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Original Article

Improvement effects of *Astragalus* polysaccharide enzymatic hydrolyzates on adenine-induced peroxidative and renal failure in rats

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Abstract: Two *Astragalus* polysaccharide enzymatic hydrolyzates (APSEH1 and APSEH2) were from the hot water extract of *Radix Astragali* and enzymatic hydrolyzed by cellulase or galactosidase. Their average molecular weight was 78.25 and 10.14 kDa. In this present research, the effects of three *Astragalus* polysaccharide (APS) fractions with different molecular weight on adenine induced renal failure were investigated. In vivo experiments showed that APSEH could significantly decrease the serum contents of serum urea nitrogen (SUN), serum creatinine (SCR), albumin (ALB) and urinary protein (URI) and kidney weight, kidney index. Meanwhile, APSEH could obviously reduce malondialdehyde (MDA) contents and increase catalase (CAT), GSH peroxide (GSH-Px) and superoxide dismutase (SOD) activities in kidney tissues. These results suggest that the lower molecular weight APSEH has exerted better reno-protective effects and antioxidant activity on ADN-induced renal failure in rats. The improvement effect may be related to its antioxidant activity.

Keywords: Renal failure, enzymatic hydrolyzates, different molecular weight

Introduction

The chronic renal failure model can be induced in rats by periodically adenine (ADN) intragastric administration [1], it could increase serum creatinine, uric acid and urea nitrogen by reducing the urinary excretion [2]. This disease could cause serious complications such as stroke, diabetes, cardiovascular and so on [3].

The *Radix Astragali*, one of most popular Chinese herb medicines that widely used in China for thousands of years [4]. In recently years, more and more natural polysaccharides have been investigated and proved to exhibit different biological activities [5]. Pharmacology researches have demonstrated that *Astragalus* polysaccharide (APS) exhibited various biological activities, such as immunomodulation [6], antitumor [7, 8], anti-diabetes [9], antiviral [10], antioxidant [11], hepatoprotection [12] and renal protective effect [13]. But few researches mainly focused on the enzymatic hydrolysis of APS and the biological activities of APSEH.

According to the literature, the bioactivity of polysaccharides was dependent on structure [7], viscosity [14] and molecular weight [15]. The molecular weight of polysaccharide has much effect on their bioactivity. For example, the literature have reported that the polysaccharide with low-molecular weight exhibit significant renoprotective [16, 17] and higher antioxidant activity, which was about 29 kDa [18].

There were several physical and chemical ways to degrade the polysaccharides, including ultrasonic degradation [19], oxidative degradation [20], and enzymatic hydrolysis [21]. Among the different ways employed, enzymatic hydrolysis was proved to be an effective way for removing the glycosidic bonds of the polysaccharide [22].

The purpose of this research was to prepare different molecular weight of APS by enzymatic hydrolysis in order to further understand the relationship between molecular weight and renal protective effects of APSEH. In addition, the present work was conducted to investigate the reno-protective and antioxidative effects of

Table 1. Molecular weight and total polysaccharides of three APS fractions

Sample	Mw (kDa)	Total polysaccharide (%)
APS	135.12	85.98 ± 1.69
APSEH1	78.25	86.27 ± 0.87
APSEH2	10.14	85.71 ± 1.02

the two APSEH on renal failure and hyperuricemia rats induced by adenine-intragastrically.

Materials and methods

Chemicals and materials

The dried *Radix Astragali* were purchased from An Guo Zhong Sheng Medicinal Material LTD. The diagnostic kits for antioxidant indicators and biochemical analysis were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Cellulase and galactosidase were purchased from Sigma Chemical Company. All other chemical reagents with analytical grade were purchased from Sinopharm Chemical Reagent Co., Ltd.

