



Selenylation modification: enhancement of the antioxidant activity of a *Glycyrrhiza uralensis* polysaccharide

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Abstract

Selenium (Se) is an essential trace element for human beings and many other forms of life. Organic selenium from natural foods has greater bioavailability and is safer than inorganic selenium species. In this article, the structural properties and antioxidant activities of a *Glycyrrhiza uralensis* polysaccharide (GUP) after selenylation modification were investigated. The GUP was extracted by water decoction and ethanol precipitation and purified via protein elimination using the trichloroacetic acid method and column chromatography. The purified product was subsequently modified by the nitric acid-sodium selenite (HNO₃-Na₂SeO₃) method. The selenized GUP (SeGUP) product was characterized by Fourier transform-infrared (FT-IR) spectroscopy, and its thermal stability, particle size, and antioxidant activities were investigated. FT-IR analysis indicated that the selenium in SeGUP existed mainly as O-Se-O. The thermal stability and particle size of SeGUP differed significantly from those of GUP. Moreover, compared to GUP, SeGUP exhibited greater antioxidant activities *in vitro* and *in vivo*. These results indicate that selenylation modification significantly enhances the antioxidant activity of SeGUP, increasing its potential for application as an antioxidant.

Keywords Selenylation modification · *Glycyrrhiza Uralensis* · Polysaccharide · Antioxidant activity

Introduction

Glycyrrhiza uralensis is a perennial herb that belongs to the Leguminosae family of plants. It is an important windbreak and sand fixation plant in desert areas as well as an authentic herb commonly used in Asia as a sweetener. In Chinese medicine, it is highly valued as an analgesic and for treating respiratory, inflammatory, skin, and liver diseases. Furthermore, its polysaccharides are important active substances that are involved in a variety of biological functions, demonstrating strong immune activity [1] and anti-inflammatory [2], anti-tumor [3], and antioxidant properties [4]. The extensive pharmacological effects of these polysaccharides show great potential for application in the pharmaceutical and functional food industries.

Selenium is an essential trace element in the human body and an important component of selenium-containing enzymes

(e.g., glutathione peroxidase and thioredoxin reductase) [5]. It also exhibits anti-carcinogenic [6, 7], immuno-regulatory [8], and antioxidant properties [9–11]. At present, two types of selenium supplements are available: inorganic selenium and organic selenium. Inorganic selenium has accumulative toxicity and mutagenicity, and its dosage is difficult to control. Currently, out of all the selenium supplements available for purchase, organic selenium polysaccharides are increasingly popular because of their low toxicity and high efficiency. Selenium polysaccharides have also demonstrated high immunological activity and biocompatibility [12].

Moreover, selenium polysaccharides can improve the bioavailability of selenium, an essential trace element in various physiological functions; hence, the body can rapidly absorb it. These compounds exhibit many biological activities including antioxidant, anti-tumor, immunity enhancement, and lipid-lowering properties; the former two activities are particularly prominent. Common selenium polysaccharides include native selenium polysaccharides, selenium polysaccharides produced by enrichment of the medium, and synthetic nano-selenium and organic selenium polysaccharides. Synthetic selenium polysaccharides have drawn much attention because of their high selenium content, short production cycles, high

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yields, and effectiveness. To date, scholars worldwide have synthesized selenium polysaccharides including *Castanea mollissima Blume* [13], *Angelica* [14], and *Lycium barbarum* [15] polysaccharides from synthetic chemical procedures.

One notable feature of selenium is its interactions with free radicals, which have been proposed to cause disease and aging [16]. Selenium exhibits free radical scavenging activity and selenium modification can effectively improve such activities in polysaccharides [17]. Therefore, this is an active area of study for researchers seeking selenium-containing antioxidants with high efficiency and low toxicity. In this work, a *Glycyrrhiza uralensis* polysaccharide (GUP) was extracted and purified, and the nitric acid-sodium selenite ($\text{HNO}_3\text{-Na}_2\text{SeO}_3$) method was used to produce a selenized polysaccharide (SeGUP). The characteristic substances and functional groups of SeGUP were investigated by Fourier transform-infrared (FT-IR) spectroscopy, and the weight loss trend of the heating process was studied by thermogravimetric analysis (TGA). The molecular weight, selenium content, and particle size were determined, and *in vitro* and *in vivo* antioxidant activity was also preliminarily studied. The aims of this study are to investigate the effect of selenium modification on the antioxidant activity of GUP and to provide a theoretical basis for the development of a polysaccharide antioxidant.

