# PPAR γ Protects Against Hypoxic Renal Tubular Epithelial Cell Injury in Rat Model: A Randomized Controlled Trial

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**Research Article**

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## Abstract

# Background

To explore t function of peroxisome proliferator-activated receptor γ(PPARγ) in renal tissue in acute hypoxic renal rat model injury.

# Methods

24 male SD rats were randomly divided into normal control group, PPARγ agonist group (rosiglitazone 10 mg/kg.d), PPARγ inhibitor group（GW9662,1mg/kg·d）and hypoxia injury group, with six rats in each group. The normal control group without any treatment, the other three groups were exposed to 7500 m altitude for seven hours of acute hypobaric hypoxia. The mRNA and protein expressions of PPARγ, superoxide dismutase (SOD), interleukin-1 β（IL-1β） and renal endothelin(ET-1) in renal tissue were detected by using real-time fluorescence quantitative polymerase chain reaction (RT-qPCR) and western blotting. The kidney's morphology was observed by light microscope and electron microscope.

# Results

The mRNA and protein expression levels of PPARγ and SOD in hypoxia injury group decreased significantly ($P < 0.05$), while the mRNA and protein expression levels of IL-1 β and ET-1 increased significantly compared with the normal control group ($P < 0.05$). After intervention with PPAR agonists, the PPARγ and SOD were elevated significantly, while IL-1 β and ET-1 were decreased significantly compared to the hypoxia injury group. The renal tubule epithelial cells (RTEC) were less damaged and abscission was reduced.

# Conclusions

PPARγ protect renal tubular epithelial cells from hypoxia-induced injury. PPARγ agonists can be used as potential target interventions to alleviate acute hypoxic kidney injury.

## Introduction

The kidney is very sensitive to hypoxia; once the hypoxia stress is irreversible, it will lead to kidney damage. Renal hypoxia is considered to play an important role in the development of various renal diseases[1]. Acute kidney injury (AKI) or chronic kidney disease (CKD) in different diseases are induced by alterations in renal blood flow [2].

Peroxisome proliferator-activated receptors (PPARs) are a family of nuclear transcription factors, which belong to the type Ⅱ nuclear hormone receptor superfamily. According to the different encoding genes,

PPARs can be divided into three cell subtypes, namely PPARα, PPARβ and PPARγ[3]. Each subtype has its specific agonists[4], and there are significant differences in the expression distribution of these three subtypes. PPARγ expression is distributed in kidney, adipose tissue and immune system, and is involved in normal kidney development and lipid metabolism. PPARγ has physiological functions such as regulating water and salt reabsorption, regulating renal blood flow and activating renin angiotensin system[5, 6]. In recent years, it has been found that the change of PPARγ receptor expression level is involved in the occurrence and development of various kidney diseases. But the PPARγ in hypoxia-induced renal injury was few reported. In this study, a rat model of acute hypoxia-induced renal injury was established, and PPARγ receptor expression was interfered with by PPARγ agonist rosiglitazone and inhibitorGW9662. To exploret role of PPARγ in renal tissue in acute hypoxic renal rat model injury.

## Methods

# Animal grouping and intervention

24 rats were randomly divided into normal control group, PPARγ agonist group, PPARγ inhibitor group and hypoxia injury group, with six rats in each group. PPARγ agonist group and PPARγ inhibitor group were given rosiglitazone 10mg/kg·d, GW9662,1mg/kg·d respectively, intraperitoneal injection three days before hypoxia until the end of the experiment. Normal control group did not receive any intervention treatment, and was given food or water. The other three groups were placed in the low-pressure and hypoxic animal chamber, and the simulated altitude in the chamber was raised to 7500 m (oxygen concentration was about 9.0%). The ambient temperature in the simulated cabin was 25 ± 2℃, the humidity was measured at 55%, and the subjects were given food and water in the cabin. After seven hours of hypoxia, the rats were put down. After the renal capsule and renal fascia were removed from the left kidney tissue, part of the tissue was placed in 4% paraformaldehyde for pathology, and the other part was placed at -80℃ for subsequent experiments.

