

Article

Renoprotective effect of *Laminaria japonica* polysaccharide in adenine-induced chronic renal failure

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Abstract: Chronic renal failure (CRF) is a major public health problem worldwide. In this work, we investigated the effects of a purified *Laminaria japonica* polysaccharide (LJP61A) on the renal function using adenine-induced CRF mice model. Results exhibited that adenine treatment caused serious renal pathological damages and elevation of serum creatinine and blood urea nitrogen of mice. However, these changes could be significantly reversed by the administration of LJP61A in a dose-dependent manner. Additionally, LJP61A could dramatically reduce the weight loss, improve the urine biochemical index, and regulate the electrolyte disturbance of CRF mice. These results suggested that the renal functions of adenine-induced CRF mice could be improved by LJP61A, which might be developed to a potential therapeutic agent for CRF patients.

Keywords: *Laminaria japonica*; Polysaccharide; Chronic renal failure

1. Introduction

Chronic renal failure (CRF) is a type of kidney disease characterized by a slow and progressive renal function decline associated with irreversible kidney sclerosis and nephrons loss [1]. In recent years, the morbidity and mortality of CRF is rising markedly, which has brought a heavy financial burden to our society [2, 3]. In China, there are 119.5 million CRF patients in 2012, with an overall prevalence of 10.8% [4]. The main therapeutic methods of end-stage CRF are hemodialysis and kidney transplantation [5]. However, the patients treated with hemodialysis are reported to have a higher overall risk of developing cancer when compared to the general population [6]. Additionally, although there are advances in kidney transplantation, limited available kidney sources restrict its application [5]. Therefore, it is imperative to develop new and effective agents from herbs or edible-medicinal materials to treat or alleviate CRF.

Laminaria japonica, a common economic edible-medicinal marine vegetable, has long been used as an important therapeutic agent for phlegm elimination and detumescence in China [7,8]. In recent years, the functional components of *L. japonica* have been widely reported by chemists and pharmacologists [9,10]. Among these components, polysaccharides have been considered as the main active components, which appear many bioactivities, such as anti-tumour, anti-virus, anti-oxidant and anti-radiation [11, 12]. In previous works, we have isolated a purified polysaccharide (LJP61A) from *L. japonica*, which was characterized by a repeating unit consisting of

$\rightarrow 3,6$)- α -D-Manp-(1 \rightarrow , \rightarrow 4)- α -D-Manp-(1 \rightarrow , \rightarrow 4)-2-O-acetyl- β -D-Glcp-(1 \rightarrow , \rightarrow 4)- β -D-Glcp-(1 \rightarrow , \rightarrow 6)-4-O-SO₃- β -D-Galp-(1 \rightarrow , \rightarrow 6)- β -D-Galp-(1 \rightarrow , \rightarrow 3)- β -D-Galp-(1 \rightarrow , and a terminal residue of α -D-Glcp-(1 \rightarrow (Figure 1) [13]. LJP61A has been proved to suppressing atherosclerosis via the regulation of cellular lipid metabolism, inhibition of cellular inflammation and alleviation of insulin resistance [13, 14]. Additionally, we have demonstrated that LJP61A could ameliorate vascular calcification via preventing osteoblastic differentiation of vascular smooth muscle cells [15]. Because atherosclerosis and vascular calcification are closely related with the development of CRF [16, 17], we speculated that LJP61A might have the potential of treating or alleviating CRF. Therefore, the protective effects of LJP61A on the renal function were investigated in the present work using adenine-induced CRF mice model.

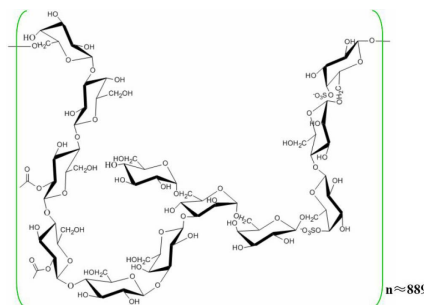


Figure 1. The chemical structure of LJP61A.

