

Research Article



PPAR γ Role on Ameliorating the Effects of Losartan in LPS-Induced Lung and Systemic Injuries in Mice

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Abstract

Background: The effects of losartan (Los) and GW9662 on lipopolysaccharides (LPS)-induced acute lung injury (ALI) and systemic inflammation were examined.

Methods: Mice were administered saline or LPS (0.250 mg/kg) for seven days and were treated with Los (1 mg/kg), GW9662 (1 mg/kg), or their combination for ten days (n=7 per group). Total and differential white blood cells (WBC) in the blood and the bronchoalveolar lavage fluid (BALF), oxidant and anti-oxidant markers including malondialdehyde (MDA), total thiol, superoxide dismutase (SOD), and catalase (CAT) levels were measured in serum, the levels of interleukin-4 (IL-4), interferon gamma (IFN- γ), and transforming growth factor beta (TGF- β 1) in the BALF, and lung histopathological changes were assessed.

Results: In the LPS group, SOD, CAT, and thiol in serum, and TGF- β 1 and IFN- γ levels and INF- γ /IL-4 ratio in the BALF were decreased, but WBC count in the blood and BALF, and serum MDA, BALF IL-4 level and lung pathological changes were significantly increased. Most variables were improved in the LPS group treated with Los or its combination with GW9662. Treatment with GW9662 alone did not change any variable.

Conclusion: Although the preventive effect of Los may not be entirely mediated through the PPAR- γ receptor, the results indicate that the Los effect is partially reversed with GW9662 treatment, suggesting at least a partial involvement of the PPAR- γ pathway.

Introduction

Several factors such as environmental pollutants, and bacterial and viral infections may induce lung inflammation leading to chronic obstructive pulmonary disease (COPD).^{1,2} Inflammatory markers including cytokines³⁻⁵ and growth factors can induce pathological changes in lung tissue by promoting tissue fibrosis⁶ which causes acute lung injury (ALI).⁷ It was shown that tobacco products⁸ and pollutants⁹ are potential sources of lipopolysaccharide (LPS).

LPS is the main biological component of the cell membrane of Gram- negative bacteria and it can induce ALI by increasing pro-inflammatory mediators and oxidative stress markers.¹⁰ The levels of interleukin one beta (IL-1 β), tumor necrosis factor-alpha (TNF- α), IL-6, and autophagy were shown to increase in LPS-induced ALI in mice and A549 epithelial cells of human alveoli.¹¹ Inducing ALI by LPS via stimulating pro-inflammatory cytokine production and activation of Toll-like receptor (TLR4) and TLR2 dependent signaling

pathways was indicated.⁷ In the LPS-induced ALI mice, miR-126-5p plays a crucial role in down-regulating vascular endothelial growth factor-A (VEGFA), which is typically overexpressed during ALI and contributes to increased vascular permeability and inflammation; thus, the downregulation of miR-126-5p leads to VEGFA overexpression that exacerbates lung injury, while restoration of miR-126-5p alleviates ALI by maintaining endothelial integrity and reducing inflammation.¹² Moreover after LPS administration, fibrotic factors including TGF- β 1, collagen type I and III, and α -smooth muscle-actin (α -SMA) levels were reported to increase.¹³

Losartan (Los), as an angiotensin II receptor antagonist, is a medication mainly used to treat cardiac failure and hypertension which are common comorbidities in COPD patients. This drug showed therapeutic effect on COPD by decreasing pneumonia and exacerbation factors which reduced the mortality rate in COPD.¹³⁻¹⁸ Treatment with Los also decreased inflammation, oxidative stress, and nuclear factor kappa B (NF- κ B) protein expression in

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COPD rats with ALI induced by tobacco smoke.¹⁹

In addition, the ameliorative effect of PPAR- γ agonist on LPS-induced neuro-inflammation was demonstrated. It was indicated that treatment with PPAR- γ agonist decreases the expression of inflammatory mediators and the expression of their related signaling proteins such as I κ B and NF- κ B.²⁰

Therefore, the present study aimed to examine the effects of Los alone or in combination with a PPAR- γ -receptor antagonist on ALI and inflammation induced by LPS. This aim was achieved by assessing the therapeutic effects of an angiotensin II receptor antagonist, Los alone, and in the presence of a PPAR- γ receptor antagonist, GW9662 on LPS-induced ALI.

Materials and Methods

Animals

In the animal house of Mashhad University of Medical Sciences, Mashhad, Iran, BALB/c mice (male, 30-35 g) were kept in cages (temperature of 22 ± 2 °C, humidity of $54 \pm 2\%$ and light/dark cycle 12 h). Animal experimental protocols (code 951071) were approved by the ethics committee of Mashhad University of Medical Sciences. National Laws regarding the use and care of laboratory animals complying with the ARRIVE guidelines by either the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, the EU Directive 2010/63/EU for animal experiments, or the National Research Council's Guide for the Care and Use of Laboratory Animals were followed.

Experimental Groups

Seven groups of mice in two main categories were included in the study ($n=7$ in each group) as follows:

- A) Saline-administered groups including:
 1. Non-treated or control (Ctrl) group
 2. Los (1 mg/kg/day, 10 days)-treated (Los) group
 3. GW9662, a PPAR- γ receptor antagonist (1 mg/kg/day)-treated (GW) group
- B) LPS-administered (0.25 mg. kg/day, 7 days) groups including:
 4. Non-treated (LPS) group
 5. Group treated with Los (LPS-Los)
 6. Group treated with GW9662 (LPS-GW)
 7. Group treated with a combination of GW9662

and Los (LPS-GW-Los)

Los (1 mg/kg) and GW9662 (1 mg/kg) were administered for ten days intraperitoneally (i.p.) and saline or LPS (250 μ g/kg, i.p.) were injected during the last seven days (18) (Figure 1).