# Renal Histopathological Examination By Light Microscope And Transmission Electron Microscopy (Tem)

The kidney tissues of different parts, about the size of soybeans, were fixed with 10% neutral formalin and made into 3 ~ 4μm thick paraffin sections according to the conventional method. After staining with haematoxylin and eosin (HE), the kidney tissues were observed under ordinary light microscope. The lateral section of the removed left kidney was dissected, and the tissue with a diameter of 1μm was cut from the renal cortex. The tissue was fixed with 3.1% glutaraldehyde, bathed in buffer solution, fixed with 1% osmium acid, dehydrated by acetone and embedded with Epon812, and then prepared a semi-thin section. They were positioned under a light microscope, then cut into ultra-thin sections, dyed with uranium acetate and aluminum citrate, and observed under TEM.

# Quantitative Real-time Fluorescent Pcr

Total RNA was extracted from kidney tissue by NucleoZOL (Macherey-Nagel, Germany), the mRNA was reversely transcribed into cDNA according to the instructions of TaKaRa reverse transcription kit, and amplified by ABI 7500 PCR machine. The primers were designed and synthesized by Sangon Bioengineering (Shanghai) Co., LTD., and their sequences are shown in Table 1. Total PCR reaction system: forward and reverse primer 0.4μL, Mix 10.0 μL, plate 2.0 μL, adding ddHO to 20.0 μL. Reaction conditions: 95 ℃, 30s, one cycle; 95 ℃, 10 s, 60 ℃, 30 s, 40 cycles; 95 ℃, 15s, 60 ℃, 60 s, 95 ℃, 15s, cycle once. The relative mRNA expression was normalized to β-actin and calculated using the $2^{-\triangle\triangle Ct}$ formula.

Table 1
The real-time PCR primer of rat mRNA

| Gene | Forward primer (5′→3′) | Reverse primer (5′→3′) |
|---|---|---|
| PPARγ | CCATCGAGGACATCCAAGACAACC | GTGCTCTGTGACAATCTGCCTGAG |
| IL-1β | CTCACAGCAGCATCTCGACAAGAG | TCCACGGGCAAGACATAGGTAGC |
| ET-1 | TGCTCCTGCTCCTCCTTGATGG | TCGCTTAGACTTAGAAGGGCTTCC |
| SOD | CCACATCGGCCTGTGTATATCCCAG | CGTGAAGCTGGAGAAGGAGAAGCTG |
| B-actin | AGTGTGACGTTGACATCCGTA | GCCAGAGCAGTAATCTCCTFCT |

# Western blot analysis

Total protein was extracted from kidney tissue with protein lysate. After boiling and denaturation, 10 μg of protein was added to sample, and protein was isolated by sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE). Protein was transferred to polyvinylidene fluoride (PVDF) membrane by wet transfer method. The membranes were blocked with 1×TBST containing 5% nonfat milk for 1.5 h at room temperature. Subsequently, the membrane was added with primary antibody PPARγ (1:1,000), IL-1β (1:1,000), ET-1 (1:1,500) and SOD (1:2,000) and incubated overnight in a refrigerator at 4 ℃. Washed the membrane with TBST three times for 10 minutes each. The membranes were added with horseradish peroxidase labeled secondary antibody (1:5 000, SAB Company) and incubated at room temperature for 1.5 h and washed three times for 10 min each. The membrane was scanned using fluO-RChem ®HD2 imaging system and the results were analyzed using ImageJ software. The ratio of gray value of target protein band to gray value of internal reference β-actin protein band was used as the relative expression level of target protein.

# Statistical analysis

Statistical analyses were carried out using the software package SPSS version 25. All experimental data are shown as the Mean ± The standard deviation. The significant differences between two groups were evaluated using the independent—samples test. Moreover, the significant differences among groups were

examined using one-way analysis of variance (ANOVA) with post-hoc Fisher's LSD, $P < 0.05$ was considered to indicate a statistically significance.

## Results

# Pathological changes of renal tissue

Hematoxylin-eosin staining showed that there was no significant change in renal histopathology in normal control group. Compared with the normal control group, the RTEC were swollen and exfoliated in both PPARγ inhibitor group and hypoxia injury group, but the PPARγ inhibitor group showed more severe cell damage. The swelling degree of RTEC was reduced in PPARγ agonist group compared with hypoxia injury group. TEM showed mitochondrial swelling and stromal focal edema of renal tubular epithelial cells and glomerular podocytes in both PPARγ inhibitor group and hypoxia injury group compared with the normal control group. The mitochondrial swelling and stromal focal edema was remission in the PPARγ agonist group (Figure 1).

