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Carboxymethylation of an exopolysaccharide from *Lachnum* and effect of its derivatives on experimental chronic renal failure

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ABSTRACT

Carboxymethylated polysaccharide CLEP-1b was prepared from a single component (LEP-1b) of *Lachnum* YM281 exopolysaccharides by molecular modification with a degree of substitution (DS) of 0.286. Infrared result proved that the carboxymethylation of LEP-1b succeeded and ^{13}C NMR result showed that the carboxymethyl group (—CH_2COOH) was chemically linked to an oxygen (O) atom of the hydroxyl on C-3 of LEP-1b. LEP-1b could improve the histopathological status of kidney and significantly reduce the contents of serum creatinine (Scr) and blood urea nitrogen (BUN), and increase the contents of total protein and albumin. It could also enhance the activity of SOD, GSH-PX, CAT, GSH and decrease MDA contents in the nephridial and hepatic tissues. What's more, CLEP-1b showed more significant effects than LEP-1b at the same dosage. The research indicated that LEP-1b and CLEP-1b could mitigate the chronic renal failure of mice and the effects were closely associated with antioxidant activity.

1. Introduction

Kidney is an important excretory organ of human body, with the main functions of filtering and forming urine and discharging metabolic wastes. It also regulates electrolytes and the acid–base balance in human body. Chronic renal failure (CRF) is the progressive renal injury resulted from primary renal diseases or secondary diseases. Clinical syndrome represents mainly as metabolite retention, water and electrolyte disturbance, acid-base disturbance or damaged systems in body. The CRF has become a global public health problem (Bandara, Wijewardena, Liyanege, Upul, & Bandara, 2010). Hemodialysis and renal transplantation are effective ways to cure end-stage CRF, but they cost too much. Haikunshenxi capsules (Fucoidan), as a commonly used medicine, long-term use of which may result in hepatic injury, nausea, emesis or other adverse reactions (Shi, Xu, & Zhou, 2013; Wang, Zhang, Jin, Niu, & Zhang, 2011; Yin, Lv, & Chen, 2007). Thus, it is of great significance to explore and develop new natural drugs without toxic side effects to treat the CRF.

Fungal polysaccharide is a kind of natural polymer which is composed of monosaccharides via glycosidic bonds. It shows the abilities of antioxidant, anti-aging and mitigating the CRF. For example, *Atractylodes rhizom* polysaccharide was revealed a promising preventive effect on renal failure in rats (Feng et al., 2010). And, *Cordyceps* polysaccharide had a therapeutic and preventive effect on adenine-induced CRF (Yin et al., 2007). Thus, fungal polysaccharide can be used as immunopotentiator and immune activator, which is also reputed as the "biological response modifier" (Luo, Xu, Yu, Yang, & Zheng, 2008). The activity of fungal polysaccharide depends on its molecular structure, so its activity can be affected by molecular modifications, such as sulfation, phosphorylation, carboxymethylation and acetylation (Ye, Yuan, He, Du, & Ma, 2013). Furthermore, substituent groups, substitution positions and degree of substitution (DS) essentially alter the activity of fungal polysaccharide derivatives (Kawashima, Murakami, Nishimura, Nakano, & Obata, 2012; Ye et al., 2013). Carboxymethylation of polysaccharide means to introduce carboxymethyl groups to the chains of polysaccharide so as to bring new activities or to enhance the intrinsic activity of polysaccharide (El-Sherbiny, 2009; Magnani, & Calliari, 2009). The antioxidant activity of carboxymethylated *Coprinus comatus* polysaccharide was much better than before carboxymethylation (Zhou, Tian, Zhang, & Liu, 2010). *Poria sclerotium* polysaccharide without anti-gastric adenocarcinoma activity showed strong anti-gastric adenocarcinoma activity after carboxymethylation (Li, Hou, Wang, & Zhang, 2012). However, research on mitigating the CRF of carboxymethylated polysaccharide has not been reported so far.

Lachnum is a kind of saprophytic fungus and produces a great deal of active polysaccharide by submerged fermentation. The polysaccharide showed antioxidant, anti-aging, lipid lowering and

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liver protecting activities (Qiu, Ma, Ye, Yuan, & Wu, 2013; Ye et al., 2012, 2013). Furthermore, phosphorylation modification of *Lachnum* polysaccharide could improve the anti-tumor activity (Ye et al., 2013). Previous research showed that the main chain of LEP-1b (separated from *Lachnum* YM281 exopolysaccharide) is composed of (1 → 3)-β-D-glucan and its branch chain is (1 → 3) and (1 → 6)-β-D-glucan (Qiu et al., 2013). In this research, carboxymethylation of LEP-1b (CLEP-1b) was conducted and the effects of LEP-1b and CLEP-1b on experimental CRF were assessed.