Materials and methods

Reagents and instruments

Glycyrrhiza uralensis was acquired from Shihezi Guohao Chinese Herbal Medicine Chemical Reagent Co. Ltd.; 1,1-diphenyl-2-picrylhydrazyl (DPPH), trichloroacetic acid, and potassium ferricyanide ($\text{K}_3\text{Fe}(\text{CN})_6$) were purchased from Shanghai Yuanye Chemical Reagent Ltd., nitric acid (HNO_3) was purchased from Chengdu Kelong Chemical Reagent Co. Ltd.; ascorbic acid (Vc), sodium selenite, and trichloroacetic acid were acquired from Tianjin Fuchen Chemical Reagent Co. Ltd.; potassium bromide (KBr) was purchased from Sinopharm Chemical Reagent Co. Ltd. All reagents used were of analytical grade. Nitroblue tetrazolium chloride (NBT), β -nicotinamide adenine dinucleotide (NADH), phenazine methosulfate (PMS), Sephadex G-100, and the dialysis membrane (Viskase, USA) were supplied by Beijing Biotopped Science & Technology Co. Ltd., China. Potassium ferricyanide was purchased from Sinopharm Chemical Reagent Co., Ltd. Dextran T-20000 [molecular weight (Mw) = 2,000,000], Dextran T-150 (Mw = 133,800), Dextran T-40 (Mw = 36,800), Dextran T-10 (Mw = 9700), and Dextran T-5 (Mw = 2700) were purchased from Sigma-Aldrich (USA). SOD, MDA, and GSH-Px ELISA kits were the products of the Nanjing Institute of Biological Engineering.

The equipment used in this study includes: Nicolet iS50 FT-IR (Thermo Fisher Scientific, USA); inductively coupled plasma-optical emission spectrometer iCAP 6300 (ICP-OES; Thermo Fisher Scientific, USA); Zetasizer Nano ZS90 nanoparticle size and zeta potential analyzer (Malvern, UK); TGA Q50 V20, 13 Build 39 (Universal V4.5A TA Instruments, USA); Waters 1525 high-performance gel filtration chromatography (HPGFC) system equipped with a Waters 2410 Refractive Index Detector (RID), and Ultrahydrogel™ Linear columns (300×7.8 mm, Waters, USA).

Methods

Extraction and purification of GUP

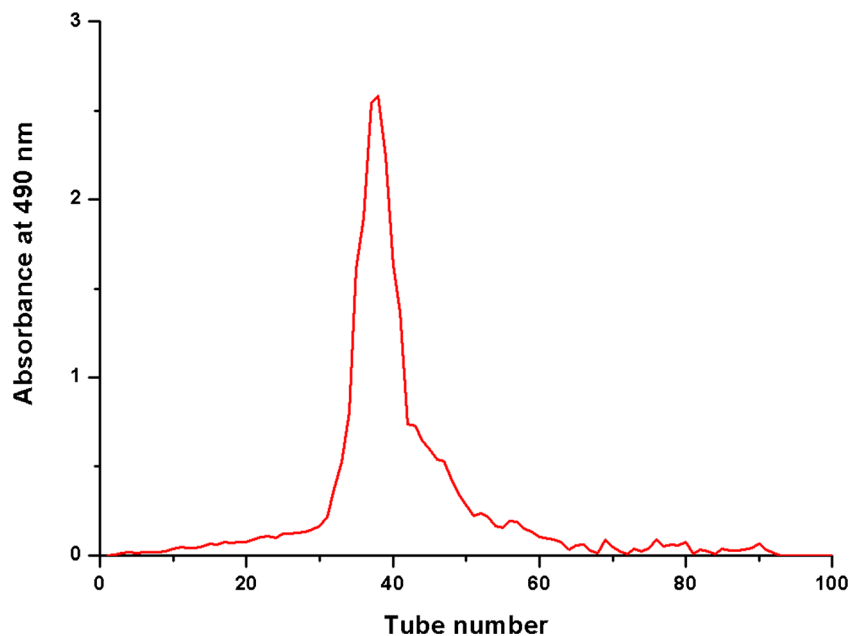
Dried *Glycyrrhiza uralensis* (1000 g) was soaked in 95% ethanol for 12 h. The resultant product was decocted with nine-fold distilled water three times each for 4 h, 2 h, and 1 h. The solution was filtered and its volume reduced by rotary evaporator (RE-52, Shanghai Yarong Biochemistry Instrument factory, China) to 1000 mL. Next, 95% absolute ethanol was added until a final concentration of 80% was reached. This solution was stirred evenly and allowed to stand overnight. The precipitate was washed with distilled water and centrifuged at $3000 \times g$ for 15 min to remove any insoluble material. The supernatant was freeze-dried to afford the crude product.