# The Mrna Expression Levels Of Pparγ, Sod, Il-1β And Et-1 By Rt-pcr

To investigate the effects of rosiglitazone, a PPARγ agonist, on acute hypoxic renal injury in rats, we detected the expression levels of PPARγ, SOD, IL-1β and ET-1. Compared with normal control group, the mRNA expressions of PPARγ and SOD in hypoxia injury group were significantly decreased (Fig. 2, $P < 0.05$), while the mRNA expressions of IL-1β and ET-1 were significantly increased (Fig. 2, $P < 0.05$). Compared with hypoxia injury group, the mRNA expression levels of PPARγ and SOD were significantly increased (Fig. 2, $P < 0.05$), while the mRNA expression levels of IL-1β and ET-1 were significantly decreased (Fig. 2, $P < 0.05$) in the PPARγ agonist group. In the PPARγ inhibitor group, the mRNA and protein expressions of PPARγ and SOD were more decreased, and IL-1β and ET-1 were more increased compare to the hypoxia injury group (Fig. 2, $P < 0.05$).

# The Protein Expression Levels Of Pparγ, Sod, Il-1β And Et-1 By Wester-blot

The wester-blot result showed that protein expression levels of PPARγ, SOD, IL-1β and ET-1. Compared with normal control group, the protein expressions of PPARγ and SOD in hypoxia injury group were significantly decreased (Fig. 3, $P < 0.05$), while the IL-1β and ET-1 were significantly increased (Fig. 3, $P < 0.05$). Compared with hypoxia injury group, the PPARγ and SOD were significantly increased (Fig. 3, $P < 0.05$), while IL-1β and ET-1 were significantly decreased ($P < 0.05$) in the PPARγ agonist group. In the PPARγ inhibitor group, the PPARγ and SOD were more decreased, and IL-1β and ET-1 were more increased compare to the hypoxia injury group (Fig. 3, $P < 0.05$).

# Discussion

Studies have shown that hypoxia is one of the most common causes of renal tubular epithelial cell injury, so oxidative injury plays an important role in the occurrence and development of renal diseases[7, 8]. This present study showed that low oxygen induced renal injury in rats with acute hypoxia model, using low pressure hypoxic animals lab to make oxygen, establish acute kidney injury model of rats induced by hypoxia. HE staining showed that the epithelial cells of renal tubules were swollen and exfoliated in the model group. And TEM showed that RTEC and glomerular podocyte mitochondria were significantly swollen after hypoxia compared with normal control group, suggesting that the model was successfully constructed. The result was similar to the Chhabra[9] study, in which the renal tissue structural damage was observed in the acute hypoxia group for one day. Mitochondria are the main organelles eukaryotic cells powered, renal tubular epithelial cells in high-energy mitochondrial content is very rich. The causes of renal hypoxia include systemic hypoxia caused by hypoxic environment and local oxygen deficiency of kidney caused by different diseases such as atherosclerosis and hypertension. Current research models mainly focus on local renal hypoxia, which changes renal blood flow. The mechanism of systemic hypoxia in renal injury remains unclear. This study was conducted with the model of systemic hypoxia in rats.

In the present study, expressions of PPARγ was decreased and RTEC was injure in hypoxia injury group and PPARγ inhibitor group. But the PPARγ agonist relieved the injury degree of RTEC and glomerular podoccytes, suggesting that the change of PPARγ expression level may be related to the injury of RTEC.Activated PPAR-γ can inhibit adhesion molecules, chemokines and other inflammatory factors, inhibit inflammatory response, reduce reactive oxygen species and oxidative stress[10, 11]. Mice with PPARγ gene knockout developed renal insufficiency, reduced creatinine clearance, fibrosis, and tubular dilation[12]. It was reported that pioglitazone, a PPARγ agonist, alleviated renal tissue injury by up-regulating the expression of PPARγ and increasing antioxidant capacity in rats with ischemia-reperfusion injury[13–15]. In diabetic mice, the activation of amino-terminal kinase (JNK) pathway up-regulates the expression of PPARγ and inhibits the apoptosis of islet cells and mesangial cells, thereby improving insulin resistance and delaying renal injury[16]. In the RTEC model induced by sodium urate crystals, the expression of intracellular inflammatory bodies and interleukin-1 β decreased significantly after PPARγ activation, and the cell damage was alleviated[17]. In hypoxia-induced RTEC injury, the agonist rosiglitazone can up-regulate the protein expression of PPARγ and reduce RTEC injury. PPARγ expression in damaged RTEC was inhibited in the hypoxia/reoxygenation (HR) model.