On day 11 (the end of the experiment), ketamine (50 mg/kg, i.p.)²¹ and xylazine (5 mg/kg, i.p.)²¹ were injected and animals were sacrificed without pain and stress. The lungs were removed, the left lung was clamped and the right lung was washed five times through a tracheal cannula, each time with 1 mL saline (5 mL, total) for preparing BALF. To measure the cytokine levels (IL-4, IFN- γ , and TGF- β) as well as oxidative stress markers, the BALF was centrifuged for 10 min at 2500 rpm, at 4 °C and the supernatant was stored at -70 °C. After animals were sacrificed, a blood sample (2.5 mL) was prepared from the heart, its serum was isolated, and total and differential WBC were counted according to a previous method.²² To measure oxidative stress markers, the BALF samples were prepared and stored at -70 °C.

Counting White Blood Cells (WBC)

Total WBC was counted in a hemocytometer (Burker chamber) in the blood and the BALF. The smear of blood was stained with Wright-Giemsa and differential WBC was counted based on a previous method.²¹

Assessment of Oxidative Stress

Oxidant and anti-oxidant markers including MDA, total thiol, SOD, and CAT levels were measured in serum samples stored at -70 based on a previously reported method^{21,23} and described below.

MDA was measured using, 2 mL reagent of thiobarbituric acid (TBA)/trichloroacetic acid (TCA)/HCl added to 1 mL serum and heated in a water bath for 40 min and cooled and centrifuged at $1000 \times g$ for 10 min. At 535 nm, the absorbance was measured and MDA concentration (C) in nM was calculated using $C = \text{Absorbance} / (1.56 \times 105)$.

Measurement of the activity of SOD was done by the generation of superoxide by pyrogallol auto-oxidation and the inhibition of superoxide-dependent reduction of the tetrazolium dye, MTT (3-(4, 5-dimethylthiazol-2-yl, 2, 5-diphenyltetrazolium bromide) to its formazan by SOD. At 570 nm, SOD activity was measured and expressed as unit (U)/mL.

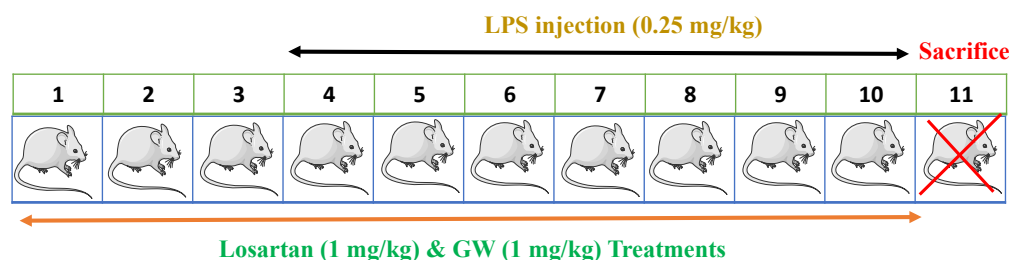


Figure 1. Time table of the study and protocol of lipopolysaccharide (LPS)-induced lung injury and treatment (The form of the rat used on the site <https://smart.servier.com/> and final drawing of the software Power Point).

Measurement of the activity of CAT was done by determination of the rate constant, k , (dimension: s^{-1} , k) of hydrogen peroxide decomposition by the reduction in absorbance at 240 nm per minute and expressed as unit (U)/mL.

Measurement of total thiol concentration was done by adding 1 ml trisethylene diamine tetraacetic acid (EDTA) buffer to 50 μ L serum in 1 ml cuvettes and read at 412 nm against Tris-EDTA buffer alone (A1). Then, 20 μ L DTNB reagents were added to the mixture and kept for 15 min and the sample absorbance was read again (A2). The absorbance of the DTNB reagent was read as a blank (B) and the following equation was used to calculate total thiol concentration (mM):

$$\text{Total thiol concentration (mM)} = (A2 - A1 - B) \times 1.07 / 0.05 \times 13.6.$$

Measurement of Cytokine Levels

The levels of IL-4 (Catalogue Number: SRP3211), IFN- γ (Catalogue Number: SRP3058), and TGF- β 1 (Catalogue Number: SRP0300) in the BALF were evaluated using specific ELISA kits (eBioscience Co, San Diego, CA, USA) based on the manufacturer's instructions and a previous method.

Evaluation of Lung Histopathological Changes

Lung histopathological change scores including interstitial inflammation, fibrosis, lymphoid infiltration, and emphysema were evaluated in the left lung based on a previous method.^{21, 24} The specimens of the left lung were fixed in 10% formalin (37%, Merck, Germany), embedded in paraffin and cut into 4 μ m sliced then they were stained with hematoxylin-eosin (H&E) solution and pathological evaluations were done under a light microscope. Lung pathologic changes were scored as: no pathologic changes=0, patchy changes=1, local changes=2, scattered changes=3 and severe changes (in the most parts of the lung)=4. Lung pathological evaluation was performed in the control, LPS, LPS-Los, LPS-GW, and LPS-GW-Los groups because there were minor changes in the other measured variables in the Saline-Los, and Saline-GW groups.

Statistical Analysis

Mean $\pm$ SEM of the data is presented and compared among groups using ANOVA (one-way analysis of variance) and Tukey post hoc test. The level of $P < 0.05$ was regarded as statistical significance.

Results

Systemic Results

Blood WBC Results

The numbers of WBC (total and differential) were significantly increased in the LPS group ($P < 0.001$ for all cases) but the Saline-Los and Saline-GW groups did not show significant changes in total and differential WBC

compared to the Ctrl group (Figure 2).

The numbers of WBC (total and differential) in the LPS-Los group were significantly decreased in comparison with the LPS group ($P < 0.01$ for neutrophils and monocytes and $P < 0.001$ for other cases) (Figure 2).

In the LPS-GW-Los group, lymphocytes ($P < 0.01$) and eosinophils were significantly decreased compared to the LPS group ($P < 0.001$) (Figure 2). The effects of GW-Los combination treatment on total and differential WBC were significantly lower than that of Los treatment except for monocytes count ($P < 0.05$ for neutrophils and $P < 0.01$ for other cases) (Figure 2).

However, the numbers of WBC (total and differential) in the LPS-GW group were not changed compared to the LPS group (Figure 2). In addition, in the LPS-GW group, total and differential WBC counts were significantly higher than the LPS-Los group ($P < 0.05$ to $P < 0.001$) (Figure 2).

