Allicin Protects Renal Function, Improves Oxidative Stress and Lipid Peroxidation in Rats with Chronic Renal Failure

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Keywords. Allyl compounds; chronic kidney disease; oxidative stress; lipid peroxidation; nuclear factor-kappa B; mitogen-activated protein kinase pathways

Introduction. The research was an attempt to explore the potential impact of allicin on lipid peroxidation and oxidative stress in rats diagnosed with chronic kidney disease (CKD), and to determine its underlying mechanism.

Methods. Sixty rats were randomly divided into sham-operated, modelling, and allicin low, medium, and high dose groups. The histopathological structure of the kidney was observed in each group. Biochemical measurements were conducted to assess kidney function, including serum creatinine (Scr) and blood urea nitrogen (BUN), and 24-hour urine protein quantification. Levels of malondialdehyde (MDA), superoxide dismutase (SOD), reactive oxidative species (ROS), and reduced glutathione (GSH) in kidney tissue were measured, and mitogen-activated protein kinase (MAPK) and NF (nuclear factor) -κB protein levels were detected by western blotting.

Results. They showed that allicin improved the pathological structure of renal tissue and protected renal function by reducing oxidative stress and lipid peroxidation via targeting the ROS/MAPK/NF-κB pathway. Allicin increased SOD and GSH levels, while decreasing Scr, MDA, ROS, BUN, and the amount of protein excreted in urine over a 24-hour in medium and high dose groups. MAPK and NF-κB protein levels in medium and high dose allicin groups were lower than the modelling group.

Conclusion. Based on the results, it can be inferred that allicin may safeguard renal function in rats with CKD and has the potential to serve as a treatment for kidney ailments.

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INTRODUCTION

Chronic kidney disease (CKD), is a chronic, progressive kidney parenchymal disorder caused by a range of factors, leading to significant kidney atrophy and loss of basic kidney functions. Antioxidant capacity and inflammatory stress are crucial factors in the development of CKD lesions.[1,2] Oxidative stress promotes the release of large amounts of inflammatory factors via signaling pathways such as Mitogen-Activated Protein Kinase (MAPK) and Nuclear Factor Kappa B (NF-κB), which disrupt the normal physiological structure of kidney cells, a key feature in the development of CKD.[3-5] According to Chinese medicine, CKD is the end result of kidney-related diseases.[6] Allicin, an organic sulfur compound, has been widely studied for its protective effects on the cardiovascular system, anti-tumor and antioxidant properties, and renal function improvement. Some studies[7] have suggested that allicin can improve

renal function, inhibit renal tissue destruction, and play an antioxidant role in CKD rats. Other studies have shown that hydrogen sulfide can inhibit apoptosis and inflammatory responses through the ROS/MAPK/NF-κB signaling pathway in CKD rats, exerting a protective effect on renal function. It is therefore speculated that allicin may also improve renal function in CKD rats through antioxidant stress mediated by the ROS/MAPK/NF-κB signaling pathway. This research was an attempt to explore the potential impact of allicin on lipid peroxidation and oxidative stress in rats with CKD, as well as investigating the underlying mechanism. The aim of this research is to establish a comprehensive/acceptable scientific foundation for the effective management of CKD.

MATERIALS AND METHODS

Experimental reagents and instruments

Allicin injection (specification: 10 mL: 30 mg) was purchased from Chenxin Pharmaceutical Co., LTD. (Approval No.: National Drug Approval H2005402); Hematoxylin-eosin staining (HE) available from Nanjing Quanlong Biotechnology Co., LTD. (Nanjing, China). Superoxide dismutase (SOD), Maleicdialdehyde (MDA), reactive oxygen species (ROS) and glutathione (GSH) assay kits were available from Biyuntian Biotechnology Co., LTD. (Shanghai, China). Rabbit monoclonal antibody against human MAPK and NF-κB was purchased from Santa Cruz (Santa Cruz, CA, USA). Electrochemiluminescence (ECL) Chemiluminescence Detection Kit was purchased from Shanghai Jizhi Biochemical Technology Co., LTD. (Shanghai, China). Automatic biochemical analyzer and automatic urine protein analyzer were purchased from Yixing Xinyi Instrument Co., LTD. (Yixing, China), and Gel electrophoresis analysis system was available from Qingdao Jiading Analytical Instrument Co., LTD. (Qingdao, China).