2. Results and Discussion

2.1. LJP61A regulates body weight, water and food intake of CRF mice

As shown in Figure 2A, the body weights of CRF mice were significantly reduced by adenine when compared with those of control group. However, the weight loss of CRF mice could be attenuated by LJP61A. In addition, it could be found that adenine treatment remarkably increased the water intake of CRF mice, and reduced the food intake (Figure 2B-C). However, these alterations of CRF mice were significantly reversed by LJP61A. These results indicated the physiological state of adenine-induced CRF mice could be enhanced by LJP61A.

2.2. LJP61A ameliorates kidney injury of CRF mice

As shown in Figure 3A, the kidneys of mice in control group are reddish brown and glossy, while those of mice in CRF group are gray white and uneven. However, the gray white and uneven kidneys of CRF mice could be ameliorated by the treatment of LJP61A, which indicated LJP61A could mitigate the kidney injury of adenine-induced CRF mice. To further support the viewpoint above, H&E and MT staining were performed. In the CRF group, kidney sections showed several signs of diffuse injury and interstitial fibrosis (Figure 3B, C). However, it could be found that the renal pathological damages of CRF mice were remarkably reversed by the treatment of LJP61A in a dose-dependent manner (Figure 3D, E). When LJP61A dosage reached 200 mg/kg/day, the areas of injury and fibrosis were suppressed by 44.7% and 43.8% compared to those of CRF group, respectively. These results further supported the conclusion that the damaged kidney of adenine-induced CRF mice could be repaired by LJP61A.