In this study, we established a rat model of acute hypoxic kidney injury and used PPARγ agonist rosiglitazone to intervene the expression of PPARγ. The results showed that after PPARγ agonist intervention, the expressions of PPARγ and SOD were significantly increased, while the expressions of IL-1β and ET-1 were significantly decreased, suggesting that up-regulation of PPARγ expression can alleviate hypoxia-induced renal injury in rats. SOD, IL-1β and ET-1 were involved in the process of hypoxic kidney injury. SOD is the most important antioxidant enzyme in the body, which is involved in maintaining

cell integrity and metabolic process. The level of SOD consumption in kidney tissue reflects the degree of oxidative damage in kidney tissue. Studies have shown that in cisplatin-induced liver injury model, SOD and PPARγ expression in liver were decreased[18]. The result was similar in our acute hypoxic kidney injury rat. Acute hypoxia rapidly activates endothelial cells and triggers an inflammatory response[19]. IL-1β is the most common proinflammatory factor and plays an important role in inflammatory response. If the renal tubule injury, endothelial cell function can be impaired by the production of inflammatory mediators (such as IL-1β). PPAR-γ agonists can inhibit IL-1β induced expression of vascular smooth muscle cells to secrete hemagglutinin-like receptor 1[20]. ET-1 is secreted by vascular endothelial cells. Hypoxia can increase ET synthesis. Renal vessels are known to be 10 times more sensitive to the vasoconstriction effect of ET than other vascular beds. It has been reported that the secretion of ET-1 is increased in PPARγ -knockout mice[21]. It is suggested that PPARγ may be directly involved in inhibiting the secretion of ET-1. Our study suggested that the decline of PPARγ was involved in the development of hypoxic kidney injury, PPARγ agonists maybe protect against hypoxic kidney injury by inhibiting the ET-1 and IL-1β.

# Conclusions

PPARγ expression was highly correlated with acute hypoxia kidney injury in rat model. PPARγ agonist can alleviate hypoxic kidney injury in a possible pathway that inhibited the ET-1 and IL-1β expression.

# Abbreviations

AKI
Acute kidney injury
ANOVA
Analysis of variance
CKD
Chronic kidney disease
ET
Endothelin
HE
Hematoxylin and eosin
IL
interleukin
PPAR
peroxisome proliferator-activated receptor
PVDF
polyvinylidene fluoride
RTEC
renal tubule epithelial cells

SD
Sprague-Dawley
SDS-PAGE
sodium dodecyl sulfate polyacrylamide gel
SOD
superoxide dismutase
TBST
TBS (tris buffered saline) + Tween
TEM
transmission electron microscopy

# Declarations

### Ethics approval and consent to participate

The study was carried out in compliance with the ARRIVE guidelines 2.0. The animal usage and protocol were reviewed and approved by Guangxi Medical University Ethical Review Committee. Animal Use License No. : SYXK GUI 2020-0004; Animal production License No. : SCXK GUI 2020-0003.And the experimental operations and procedures were in accordance with the regulations of the People's Republic of China on the administration of experimental animals and the ethical requirements.

### Consent for publication

Not applicable.

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. Our data is deposited in 10.6084/m9.figshare.19233777.

### Competing interests

The authors declare that they have no competing interests.


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### Author’s contributions:

SQZ and JSZ conceived the study and contributed equally to the paper, SQZ, JSZ, LT and SJJ collected and analyzed the data, SQZ wrote the manuscript. XQC revised the final paper. YHQ contributed to the conception and design of the study. All authors read and approved the final manuscript.

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Not applicable.

