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Optimization of selenylation conditions for lycium barbarum polysaccharide based on antioxidant activity

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ABSTRACT

Lycium barbarum polysaccharide (LBP) was modified by HNO_3–Na_2SeO_3 method according to $L_9(3^4)$ orthogonal design to obtain nine selenizing LBPs (sLBPs), $sLBP_1$–$sLBP_9$. Their antioxidant activities in vitro were compared by free radical-scavenging test. $sLBP_6$, $sLBP_8$ and $sLBP_9$ presented stronger activity. In vivo test, 14-day-old chickens were injected respectively with $sLBP_6$, $sLBP_8$ and $sLBP_9$ taking LBP as control, and serum GSH-Px and SOD activities and MDA content were determined. The results showed that three sLBPs could significantly enhance GSH-Px and SOD activities and decrease MDA content. The actions of sLBPs were significantly stronger than that of unmodified LBP. These results indicated that selenylation modification could significantly enhance the antioxidant activities of LBP, $sLBP_6$ possessed the best efficacy and could be exploited into an antioxidant. The optimal modification conditions were 400 mg of sodium selenite for 500 mg of LBP, reaction temperature of 70 °C and reaction time of 6 h.

1. Introduction

In the oxidative metabolism process, organism can continuously produce various kinds of reactive oxygen species (ROS), including hydroxyl radical (·OH), superoxide anion ($O_2^{-}\cdot$), hydrogen peroxide (H_2O_2) and so on (Gotz, Kunig, Riederer, & Youdim, 1994; Afonso, Champy, Mitrovic, Collin, & Lomri, 2007). Normally, the oxidation and antioxidation of body maintain dynamic equilibrium. Under the balanced state, these free radicals play an important role in anti-bacterial, anti-inflammatory and inhibiting tumor. Once this balance is broken, e.g. when the body is attacked by disease or certain exogenous drugs and poisons, ROS will increase and cause powerful injury, such as lipid peroxidation of biofilm, destruction of enzymes, amino acids and protein, impact on the structure and function of internal organs and immune system, thus lead to illness even death (Halliwell & Gutteridge, 1999; Sies, Stahl, & Sevanian, 2005). The present study has found that the development of many diseases are associated with free radicals. Therefore, increasing attentions have been paid to researches of improving defense mechanism and antioxidative damage drugs (Marx, 1987).

Many studies showed that a lot of polysaccharide compounds isolated from the natural product can scavenge free radicals, inhibit lipid peroxidation and linoleic acid oxidation (Yu, Zhu, Dai, Wang, & Mao, 2008). For example, lycium barbarum polysaccharide (LBP) is the main active ingredient of lycum barbarum, and possesses wide pharmacological effects, such as, antioxidation, anti-aging, anti-tumor, immunoregulation, hepatoprotection and so on (Li, Wu, Yu, Zhu, & Song, 2013). In human and animal, selenium is an essential trace element and participates in many important physiological activities. It cannot only enhance the animals' antioxidant levels via glutathione peroxidase (GSH-Px), but also can improve immune function by maintaining the integrity of membrane structure (Fu, Liu, Wu, Li, & Cao, 2013). Therefore, the combination of selenium with polysaccharide into organic selenium compounds, selenizing polysaccharides, which will possess duple and higher biological activity in comparison with selenium and polysaccharide. It is easily absorbed and utilized by the organism as well (Staaf, Yang, Huttunen, & Widmalm, 2000). Selenium polysaccharide has been used more and more widely in antioxidation, immunomodulation, antitumor, anti-aging and so on (Li, Zhao, Liu, Song, & Tie, 2010). Our

Abbreviations: LBP, lycium barbarum polysaccharide; sLBP, selenizing lycium barbarum polysaccharide; HNO_3, nitric acid; Na_2SeO_3, sodium selenite; BC, blank control; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; MDA, malondialdehyde; ROS, reactive oxygen species.

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previous study also confirmed that selenylation modification could enhance immune-enhancing and antioxidant activities of Chinese angelica polysaccharide (Qin, 2013).