The crude GUP was re-dissolved in distilled water, treated with trichloroacetic acid [18], and subsequently dissolved in distilled water (0.05 g/mL). The resulting solution was poured into a Sephadex G-100 column (2 cm \times 80 cm) and eluted with distilled water (flow rate = 12 mL/h; 5 mL per tube) to generate an elution curve (one peak, Fig. 1). The purified GUP was obtained after lyophilization and its polysaccharide content was then measured by the phenol-sulfuric acid method [19, 20].

Selenylation modification of GUP

The polysaccharide GUP was selenized by a slightly modified version of a previously reported nitric acid-sodium selenite method [21]. The purified GUP dry powder (500 mg) was added to 50 mL HNO_3 (0.5%, v/v) and stirred at room temperature (25 °C) until it was completely dissolved. Next, 200 mg Na_2SeO_3 were added and stirred at 70 °C in a water bath for 8 h. After the reaction was complete, the solution was cooled to room temperature and adjusted to a pH ranging between 5 and 6 by the addition of anhydrous Na_2CO_3 . After centrifugation (10 min, $3000 \times g$), the reaction solution was transferred to a 1 KD dialysis membrane in distilled water for 24 h; the free sodium selenite was measured every 6 h by ascorbic acid. The dialysis was stopped when the color of the

Fig. 1 Elution curve of *Glycyrrhiza uralensis* polysaccharide (GUP) purification



dialysis solution was no longer red. SeGUP was produced after freeze-drying (yield: 16% w/w; yellow powder).

Identification of SeGUP

Determination of the selenium content and molecular weight

The Se content of SeGUP was determined by ICP-OES. The Mw values of SeGUP and GUP were determined by HPGFC. This was performed with two successively linked columns and a 0.1 mol/L NaNO₃ aqueous solution as the mobile phase. The analysis was performed at 30 °C with an injection volume of 20 µL and a flow rate of 0.8 mL/min. The HPGFC system was calibrated with T-series Dextran standards.

FT-IR analysis

SeGUP and GUP FT-IR spectra were recorded at a wavenumber range of 4000–500 cm⁻¹ by the KBr pellet method with an FT-IR spectrometer.

Detection of particle size distribution

The sizes of the SeGUP and GUP particles in distilled water (0.2 mg/mL) were measured using a nanoparticle size and zeta potential analyzer. All data were analyzed by particle size distribution software (DTS5.00).

TGA analysis

SeGUP and GUP were tested by TGA at the following conditions: heating rate = 20 °C/min, temperature range = 25–700 °C, N₂ gas; reference name = Al₂O₃.

In vitro antioxidant activity

Superoxide anion radical assay

Superoxide anion scavenging activities were determined according to the method described by Yen and Chen [22] and Chen and Yen [23], with slight modifications. Taking Vc as the control, SeGUP and GUP were prepared from a series of different concentrations (0.1–3.2 mg/mL) of the sample solution. The sample reaction solution consisted of equal volumes (1.5 mL) of the sample and reaction solutions (0.5 mL of 0.3 × 10⁻³ mol/L NBT, 0.5 mL of 0.468 × 10⁻³ mol/L NADH, and 0.5 mL of 0.6 × 10⁻⁴ mol/L PMS). The reaction solution was kept at 25 °C for 5 min; absorbance wavelength = 560 nm. Here, 1.5 mL of distilled water and 1.5 mL of phosphate-buffered saline solution with a pH of 7.4 were used as the blank and control, respectively. The scavenging rate was calculated by the equation:

$$\text{Scavenging rate (\%)} = 1 - [(A_{\text{sample}} - A_{\text{blank}}) / A_{\text{control}}] \times 100\%$$

where A_{sample} = absorbance of the sample group, A_{blank} = absorbance of the blank group, and A_{control} = absorbance of the standard control group.