Experimental animals and grouping

We used 60 SD rats for our experiment, all of which were between 6-8 weeks old and had a body weight of approximately 220 ± 20 g. We purchased SD rats from Hangzhou Qizhen Experimental Animal Science & Technology Co., Ltd. (Hangzhou, China), Licence No. SCXK (Zhe) 2022-0005; housed in separate cages in a standard environment, free to feed and drink. This research received the approval (NO.ZJKF20211224034) from the Animal Ethics Committee of Zhangjiakou City First Hospital Animal Center.

The study included sixty rats, which were categorized into separate groups for experimentation purposes. These groups comprised the sham operation, model, and three allicin treatment groups, namely low-dose (5 mg/kg), medium-dose (10 mg/kg), and high-dose (20 mg/kg). Each group had 12 rats. CKD models of the model group and allicin groups with different doses were established by referring to existing literature.[9] In the sham operation group, only dorsal incision was performed without nephrectomy. No rats died during the experiment. On the third day after modeling, allicin low-dose, medium-dose and high-dose groups were intraperitoneally injected with 2 mL allicin solution at levels of 5 mg/kg, 10 mg/kg and 20 mg/kg; equal amounts of saline were injected intraperitoneally in the sham and model groups.

Sample collection

After gavage, the rats were anaesthetized and 3 mL of blood samples were collected from the rats' abdomen and centrifuged (3500 r/min) for 15 min. The supernatant was taken and placed in a -20°C refrigerator for use. The rats were sacrificed by neck dissection, and kidney tissue was obtained by surgery. A portion of the kidney tissue was identified by using histopathological methods, while the remaining portion was preserved in a refrigerator at a temperature of -80°C to be utilized at a later time.

Histopathological examination of kidney in rats

A portion of the kidney tissue was paraffin-embedded, sectioned by conventional means, stained with HE and placed under a light microscope to observe the histopathological changes in each group.

Detected renal function and routine indicators

Serum creatinine (Scr) and blood urea nitrogen (BUN) were detected by automatic biochemical analyzer. Urine was collected from rats for 24 hours and its protein was quantified by urine protein analyzer.

Oxidative stress and lipid peroxidation

A portion of kidney tissue was taken in a

refrigerator at -80°C and ground well for the determination of MDA by the barbiturate thiosulfate method. SOD was detected by colorimetric method. ROS levels were detected by using ROS assay kits. GSH was detected by dithio-dinitrobenzoic acid method.

Western blot detection of MAPK and NF-κB protein

The kidneys of the rats in all experimental groups were pulverized and homogenized. The renal tissues were lysed with RIPA protein lysate, followed by ice lysis for 30 min and centrifugation for 15 min. Total protein was abstracted and protein concentrations were quantified by a BCA kit (Pierce, Rockford, IL, USA). After electrophoresis on a 10% sodium dodecyl sulfate-polyacrylamide gel and the transfer to a PVDF membrane, the proteins were blocked with skimmed milk powder for 1 hour. Primary antibodies (MAPK: 1:1,000, NF-κB: 1:2,000) were added and incubated overnight at 4°C. The membrane was washed 3 times with PBS. HRP-labelled secondary antibody was added and incubated at room temperature for 2 hours. The membrane was washed 3 times with PBS. A final drop of ECL was added for color development and the protein bands were imaged on a gel image imaging system using GAPDH (1:2 000) as an internal reference.