2. Materials and methods

2.1. Materials

LEP-1b is a homogeneous component of exopolysaccharide purified from *Lachnum* YM281 and the purity of LEP-1b is chromatographic grade. LEP-1b is a white powder and readily soluble in water, which is preserved in Microbial Resource and Application Research Center of Hefei University of Technology.

2.2. Laboratory animals

Seventy Kunming male mice, with individual weight of 20 ± 2 g, were purchased from the Laboratory Animal Center of Anhui Medical University (Certificate number: Experimental Animal Standard No. 1 of Anhui Hospitals). Growth conditions for these mice: temperature 23 ± 2 °C, humidity 55 ± 5%, alternative feeding with 10 h light and 10 h darkness.

All animals' treatments were strictly in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.3. Reagents and instruments

Adenine was purchased from Luoyang Desheng Chemical Co., Ltd. (Henan, China), Haikunshenxi capsules from Huinan Changlong Biochamical Pharmacy Co., Ltd (Jilin, China), serum creatinine (Scr) kit, blood urea nitrogen (BUN) kit total protein (TP) kit and albumin (ALB) kit purchased from Nanjing Jiancheng Bioengineering Institute (Jiangsu, China), Superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), catalase (CAT), glutathione reductase (GSH) and malonaldehyde (MDA) purchased from Nanjing Jiancheng Bioengineering Institute (Jiangsu, China). All the other reagents used were analytically pure and bought from Shanghai Zhenqi Chemical Reagent Co., Ltd. (Shanghai, China).

Fourier transfer infrared spectroscopy Nexus 670 (Thermo Nicolet Co., U.S.), NMR spectrometer Bruker Avance AV-500 (Bruker Co., Germany), Refrigerated centrifuge 4K15C (Sigma Co., U.S.), iMark Microplate absorbance reader Model 680 (Bio-Rad Laboratories Inc., U.S.).

2.4. Preparation of carboxymethylated Lachnum polysaccharides

Carboxymethylation was performed according to the method of Ohno, Kurachi, and Yadomae (1988). One gram LEP-1b was dissolved in 50 mL 2.0 mol/L NaOH solution, and then 5 g chloroacetic acid was added. The reaction was performed at 60 °C for 3 h. Then the pH was adjusted to 7.0 by glacial acetic acid after cooling down to room temperature. Before freeze drying, the obtained sample was dialyzed in dialysis bag (with molecular weight cutoff of 13,000 Da) with distilled water for 24 h.

$$\text{CLEP-1b yield}\,(\%) = \frac{m_1}{m_2} \times 100\% \quad (1)$$

m_1 was the mass of CLEP-1b and m_2 was the mass of LEP-1b.

2.5. Structure verification of the carboxymethylated derivative CLEP-1b of polysaccharide

2.5.1. Determination of the degree of substitution of carboxymethyl in CLEP-1b

The method by Zhou et al. (2010) was as follows. 0.05 g CLEP-1b was dissolved in 20 mL 0.1 mol/L HCl standard solution. Later, the solution stirred thoroughly. The solution was titrated with 0.1 mol/L NaOH standard solution and the volumes (V_1 and V_2) of NaOH standard solution consumed at the time of pH 2.1 and pH 4.3 were recorded. The degree of substitution (DS) was calculated as follows:

$$\text{DS} = \frac{0.203\ \ \text{A}}{1 - 0.058\ \ \text{A}} \quad (2)$$

$A = (V_2 - V_1) \times c/m$, DS was the degree of substitution; m was the mass (unit: g) of the sample and c was the concentration (unit: mol/L) of the NaOH standard solution.

2.5.2. Infrared spectrum of LEP-1b and CLEP-1b

LEP-1b and CLEP-1b (6 mg) were, respectively, dispersed in KBr (100 mg) pellets. IR scanning with Fourier transform infrared spectrometer was then conducted at 4000–400 cm^{-1}.