Preparation of APS and APSEH

The dried *Astragalus* Radix powder (200 g) was extracted with water (1.5 L) for 1h at 90°C and for three times. The extract was centrifuged and filtered to obtain the supernatant. Supernatant was precipitated with 80% ethanol at 3°C for 12 h after concentration under vacuum. The crude polysaccharide (CP) was obtained by centrifugation, lyophilized, and then deproteinated by Sevag method [23]. Thereafter, the CP was dissolved in deionized water and further purified by dialysis at 3°C for 72 h with a cut-off 3.5 kDa. Finally, the high molecular weight polysaccharide was lyophilized and labeled as APS. The cellulase and galactosidase were selected to catalyze APS hydrolysis under the condition of 50°C and pH 4.5, and incubation for 5 h. Thereafter, the reaction was terminated in an ice water bath for 10 min [24]. The reaction mixtures were centrifuged and collected the supernatant. Finally, the supernatant were lyophilized after dialysis at 3°C for 72 h with a cut-off 3.5 kDa, labeled as APSEH1 and APSEH2, respectively. The total polysaccharides content was tested by the phenol-sulfuric acid method using glucose as the standard substance to a calibration curve [25].

GPC-MALLS-RI measurement

The weight-average molecular weights (Mw) and chain conformation of APS/APSEH were measured by gel permeation chromatography (GPC) combined with multi-angle laser light scattering (MALLS) detector (Wyatt Technology Co) and a refractive index detector. GPC-MALLS-RI was performed with a Shodex-OHpak SB-804 HQ column (8.0 mm × 300 mm), the injection volume was 0.1 mL and all samples (1 mg/ml) were dissolved in 0.1% sodium chloride which was used as the mobile phase at 0.75 ml/min at 30°C [26]. Software (Version Astra 6.1.1.17) was used for the data analysis and acquisition.

Animal experiments

The animal experiments were approved by the ethics committee, under the guidelines of local institutional and governmental regulations. The male SD rats (178.25 ± 20 g) were purchased from Changsha Tianqin Biotechnology Company and were housed under conditions of humidity (45 ± 5%), temperature (21 ± 2°C) and a 12 h light/dark cycle. After seven days of accommodation, the renal failure modes were induced by adenine-intragastrically for 28 days (70 mg/kg/d bodyweight, freshly prepared in distilled water) except normal control (NC, n=10). We detected the serum creatinine (SCR) albumin (ALB), urinary protein (URI) and serum urea nitrogen (SUN) in the blood serum to confirm whether the renal failure model was successful in last day. The renal failure rats were randomly divided into 7 groups (n=10). One was model control group; others were six dosage groups: APSL (APS 60 mg/kg/d), APSH (APS 120 mg/kg/d), APSEH1L (APSEH1 60 mg/kg/d), APSE H1H (APSEH1 120 mg/kg/d), APSEH2L (APS EH2 60 mg/kg/d), APSEH2H (APSEH2 120 mg/kg/d) were administered orally for four weeks, respectively [27]. The NC and MC rats were administered orally with the same amount of distilled water. All rats were weighted and anaesthetized on the 28th experimental day, the blood samples were centrifuged at 9000 r/min for 15 min at 4°C after sampled from retrobulbar vein, the serum was collected and stored at -20°C for further test the levels of ALB, URI,