Increased total and differential WBC in the blood in the LPS group indicated LPS-induced systemic inflammation. The reduction in systemic inflammation (total and differential WBC) observed in the LPS-treated group receiving both Los and GW highlights the therapeutic effect of Los, which is further enhanced by the additive effect of GW.

Serum Oxidant and Anti-Oxidant Markers

Significantly decreased serum CAT and SOD activities and thiol levels but increased MDA levels, were observed in the LPS group compared to the control group ($P < 0.001$ for all cases). However, there were no significant changes in oxidative stress markers in the Saline-Los and Saline-GW groups in comparison to the Ctrl group (Figure 3).

All serum oxidative stress marker levels in the LPS-Los and LPS-GW-Los groups were significantly improved in comparison with the LPS group except MDA in the LPS-GW-Los group ($P < 0.05$ to $P < 0.001$) (Figure 3). The improvement of all oxidative stress markers in the LPS-Los group was significantly greater than that of the LPS-GW, and the improvement of SOD and CAT activities was higher than that of the LPS-GW-Los group ($P < 0.05$ to $P < 0.01$) (Figure 3).

The LPS-GWs groups did not show any significant improvement in oxidative stress markers compared to the LPS group (Figure 3).

The alterations in serum oxidative stress markers in the LPS group confirmed LPS-induced systemic oxidative stress. Treatment with Los improved these markers, and the combination of Los and GW resulted in even greater improvements, demonstrating both the protective effect of Los and the additive benefit of GW against LPS-induced oxidative stress.

Lung-Specific Results

WBC Results

In the LPS group, the numbers of WBC (total and differential) were increased in the BALF in comparison

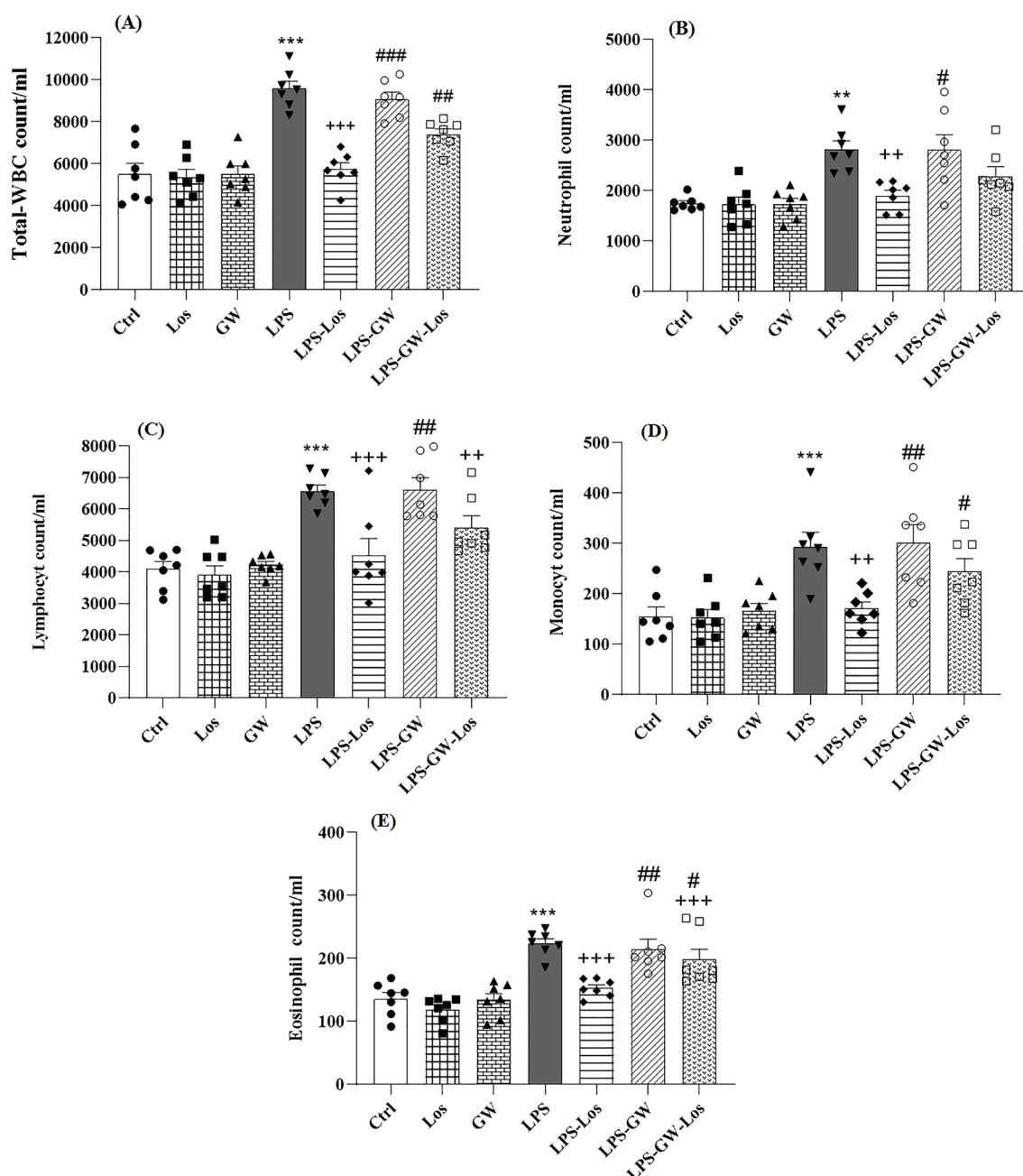


Figure 2. Total WBC (A) neutrophil (B) Lymphocyte (C) Monocyte (D), and Eosinophil (E) counts in the blood examined by cell counting of the control (Ctrl), saline + losartan (1 mg/kg)-treated (Los), saline + GW9662 (1 mg/kg)-treated (GW), lipopolysaccharide-administered (LPS), LPS + Los (1 mg/kg)-treated (LPS-Los), LPS + GW9662 (1 mg/kg)-treated (LPS-GW), and LPS + Los + GW9662 combination-treated (LPS-GW-Los) groups. Data are shown as mean $\pm$ SEM ($n=7$ per group). **, $P<0.01$ and ***, $P<0.001$ compared to the control group, ++, $P<0.01$ and +++, $P<0.001$ compared to the LPS group, #, $P<0.05$ and ##, $P<0.01$, ###, $P<0.001$ compared to the LPS-Los group. Statistical analysis were performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

with the Ctrl group ($P<0.001$ for all cases) but in the Saline-Los and Saline-GW groups, the numbers of WBC (total and differential) were not significantly changed in comparison with the Ctrl group (Figure 4).

