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Exploring the Protective Effect of the Ethanolic Extract of *Rosa laevigata* Michx. Fruit on Rats with Mesangial Proliferative Glomerulonephritis Based on NLRP3 Inflammasome Pathway

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Abstract: *Objective:* To investigate the effect of the ethanolic extract of *Rosa laevigata* Michx. fruit on rats with mesangial proliferative glomerulonephritis based on the NLRP3 inflammasome pathway. *Methods:* Thirty Wistar rats were divided into three groups, a blank control group, a diabetic nephropathy (DN) model group, and an ethanolic extract intervention group, according to the random number table method, with 10 rats in each group. One day before the experiment, basic feeding was initiated for all the rats; the changes in activity and weight of each group of rats were observed and recorded after 7 d, and a rat model of renal function injury was established after 1 d. *Results:* Compared with the control group, the model group had significantly higher kidney/body ratio, 24 h urine protein, serum creatinine (SCr), blood urea nitrogen (BUN), glomerular mesangial cell (GMC) count, and extracellular matrix (ECM) positive area ratio ($P < 0.05$); the same indicators were significantly lower in the intervention group than in the model group ($P < 0.05$). The NLRP3 inflammasome pathway in renal intrinsic cells was activated in the intervention group. The overactivation of NLRP3 inflammasome is known to promote interleukin (IL)-1β release, which was inhibited in the intervention group. *Conclusion:* The ethanolic extract of *Rosa laevigata* Michx. fruit has a protective effect on renal intrinsic cells and may be related to NLRP3 inflammasome pathway, suggesting that the fruit of *Rosa laevigata* Michx. has a potential role in protecting renal intrinsic cells from inflammatory damage. NLRP3 inflammasomes are involved in the development of various chronic inflammatory diseases, such as acute and chronic glomerulonephritis and renal fibrosis.

Keywords: Ethanolic extract of *Rosa laevigata* Michx. fruit; Glomerulonephritis; NLRP3

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1. Introduction

Glomerulonephritis is a common pathological type of chronic kidney disease, and its occurrence is associated with excessive activation of renal lamina propria cells. In clinical practice, NLRP3 inflammasomes have been recognized as a new immunomodulatory factor. *Rosa laevigata* Michx., of which its fruit is known as wild golden cherry, hawthorn seed, *etc*., is a plant of the Rosaceae family and is widely distributed across the country. In recent years, despite the numerous studies on the ethanolic extract of wild golden cherry, there very few studies on its mechanism of action. In China, about 20%–40% of patients

with mesangial proliferative glomerulonephritis developed end-stage renal disease. Timely monitoring and individualized treatment can effectively stop or delay the progression of this condition. Since the common feature of all mesangial proliferative glomerulonephritis is the proliferation of glomerular mesangial cells, which leads to functional kidney damage, biomarkers of these cells can be used to monitor the disease [1-8]. We used immunohistochemistry, reverse transcription polymerase chain reaction (RT-PCR), and Western blot to study the protective effect of the ethanolic extract of wild golden cherry on rats with mesangial proliferative glomerulonephritis and the role of NLRP3 inflammasome pathway.

2. Methods

2.1. Animal model and experiment

In this study, 30 Wistar rats were divided into three groups, a blank control group, a diabetic nephropathy (DN) model group, and an ethanolic extract intervention group, according to the random number table method, with 10 rats in each group. One day before the experiment, basic feeding was initiated for all the rats. After 7 d, changes in activity and weight of the rats in each group were observed and recorded, and a rat model of renal function injury was established after 1 d. In order to establish a rat model of kidney injury, the animals were anesthetized and fixed on a collagen membrane with 1% glutaraldehyde; the model group was given saline instead of urea, the control group was given a high-salt diet, and the intervention group was given sodium citrate instead of saline. After the experiment, the kidney specimens of each group were examined by immunohistochemistry. Immunohistochemical staining under light microscope was performed to observe the changes in glomerular pathology before and after the intervention. The kidney specimens were incubated with 5% carbon dioxide (CO_2) for 30 min, washed with phosphate-buffered saline (PBS) 5 times, and further incubated for another 10 min with new samples to ensure no contamination of samples. The kidney sections were fixed with 2% silver nitrate for 6 h and then placed into 10% neutral formalin solution for storage. The prepared specimens were subsequently incubated in 5% CO_2 for Western blot analysis on 96-well plates.

2.2. Observation indicators

(1) *Rosa laevigata* Michx. fruit extract: ethanolic extract was extracted from fresh *Rosa laevigata* Michx. fruit, and the content was 100%. (2) Urine: fresh Wistar rat urine was collected; bacterial culture was performed; and 100 μL of urine sample was taken for analysis after isolation, inactivation, and dilution. (3) Protein extraction: ultra-high temperature instantaneous rotating disk centrifugal method and ultra-high-performance liquid chromatography (UPLC) were used to isolate each group of proteins in rat serum and determine the content of each component in each group of samples (purity 100%), respectively. (4) Cytomorphological examination: rat glomeruli were stained by immunohistochemical staining to observe the morphological changes in the glomeruli. (5) Determination of urinary protein by enzyme standardization: samples were measured by A and B wavelength absorbance meter at 600 nm with enzyme label; according to the calculation of urine protein volume, albumin and total cholesterol in urine were determined by automatic colorimetry and radioimmunoassay (RIA), respectively. (6) Electron micrographs were taken by laser confocal microscopy to observe pathological changes in the glomeruli.