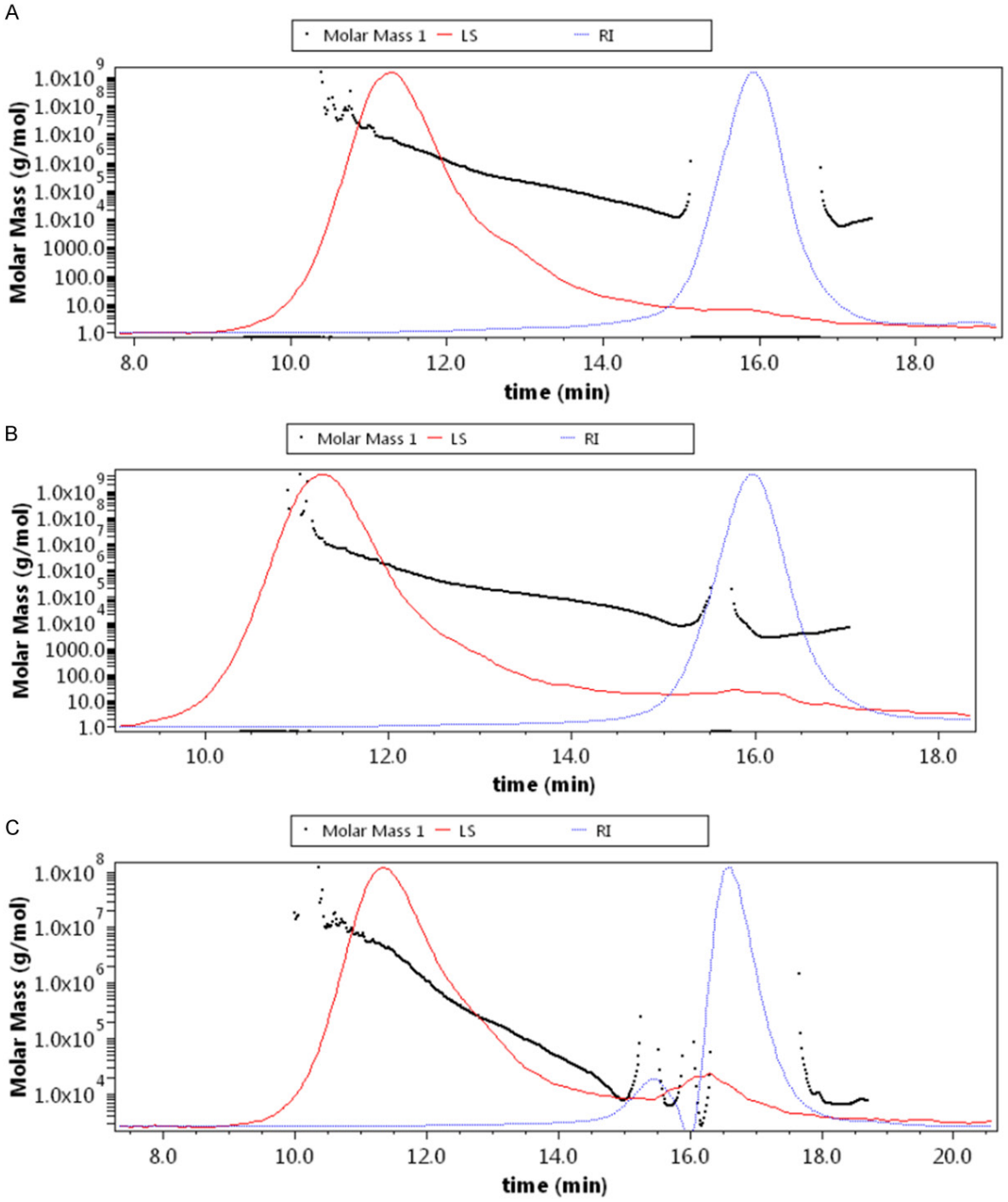

Figure 1. The RI and LS chromatograms for APS (A), APSEH1 (B) and APSEH2 (C).

SUN and SCR. The kidneys were quickly collected, weighted and homogenized with ice physiological saline. Thereafter, the homogenate was centrifuged at 4000 r/min for 15 min at 4°C and the supernatant was collected for further analysis the SOD, GSH-Px and CAT activities and MDA, total protein contents [28].

Kidney index = (kidney weight/body weight) × 100.

Biochemical analysis

The levels serum of ALB, URI, SUN, SCR, SOD, GSH-Px, CAT, MDA, and total protein were measured with commercial assay kits on the basis of the manufacturer specification.