Treatment with Los significantly reduced the numbers of WBC (total and differential) and treatment with the combination of GW-Los reduced monocyte and eosinophil counts ($P<0.01$ to $P<0.001$) in comparison with the LPS group (Figure 4). However, the effects of Los treatment on total and all differential WBC were significantly greater than GW-Los and combination

treatment groups ($P<0.05$ to $P<0.01$) (Figure 4).

The numbers of WBC (total and differential) in the LPS-GW group were not changed in comparison with the LPS group (Figure 4). In the LPS-GW group, differential WBC counts were significantly greater than the LPS-Los group ($P<0.01$ for monocyte and $P<0.001$ for other cases) (Figure 4).

In the LPS group, total and differential WBC in the BALF were increased, indicating LPS-induced lung inflammation in terms of increased inflammatory cells. However, in the LPS group treated with Los, total and

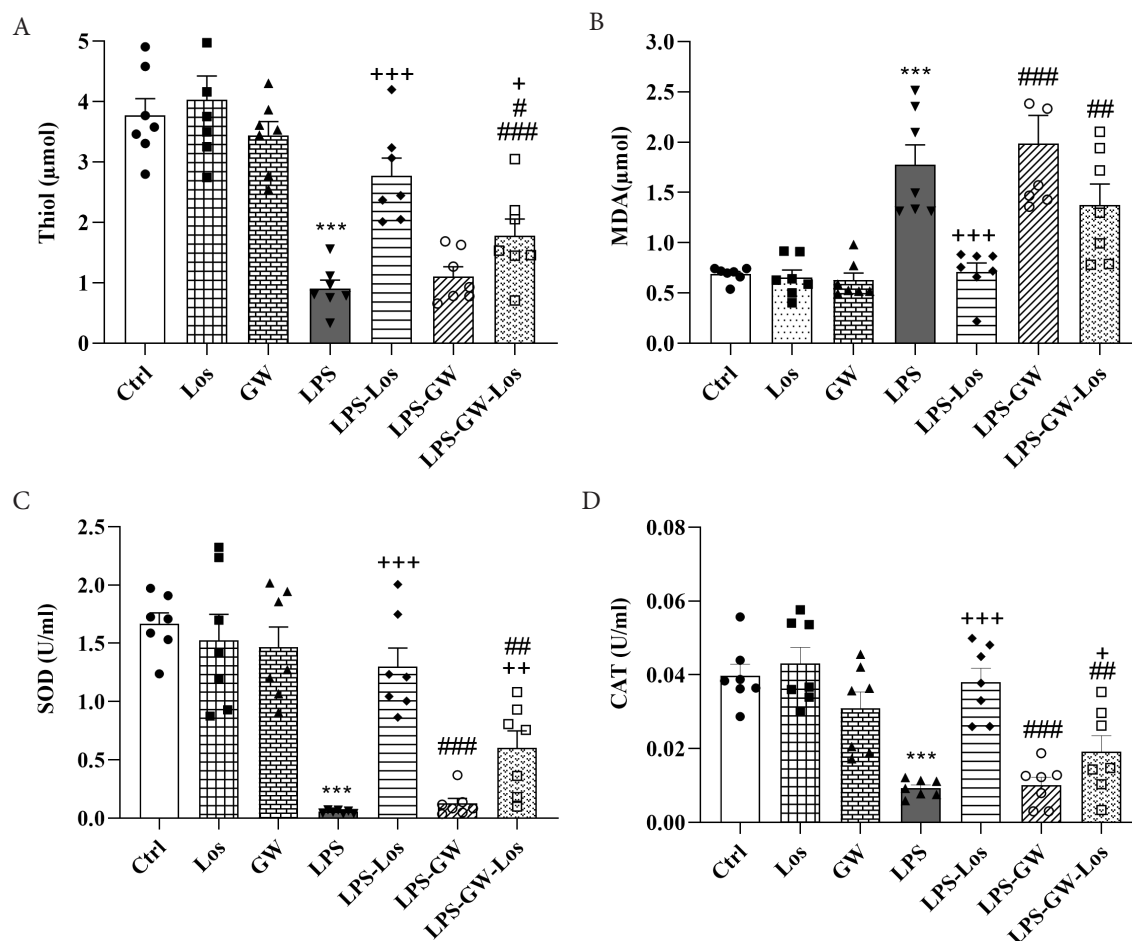


Figure 3. The levels of total thiol (A) MDA (B) SOD (C) and CAT (D) in the serum examined by cell biochemical methods of the control (Ctrl), saline + losartan (1 mg/kg)-treated (Los), saline + GW9662 (1 mg/kg)-treated (GW), lipopolysaccharide-administered (LPS), LPS + Los (1 mg/kg)-treated (LPS-Los), LPS + GW9662 (1 mg/kg)-treated (LPS-GW), and LPS + Los + GW9662 combination-treated (LPS-GW-Los) groups. Data are shown as mean $\pm$ SEM (n=7 per group). ***, $P < 0.001$ compared to the control group, +, $P < 0.05$ and ++, $P < 0.01$, +++ $P < 0.001$ compared to the LPS group, #, $P < 0.05$ ##, $P < 0.01$ and ###, $P < 0.001$ compared to the LPS-Los group. Statistical analysis were performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

differential WBC were reduced which demonstrated the effect of Los on LPS-induced increased inflammatory cells in the lung.