In this study, LBP was modified by HNO_3–Na_2SeO_3 method (Yang, Huang, Jiang, Zhu, & Han, 2009) under nine kinds of modification conditions according to $L_9(3^4)$ orthogonal design of three factors, usage amount of sodium selenite, the reaction temperature and reaction time, and three levels (Li, Miu, & Liu, 2001). Nine selenizing LBPs (sLBPs), $sLBP_1$–$sLBP_9$ were obtained. First by experiment in vitro, the hydroxy and superoxide anion scavenging ability of nine sLBPSs were compared. Three sLBPs with better action were picked out. Then by test in vivo, the effects of three sLBPs on serum GSH-Px and SOD activities and MDA contents were determined. The aim of this study is to explore the probability of selenylation modification to improve the antioxidant activity of LBP, selected out the best sLBP and modification condition, and offer theoretical evidence for the development of polysaccharide antioxidants.

2. Materials and methods

2.1. Drug and reagents

Lycium barbarum was purchased from Yongfuyuan Trade Ltd. and yielded form Ningxia Hui Autonomous Region of China.

Sodium selenite bought from Shanghai Lingfeng Chemical Reagent Ltd. was dissolved into 0.05 g mL^{-1} with ultrapure water. Standardry selenium stored solution at 100 μg mL^{-1} supplied by National standard substance research center was accurately diluted into 1 μg mL^{-1} of standardry selenium solution. Nitric acid (HNO_3) was the product of Shanghai Lingfeng Chemical Reagent Ltd. Pyrogallol, Sinopharm Chemical Reagent Co., Ltd. Phenanthroline, ferrous sulfate, hydrochloric acid, hydrogen peroxide and other reagents were of analytical grade.

Reagent kits of GSH-Px, SOD and MDA were bought from Nanjing Jiancheng Bioengineering Institute.

2.2. Preparation of sLBP

2.2.1. Extraction and purification of LBP

Lycium barbarum was soaked in water for 3 h, extracted three times in 90 °C water bath, each time 1.5 h. The filtrate was concentrated and centrifugated at 3000 rpm for 30 min. The supernatant was slowly added with 95% alcohol till the ethanol content up to 80%. After standing overnight the precipitate was collected, washed twice with acetone, dried to obtain crude LBP (Ramesh & Tharanathan, 1999) and protein-eliminating purified by trichloroacetic acid method (Li & Wang, 2004) to obtain purified LBP. Its carbohydrate content was 87.1% measured by phenol-sulfuric acid method (Li & Wang, 2008; Yu, Yang, Liu, & Liu, 2009).

2.2.2. Selenylation modification of LBP

Nine groups of modification conditions were designed according to $L_9(3^4)$ orthogonal test taking three factors respectively at three levels as indexes, the usage amount of sodium selenite at 200, 300 and 400 mg for 500 mg of LBP (A), the reaction temperature at 50, 70 and 90 °C (B), and the reaction time for 6, 8 and 10 h (C) (Table 1). After the reaction was finished, the mixture was cooled into room temperature, pH was adjusted into 5–6 with saturated sodium carbonate solution, and dialyzed in dialysis sack with 1 kDa ultrafiltration membrane against tap water and till no free sodium selenium was detected by ascorbic acid method (Li, Miu, Liu, 2001). The polysaccharide solution was concentrated and freeze-dried. Nine selenizing LBPs respectively named $sLBP_1$–$sLBP_9$ were obtained. Their selenium concents were determined by atomic fluorescence spectrometry (Gao, Qin, & Huang, 2006) with atomic fluorescence spectrometer (Model AFS-930, Beijing Jitian instrument Co., Ltd.), and the carbohydrate contents were determined by the phenol-sulfuric acid method.

2.3. Infrared spectroscopy analysis

After dried for 2 h in oven, sLBP or LBP of 1 mg was put into an agate mortar, added with dried KBr 100–200 mg, finely ground, pressed into thin slices, and the infrared spectroscopy in wavenumber range of 4000–400 cm^{-1} was recorded by FT-IR920 Fourier transform infrared spectrometer (Tianjin Tuopu Instrument Co., Ltd.).