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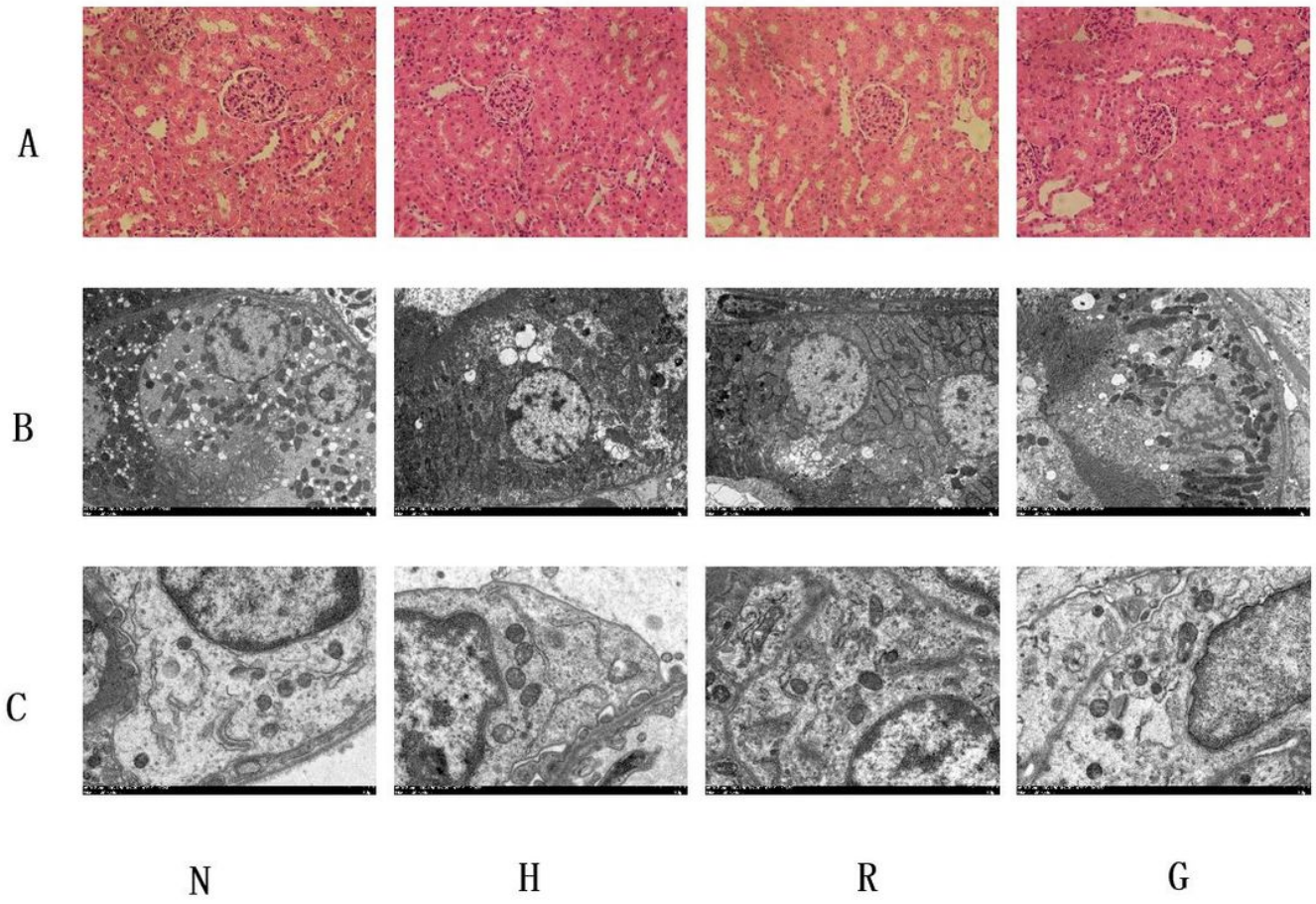
# Figures




## Figure 1

**Pathological changes of renal tissue.** (A) Pathological changes of rat kidney with HE staining under light microscope (×400). (B-C) Under the transmission electron microscopy of rat renal tubular epithelial cells (×1.5 k), and rat glomerular podocytes (×6 k). N: normal control group; H: hypoxia injury group; R: PPARγ agonist group;G: PPARγ inhibitor group.