Statistical analysis

All data were analyzed by using Statistical Product and Service Solutions (SPSS) 22.0 statistical software (IBM, Armonk, NY, USA). One-way ANOVA (one-way ANOVA) was performed to compare differences between groups. We employed the LSD-t test to make pairwise comparisons among the separate groups. At $P < .05$, the data were considered to have statistical differences.

RESULTS

Effect of Allicin on renal function in CKD rats

Compared with sham operation group, Scr and BUN levels and 24 hours urinary protein in model group were elevated. However, the levels of serum Scr, BUN and 24 h urinary protein in allicin medium and high dose groups decreased. (Table 1).

Effects of Allicin on SOD, MDA, GSH and ROS levels in renal tissues of CKD rats

Results also showed that SOD and GSH in the model group significantly decreased, while the levels of MDA and ROS were significantly upregulated ($P < .05$). Levels of SOD and GSH in medium and high dose allicin groups increased, while the levels of MDA and ROS markedly reduced (Table 2).

Effect of Allicin on histopathological changes in the kidneys of various groups of rats

The glomeruli of the sham-operated rats were morphologically normal, with neatly arranged and intact cells. In the group of models, we observed the expansion and shrinkage of the renal tubules, as well as the presence of inflammatory granulocyte

Table 1. Effect of Allicin on Renal Function of CKD Rats ($\bar{x}$ ± s, n = 12)

Groups	Scr (μmol/L)	Mmol /L	24 h Urine Protein (mg/h)
Sham	8.75 ± 0.88	10.69 ± 1.13	22.34 ± 2.31
Model	29.12 ± 3.10*	20.82 ± 1.93*	97.63 ± 9.83
Allicin Low Dose	28.73 ± 3.02	19.93 ± 1.86	96.32 ± 9.71
Allicin Medium Dose	21.34 ± 2.31$^{\#}$	16.58 ± 1.74$^{\#}$	67.82 ± 6.85
Allicin High Dose	11.63 ± 1.03$^{\#\Delta}$	14.29 ± 1.53$^{\#\Delta}$	55.64 ± 5.67

Note: Compared with the sham group,*$P < .05$; Compared with the model group,$^{\#}$ $P < .05$, compared with allicin low-dose group, $^{\Delta}$ $P < .05$.

Table 2. Effects of Allicin on Oxidative Stress and Lipid Peroxidation Indexes in Renal Tissues of CKD Rats ($\bar{x}$ ± s, n = 12)

Groups	SOD (mU/mg)	MDA (μmol/mg)	GSH, mg/g	ROS (ng/mg)
Sham	108.36 ± 11.22	4.79 ± 0.53	20.98 ± 2.13	358.73 ± 36.47
Model	93.47 ± 9.41*	12.08 ± 1.31*	9.73 ± 1.01*	461.51 ± 46.53
Allicin Low Dose	94.16 ± 9.53	12.24 ± 1.44	10.03 ± 1.08	468.96 ± 47.33
Allicin Medium Dose	99.43 ± 10.13$^{\#}$	7.12 ± 0.81$^{\#}$	14.47 ± 1.56$^{\#}$	418.37 ± 42.68
Allicin High Dose	103.65 ± 10.42$^{\#\Delta}$	9.84 ± 1.16$^{\#\Delta}$	16.34 ± 1.72$^{\#\Delta}$	388.72 ± 39.43

Note: Compared with the sham group, *$P < .05$; Compared with the model group,$^{\#}$ $P < .05$, compared with allicin low-dose group, $^{\Delta}$ $P < .05$.

infiltration in the renal interstitium. With the administration of allicin and increasing its dose, glomerular dilation and atrophy were gradually relieved, and inflammatory cell infiltration gradually reduced. The improvement effect was most obvious at high dose of allicin (Figure 1).