Finally nine sLBPs and LBP were dissolved into 1 mg mL^{-1} with distilled water for the test in vitro. Based on the experimental results in vitro, the diluted preparations of $sLBP_6$, $sLBP_8$, $sLBP_9$ and LBP were sterilized by pasteurization and detected for endotox in by pyrogen tests. When the endotoxin amount was up to the standard of Chinese Veterinary Pharmacopoeia (less than 0.5 EU mL^{-1}) (Veterinary Pharmacopoeia Commission of the People's Republic of China, 2010), they were stored at 4 °C for the test in vivo (Kong, Hu, Rui, Wang, & Li, 2004).

2.4. Comparision of antioxidative activities in vitro

2.4.1. Hydroxyl radical-scavenging ability

Forty eight test tubes were assigned into damage group, non-damage group and 10 polysaccharide groups (nine sLBPs and LBP), four tubes per group. First, all test tubes were added with 5 mmol L^{-1} of phenanthroline 0.5 mL and pH7.4 phosphate buffer 2.0 mL, shaken well, again added with 7.5 mmol L^{-1} of $FeSO_4$ 1.0 mL, shaken well immediately. Second, the tubes of 10 polysaccharide groups were added respectively with 1 mL of nine sLBPs and LBP and 0.2% H_2O_2 solution 1.0 mL, the tubes of damage group were added with 0.2% H_2O_2 solution 1.0 mL. Finally, all tubes were added with distilled water up to 10 mL, reacted for 1 h in 37 °C water bath, and the absorbances at a wave length of 536 nm (A_{536}) were measured by 754 UV spectrophotometer

Table 1
Modification conditions, yields and contents of selenium and carbohydrate of sLBPs.

sLBP	*A* Na_2SeO_3 (mg)	*B* temperature (°C)	*C* time (h)	Yeild (%)	Se content (mg g^{-1})	Carbohydrate content (%)
$sLBP_1$	200	50	6	40.95	7.65	35.4
$sLBP_2$	300	50	8	34.24	8.84	34.9
$sLBP_3$	400	50	10	30.56	10.44	33.2
$sLBP_4$	200	70	8	29.94	8.52	34.3
$sLBP_5$	300	70	10	24.75	11.76	30.4
$sLBP_6$	400	70	6	35.84	13.66	47.5
$sLBP_7$	200	90	10	21.70	8.73	19.2
$sLBP_8$	300	90	6	30.42	9.47	44.6
$sLBP_9$	400	90	8	32.76	11.85	42.4

(Shanghai Precision & Scientific Instrument Co., Ltd.). The hydroxyl radical-scavenging rate was calculated according to the equation (Olabinri et al., 2010): Hydroxyl radical-scavenging rate = $(\bar{A}_{\text{polysaccharid group}} - \bar{A}_{\text{damaged group}})/(\bar{A}_{\text{non-damaged group}} - \bar{A}_{\text{damage group}}) \times 100\%$ ($\bar{A}$ was an average value of four tubes).

2.4.2. Superoxide anion-scavenging ability

Superoxide anion-scavenging ability was measured by pyrogallol autoxidation method (Marklund & Marklund, 1974). 44 test tubes were assigned into 10 polysaccharide groups (nine sLBPs and LBP) and blank control (BC) group, four tubes per group. First, all tubes were added with 2.4 mL of pH 8.2 Tris–HCl buffer. Second, the tubes of 10 polysaccharide groups were added with respectively with 1 mL of 9 sLBPs and LBP 1 mL, the tubes of blank control group, with equal volume of distilled water, mixed well. Finally, all tubes weres added with 7 mmol L^{-1} pyrogallol 0.3 mL begining reaction, and at the fourth minute of reaction was added with one drop of 10 mol L^{-1} concentrated hydrochloric acid to stop the reaction, and the absorbances at a wave length of 325 nm (A_{325}) were measured by 754 UV spectrophotometer. The superoxide anion-scavenging rate was calculated according to the equation (Marklund & Marklund, 1974): superoxide anion-scavenging rate = $(\bar{A}_{\text{B group}} - \bar{A}_{\text{polysaccharide group}})/\bar{A}_{\text{BC group}} \times 100\%$ ($\bar{A}$ was an average value of four tubes).