2.5.3. ^{13}C NMR spectrum of LEP-1b and CLEP-1b

LEP-1b and CLEP-1b 20.0 mg were, respectively, dissolved in 1 ml D_2O. Later, the solutions were analyzed by NMR spectrometer Bruker Avance AV 500 at 90 °C.

2.6. The effect of LEP-1b and CLEP-1b on the experimental CRF

2.6.1. Establishment, grouping and oral administration of mice models with the CRF

The method by Yokozawa, Zheng, Oura, and Koizumi (1986) was referred to. Adenine intragastric administration was adopted to build the models of mice with the CRF. After being adaptively fed for one week, 10 of the 70 healthy male Kunming mice were selected randomly as the normal group (control) and later intragastrically administered with normal saline at the rate of 10 mL/kg. All the other mice were intragastrically administered with adenine at the rate of 300 mg/kg d for 21 days (once per day). Then Scr and BUN contents in the mice were measured. Mice reached modeling requirements were randomly divided into 6 groups (10 mice per group), including: model group (normal saline: 10 mL/kg), positive group (Haikunshenxi capsule: 150 mg/kg), low-dosage LEP-1b group (LEP-1b: 100 mg/kg), high-dosage LEP-1b group (LEP-1b: 200 mg/kg), low-dosage CLEP-1b group (CLEP-1b: 100 mg/kg) and high-dosage CLEP-1b group (CLEP-1b: 200 mg/kg).

2.6.2. Determination of food consumption, weight and kindey index

Food consumption was measured everyday during the dosing period and average value was calculated. Mice were weighed after the last dosage and then killed by cervical dislocation. The kidneys were taken out, weighed and then the kidney index was calculated (Feng et al., 2010).

$$\text{Kidney index} = \frac{\text{mass of kidney}}{\text{mice weight}} \times 100\%$$

2.6.3. Histopathological examination of the kidneys

Kidney was fixed in AFF stationary liquid. Later, the embedded kidney was cut in O.C.T. embedding agent (optimum cutting temperature compound) into slices, which was then conducted HE (Haematoxylin and Eosin) dyeing, and finally observed and taken picture under an optical microscope.