3. Results

3.1. Comparison of kidney/body ratio and 24 h urine protein level of each group

Compared to the control group, the model group had significantly higher kidney/body ratio and 24 h urine protein level ($P < 0.05$); compared with the model group, the intervention group had significantly lower kidney/body ratio and 24 h urine protein level ($P < 0.05$), as shown in **Table 1**.

Table 1. Comparison of kidney/body ratio and 24 h urine protein level of each group

Group	Kidney/body ratio	24 h urine protein (mg)
Control group	4.92 ± 0.32	2.20 ± 0.21
Model group	10.04 ± 0.49[a]	8.75 ± 0.37[a]
Intervention group	7.43 ± 0.56[b]	5.41 ± 0.46[b]

Data are given as mean ± SD, n = 10; [a]$P < 0.05$, compared with the control group; [b]$P < 0.05$, compared with the model group

3.2. Comparison of serum creatinine and blood urea nitrogen levels of each group

Compared with the control group, the model group had significantly higher SCr and BUN ($P < 0.05$); compared with the model group, the intervention group had significantly lower SCr and BUN levels ($P < 0.05$), as shown in **Table 2**.

Table 2. Comparison of serum creatinine (SCr) and blood urea nitrogen (BUN) of each group

Group	SCr (μmol/L)	BUN (μmol/L)
Control group	16.32 ± 3.96	8.96 ± 2.19
Model group	32.36 ± 4.49[a]	21.54 ± 3.65[a]
Intervention group	22.94 ± 5.99[b]	14.03 ± 4.96[b]

Data are given as mean ± SD, n = 10; [a]$P < 0.05$, compared with the control group; [b]$P < 0.05$, compared with the model group

3.3. Comparison of glomerular mesangial cell (GMC) count and extracellular matrix (ECM) positive area ratio of each group

Compared with the control group, the model group had significantly higher GMC count and ECM positive area ratio ($P < 0.05$); compared with the model group, the intervention group had significantly lower GMC count and ECM positive area ratio ($P < 0.05$), as shown in **Table 3**.

Table 3. Comparison of GMC count and ECM positive area ratio of each group

Group	GMC count (pcs)	ECM positive area ratio
Control group	41.36 ± 0.32	0.13 ± 0.21
Model group	72.39 ± 0.49[a]	0.25 ± 0.37[a]
Intervention group	47.63 ± 0.56[b]	0.16 ± 0.46[b]

Data are given as mean ± SD, n = 10; [a]$P < 0.05$, compared with the control group; [b]$P < 0.05$, compared with the model group

4. Discussion

4.1. Ethanolic extract of *Rosa laevigata* Michx. fruit

Rosa laevigata Michx. is a deciduous tree or shrub of the Rosaceae family. In recent years, research has found that it contains a variety of nutrients, bioactive substances, and chemical components. As a traditional Chinese herbal medicine for more than a millennium, wild golden cherry is mainly distributed in northeast, north, and northwest China, among which its cultivation is most prevalent in northeast China. The ethanolic extract of wild golden cherry is a modern traditional Chinese medicine. The ethanolic extract, which is derived from the dried mature fruit of *Rosa laevigata* Michx., has strong antioxidant activity, anti-aging effect, as well as preventive and therapeutic effects on various diseases and can exert pharmacological activity by reducing the level of reactive oxygen species and inhibit various pathogenic factors. The results of the present study showed that the ethanolic extract of wild golden cherry could significantly inhibit the

increase in intracellular reactive oxygen species, downregulate the activity of several intracellular signaling pathways, regulate cell metabolism and gene expression, as well as significantly reduce the level of inflammatory factor IL-1β [9-11].

4.2. Conclusion

In recent years, the morbidity and mortality of chronic kidney disease (CKD) have been on the rise. Hypertension, dyslipidemia, and hyperuricemia are the main mechanisms of which CKD occurs. As an important metabolic organ in the human body, the kidney undertakes an important task of removing excess water and toxins from the blood and maintaining normal circulation and metabolism in the body. The NLRP3 inflammasome pathway of renal intrinsic cell is one of the key links in the body's anti-inflammatory response and plays a vital role in autoimmune diseases, such as IgA nephropathy and nonalcoholic steatohepatitis. The results of the present study showed that the therapeutic effect of the ethanolic extract on the rat model of glomerulonephritis was significantly better than that observed in the blank control group and the DN group; the ethanolic extract of wild golden cherry also demonstrated a significant inhibitory effect on the expression levels of inflammatory factors IL-1β and IL-18 in kidney tissues. The activation of NLRP3 inflammasome pathway leads to the secretion of a large amount of proinflammatory factors by glomerular mesangial cells, which activate nuclear factor kappa B (NF-κB), mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K)/Akt, and other pathways to produce a large amount of profibrotic factors. These profibrotic factors promote the development of renal fibrosis. The ethanolic extract of wild golden cherry inhibits NLRP3 inflammasome activity and thus inhibits NLRP3 inflammasome pathway and NF-κBp65 entry into the nucleus to achieve anti-inflammatory effects.