2.5. Assay of antioxidative activity in vivo

2.5.1. Animals and experimental design

One-day-old White Roman chickens (male) were purchased from Tangquan Poultry Farm, routine vaccination of ND vaccine (La Sota strain, No. 119082, purchased from Nanjing Tianbang Bioindustry Co., Ltd.). At 14-day-old 150 chickens were selected and randomly assigned into 5 groups. The chickens in four polysaccharide groups were intramuscularly injected respectively with 0.5 mL of $sLBP_6$, $sLBP_8$, $sLBP_9$ and LBP, in blank control (BC) group, with equal volume of physiological saline, once a day for three successive days.

2.5.2. Assay of serumal GSH-Px and SOD activities and MDA content

On days 7 (D_7), 14 (D_{14}), 21 (D_{21}) and 28 (D_{28}) after the first injection, the blood samples of six chickens randomly from each group were collected for the determination of GSH-Px activity, SOD activity and MDA content in serum.

2.6. Statistical analysis

Data were expressed as means ± SE and the Duncan's multiple range test was used to analyze the difference among polysaccharides and control groups. Significant differences between means were considered at $P < 0.05$.

3. Results

3.1. The modification conditions, yields and contents of selenium and carbohydrate of sLBPs

The modification conditions, yields and contents of selenium and carbohydrate of sLBPs are listed in Table 1. The yield of $sLBP_1$ was the highest, up to 40.95%, and next were $sLBP_6$, $sLBP_2$ and $sLBP_9$. The selenium content of $sLBP_6$ was the highest (13.66 mg g^{-1}), and next were $sLBP_9$, $sLBP_5$ and $sLBP_3$. The carbohydrate content of $sLBP_6$ was the highest (47.5%), and next were $sLBP_8$, $sLBP_9$ and $sLBP_1$.

3.2. The infrared spectroscopy characteristic of sLBP

The FT-IR spectra of LBP and sLBP in the ranges of 4000–400 cm^{-1} are illustrated in Fig. 1(A and B). In the spectra of LBP and sLBP, there were two bands, one appeared in the region of 3600–3200 cm^{-1} corresponding to the hydroxyl stretching vibration and another in the region of 1400–1000 cm^{-1} corresponding to C–O–C stretching vibration. This indicated that the LBP and sLBP were polysacaccharides. In comparision with the spectrum of LBP, the spectrum of sLBP presented two characteristic absorption bands, one appeared at 1026.71 cm^{-1} describing an symmetrical O–Se–O stretching vibration and another at 764.64 cm^{-1} describing an Se=O stretching vibration (Fig. 1B), which indicated that sLBP was successfully modified in selenylation.

3.3. The changes of hydroxyl radical-scavenging rate

The hydroxyl radical-scavenging rates in each group are illustrated in Fig. 2. The hydroxyl radical-scavenging rate in all sLBPs groups were significantly or numerically higher than that in LBP group, in $sLBP_6$ group was the highest (62.01%), the next were in $sLBP_9$ (57.30%), $sLBP_8$ (53.03%), $sLBP_3$ (50.27%), $sLBP_5$ (49.29%), $sLBP_7$ (49.20%), $sLBP_2$ (48.91%) and $sLBP_4$ (47.50%) groups, and in these eight groups were significantly higher than that in LBP (46.29%) group ($P < 0.05$).

3.4. The changes of superoxide anion-scavenging rate

The superoxide anion-scavenging rates in each group are illustrated in Fig. 3. The superoxide anion-scavenging rate in all sLBPs groups were significantly or numerically higher than that of LBP group, in $sLBP_9$ group was the highest (59.78%), the next were in $sLBP_6$ (54.55%), $sLBP_8$ (52.34%), $sLBP_5$ (48.30%), $sLBP_3$ (45.76%), $sLBP_7$ (45.53%) and $sLBP_4$ (41.17%) group, and in these seven groups were significantly higher than that in LBP (38.40%) group ($P < 0.05$).

3.5. The changes of serum GSH-Px activity

The serum GSH-Px activities in each group are listed in Table 2. At all time points after injection, the serum GSH-Px activities in three sLBPs groups were significantly higher than those in BC group

Table 2
The serum GSH-Px activities in each group.