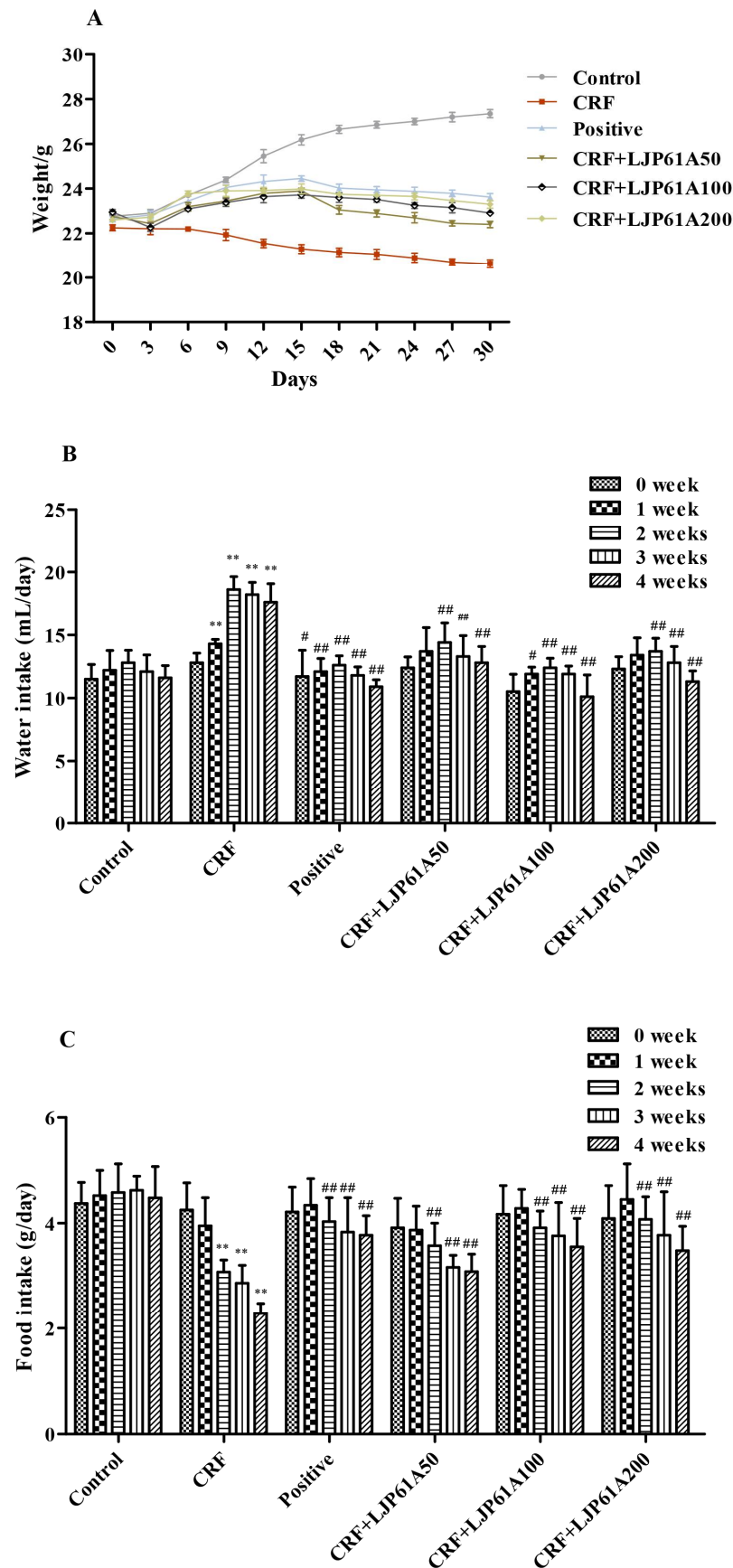


Figure 2. The effects of LJP61A on the body weight (A), water (B) and food (C) intake of adenine-induced CRF mice. * $p < 0.05$, ** $p < 0.01$ (vs. Control group); # $p < 0.05$, ## $p < 0.01$ (vs. CRF group).

