LPS-induced AKI. In this respect, TLR4 is the main receptor for LPS and activation of TLR4 by LPS could activate NF-κB downstream cascade. NF-κB itself is regarded as the primary transcription factor for regulation of cytokines production during inflammation [64,65]. In the present study, obtained results indicated that SAC pretreatment of LPS-challenged mice dose-dependently mitigated inflammatory cytokines in kidney tissue. To demonstrate anti-inflammatory effect of SAC on LPS-induced AKI, we showed that SAC decreased LPS-induced TLR4 expression and NF-κB activation. Thus, part of protective effect of SAC against LPS-induced AKI is linked to its mitigation of renal inflammation. Consistent with our results, it has been found out that SAC is capable to alleviate inflammation via modulating the expression level of inflammation-triggering molecules like NF-κB in chromium-induced hepatotoxicity in rats [66]. Additionally, Park et al. in 2014 showed that SAC could alleviate non-steroidal anti-inflammatory drug-induced gastric mucosal damage through appropriate modulation of Cox2, heme oxygenase-1, and histone deacetylation [31].

MMP is an important indicator of mitochondrial function that its reduction is suggestive of cell damage and death [17,67]. In our study, SAC was capable to prevent MMP decrease following LPS, indicating its ability to maintain mitochondrial function. Like our finding regarding MMP, It has shown that a derivative of SAC is capable to prevent mitochondrial dysfunction in isoproterenol induced cardiotoxicity in rats [68]. In addition, apoptosis is also enhanced as a pathogenic factor of LPS-induced AKI [57,69] and agents with a potential to inhibit inflammation and apoptosis are promising to prevent AKI [69]. In line with our findings, part of protective effect of SAC has been attributed to its anti-apoptotic effect. In this respect, Javed et al. showed that SAC inhibits apoptotic parameters like DNA fragmentation and could modulate expression of Bcl2 and p53 in streptozotocin-induced diabetic condition [24].

Collectively, SAC is capable to alleviate LPS-induced AKI through mitigation of renal oxidative stress, inflammation, and apoptosis in addition to preservation of mitochondrial integrity and its favorable effect exhibits a dose-dependent pattern.

Acknowledgement

This research project was financially supported in 2017 by a research grant (no. 958692) from National Institute for Medical Research Development (NIMAD) of Iran.

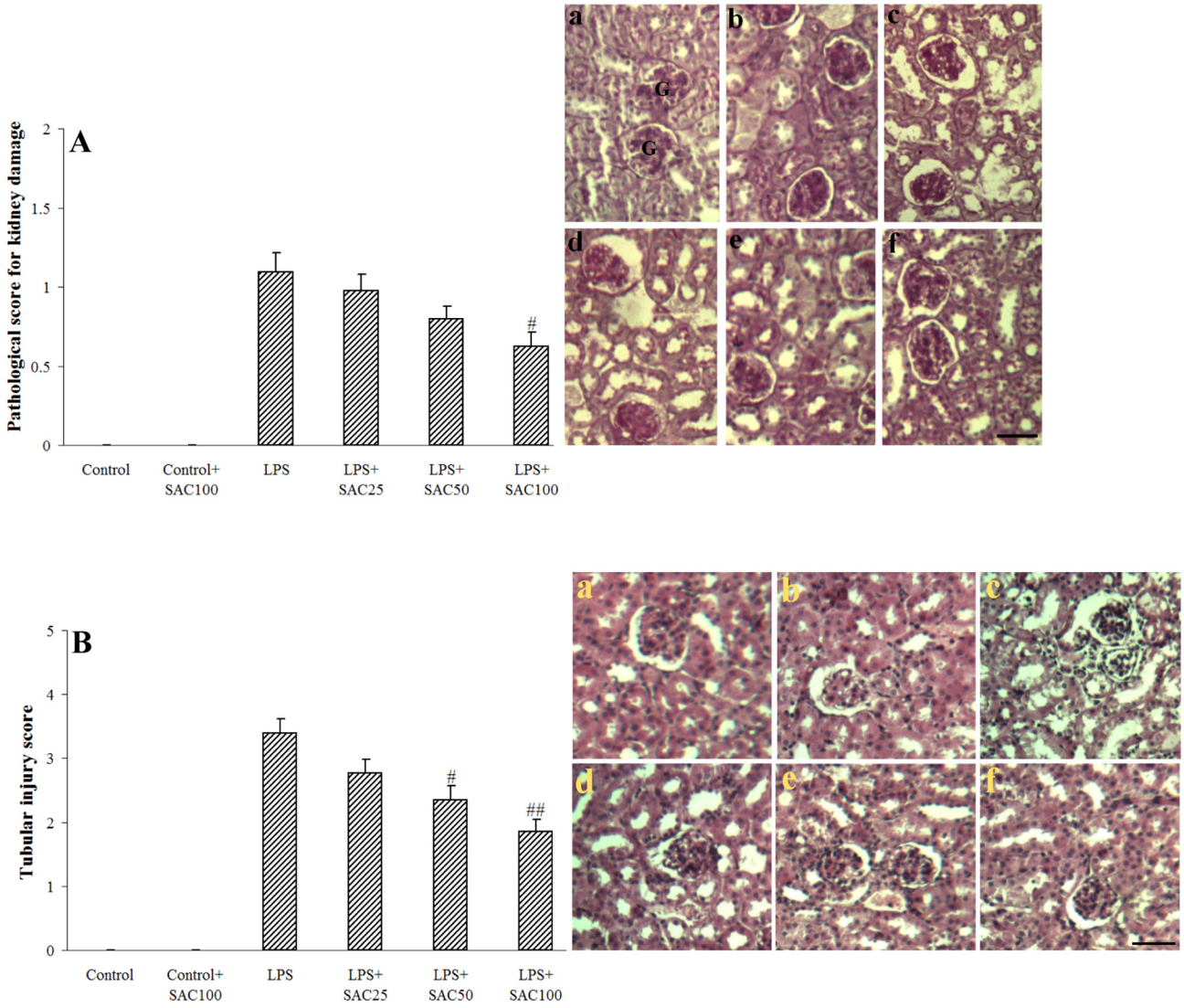

Fig. 6. Averaged pathologic scores of renal tissue damage in PAS staining (A) and renal tubular injury severity in H&E staining (B) and representative photomicrographs of control (a), control + SAC100 (b), LPS (c), LPS + SAC25 (d), LPS + SAC50 (e), and LPS + SAC100 (f) groups. G indicates glomerulus. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ (relative to LPS). n = 4 per group. Scale bar = 60 μm.

Conflict of interest

The authors declare that they have no competing interests.

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