NLRP3 inflammasomes play an important role in regulating autoimmune diseases and the body's inflammatory response. Recent studies have revealed that NLRP3 inflammasomes are involved in the development of kidney diseases [12,13]. It has been demonstrated that NLRP3 messenger RNA (mRNA) and protein expressions are low in normal kidney tissues but significantly higher in diseased kidney tissues, thus suggesting that NLRP3 inflammasomes may be involved in the development of kidney diseases. In addition, it has been reported that the activation of NLRP3 inflammasome pathway participates in the development of kidney injury by promoting the production of IL-13 and IL-6, both of which are important inflammatory mediators in kidney injury. Wang *et al*. [14] showed that the inhibition of NLRP3 inflammasome activation can reduce kidney injury and fibrosis in patients with chronic kidney disease. In addition, a study has shown that caspase-1 protease inhibitors and NLRP3 knockdown can inhibit neutrophil infiltration in acute kidney injury caused by sepsis [15]. However, the role of NLRP3 inflammasomes in renal tissue injury among rats with mesangial proliferative glomerulonephritis has not been investigated. In the present study, we found that the expression of NLRP3 protein was significantly higher in the renal tissues of rats with mesangial proliferative glomerulonephritis compared with the control group; moreover, the expression of NLRP3 positive cells in renal tissues was also significantly higher in rats with mesangial proliferative glomerulonephritis. However, the amount of NLRP3 protein and positive cells in renal tissues significantly decreased after treatment with the ethanolic extract of wild golden cherry. Therefore, we speculate that NLRP3 inflammasomes may be involved in the occurrence of renal injury in mesangial proliferative glomerulonephritis and suggest that ethanolic extract of wild golden cherry could alleviate renal tissue injury by inhibiting NLRP3 expression.

NLRP3 is a deacetylase-like inflammatory factor that regulates various processes, such as aging, cancer, glucose metabolism, and energy homeostasis. An imbalance in NLRP3 expression increases the risk for inflammatory diseases and autoimmune disorders. NLRP3 is widely expressed in renal cells, including glomerular mesangial cells. NLRP3 overexpression has been found to promote damage by a variety of factors, including oxidative stress, apoptosis, and renal inflammatory stimuli. By promoting transforming

growth factor-β1 (TGF-β1) signaling, shortening fibrosis progression, and increasing proteinuria, NLRP3 exerts a significant renal damaging effect. Studies have shown that NLRP3 promotes the expression of TGF-β1 and fibronectin (a key component of extracellular matrix deposition); in addition, it accelerates diabetic kidney injury and delays the fibrotic process. NF-κB is one of the important transcription factors involved in inflammatory response and an important component of the NLRP3 inflammasome pathway that induces the expression of a large number of inflammatory cytokines released extracellularly as early endogenous alerts of inflammation after injury. NF-κB is released earlier than other pro-inflammatory cytokines and acts as an "early mediator" of sepsis. Blocking NF-κB activity reduces mortality in animal models of endotoxemia. Unactivated NF-κB is present as a polymer with IκB or as two polymers with precursor proteins. In response to stimulation by inflammation-inducing factors, NF-κBp65 is transferred from the cytoplasm to the nucleus to regulate the expression of inflammatory cytokines.

Our results showed that the ethanolic extract of wild golden cherry has a protective effect on renal intrinsic cells and may be related to NLRP3 inflammasome pathway, suggesting a potential protective effect of wild golden cherry

against inflammatory damage in renal intrinsic cells. NLRP3 inflammasomes are involved in the development of various chronic inflammatory diseases, such as acute and chronic glomerulonephritis and renal fibrosis. As a natural herbal medicine with high anti-inflammatory, antioxidant, and anti-tumor activities, wild golden cherry provides a new path for further exploration of its physiological functions and mechanisms of action in various diseases. For example, wild golden cherry has been used in the treatment of cardiovascular diseases, tumors, *etc*.; the active ingredient in its extract has preventive and alleviating effects on lesions of the cardiovascular system, urinary system, and nervous system. The ethanol extract of wild golden cherry may play an important role in the protection of kidney cells by improving microcirculation, inhibiting the release of inflammatory factors and oxidative stress, as well as regulating immune function.

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Disclosure statement

The authors declare no conflict of interest.

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