Group	D_7	D_{14}	D_{21}	D_{28}
$sLBP_6$	1875.04 ± 37.25^{b}	1974.21 ± 34.99^{ab}	2149.42 ± 16.53^{a}	2086.62 ± 31.88^{a}
$sLBP_8$	1818.84 ± 41.29^{b}	1891.57 ± 54.42^{bc}	1964.30 ± 29.38^{b}	1855.21 ± 34.51^{c}
$sLBP_9$	1994.04 ± 38.12^{a}	2037.02 ± 31.88^{a}	2063.47 ± 46.28^{ab}	1957.69 ± 34.83^{b}
LBP	1703.14 ± 14.41^{c}	1841.98 ± 23.14^{c}	1792.40 ± 49.37^{c}	1670.08 ± 17.18^{d}
BC	1564.30 ± 23.84^{d}	1656.86 ± 17.49^{d}	1686.61 ± 38.12^{c}	1655.86 ± 23.84^{d}

Column data without the same superscripts (a–d) differ significantly ($P < 0.05$).

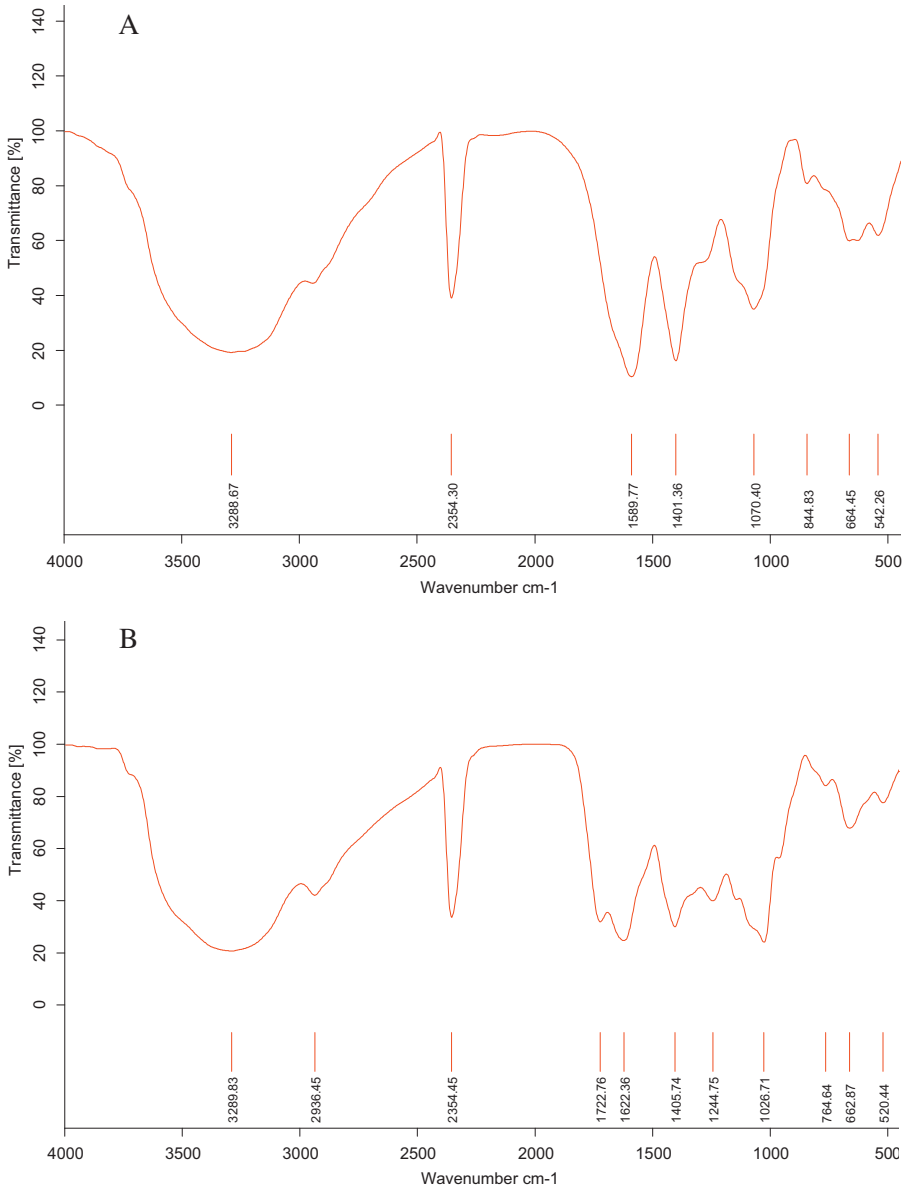

Fig. 1. Infrared spectra of LBP and selenylated LBP (A) LBP; (B) sLBP.