BALF Cytokine Levels

In the LPS group, the levels of IFN- γ and TGF- β and INF- γ /IL-4 ratio in the BALF were significantly increased but the IL-4 level was decreased in comparison with the Ctrl group ($P < 0.001$ for all cases). In addition, the cytokine levels in the Saline-Los and Saline-GW groups did not change in comparison with the Ctrl group (Figure 5).

Cytokines levels and INF- γ /IL-4 ratio were improved in the LPS-Los and LPS-GW-Los groups in comparison with the LPS group ($P < 0.01$ for TGF- β in the LPS-GW-Los group, and $p < 0.001$ for other cases), (Figure 5). The effect of GW-Los combination treatment on all measured cytokines and INF- γ /IL-4 ratio was significantly lower than Los alone ($P < 0.05$ to $P < 0.001$), (Figure 5).

There was no significant difference in cytokine levels in the LPS-GW group in comparison with the LPS groups (Figure 5).

Increased IFN- γ , TGF- β , and INF- γ /IL-4 ratio in the

BALF but decreased IL-4 in the LPS group, are other important indicators of LPS-induced lung inflammation. The improvements in the cytokine levels and INF- γ /IL-4 ratio in the LPS group treated with Los have demonstrated the effect of Los on LPS-induced lung inflammation.

Lung Pathological Evaluation

Figure 6 illustrates photographs of lung histology in the Ctrl, LPS-Los, LPS-GW, and LPS-GW-Los groups. In the LPS group, interstitial inflammation, emphysema, lymphoid infiltration, and peri-bronchitis were significantly higher than in the Ctrl group ($P < 0.001$ for all cases) (Figure 6).

All lung pathological changes were decreased in the LPS-Los group compared to the LPS group ($P < 0.001$ for all cases). The improvement of all pathological changes in the LPS-Los group was higher than in the LPS-GW-Los and LPS-GW groups ($P < 0.05$ to $p < 0.001$) (Figure 6).

The lung pathological changes in the LPS group showed LPS-induced ALI and their improvements in the LPS group treated with Los demonstrated the therapeutic effect of Los on LP-induced ALI.²²

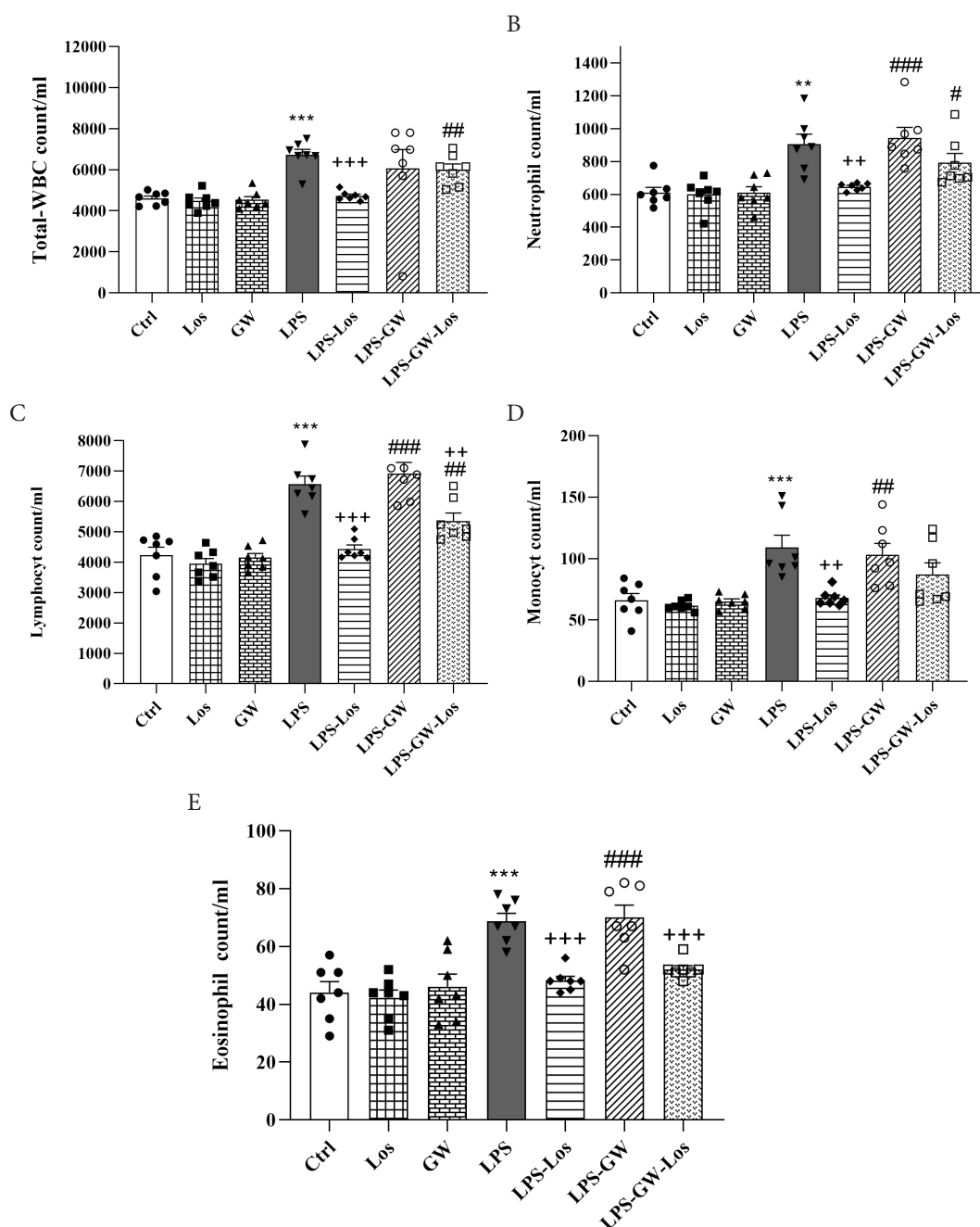


Figure 4. Total WBC (A) Neutrophil (B) Lymphocyte (C), Monocyte (D), and Eosinophil (E) counts in the BALF examined by cell counting of the control (Ctrl), saline+losartan (1 mg/kg)-treated (Los), saline+GW9662 (1 mg/kg)-treated (GW), lipopolysaccharide-administered (LPS), LPS+Los (1 mg/kg)-treated (LPS-Los), LPS+GW9662 (1 mg/kg)-treated (LPS-GW), and LPS+Los+GW9662 combination-treated (LPS-GW-Los) groups. Data are shown as mean±SEM (n=7 per group). **, $P<0.01$ and ***, $P<0.001$ compared to the control group, ++, $P<0.01$ and +++, $P<0.001$ compared to the LPS group, #, $P<0.05$, ##, $P<0.01$ and ###, $P<0.001$ compared to the LPS-Los group. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