The data statistical analysis

All experiment results were reported as the means ± SD and the experiment were replicat-

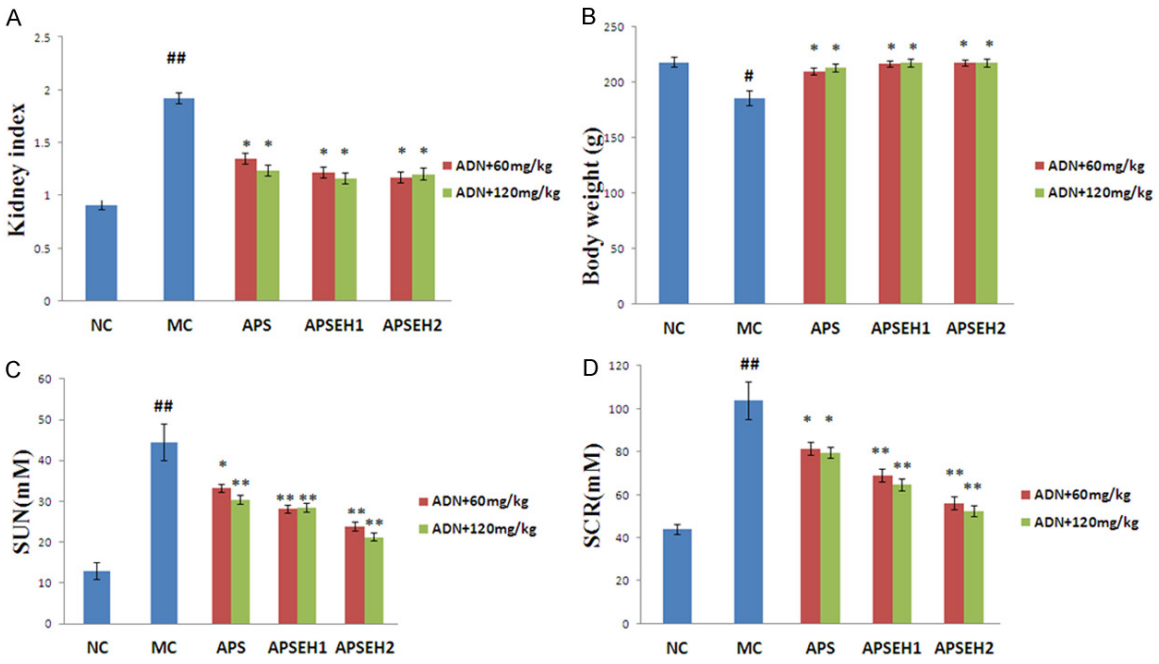

Figure 2. Effects of three APS fractions on kidney index (A), body weight (B), serum levels of SUN (C) and SCR (D). The data were reported as the mean ± SD of ten rats per group. ##*P*<0.01 (vs NC group) and **P*<0.05 (vs MC group).