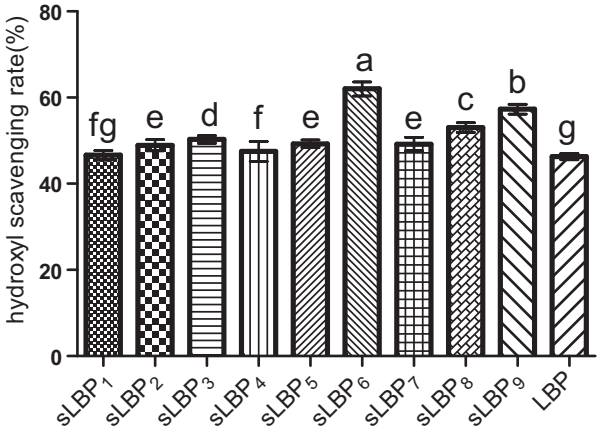

Fig. 2. The hydroxyl radical-scavenging rate in each group. Bars without the same superscripts ((a)–(g)) differ significantly ($P < 0.05$).

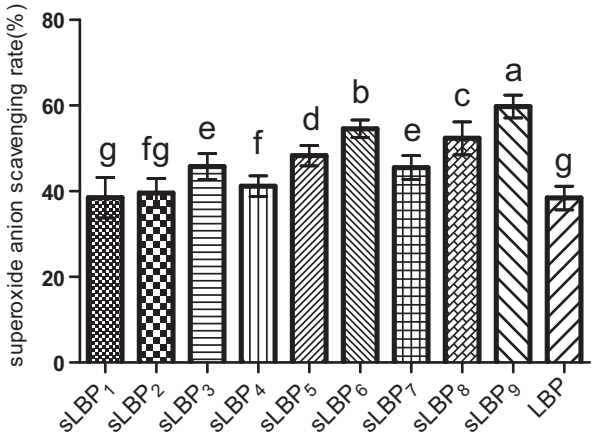

Fig. 3. The superoxide anion-scavenging rate in each group. Bars without the same superscripts ((a)–(g)) differ significantly ($P < 0.05$).

Table 3
The serum SOD activities in each group.

Group	D_7	D_{14}	D_{21}	D_{28}
$sLBP_6$	181.19 ± 1.71^a	189.61 ± 2.03^a	190.45 ± 1.40^a	185.12 ± 2.71^a
$sLBP_8$	176.41 ± 4.49^a	179.78 ± 2.68^b	174.72 ± 2.50^b	173.88 ± 1.97^b
$sLBP_9$	187.65 ± 3.51^a	186.52 ± 3.90^{ab}	183.15 ± 3.93^{ab}	175.01 ± 2.12^b
LBP	160.96 ± 4.25^b	164.33 ± 1.97^c	161.80 ± 2.68^c	155.34 ± 1.71^c
BC	149.72 ± 3.51^b	148.60 ± 1.49^d	154.78 ± 4.38^c	148.60 ± 2.03^d

Column data without the same superscripts (a–d) differ significantly ($P < 0.05$).

Table 4
The MDA contents in each group.

Group	D_7	D_{14}	D_{21}	D_{28}
$sLBP_6$	3.92 ± 0.23^b	3.28 ± 0.11^c	2.54 ± 0.15^d	2.97 ± 0.09^d
$sLBP_8$	4.05 ± 0.19^b	3.97 ± 0.07^b	3.32 ± 0.07^c	3.84 ± 0.15^c
$sLBP_9$	4.05 ± 0.23^b	3.88 ± 0.16^b	3.02 ± 0.11^c	3.53 ± 0.11^c
LBP	4.53 ± 0.23^{ab}	4.18 ± 0.16^b	3.88 ± 0.04^b	4.27 ± 0.09^b
BC	5.22 ± 0.23^a	4.61 ± 0.07^a	4.40 ± 0.19^a	4.96 ± 0.09^a

Column ata without the same superscripts (a–d) differ significantly ($P < 0.05$).