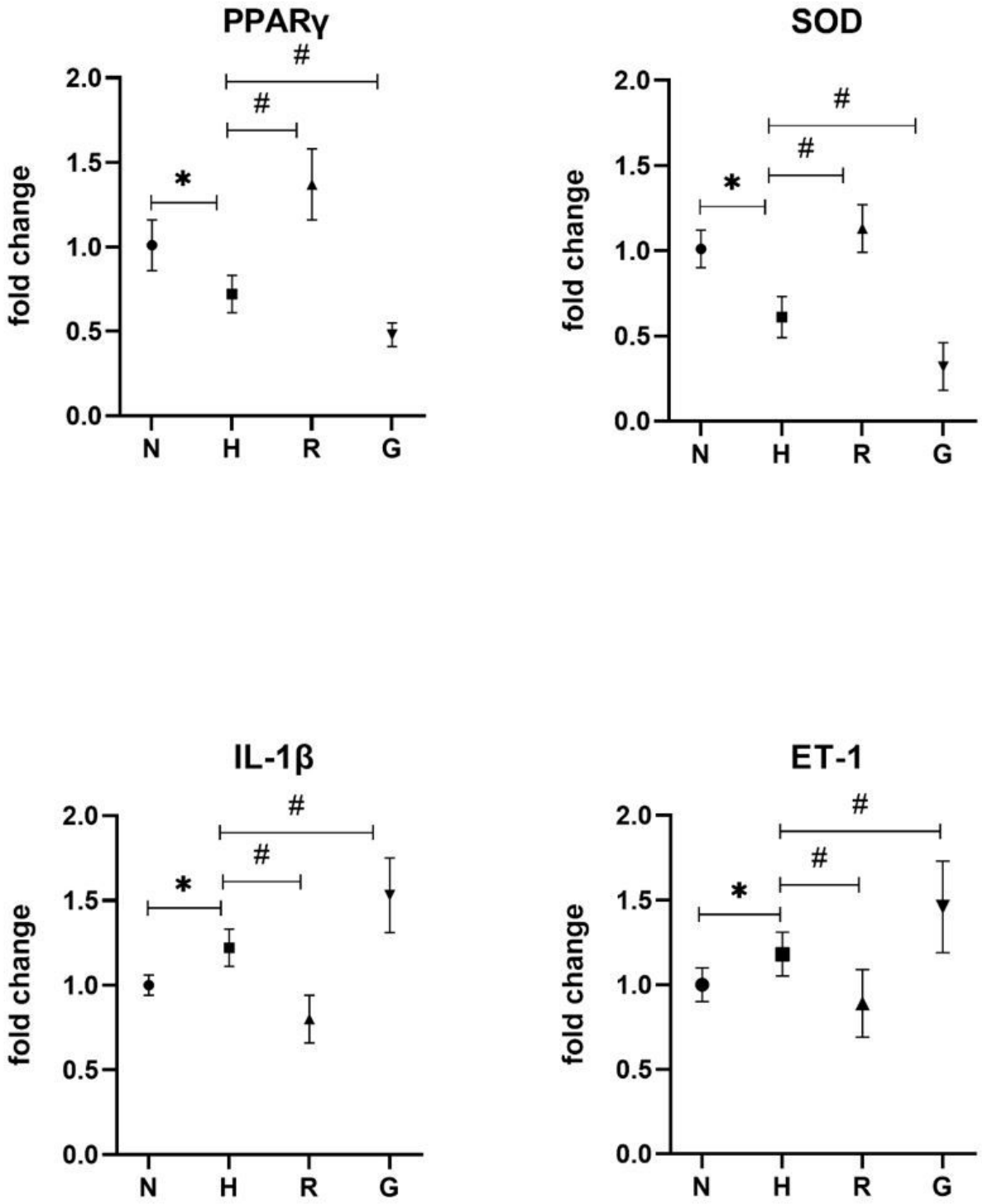



Figure 2

**The mRNA expression levels of PPARγ、SOD、IL-1β and ET-1.** RT-PCR analysis of expressions of PPARγ、SOD、IL-1β and ET-1 in rat kidney tissue. *P < 0.05, compared with normal control group; # P < 0.05, compared with hypoxia injury group. N: normal control group; H: hypoxia injury group; R: PPARγ agonist group;G: PPARγ inhibitor group.