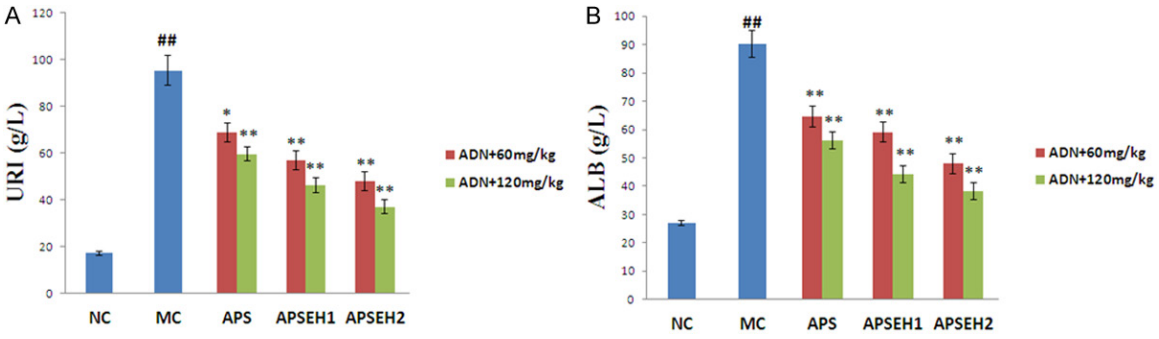

Figure 3. Effects of three APS fractions on serum levels of URI (A) and ALB (B). The data were reported as the mean ± SD of ten rats per group. ##*P*<0.01 (vs NC group) and ***P*<0.01, **P*<0.05 (vs MC group).

ed twice. The differences between groups were estimated by one-way ANOVA and using SPSS software (version 16.0); *P*<0.05 was usually considered as statistically significant.

Results

Molecular weight and total polysaccharides for APS and APSEH

As shows in **Table 1**, the Mw and total polysaccharides contents of three APS fractions were tested. The total polysaccharides contents in APS, APSEH1 and APSEH2 tested by phenol-sulfuric acid method were up to 85.98%, 86.27% and 85.71%, respectively. The Mw of three APS fractions were measured by GPC-MALLS-RI, among the three APS fractions, the Mw of APS was 135.12 kDa, which was higher than APSEH1 and APSEH2. The different Mw of APSEH was obtained by different enzymatic hydrolysis conditions using cellulase or galactosidase. As were shown in **Figure 1A-C**, the differential signals from MALLS and refractive index detector (RI) of three APS fractions in 0.1% sodium chloride at 30°C.

Effects of three APS fractions on kidney index and body weight

All results of kidney index in different groups were shown in **Figure 2A**. The kidney index in