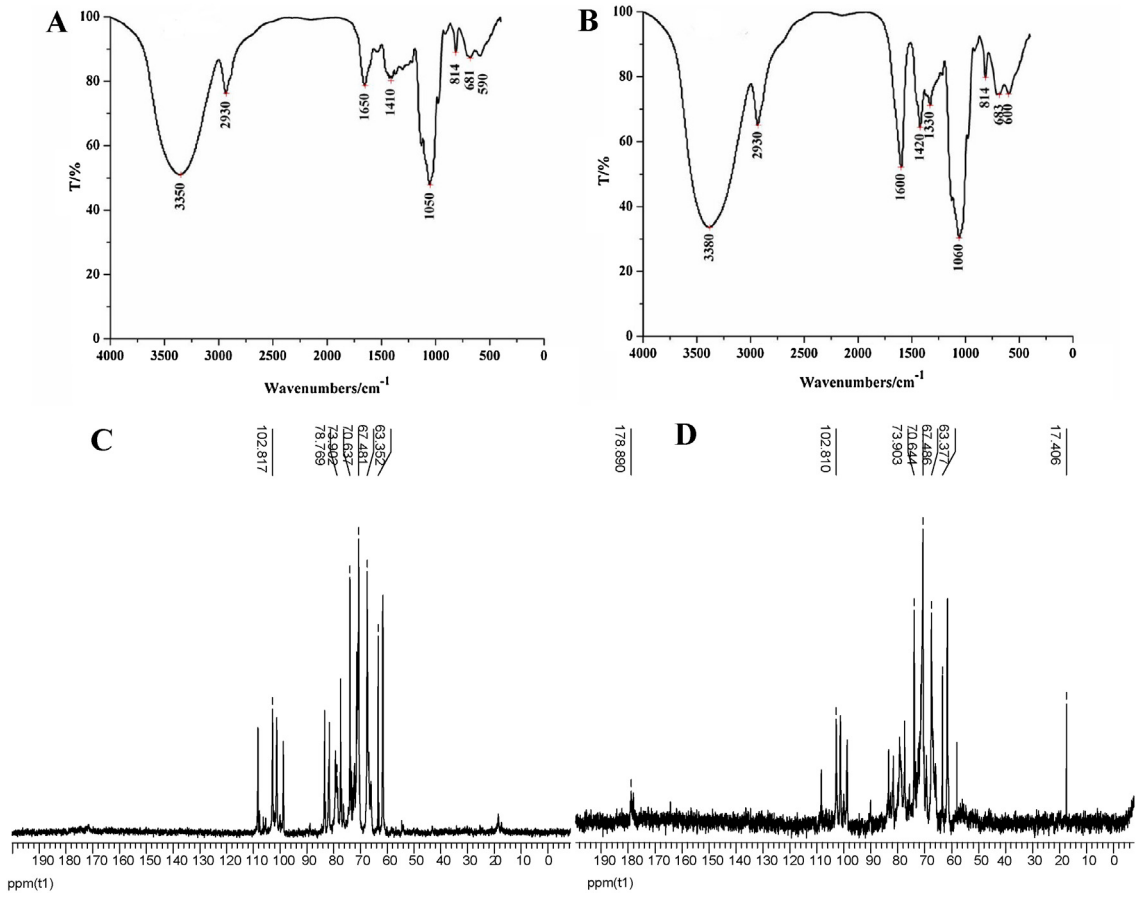

Fig. 1. IR Spectrum of LEP-1b (A) and CLEP-1b (B); 13C NMR spectrum of LEP-1b (C) and CLEP-1b (D).

2.6.4. Determination of blood biochemical indexes of mice with CRF

Before the cervical dislocation of the mice, blood was sampled from the mice eyes and kept at room temperature for half an hour, meanwhile, it coagulated naturally. Later, coagulated blood was centrifuged for 5 min at a speed of 5000 r/min. Then, the serum was taken out and kept at 4 °C for the determination of the blood biochemical indexes of the mice, including BUN, Scr, TP and ALB, which were, respectively, detected with diacetyl oxime method, picric acid colorimetric method, coomassie brilliant blue method and bromocresol green colorimetric method according to the kit instructions.

2.6.5. Determination of antioxidant enzymes' activity of the kidney and liver tissue

The kidney and liver tissue of mice were weighed precisely and homogenate medium (0.9% normal saline) at a rate of 1 g: 10 mL (weight: volume). The solution was homogenized mechanically in ice–water bath and centrifuged for 10 min at a speed of 3000 r/min. The supernatant (namely the 10% tissue homogenate) was obtained. Contents of SOD, GSH-PX, CAT, GSH and MDA in the homogenate solution were determined, respectively, with hydroxylamine method, colorimetric method, visible spectrometry, and colorimetric method and TBA method according to the kit instructions.

2.7. Statistical analysis

All data were presented as mean ± standard deviation after being statistically processed. Software SPSS 13.0 was used for *t*-test analysis of the intergroup deviation. $p < 0.05$ means significant difference while $p < 0.01$ means very significant difference.

3. Results and discussions

3.1. Acquisition and structure verification of CLEP-1b

It is clear from Formulas (1) and (2) that the yield of CLEP-1b was 78.4% while DS was 0.286.

It can be seen from Fig. 1A that in the infrared spectrum of LEP-1b, the absorption peak at 3350 cm^{-1}, 2930 cm^{-1}, 1650 cm^{-1} and 1050 cm^{-1} were resulted by O—H stretching vibration, C—H stretching vibration, water bending vibration and C—O—C asymmetry stretching vibration (Chen et al., 2012, 2013). Thus, it is clear that LEP-1b shows absorption peaks with typical (1→3)-,(1→6)-β-D-glucan characteristics. This result is similar to the result of the previous research by Qiu et al. (2013). Furthermore, in Fig. 1B, the absorption peak of CLEP-1b at 1650 cm^{-1} disappeared and new absorption peaks appeared at 1600 cm^{-1}, 1420 cm^{-1} and 1330 cm^{-1}, resulted by C=O asymmetry stretching vibration, C—O stretching vibration and methylene vibration in the carbonyl, respectively (Manrique & Lajolo, 2002; Zhang, Wang, Yang, Yang, & Wang, 2011). Thus, the carboxymethylated modification of LEP-1b was successful.

As shown in Fig. 1C, the anomeric carbon signal with chemical shift at 102.82 ppm in the ^{13}C NMR spectrum of LEP-1b was generated by C-1 of →3)-β-D-Glcp-(1→, which indicated that the sugar ring configuration was pyran type (Sun, Liu, Yang, & Kennedy, 2010); chemical shifts of C-2, C-3, C-4, C-5 and C-6 were 70.64 ppm, 78.77 ppm, 67.48 ppm, 73.90 ppm and 63.35 ppm, respectively. In

Table 1
Effects of LEP-1b and CLEP-1b on food intake, weight and kidney index of mice with CRF.