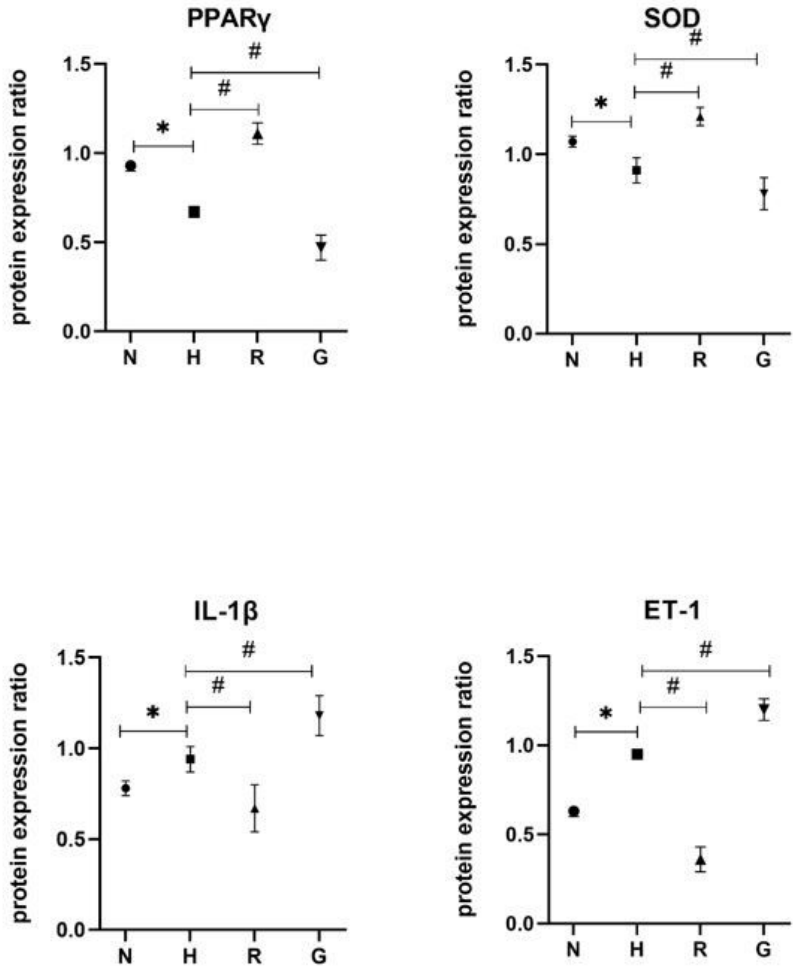



Figure 3-1

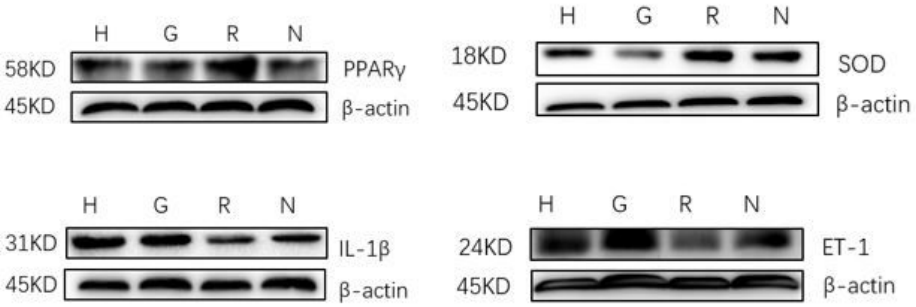



Figure 3-2

## Figure 3

**The protein expression levels of PPARγ、SOD、IL-1β and ET-1.** Western blot that was cropped analysis of expressions of PPARγ、SOD、IL-1β and ET-1 in rat kidney tissue. *P < 0.05, compared with normal control group; # P < 0.05, compared with hypoxia injury group. N: normal control group; H: hypoxia injury group; R: PPARγ agonist group;G: PPARγ inhibitor group. The blots were cropped, full-length blots are presented in Supplementary Figure 1.

# Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigure1.docx