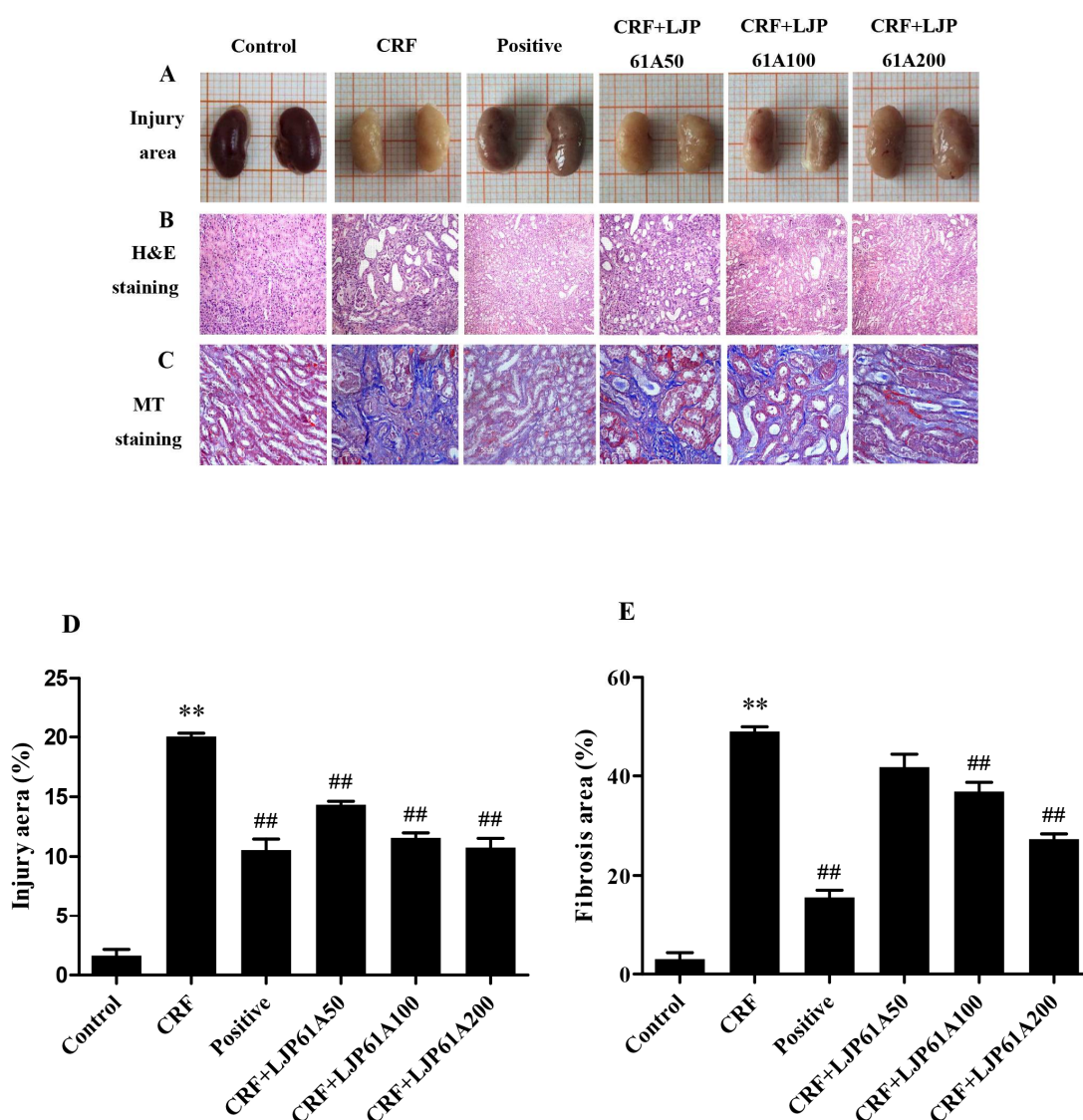


Figure 3. LJP61A ameliorates kidney injury in adenine-induced CRF mice. (A) Renal morphology; (B) H&E staining; (C) MT staining; (D) injury area; (E) fibrosis area. ** $p < 0.01$ (vs. Control group); ## $p < 0.01$ (vs. CRF group).

2.3. LJP61A regulates blood biochemical index of CRF mice

SCr and BUN, two main end products of protein metabolism, are mainly excreted by glomerular filtration. They are the most important indexes of renal function [18,19]. As shown in Figure 4A-B, the SCr and BUN levels of mice in CRF group were significantly elevated by the treatment of adenine when compared with those of control group. However, these enhancements were remarkably inhibited by LJP61A in a dose-dependent manner. When the dosage reached 200 mg/kg/day, the levels of SCr and BUN were decreased to the 73.41% and 44.62% of CRF group, respectively. These results confirmed that LJP61A could improve the kidney function of adenine-induced CRF mice.

2.4. LJP61A regulates urine biochemical index of CRF mice

Besides SCr and BUN, UCr and UP were also considered as the key indexes of kidney function [20]. As shown in Figure 5, the UCr level of mice in CRF group was significantly reduced by adenine when compared with that of control group, while UP was increased.

However, these alterations of CRF mice were significantly reversed by LJP61A, which further confirmed LJP61A could improve the kidney function of adenine-induced CRF mice.

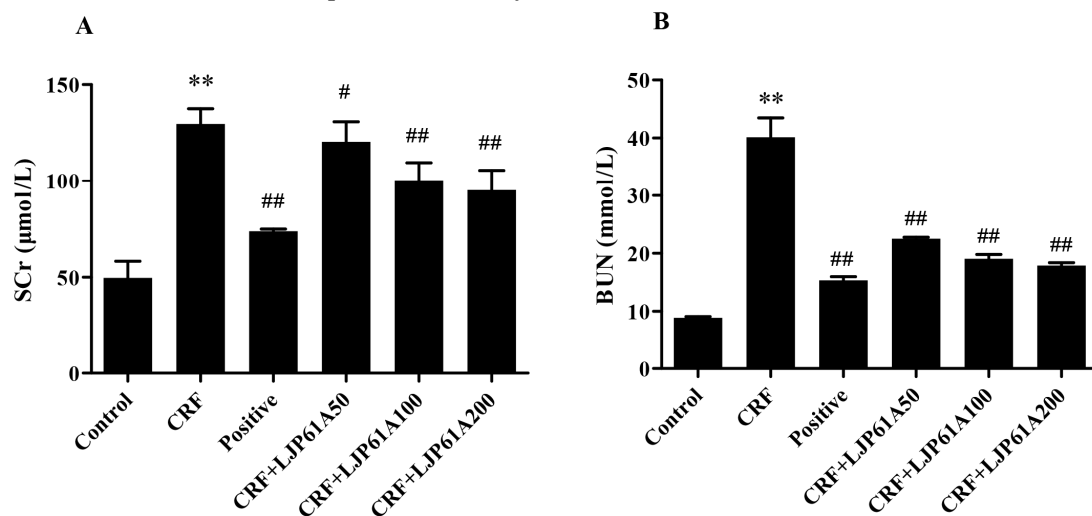


Figure 4. The effects of LJP61A on the SCr (A) and BUN (B) levels of adenine-induced CRF mice. * $p < 0.05$, ** $p < 0.01$ (vs. Control group); # $p < 0.05$, ## $p < 0.01$ (vs. CRF group).