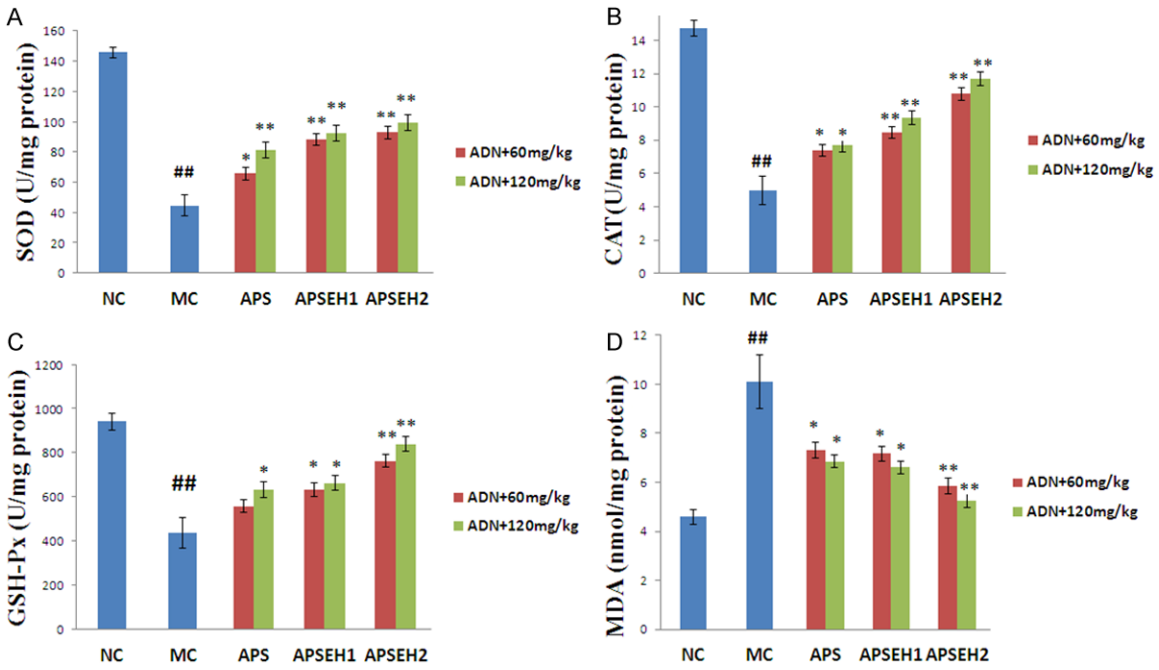

Figure 4. Effects of three APS fractions on SOD (A), CAT (B), GSH-Px (C) activities and MDA (D) in kidney tissue. The data were reported as the mean ± SD of ten rats per group. ##P<0.01 (vs NC group) and **P<0.01, *P<0.05 (vs MC group).

MC group was significantly higher (P<0.01) than those in NC group. However, the kidney index of APS, APSEH1 and APSEH2 groups were lower than the MC group (P<0.05), the increase of kidney index could be mitigated by oral administration the three APS fractions. Changes in body weight were recorded in all groups at the end of experiment (**Figure 2B**). Significantly decreased in body weight was observed in MC group when compared with that in NC group. However, the decline of body weight could be mitigated by oral administration of APS fractions at different dosage (P<0.05). The experimental results implied that the ADN-induced renal failure could be improved to some extent by the treatment with those APS fractions.

Effects of three APS fractions on serum biochemical parameters

Clinically, the serum levels of ALB, URI, SUN and SCR were usually used as biochemical markers for renal failure. As shown in **Figures 2C**, **2D**, **3A**, **3B**, the URI, ALB, SUN ADN SCR levels of ADN-induced renal failure rats were significantly increased when compared with those in the normal rats (P<0.01). However, after oral administration of APS fractions for four weeks at different dosage, the increase of URI, ALB, SUN ADN SCR could be assuaged (P<0.05, P<0.01). Meanwhile, the levels of URI, ALB, SUN and SCR in APSEH1 and APSEH2 groups were evidently lower (P<0.01) than those in APS group at the same dosage.

Effects of three APS fractions on SOD, CAT, GSH-Px activities and MDA contents in kidney tissue

In current work, the four biochemical indices that related to antioxidant activities were analyzed, in order to investigate the antioxidant activities of APS, APSEH1 and APSEH2 in ADN-induced renal failure rats (**Figure 4A-D**). As displayed in **Figure 4A-C**, the SOD, CAT and GSH-Px activities of ADN-induced renal failure rats were significantly decreased when compared with the normal rats (P<0.01). However, after the four weeks of treatment, the decline of those antioxidant enzyme activities could be alleviated after the treatment with APSEH1 and APSEH2 at different dosages compared with that in MC group (P<0.01). As shown in **Figure 4D**, the increased contents of MDA were observed in kidney tissue in MC group when compared to the NC group (P<0.01). However, the contents of MDA in kidney were signifi-

cantly decreased in APS, APSEH1 and APSEH2 group, respectively ($P<0.05$ and $P<0.01$).

Discussions

The serum levels of SCR manifest the ability of renal tissue to remove the creatinine from blood and gather it in the urine. However, kidney dysfunction could lead to the levels of SCR increased through weaken the ability to filter creatinine. In addition, the injured kidneys could cause the levels of SUN elevated on account of the kidney tissue have no ability to remove the urea from the blood [29]. ADN feeding could successfully established the renal failure model as assessed by significantly increased the weight of renal tissue and urine production, markedly declined the clearance rate of creatinine and the body weight [30, 31]. In present work, ADN-induced renal failure of rats showed the significantly decrease in body weight, markedly increased the levels of SUN and SCR, indicating that the renal failure model was successfully established. Then, the improvement effects of three APS fractions were investigated.