Hydroxyl radical assay

Hydroxyl radical scavenging activities were measured by the method described by Halliwell *et al.* [24] with slight modifications. Taking Vc as the control, SeGUP and GUP were prepared in a series of different concentrations (0.1–3.2 mg/mL) of the sample solution. Next, 1.5 mL of 1 mmol/L phenanthroline-ethanol was added to 2 mL PBS (pH 7.4, 0.2 mmol/L); 1.0 mL of 1.5 mmol/L FeSO₄, 1 mL sample solution, and 1 mL H₂O₂ (0.1%, v/v) were then added. For this assay, 1 mL and 2 mL water samples were used as the blank and standard control groups, respectively. The reaction was kept at 37 °C for 60 min; absorbance wavelength = 536 nm. The scavenging rate was calculated from the following equation:

$$\text{scavenging rate(\%)} = [(A_2 - A_1) / (A_0 - A_1) \times 100\%]$$

where A₂ = absorbance of the sample group, A₁ = absorbance of the blank group, and A₀ = absorbance of the standard control group.

DPPH assay

The scavenging activity of DPPH radicals was determined according to measurements described by Yokozawa *et al.* [25] and Larrauri *et al.* [26] with slight modifications. Taking Vc as the control, SeGUP and GUP were prepared in a series of different concentrations (0.1–3.2 mg/mL) of the sample solution. Next, 2 mL of each sample solution and 2 mL of 0.2 × 10⁻³ mol/L DPPH in ethanol were mixed together. The resultant mixtures were kept in the dark for 30 min and the absorbance was measured at 517 nm. The scavenging rate was calculated from the following equation:

$$\text{scavenging rate (\%)} = 1 - [(A_{\text{sample}} - A_{\text{blank}}) / A_{\text{DPPH}}] \times 100\%$$

where A_{sample} = absorbance of 2 mL sample solution + 2 mL DPPH, A_{blank} = absorbance of 2 mL sample solution + 2 mL absolute ethanol, and A_{DPPH} = absorbance of 2 mL DPPH + 2 mL absolute ethanol.

Reducing power assay

The reducing power was estimated according to the method described by Tsai *et al.* [27] with slight modifications. Different concentrations of the SeGUP and GUP solutions (1 mL; 0.1–3.2 mg/mL) were added to 2.5 mL PBS (pH 6.6, 0.2 mol/L) and 2.5 mL K₃Fe(CN)₆ (1%, w/v). The resultant mixture was kept at 50 °C in a water bath for 20 min. After rapid cooling, 2.5 mL trichloroacetic acid (10%, w/v) were added and the sample was centrifuged at 3000×g for 10 min. Subsequently, 0.2 mL of FeCl₃ (0.1%, w/v) and 1 mL of distilled water were added to 1 mL of the supernatant and

allowed to stand for 10 min; absorbance was measured at 700 nm.

In vivo antioxidant activity

Acute toxicity test

Healthy Kunming mice (weighing 25 ± 2 g, 6 weeks old) were purchased from the animal laboratory center of Shihezi University, including 35 males and 35 females. All experiments related with animals were approved by the Animal Ethics Committee of Shihezi University (Approval No. AECSU2013–17). All mice were divided into seven groups (n = 10), with each group including 5 males and 5 females. The control group received physiological saline (10 mL kg⁻¹). The other groups were administered intragastrically with SeGUP (0.15 mL/10 g) at approximately the same time interval of 24 h for 14 consecutive days [28] with specific dosages of 100, 150, 228.95, 347.42, 527.23, and 800 mg/kg.

Animal preparation and experimental design

Equal numbers of male and female healthy Kunming mice (weighing 20 ± 2 g) were purchased from the animal laboratory center of Shihezi University. Breeding mice and related experiments were carried out strictly in accordance with the related requirements of the Animal Research Committee guidelines of Shihezi University. After acclimation for 7 days, the mice were randomly assigned to eight groups (10 in each group, 5 per sex). The normal control (NC) mice and the Vc group received water and Vc by intragastric administration, respectively; normal mice received SeGUP by intragastric administration (SeGUP-L: SeGUP-Low-dose group, SeGUP-M: SeGUP-Medium-dose group, SeGUP-H: SeGUP-High-dose group); normal mice received GUP by intragastric administration (GUP-L: GUP-Low-dose group, GUP-M: GUP-Medium-dose group, GUP-H: GUP-High-dose group). All groups were administered intragastrically once per day at approximately the same time interval of 24 h for 30 consecutive days, as in Liu *et al.* [29].