Groups	Food consumption (g/mouse/d)	Weight (g)	Renal index (%)
Normal group	3.65 ± 0.34	38.93 ± 1.78[b]	0.6884 ± 0.012[b]
Model group	2.97 ± 0.16	33.25 ± 1.87	1.7353 ± 0.028
Positive group	3.12 ± 0.21	38.44 ± 1.29[b]	0.6374 ± 0.035[b]
Low-dosage LEP-1b group	3.23 ± 0.24	34.39 ± 1.22[b]	1.0584 ± 0.025[b]
High-dosage LEP-1b group	3.36 ± 0.19	36.20 ± 1.71[b]	0.8536 ± 0.017[b]
Low-dosage CLEP-1b group	3.29 ± 0.27	37.91 ± 1.38[b]	0.8626 ± 0.041[b]
High-dosage CLEP-1b group	3.43 ± 0.25[a]	38.64 ± 1.56[b]	0.7593 ± 0.049[b]

[a] $p < 0.05$, significantly different from model control group.
[b] $p < 0.01$, highly significantly different from model control group.

Fig. 1D, two new absorption peaks appeared in the ^{13}C NMR spectrum of CLEP-1b. The signals peak at 178.89 ppm was assigned to anomeric carbons of C=O, and the signals peak at 17.42 ppm was caused by methylene, which indicated the $-CH_2COOH$ was present in CLEP-1b (Lawal, Lechner, & Kulicke, 2008; Saghir, Iqbal, Hussain, Koschella, & Heinze, 2008). C-3 signal peak at 78.77 ppm weakened clearly, indicating that the $-CH_2COOH$ was chemically linked to an oxygen (O) atom of hydroxyl on C-3 of LEP-1b. This result was in agreement with reports by Magnani and Calliari (2009). The chemical shifts of C-1, C-2, C-4, C-5 and C-6 in the ^{13}C NMR spectrum of CLEP-1b were not changed at all when compared to LEP-1b.

Above IR and ^{13}C NMR spectrum proved that the carboxymethyl group ($-CH_2COOH$) was chemically linked to an oxygen (O) atom of the hydroxyl on C-3 of LEP-1b, which indicated successful carboxymethylation of LEP-1b.

3.2. The effect of LEP-1b and CLEP-1b on the experimental CRF

3.2.1. Influence of LEP-1b and CLEP-1b to food consumption, weight and kindey index of mice with CRF

Compared with the model group, food consumption of mice in LEP-1b group and CLEP-1b group increased in different degrees (Table 1). And, food consumption of mice in the high-dosage CLEP-1b group increased by 9.93% when compared to the model group, marking significant difference ($p < 0.05$). Weights of mice in LEP-1b and CLEP-1b groups were significantly higher than those in the model group (Table 1, $p < 0.01$). In which, the weight of the high-dosage CLEP-1b group was close to that of normal mice. Kindey index was critical to evaluate the health state of mice kidney. Kindey index of normal mice was generally less than 1%. However, after renal failure was induced by adenine, the kidney enlargement resulted in the increase of kindey index (Feng et al., 2010). Compared with model group, kindey index of mice in the low-dosage and high-dosage LEP-1b groups decreased significantly ($p < 0.01$) and showed dosage-effect relationship, which was in coincidence with the report by Yin et al. (2007). The kindey index of mice in CLEP-1b group was lower than that of mice in LEP-1b group when being served at the same dosage.

3.3. Pathological observation of mice kidney tissue

In the normal group, the mice had compactly arranged glomerulus and kidney tubules, without adenine metabolic sediments (Fig. 2A). In the model group, the mice had a great deal of bubbles in the kidney tissue, without any normal glomerulus or kidney tubule. In the low-dosage and high-dosage LEP-1b groups, the mice had little complete glomerulus and kidney tubules in the kidney tissue. The result was similar with that reported by Hou et al. (2009). In the low-dosage and high-dosage CLEP-1b groups, the mice had more complete glomerulus and kidney tubules than the mice in the corresponding LEP-1b group. What's more, the degree of swelling in the kidney tubules and nephrocoel expansion of mice in the high-dosage CLEP-1b group was less and the infiltration degree of interstitial inflammatory cell was lighter than those in other groups, with no obvious adenine metabolite. Thus, it was clear that LEP-1b could improve the renal pathological tissue state and CLEP-1b had more obvious effects.