Correlation Between the BALF and Serum Total and Differential WBC Counts

There were significant correlations between the BALF and blood total and differential WBC counts ($r=0.82$, 0.83 , 0.76 , 0.89 , and 0.76 for total WBC, neutrophil, lymphocyte, eosinophil, and monocyte respectively, $P<0.001$ for all cases) in the control LPS and treated groups. These correlations also suggested the parallel treatment effect of Los and GW on systemic and lung inflammation induced by LPS. These results indicate a connection between systemic and lung inflammation

and demonstrate that systemic administration of LPS can induce both systemic and lung inflammations.

Discussion

In this study, LPS administration for 7 days increased the numbers of WBC (total and differential) in the blood and BALF, and serum level of MDA as well as BALF levels of IFN- γ , TGF- β and INF- γ /IL-4 ratio, but decreased BALF level of IL-4, total thiol content, and SOD and CAT activities in the serum. LPS exposure also induced lung pathological changes including intestinal inflammation,

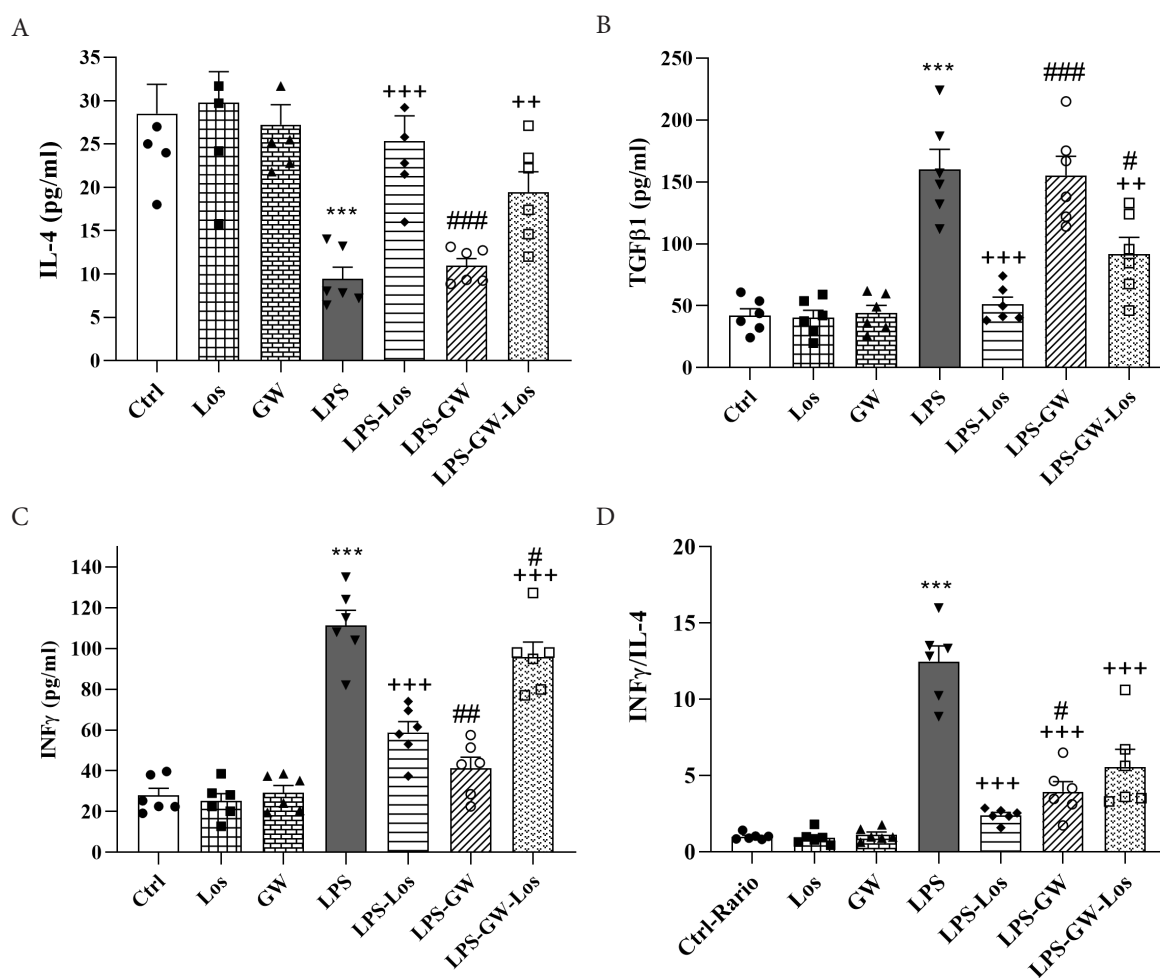


Figure 5. IL-4 (A) TGF- β (B) and IFN- γ (C) INF- γ /IL-4 ratio (D) in the BALF examined by ELISA method of the control (Ctrl), saline + losartan (1 mg/kg)-treated (Los), saline + GW9662 (1 mg/kg)-treated (GW), lipopolysaccharide-administered (LPS), LPS + Los (1 mg/kg)-treated (LPS-Los), LPS + GW9662 (1 mg/kg)-treated (LPS-GW), and LPS + Los + GW9662 combination-treated (LPS-GW-Los) groups. Data are shown as mean $\pm$ SEM (n = 7 per group). ***, $P < 0.001$ compared to the control group, ++, $P < 0.01$ and +, $P < 0.05$ compared to the LPS group, #, $P < 0.05$, ##, $P < 0.01$, ###, $P < 0.001$ compared to the LPS-Los group. Statistical analysis were performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

emphysema, lymphoid infiltration and peri-bronchitis. Our previous studies confirmed ALI shown by increased WBCs, IFN- γ and TGF- β levels, oxidant/antioxidant imbalance, reduced level of IL-4 in the BALF, and induced lung pathological changes, as well as systemic inflammation by increased numbers of WBC (total and differential) count, and oxidative stress markers, but reduced antioxidant markers in the serum of the mice following chronic systemic administration of LPS.^{21,25-27} The above studies support the results of the present study regarding LPS-induced ALI and systemic inflammation. Significant correlations between the BALF and blood numbers of WBC (total and differential) may suggest a link between the ALI and systemic inflammation. Therefore, systemic administration of LPS perhaps induces systemic inflammation and oxidative stress and these changes in turn may induce ALI.