GSH-Px and SOD activities and the MDA level in the serum and livers of mice

The mice were fasted overnight and sacrificed by cervical dislocation at the end of the experiment. Their blood was centrifuged (3000×g, 10 min, 4 °C) to obtain serum. The liver of each mouse was removed and rinsed with ice-cold physiological saline. Ten percent (w/v) liver homogenate was prepared by homogenization in ice-cold physiological saline. The supernatant of the homogenate was collected by centrifugation (3000×g, 10 min, 4 °C). The antioxidant enzyme

activities (GSH-Px, SOD) and MDA levels in the serum and livers were measured by using SOD, MDA, and GSH-Px kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China; catalogue numbers: A001–3, A003–1, A005) according to the manufacturer's instructions.

Results and discussion

Determination of Se content and molecular weight

On the basis of ICP-OES analysis, the Se content in SeGUP was determined as 1.339 mg/g; from HPGFC analysis, the Mw values for SeGUP and GUP were determined as 0.58×10^4 Da and 3.8032×10^4 Da, respectively. According to previous reports, polysaccharides cause serious depolymerization when they react for prolonged periods in a strongly acidic environment [30]. In this experiment, the main reason for the decrease in molecular weight of selenium polysaccharide is the hydrolysis of polysaccharides, and the high temperature and long-duration reactions in the strongly acidic environment may have increased their hydrolysis rate.

SeGUP and GUP IR spectra

The FT-IR spectra of GUP and SeGUP are displayed in Fig. 2. The IR spectrum of GUP showed characteristic peaks at 3405 cm^{-1} and 2931 cm^{-1} corresponding to the O-H and C-H stretching vibrations, respectively [31]. Comparing the IR spectra of GUP and SeGUP, their structures were similar. However, there was a peak at 956.32 cm^{-1} in the SeGUP spectrum, which we attribute to the O-Se-O stretching vibration [32]. This result provides strong evidence that sodium selenite combined with GUP via an esterification reaction.

Particle size distribution

The size of polysaccharide particles has a great impact on *in vivo* metabolism and pharmacological activity. A smaller particle size is considered to be more fully absorbed by the body and is thus expected to be more pharmacologically active. The particle surface charge (zeta potential) can affect the stability of the particles in dispersions by electrostatic repulsion, which has a significant effect on the biological activity of the particles. A higher absolute zeta potential value indicates a smaller amount of dispersed particles; therefore, a more stable system is present with dispersed particles. Conversely, lower absolute zeta potential values indicate larger dispersed particles, leading to a more unstable system in which the particles tend to condense or aggregate. The detected SeGUP and GUP charges were determined as -33.8 mV and -11.5 mV , respectively. These results indicate that, at the same concentration, the SeGUP particle solution tends to be dispersed whereas the GUP particles tend to condense or aggregate. Moreover, the average SeGUP particle size (185.316 d. nm) was significantly smaller than that in GUP (374.549 d. nm) at the same concentration (Fig. 3). This occurs because the polysaccharides are hydrolyzed into small molecules during selenization. The results indicate that SeGUP exhibits a higher dispersibility at the same concentration in both the *in vitro* and *in vivo* studies. Furthermore, the system with SeGUP was more stable and more easily absorbed and applicable than GUP.

Thermogravimetric analysis

The TGA results of both GUP and SeGUP are illustrated in Fig. 4. The thermogravimetric (TG) peaks of these two substances are similar in shape with four weightlessness stages in the heating process. Weightless peaks in the first stage ($0\text{--}200 \text{ }^\circ\text{C}$) are due to the loss of adsorbed water; the maximum