3.4. The effect of LEP-1b and CLEP-1b on the blood biochemical index of mice

Scr and BUN level reflect the function of glomerular filtration and increases as the function of glomerular filtration declines, which is more frequent in renal failure, renal damage or nephritis, etc. (He et al., 2009; Wang et al., 2010). Compared with the model group, Scr and BUN contents of low-dosage and high-dosage LEP-1b

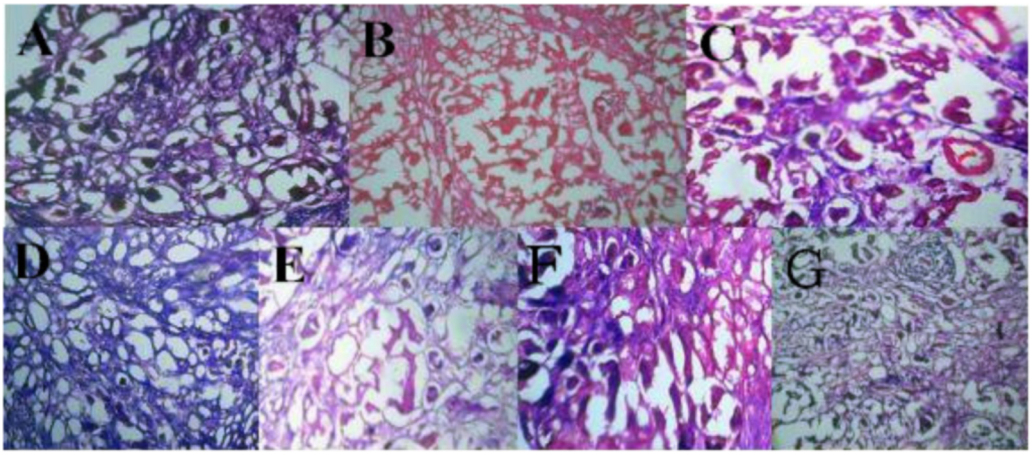

Fig. 2. Renal histopathological examination of mice (magnification 10×). (A) Normal group; (B) model group; (C) positive group; (D) low-dosage LEP-1b group; (E): high-dosage LEP-1b group; (F) low-dosage CLEP-1b group; (G) high-dosage CLEP-1b group.

Table 2
Effects of LEP-1b and CLEP-1b on Scr, BUN, TP and ALB contents of mice with CRF.

Groups	Scr (μM/L)	BUN (mM/L)	TP (g/L)	ALB (g/L)
Normal group	58.72 ± 2.25[b]	7.63 ± 0.76[b]	110.51 ± 2.37[b]	32.65 ± 0.78[b]
Model group	157.55 ± 20.87	23.65 ± 1.28	95.82 ± 1.84	26.20 ± 0.43
Positive group	70.21 ± 4.69[b]	9.34 ± 0.32[b]	106.03 ± 1.67[b]	30.61 ± 0.61[b]
Low-dosage LEP-1b group	138.34 ± 19.82[a]	21.37 ± 1.72[a]	98.48 ± 2.01[a]	27.41 ± 0.38[b]
High-dosage LEP-1b group	111.63 ± 17.82[b]	16.86 ± 1.72[b]	104.71 ± 1.93[b]	29.56 ± 0.54[b]
Low-dosage CLEP-1b group	131.47 ± 21.82[a]	14.51 ± 1.49[b]	100.24 ± 1.82[b]	28.40 ± 0.93[b]
High-dosage CLEP-1b group	104.41 ± 12.51[b]	10.98 ± 0.63[b]	109.04 ± 3.01[b]	31.55 ± 0.29[b]

[a] $p < 0.05$, significantly different from model control group.
[b] $p < 0.01$, highly significantly different from model control group.

Table 3
Effects of LEP-1b and CLEP-1b on SOD, GSH-PX, CAT, GSH and MDA contents in kidney tissue homogenate of mice with CRF.