The investigated impact of Allicin on the levels of MAPK and NF-κB proteins in the kidney tissues of all groups of rats

Compared with sham operation group, the levels of MAPK and NF-κB protein in the model group significantly increased ($P < .05$). Compared with the model group, the levels of MAPK and NF-κB protein in allicin medium and high dose groups were remarkably up-regulated (Figure 2).

DISCUSSION

Chronic kidney disease (CKD)is a prevalent and progressive condition affecting the kidneys. It is mainly characterized by renal dysfunction and systemic symptoms. This serious condition frequently places patient at risk. Currently, CKD has become a global public health problem.[10,11] Allicin is the active ingredient in garlic, possessing various physiological functions including anti-tumor, anti-myocardial fibrosis, anti-infection, lipid-lowering, cardiovascular protection, anti-oxidation, kidney protection and even more properties.[12] In this study, the levels of Scr, BUN and 24 hours urine protein in CKD model rats were significantly higher than those in sham operation group, while the levels of Scr, BUN and 24-hour urine protein in the CKD model rats were significantly lower after allicin intervention. Several studies have suggested that allicin can improve the pathological status of CKD rats and enhance the glomerular filtration ability of urinary protein. Scr and BUN are important indicators of renal function. Exceeding levels of Scr and BUN in living organisms above the normal value indicates renal injury.[13] Scr is a product of muscle metabolism and BUN is the end product of protein metabolism and are primarily eliminated through glomerular filtration and urine. The increased levels of Scr and BUN indicate kidney dysfunction. In this study, glomerular dysfunction occurred in CKD model rats, and the filtration rate decreased, resulting in accumulation of Scr, BUN and increased 24 hours urinary protein levels in rats. After allicin intervention, glomerular filtration function improved, Scr, BUN and 24 hr. urinary protein levels reduced, and kidney damage was alleviated. In addition, according to H&E staining renal tissue of the CKD model rats showed obvious hyperplasia and swelling of mesangial cells accompanied by inflammatory granulocytic

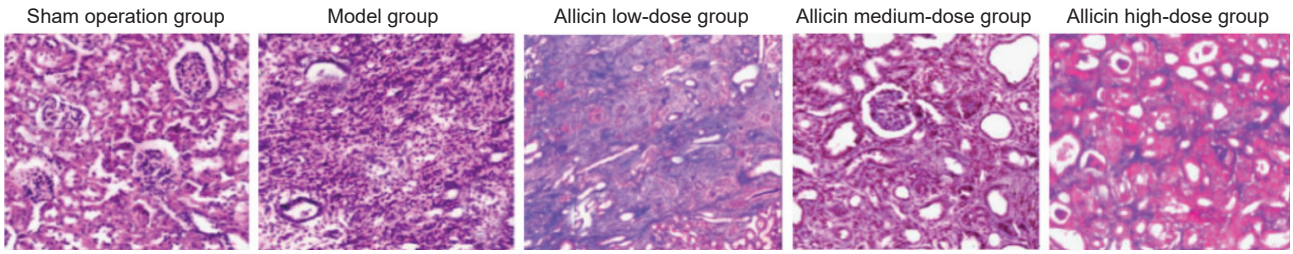

Figure 1. Comparison of Pathological Changes in Kidney Tissues of Rats in Group 1 (× 400 times)