($P < 0.05$), on D_7–D_{14} in LBP group were significantly higher than those in BC group ($P < 0.05$). The GSH-Px activities in $sLBP_6$ and $sLBP_9$ groups at all time points and in $sLBP_8$ group except D_{14} were significantly higher than that in unmodified LBP group ($P < 0.05$), and in $sLBP_9$ group on D_7–D_{14} and in $sLBP_6$ group on D_{21}–D_{28} were the highest.

3.6. The changes of serum SOD activity

The serum SOD activities in each group are listed in Table 3. At all time points, the serum SOD activities in three sLBPs groups were significantly higher than those in BC group ($P < 0.05$), in LBP group on D_{14} and D_{28} were significantly higher than those in BC group ($P < 0.05$). The SOD activities in three sLBPs groups at all time points were significantly higher than those in unmodified LBP group ($P < 0.05$), and in $sLBP_9$ group on D_7 and in $sLBP_6$ groups on D_{14}–D_{28} were the highest.

3.7. The changes of serum MDA contents

The serum MDA contents in each group are listed in Table 4. At all time points after injection, the serum MDA contents in three sLBPs groups were significantly lower than those in BC group ($P < 0.05$), in LBP group on D_{14}–D_{28} were significantly lower than those in BC group ($P < 0.05$). The MDA contents in $sLBP_6$ group on D_{14}–D_{28} and in $sLBP_8$ and $sLBP_9$ groups on D_{21}–D_{28} were significantly lower than those in unmodified LBP group ($P < 0.05$), in $sLBP_6$ group at all time points were the lowest and on D_{14}–D_{28} were significantly lower than those in $sLBP_8$ and $sLBP_9$ groups ($P < 0.05$).

4. Discussion

Hydroxyl radical is the most active among the oxygen radicals and can induce severe damage for the adjacent biomolecules (Chance, Sies, & Boveris, 1979). It has been becoming a hot spot in free radical chemical research because of its shorter existence, stronger aggressiveness, greater destructiveness (Xiao & Li, 2010). Therefore, the hydroxyl radical-scavenging ability is a main index to reflect antioxidative activity. The results of test in vitro showed that the hydroxyl radical-scavenging rate in nine sLBPs groups were significantly higher or numerically higher than that of LBP group, in $sLBP_6$ group was the highest, the next was in $sLBP_9$, $sLBP_8$, $sLBP_3$, $sLBP_5$ $sLBP_7$, $sLBP_2$ and $sLBP_4$ group, and in these eight groups were significantly higher than that in LBP group, this indicated that selenylation modification could significantly enhance the antioxidative activity in vitro of LBP, and $sLBP_6$, $sLBP_9$, $sLBP_8$ possessed stronger efficacy.

Superoxide anion is generated by autooxidation reaction of enzyme systems or electron transfer of nonenzyme to reduce molecular oxygen (Liu & Ng, 2000). Among all ROSs, superoxide anion is formed first, although it is a relatively weaker oxidant, it can play an important role in formation of other ROSs, which lead to oxidative damage of lipid, protein and DNA (Qiao et al., 2009; Wickens, 2001). Therefore superoxide anion-scavenging ability is an important index to reflect antioxidant level as well. The results of this research showed that superoxide anion-scavenging rate in nine sLBPs groups were significantly or numerically higher than that of LBP group, in $sLBP_9$ group was the highest, the next was in $sLBP_6$, $sLBP_8$, $sLBP_5$, $sLBP_3$, $sLBP_7$ and $sLBP_4$ group, in these seven groups were significantly higher than that of LBP group, this also indicated that selenylation modification could significantly enhance the antioxidative activity in vitro of LBP, and $sLBP_9$, $sLBP_8$ and $sLBP_6$ possessed the better efficacy.