2.5. LJP61A regulates electrolyte disturbance of CRF mice

Electrolyte disturbance is an important clinical manifestation of CRF [1]. Thus, the effects of LJP61A on the electrolyte disturbance of CRF mice were investigated. As shown in Table 1, compared with control group, the CRF group showed a significant increase in serum levels of chlorine, potassium, magnesium, sodium and phosphorus. Meanwhile the urine levels of calcium, phosphorus and magnesium and serum level of calcium present a significant decrease. However, these alterations of CRF mice were reversed by LJP61A, besides the serum levels of chlorine and sodium. These results indicate that LJP61A could improve the electrolyte disturbance of adenine-induced CRF mice.

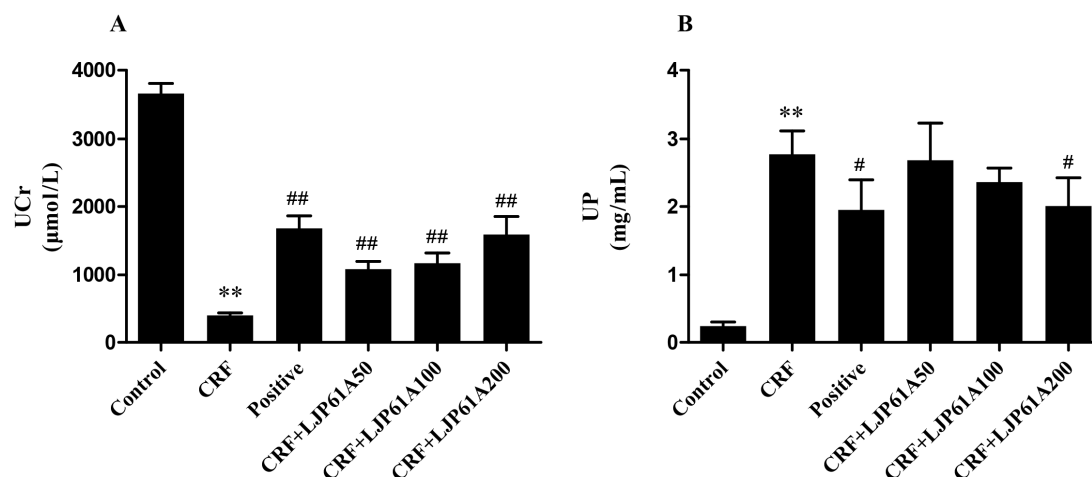


Figure 5. The effects of LJP61A on the UCr (A) and UP (B) levels of adenine-induced CRF mice. # $p < 0.05$, ## $p < 0.01$ (vs. CRF group).

Table 1. LJP61A regulates electrolyte disturbance of adenine-induced CRF mice.

Group	Serum Calcium (mmol/L)	Serum Chlorine (mmol/L)	Serum Potassium (mmol/L)	Serum Magnesium (mmol/L)	Serum Sodium (mmol/L)	Serum Phosphorus (mmol/L)	Urine Calcium (mmol/L)	Urine Phosphorus (mmol/L)	Urine Magnesium (mmol/L)
Control	3.27±0.12	85.29±0.26	9.10±0.21	1.88±0.01	146.97±0.39	3.52±0.16	2.84±0.68	14.37±2.8	4.01±0.52
CRF	2.55±0.05**	105.97±0.52**	11.83±0.12**	2.43±0.07*	162.07±1.76**	5.82±0.11**	1.69±0.30*	8.75±1.6*	2.67±1.78*
Positive	3.13±0.10▲▲	101.69±0.61	9.60±0.47▲▲	2.18±0.04▲▲	148.15±0.85	3.93±0.25▲▲	2.56±0.22▲▲	10.30±3.5▲▲	3.10±1.25▲▲
CRF+LJP61A50	2.56±0.11	105.30±0.27	10.43±0.42▲▲	2.11±0.12▲▲	158.73±1.66	5.22±0.12▲▲	1.91±0.12▲	10.26±2.1▲▲	2.89±2.01▲▲
CRF+LJP61A100	2.78±0.11▲▲	104.13±0.66	10.07±0.50▲▲	1.97±0.07▲▲	152.66±0.22	4.79±0.13▲▲	1.89±0.45▲	11.35±1.9▲▲	2.92±1.34▲▲
CRF+LJP61A200	2.98±0.12▲▲	102.00±0.90	9.97±0.22▲▲	1.89±0.06▲▲	150.73±0.55	3.72±0.22▲▲	2.49±0.27▲▲	11.84±3.0▲▲	3.05±2.70▲▲