Groups	SOD (U/mgprot)	GSH-PX	CAT (U/mL)	GSH (mg/gprot)	MDA (nmol/mL)
Normal group	43.51 ± 0.97[b]	863.89 ± 23.67[b]	81.38 ± 1.39[b]	4.94 ± 0.09[b]	2.66 ± 0.21[b]
Model group	27.56 ± 2.48	696.15 ± 21.78	53.75 ± 2.76	2.85 ± 0.11	4.34 ± 0.33
Positive group	42.47 ± 2.72[b]	833.01 ± 28.72[b]	76.07 ± 3.03[b]	4.01 ± 0.07[b]	2.87 ± 0.23[b]
Low-dosage LEP-1b group	30.09 ± 2.73[a]	712.63 ± 29.45	60.35 ± 2.49[b]	2.97 ± 0.03[b]	3.74 ± 0.13[a]
High-dosage LEP-1b group	37.55 ± 01.93[b]	755.36 ± 35.72[b]	63.24 ± 2.87[b]	3.23 ± 0.05[b]	3.54 ± 0.25[b]
Low-dosage CLEP-1b group	32.99 ± 2.98[b]	791.82 ± 16.31[b]	69.21 ± 2.59[b]	3.85 ± 0.01[b]	3.17 ± 0.17[b]
High-dosage CLEP-1b group	41.18 ± 1.23[b]	852.87 ± 31.45[b]	79.53 ± 4.02[b]	4.19 ± 0.05[b]	2.97 ± 0.27[b]

[a] $p < 0.05$, significantly different from model control group.
[b] $p < 0.01$, highly significantly different from model control group.

groups decreased to different degrees ($p < 0.05$ or $p < 0.01$, Table 2), which was in coincidence with that reported by Wang et al. (2010). Scr and BUN contents of mice in CLEP-1b group were lower than those in LEP-1b group at same dosage.

CRF always results in the decrease of TP and ALB level (Li, Jiao, Chen, & Liu, 2005). Compared with model group, TP and ALB contents of mice in the low-dosage and high-dosage LEP-1b groups increased at different degrees ($p < 0.01$). TP and ALB contents of mice in CLEP-1b group were higher than those in LEP-1b group with same dosage. And the TP and ALB contents of mice in the high-dosage CLEP-1b group were close to those of normal mice.

3.5. The effect of LEP-1b and CLEP-1b on the antioxidant enzymes' activity of mice kidney and liver tissue homogenate

Research have been proved that renal diseases are closely associated with the oxidative stress reaction and the production of peroxides and toxic superoxide anion radicals (Pawlak, Mysliwiec, & Pawlak, 2008; Wang et al., 2010, 2011). Alteration the function of antioxidant enzymatic and non-enzymatic system was observed in the mice with CRF. An increasing Scr demonstrates the diminished ability of kidney to remove creatinine from the blood and concentrate it in the urine. Diseased or damaged kidneys cause an elevated SUN because the kidneys are less able to clear urea from the bloodstream (He et al., 2009; Wang et al., 2011). A fall in the degree of SUN and Scr in mice receiving CLEP-1b further substantiates its beneficial effect. The negative correlation was found in our study SUN, Scr and antioxidant enzymes of liver and kidney homogenate of mice treated with adenine, which demonstrated that free radical production activation is highly influenced by the damage of kidney. SOD, GSH-PX, CAT and GSH can scavenge the free radicals, control the lipid peroxidation of free radicals, reduce the lipid peroxidation level and relieve the CRF process of mice (Cong, Cui, Zang, & Hao, 2013). MDA contents may reflect the severity of attack organism from free radicals indirectly. It has been found by many scholars that antioxidant enzymes' activity of patients with CRF decreased and MDA contents of those increased (Ozden, Maral, Akaydın, Cetınalp, & Kalender, 2002; Zwolińska, Grzeszezak, Szczepańska, Kilis-Pstrusińska, SZPrynger, 2006).

In the kidney homogenate of mice, compared with model group, mice in the low-dosage and high-dosage LEP-1b groups showed that the contents of SOD, GSH-PX, CAT and GSH obviously increased ($p < 0.05$ or $p < 0.01$, Table 3), but the MDA contents decreased significantly ($p < 0.01$). SOD, GSH-PX, CAT and GSH contents of mice in CLEP-1b group were obviously higher and MDA contents were lower than that of mice in LEP-1b group at same dosage, which indicated that LEP-1b could significantly improve the contents of SOD, GSH-PX, CAT and GSH and reduce MDA contents in the kidney tissue homogenate of mice, and CLEP-1b showed better effects.