In order to verify the results in vitro and select a sLBP with strongest activity and optimal modification conditions, the antioxidant activities in vivo of above-mentioned three sLBPs were further determined. GSH-Px and SOD are the major enzymes in free radical-scavenging system of organism. The thiol of GSH-Px can combine with ROS thus protect tissue cells by inhibiting the formation of lipid peroxide. SOD can dismutate $O_2^{-}\cdot$ to form H_2O_2, that is further disintegrated into H_2O under the action of GSH-Px thus scavenge the free radical (Cui, Yuan, & Zhang, 2010; Lv, Gu, Tang, & Ho, 2007). In many degenerative diseases the activity of GSH-Px and SOD are inhibited (Moron, Dipierre, & Mannervik, 1979). The results of the test in vivo showed that the serumal GSH-Px and SOD in three sLBPs groups at all time points, the GSH-Px activity in LBP group on D_7–D_{14} and the SOD activity in LBP group on D_{14} and D_{28} were significantly higher than those in BC group, this indicated that three sLBPs and LBP had stronger antioxidative activity in vivo. At all time points the GSH-Px activity in $sLBP_6$ and $sLBP_9$ groups and the SOD activity in three sLBPs groups, and on D_7 and D_{21}–D_{28} the GSH-Px activity in $sLBP_8$ group were significantly higher than that in unmodified LBP group, the GSH-Px activity in $sLBP_9$ group on D_7–D_{14} and in $sLBP_6$ group on D_{21}–D_{28}, the SOD activity in $sLBP_9$ group on D_7 and in $sLBP_6$ groups on D_{14}–D_{28} were the highest, these indicated that selenylation modification could significantly enhance the antioxidant activity of LBP in vivo, $sLBP_6$ possessed the best efficacy.

MDA is the main product of lipid peroxidation and can reflect the change of oxidative damage and antioxidation (Rotruck et al., 1973). This experimental results showed that at all time points the serum MDA contents in three sLBPs groups were significantly lower than those in BC group and in LBP group on D_{14}–D_{28} were significantly lower than those in BC group, this indicated that three sLBPs and LBP had stronger antioxidative activity in vivo. The MDA contents in $sLBP_6$ group on D_{14}–D_{28} and in $sLBP_8$ and $sLBP_9$ groups on D_{21}–D_{28} were significantly lower than those in unmodified LBP group, in $sLBP_6$ group at all time points were the lowest and on D_{14}–D_{28} were significantly lower than those in $sLBP_8$ and $sLBP_9$ groups, these also indicated selenylation modification could significantly enhance the antioxidant activity in vivo of LBP, and $sLBP_6$ possessed the best efficacy.

Our previous study confirmed that the activity of selenizing polysaccharide was correlated, but not all positively correlated, with its selenium content and carbohydrate content (Qin et al., 2013). This study found that $sLBP_6$ presented strongest antioxidant activities both in vitro and vivo, the next was $sLBP_8$ and $sLBP_9$. Among the nine sLBPs, the selenium content and carbohydrate content of $sLBP_6$ were the highest, while the selenium content and carbohydrate content of $sLBP_9$ were arranged respectively in second and third place, and the carbohydrate content and selenium

content of $sLBP_8$ were arranged respectively in second and fifth place, the antioxidant activities of $sLBP_8$ and $sLBP_9$ were lower than that of $sLBP_6$. From further analysis it could be found that selenium content of $sLBP_5$ and $sLBP_3$ were higher than $sLBP_8$, but the antioxidant activities in vitro were lower than $sLBP_8$; the carbohydrate content of unmodified LBP was much higher than those of all sLBPs, but its antioxidant activity was much lower than those of sLBPs. From this it could be seen that selenizing polysaccharide with stronger antioxidatnt activity required the combination of higher selenium content with higher carbohydrate content.

In conclusion, selenylation modification can significantly enhance the antioxidant activity of LBP, $sLBP_6$ presented best efficacy and could be as a component drug of polysaccharide antioxidants, its modification conditions could be used as the optimal conditions to enhance antioxidant activity of LBP, that was 400 mg of sodium selenite for 500 mg of LBP, the reaction temperature of 70 °C and the reaction time of 6 h.

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