*p< 0.05, **p< 0.01 (vs. Control group); ▲p< 0.05, ▲▲p< 0.01 (vs. CRF group)

3. Materials and methods

3.1. Chemicals

LJP61A was extracted and purified as described previously [14]. Adenine was purchased from Biosharp (Hefei, China). Cinacalcet tablets were purchased from Kyowa Hakko Kirin Co., Ltd. (Tokyo, Japan). All other reagents were analytical grade and obtained locally.

3.2. Animals

Eight-week-old male C57/BL6 mice (20±2 g) were purchased from the Laboratory Animal Center of Anhui Medical University. The mice were maintained under specific pathogen-free conditions with a 12:12 h light-dark cycle at 25 ± 2 °C and 40% relative humidity. All animal handling procedures were performed strictly in accordance with the P.R. China Legislation on the Use and Care of Laboratory Animals.

3.3. Experimental procedure

After an acclimatization period of one week, mice were randomly divided into six groups (20 mice per group), including control group, CRF group, positive group, LJP61A groups (CRF+LJP61A50, CRF+LJP61A100 and CRF+LJP61A200). The control group was fed a control diet, while the others groups were fed the diet supplemented with adenine at the dose of 0.2% (w/w). The positive group was administered with Cinacalcet tablet at the dosage of 150 mg/kg/day. The control and CRF groups were administered with the same volume of physiological saline. The LJP61A groups were orally administered with LJP61A by 50, 100 and 200 mg/kg/day, respectively. Meanwhile, all mice were weekly weighed during the experimental period. After five weeks feeding, all mice were euthanized with CO₂. Serum and kidneys were collected for the analysis of blood biochemistry and pathology.

3.4. Water and food intake

Five mice were randomly selected to be placed in the metabolic cage every week. 15g pellet feed was put into the trough and 50mL water was put into the water hole. Meanwhile, the urine cup and the fecal cup were placed in the cage. After 24h, the residual feed and water were weighed to analyze the food and water intake.

3.5. Kidney pathological examination

The kidneys were fixed in 4% paraformaldehyde, dehydrated in increasing concentrations of ethanol, cleared with xylene and embedded in paraffin. From the paraffin blocks, five micrometer sections were prepared and stained with hematoxylin and eosin (H&E) and Masson Trichome (MT) [21].

3.6. Serum parameters

The serum creatinine (SCr), blood urea nitrogen (BUN), calcium, chlorine, potassium, magnesium, sodium and phosphorus were measured using automated analyzer (Accu-check Performa, Roche, Germany).

3.7. Urine parameters

The contents of urine protein (UP), urine creatinine (UCr), calcium, phosphorus and magnesium were tested by inspection center of the First Affiliated Hospital of Anhui University of Traditional Chinese Medicine.

3.8. Statistical analysis

Results are expressed as the mean \pm SD. Differences between groups were assessed by one-way ANOVA. Statistical tests were performed using SPSS software. Difference was considered statistically significant at $p < 0.05$ and $p < 0.01$. * $p < 0.05$, ** $p < 0.01$ versus control group; # $p < 0.05$, ## $p < 0.01$ versus CRF group.

4. Conclusions

In summary, experimental evidence in the present work confirmed that LJP61A has the ability to improve the renal function of adenine-induced CRF mice. Based on the current investigation, it is probable that the purified *L. japonica* polysaccharide LJP61A might be developed to a new therapeutic agent or functional food supplement to delay CRF in the future.

Author Contributions: M. Long and Q.-M. Li carried out the experimental work. Q. Fang, L.-H. Pan, X.-Q. Zha and J.-P. Luo provided oversight. M. Long, Q.-M. Li, X.-Q. Zha and J.-P. Luo conceived the experiments and wrote the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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