In the saline-treated groups, Los and GW9662 did not affect systemic or lung inflammation parameters. These results indicated that these two agents do not have a modulatory effect on the immune system or oxidative stress markers of healthy animals.

The present findings also indicate that treatment with Los improves the systemic and pulmonary complications induced by LPS exposure. Administration of Los significantly reduced the blood numbers of WBC (total and differential), and improved oxidant/antioxidant balance in the serum, indicating the protective effect of Los against LPS-induced systemic oxidative stress and inflammation. The numbers of WBC (total and differential), the levels of IL-4, IFN- γ , and TGF- β and IFN- γ /IL-4 ratio in the BALF as well as lung pathological changes were also improved by Los treatment similarly indicating its therapeutic effects on LPS-induced ALI. In contrast, treatment with GW9662 had no effect on inflammatory or oxidative parameters in the blood or the BALF.

In the previous studies, administration of Los abolished the angiotensin enhanced endothelin-1-induced bovine bronchi contractions, an effect which was mediated via an angiotensin II receptor type 2 receptor (AT2R),²⁸ and improved ALI by decreasing NF- κ B activity, synthesis of TNF- α and apoptosis as well as lung pathological changes.²⁹ Los significantly diminished TNF- α in the

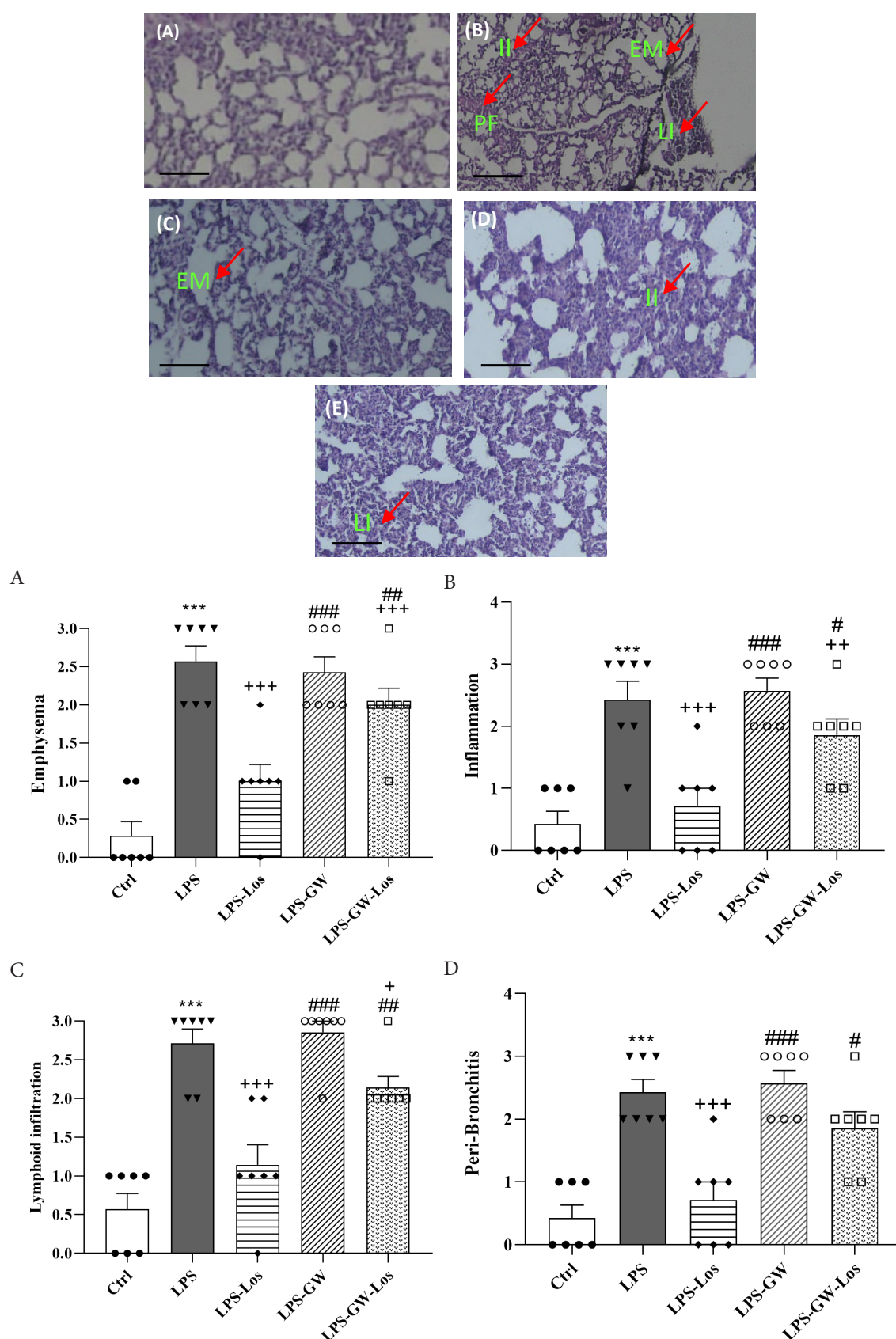


Figure 6. Photographs of a lung specimen in the control (A), LPS (B), LPS-Los (C), LPS-GW (D), and LPS-GW-Los (E) groups indicating interstitial fibrosis (PF), interstitial inflammation (II), lymphoid infiltration (LI) and emphysema (EM) (Magnification $\times 200$, Scale bar = $10\mu\text{m}$). Emphysema (A) and interstitial inflammation (B), lymphoid infiltration (C), peri-bronchitis (D) of lung tissue examined by pathological methods (upper panel) in the control (Ctrl), saline + losartan (1 mg/kg)-treated (Los), saline + GW9662 (1 mg/kg)-treated (GW), lipopolysaccharide-administered (LPS), LPS + Los (1 mg/kg)-treated (LPS-Los), LPS + GW9662 (1 mg/kg)-treated (LPS-GW), and LPS + Los + GW9662 combination-treated (LPS-GW-Los) groups and their score (lower panel). Data are shown as mean $\pm$ SEM ($n=7$ per group). ***, $P<0.001$ compared to the control group, +, $P<0.05$, ++, $P<0.01$ and +++ $P<0.001$ compared to the LPS group, #, $P<0.05$, ##, $P<0.01$, ###, $P<0.001$ compared to the LPS-Los group. Statistical analysis were performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

BALF, myeloperoxidase (MPO) activity in the lung tissues, intercellular adhesion molecule-1 (ICAM-1), caspase-3, lung pathological changes, inflammation and cell apoptosis in a rat model of ALI.³⁰ Los prevented high volume ventilation-caused ALI via reducing BALF total protein, MPO, neutrophil infiltration and pathological changes.³¹ In addition ALI induced by sepsis also improved by Los through reducing NF- κ B activation and mitogen-activated protein kinases, as well as the LPS+ATP-induced production of IL-1 β protein in the mouse macrophage induced NALP3 inflammasome activation.³² The inhibitory effect of angiotensin II receptor blockers on ROS production via the blockade of the AT1 receptor was also reported.^{33,34} The studies mentioned above reinforce the findings of this research, highlighting Los protective role in preventing ALI and mitigating systemic inflammation and oxidative stress caused by systemic LPS administration.

The results of this study showed that the effects of treatment with GW-Los combination on improvement of lymphocyte and eosinophil count in the blood and BALF, serum antioxidants, and BALF levels of IL-4, IFN- γ and TGF- β as well as lung pathological changes including emphysema, interstitial inflammation and lymphoid infiltration were lower than the effects of Los treatment. These results indicated that the effects of Los were partially abolished by GW9662 as a PPAR γ -receptor antagonist drug and suggest that Los probably suppresses inflammatory process via a PPAR γ -receptor dependent mechanism.