Liver is an important oxidation defense organ and contains a great deal of anti-oxidant enzymes (Ding et al., 2011). Researches have been proved that inflammatory factors and metabolic disorders may impair the liver function and result in changes of related enzyme activity in the liver (Wang et al., 2011). In the liver tissue homogenate of mice, SOD contents of polysaccharides groups (at different dosages) were lower than that of model group without significant difference ($p > 0.05$, Table 4), and this result was same with that reported by Wang et al. (2011). Compared with the model

Table 4
Effects of LEP-1b and CLEP-1b on SOD, GSH-PX, CAT, GSH and MDA contents in liver tissue homogenate of mice with CRF.

Groups	SOD (U/mgprot)	GSH-PX	CAT (U/mL)	GSH (mg/gprot)	MDA (nmol/mL)
Normal group	167.81 ± 4.28	263.16 ± 5.93[b]	35.67 ± 2.67[b]	3.89 ± 0.11[b]	7.51 ± 0.23[b]
Model group	168.49 ± 4.03	160.43 ± 2.41	15.74 ± 1.35	1.64 ± 0.12	16.02 ± 0.45
Positive group	155.61 ± 7.31[b]	248.76 ± 7.69[b]	34.03 ± 2.73[b]	6.77 ± 0.16[b]	13.53 ± 0.27[b]
Low-dosage LEP-1b group	165.86 ± 4.57	194.44 ± 3.78	22.44 ± 1.98[b]	2.11 ± 0.18[b]	10.92 ± 0.31[b]
High-dosage LEP-1b group	167.98 ± 6.29	256.25 ± 9.27[b]	32.72 ± 2.04[b]	3.38 ± 0.27[b]	8.14 ± 0.34[b]
Low-dosage CLEP-1b group	164.19 ± 5.29	273.65 ± 8.34[b]	28.26 ± 2.21[b]	2.73 ± 0.34[b]	9.97 ± 0.19[b]
High-dosage CLEP-1b group	166.44 ± 3.65	282.96 ± 11.98[b]	37.67 ± 3.01[b]	5.85 ± 0.23[b]	7.93 ± 0.17[b]

[a] $p < 0.05$, significantly different from model control group. [b] $p < 0.01$, highly significantly different from model control group.

group, the low-dosage and high-dosage LEP-1b had increased the GSH-PX, CAT and GSH contents of mice, with the dosage-effect relationship (Table 4), but decreased the MDA contents with highly significant difference ($p < 0.01$). The results were similar to the reports by Ozden et al. (2002). Contents of GSH-PX, GSH and CAT of mice in CLEP-1b group were higher than those in LEP-1b group at same dosage, and MDA contents lower than those correspondingly.

All the above results proved that LEP-1b and CLEP-1b could mitigate the renal failure by regulating the SOD, GSH-PX, CAT, GSH and MDA contents in the kidney tissue homogenates of mice. They could also remit the impairment of liver tissue by regulating the GSH-PX, CAT, GSH and MDA contents in the liver tissue homogenate of mice and reinforce the immune capacity of liver and then consequently protect the kidney of mice with renal failure. So, the effects of mitigating the CRF of LEP-1b and CLEP-1b were closely associated with their antioxidant activities.

Compared with LEP-1b, CLEP-1b showed significant antioxidant activity probably related to the introduction of carboxymethyl groups which changed the structure of LEP-1b and decreased the intermolecular/intramolecular hydrogen bond (Xu et al., 2009). Moreover, CLEP-1b may enhance the ability of scavenging free radicals and improving the antioxidant enzyme activity (Yang et al., 2011). Therefore, the related mechanisms remain to be studied further.

4. Conclusions

CLEP-1b was obtained by carboxymethylation modification of LEP-1b, with the DS of carboxymethyl reaching 0.286. The infrared result showed successful carboxymethylation of polysaccharide and ^{13}C NMR indicated that $-CH_2COOH$ was connected with O of the hydroxyl at C-3 in LEP-1b. LEP-1b at both low and high dosages could improve the renal pathological tissue status, enhance the contents of Scr, BUN, TP and ALB significantly, strengthen the activity of antioxidant enzymes and decrease the lipid peroxidation level in the kidney and liver tissue homogenate of mice. Compared with LEP-1b, CLEP-1b showed better effects at the same dosage. Thus, it was clear that LEP-1b and CLEP-1b could mitigate the CRF of mice, which was closely associated with the antioxidant activity of the kidney and liver. Furthermore, LEP-1b and CLEP-1b could be used as new type of drugs or healthcare products mitigating the CRF in the future.

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