For the structural characterization of polysaccharides, the multi-angle laser light scattering technology has exhibited unique preponderance. It has been proven to be an efficient method for researching the property of biological macromolecule [32]. The GPC-MALLS-RI method could directly detect the molecular weight distribution and the absolute molecular weight of the polysaccharides without the calibration curve of polysaccharide standard. In the present work, the Mw of APS, APSEH1 and APSEH2 were successfully detected by GPC-MALLS-RI. The results of present research revealed that the significant decrease in serum levels of SCR and SUN were observed on renal failure rats after the oral administration of APS, APSEH1 and APSEH2, and the three fractions of APS appear to exert some extent reno-protective effects, particularly the APSEH2 fraction. The APSEH2 fraction of APS, with lower molecular weight, but exhibited a stronger reno-protective than the high molecular weight of APS in ADN-induced renal failure. The results appear to imply the differential of molecular weight have significant effect on the kidney protective effect.

Previously research has demonstrated that oxidative stress could cause the organ damage, which defined as imbalance between the antioxidant defenses and product of ROS [33]. In hence, mitigated oxidative stress was considered as an important contributor to the development of kidney injury. There were some complexes of antioxidant enzymes in biological tissue, including the SOD, GSH-Px and CAT, they worked together to prevent the formation of reactive oxygen species (ROS). The CAT was an enzyme which has functions to prevent ROS damaging the biological system [34]. GSH-Px was an important enzyme which has functions in decreasing the oxidized tissue components and the concentration was closely related to the degree of kidney injury. It has been proofed that the consumption of GSH-Px could cause an acute kidney failure [35]. SOD was another important antioxidant enzyme in the biological tissue. It has been proofed that the SOD can prevent free radical-initiated lipid peroxidation by effectively clear free radicals [36]. MDA, the main product of lipid peroxidation generated in the peroxidation of polyunsaturated fatty acid when lipids were assaulted by the free radicals, which indirectly reflect the degree of tissue damage [37]. In the current research, the remarkable decline of CAT, GSH-Px and SOD activities and the significant increase of MDA contents were observed in ADN-induced renal failure rats when it compared with in NC group, the results showed that serious oxidative kidney injury were occurred in MC group. However, these oxidative damages could be alleviated through the treatment with the three APS fractions. The possible mechanism was the ability of APSEH to reduce the MDA contents and remove the ROS in renal. Several literature had supported the hypothesis that the fractions of APS could exert their reno-protective effect indirectly through increasing the capability of antioxidant defense system in vivo [38, 39]. The possible mechanism of APSEH on ADN-induced renal failure was through its effect on antioxidant enzymes activities [40].

In addition, the literature has been reported that the different molecular weight was attributed to the pharmacological activities of polysaccharides [41]. Previously report has been proofed that the antioxidant activities were associated with molecular weight of polysaccharides from *Athyrium multidentatum* [42]. The *Enteromorpha prolifera* polysaccharide was hydrolyzed to lower molecular weight polysaccharides by either gluco-amylase or pectin-

ase. What's more, the research has showed that the enzymatic hydrolysates of polysaccharide exhibited stronger antioxidant activity and renoprotective effects than the original polysaccharide [43]. In the present research, the APS of 10.14~135.12 kDa all exerted significant antioxidant activity and renoprotective effects. Moreover, the 10.14 kDa of APSEH2 had exhibited the best antioxidant activity, which was accordance with the antioxidant activity of polysaccharide from *Porphyridium cruentum* with a Mw of 6.55 kDa [43, 44]. The enzymatic hydrolyzates showed superior biological properties may be related with the good water solubility of enzymatic modification polysaccharides [45].

In conclusion, the APS was successfully enzymatic hydrolyzed into two lower Mw fractions (APSEH1 and APSEH2) by cellulase and galactosidase. Moreover, the relationship between Mw and reno-protective effects was discussed. Our research manifested that the lower Mw APS exhibited relatively stronger antioxidant activity and reno-protective activity in vivo. In addition, these results showed that the APSEH from *Astragalus* polysaccharide might be become a potential drug in preventing kidney failure owing to its obvious reno-protective effects and antioxidant ability.

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Disclosure of conflict of interest

None.

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