GW9662, as a selective PPAR γ antagonist, reduced the effects of Los, demonstrating that PPAR γ activation is contribute losartan's amelioration of lung and systemic inflammation induced by LPS.

In human THP-1 macrophages, Los exhibited preventive effects against LPS-induced upregulation of pro-inflammatory genes by activating the PPAR γ . Specifically, when THP-1 macrophages are stimulated with LPS, they typically increase the expression of pro-inflammatory cytokines such as TNF- α , IL-6, and IL-1 β , contributing to the inflammatory response. Los, an angiotensin II receptor blocker, has been shown to activate PPAR γ , a nuclear receptor that plays a key role in regulating inflammation. Activation of PPAR γ by Los leads to the transcriptional repression of these pro-inflammatory genes, thereby reducing the production of inflammatory cytokines. This mechanism attenuate the inflammatory response induced by LPS in macrophages, suggesting a potential anti-inflammatory role for Los beyond its cardiovascular effects.³⁵ Treatment with Los also reduced the risk of the liver injury induced by ischemia/reperfusion injury through activation of PPAR- γ receptor.³⁶ It was also shown that EXP3179, an active Los metabolite, acts as a partial PPAR- γ agonist.³⁷ The inhibitory effect of EXP3179 on the endothelial cyclooxygenase (COX)-2 expression and its potent anti-inflammatory effect was also indicated.³⁸ Anti-

inflammatory property of ligand-activated PPAR- γ was induced by suppressing pro-inflammatory transcription factors including NF- κ B and AP-1.³⁹ Inhibitory effect of COX-2 promoter and mRNA by PPAR- γ receptor activation via interacting with the c-jun component of the AP-1 complex was also shown.⁴⁰ Studies indicate that Los can up-regulate PPAR γ expression, which in turn represses pathways such as TGF- β 1, further contributing to lung protection and reducing lung fibrosis.⁴¹ Research in other organs (liver, kidney) has shown that Los protective effects are diminished when PPAR γ is antagonized; supporting the idea, that PPAR γ activation is a key mediator of Los tissue-protective actions.^{42,43} While much of this evidence comes from hepatic and renal models, the mechanisms are relevant to lung injury as well, given the shared pathways of inflammation and oxidative stress. These studies support the findings of the present study that anti-inflammatory effects of Los is mediated at list in part, mediated through PPAR- γ receptor activation. PPAR γ activation is known to suppress the production of pro-inflammatory cytokines and inhibit inflammatory signaling pathways, such as NF- κ B, which are central to LPS-induced lung injury.⁴²⁻⁴⁴ By activating PPAR γ , Los can reduce the inflammatory response in lung tissues. PPAR γ enhances cellular antioxidant systems, thereby reducing oxidative stress, a major factor in tissue damage during ALI.^{42,43} Los activation of PPAR γ protect lung cells from oxidative damage was shown in this study. However in further studies the effect of higher doses of GW9662 on LPS-induced systemic and lung inflammation should be examined. In addition, examining the effect of labeled Los on PPAR γ receptor and detailed molecular pathway analysis also could be performed in further studies.

Conclusion

These findings demonstrated that Los as an angiotensin receptor inhibitor is able to prevent ALI as well as systemic inflammation and oxidative stress induced by LPS in mice but activation of the PPAR γ receptors only partially contribute to this effect.

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Authors' Contribution

Conceptualization: Mohammad Hossein Boskabady.

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Competing Interests

The authors declare that they have no conflict of interest.

Data Availability Statement

Data will be made available on request.

Ethical Approval

For the care and use of animals, all applicable international, national, and/or institutional guidelines were followed.

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