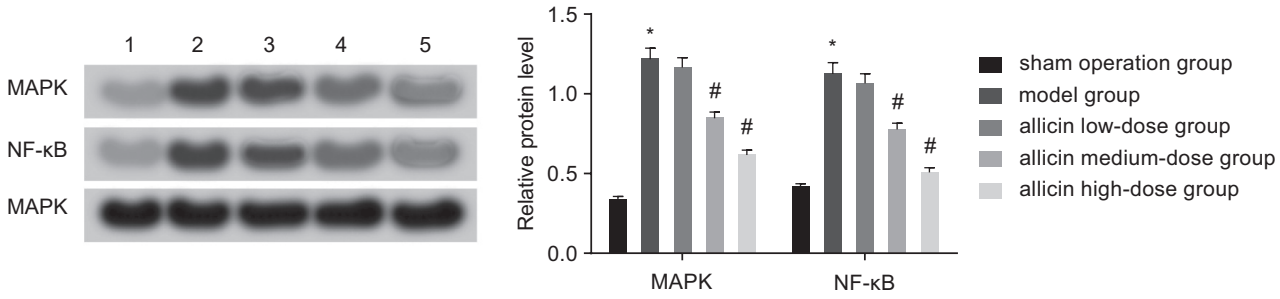

Figure 2. Changes of MAPK and NF-κB Protein Levels in Each Group (Note: 1) sham operation group; 2) Model group; 3) Allicin low-dose group; 4) Allicin medium-dose group; 5) Allicin high-*dose* group; Compared to the sham group, $^{*}P < .05$; Compared with the model group, $^{\#}P < .05$)

infiltration. Following allicin intervention, the phenomenon of glomerular hyperplasia was gradually relieved, the proliferation of mesangial cells reduced, and inflammatory cell infiltration gradually decreased, further indicating that allicin can effectively improve renal pathological morphology of the CKD rats and slowing down the advancement of the illness.

Oxidative stress injury is a pathway that induces apoptosis and plays a crucial role in the occurrence and evolution of CKD. In this study, we observed that during CKD, the levels of SOD and GSH in rat kidney tissues significantly reduced, while the levels of MDA and ROS were markedly elevated. Following allicin intervention, the levels of SOD and GSH in rat kidney tissues significantly increased, while the levels of MDA and ROS significantly decreased. Under normal circumstances, the body maintains a dynamic balance between oxidation and antioxidant cells. However, when there is physical injury or adverse stimulation, this balance can be disrupted, resulting in the release of excessive oxidation products that can damage the body tissues and organs.[14] When oxidation process is initiated, the accumulation of MDA content from lipid oxidation can damage normal cell function. SOD is the first line of body defense against oxygen free radicals and functions as a potent and effective scavenger of free radicals.[15] Studies have indicated that,[16] CKD can decrease SOD and GSH-Px activities, increase MDA content, induce oxidative stress response, and damage cell function. Previous research has shown that,[17] allicin can increase SOD activity, decrease MDA content and improve oxidative stress level of CKD dogs. Based on the findings of this research, it can be inferred that allicin can increase SOD and GSH levels, reduce MDA and ROS levels, and regulate the dynamic balance of oxidation and antioxidation in CKD rats.

It is worth noting that the MAPK/NF-κB signaling pathway participates in diverse cellular functions. In the context of CKD, the activation of this pathway can lead to renal inflammation and fibrosis, contributing to the progression of the disease. CKD may benefit from the use of allicin as a therapeutic treatment if it inhibits the MAPK/NF-B pathway. This down-regulation of the signaling pathway could have significant benefits for patients suffering from CKD.

THE STUDY LIMITATIONS

The study was conducted on rats, and the results may not necessarily apply to human beings. This research does not provide any information on potential adverse effects or toxicity of allicin, which may be a limitation in considering it as a potential therapeutic agent for renal disease.

CONCLUSION

In conclusion, allicin may regulate the oxidative and antioxidant balance in rats by attenuating the induction of the ROS/MAPK/NF-κB pathway, thereby protecting renal function and preventing the progression of CKD. However, this study only analyzed animal experiments, and clinical data were lacking. Moreover, the toxicological response of allicin was not thoroughly analyzed, which requires further research for verification in the future.

AUTHORS CONTRIBUTION

Ningning Xu and Lulu Shi designed the study and performed the experiments, Weinan Han collected the data, Guoping Yun analyzed the data, Ningning Xu and Lulu Shi prepared the manuscript. All authors read and approved the final manuscript.

DATA AVAILABILITY

The datasets used and analyzed during the current study are available from the corresponding author upon request.

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This study did not receive any funding in any form.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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