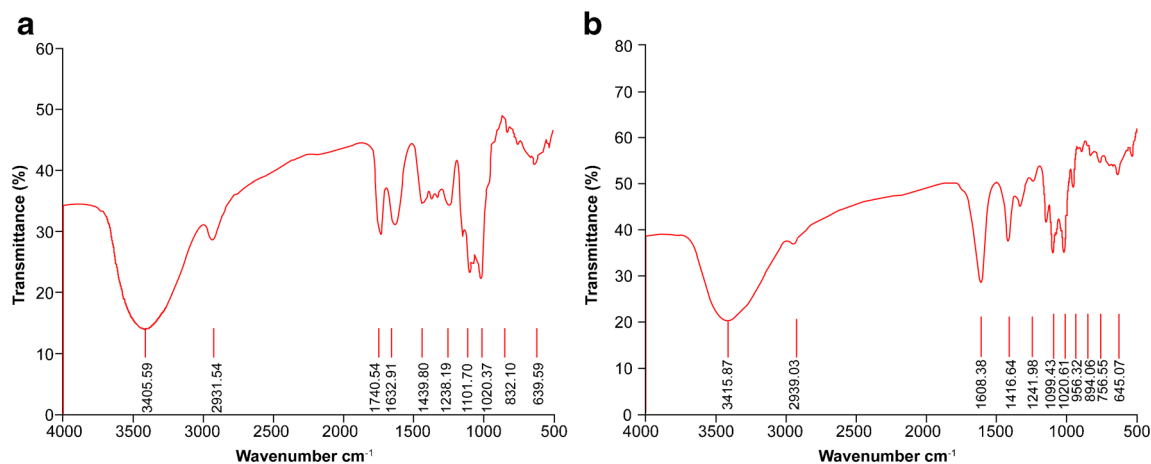


Fig. 2 Fourier transform-infrared (FT-IR) spectra of (a) *Glycyrrhiza uralensis* polysaccharide (GUP) and (b) selenized GUP (SeGUP)

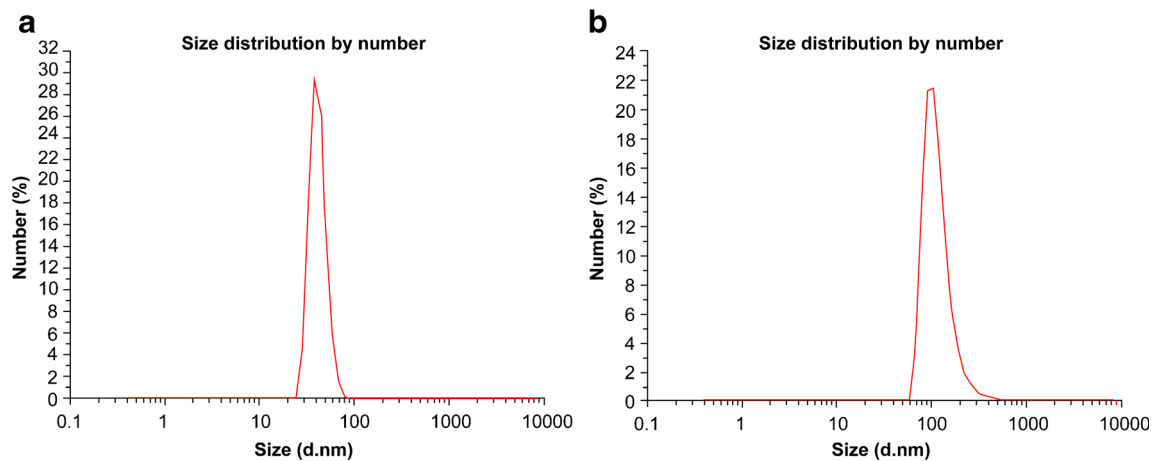


Fig. 3 Particle size distribution curves for (a) *Glycyrrhiza uralensis* polysaccharide (GUP) and (b) selenized GUP (SeGUP)

temperatures of the GUP and SeGUP decomposition rates were 77.32 °C and 79.10 °C, respectively, and the percentage weight losses during the heating process were 5.848% and 9.036%, respectively. In the second stage (200 °C–400 °C), the maximum temperatures of the GUP and SeGUP decomposition rates were 273.96 °C and 269.31 °C, respectively, and the percentage weight losses during the heating process were 58.62% and 39.07%, respectively. In the third stage (400 °C–600 °C), the maximum temperatures of the GUP and SeGUP decomposition rates were 476.24 °C and 427.02 °C, respectively, and the percentage weight losses during the heating process were 11.14% and 3.922%, respectively. Finally, in the fourth stage (600 °C–800 °C), the maximum temperatures of the GUP and SeGUP decomposition rates were 657.89 °C and 609.31 °C, respectively, and the percentage weight losses in the heating process were 6.641% and 5.960%, respectively. A comparison of the two graphs demonstrates that the weight loss peak during the heating process is steeper for SeGUP, indicating that the thermal stability of GUP is reduced after selenium acidification. Thus, we

conclude that the introduction of selenium decreased the thermal stability of GUP.

Antioxidant activity analysis

Superoxide anion radical assay

Superoxide anion free radicals not only have their own toxicity but also, through a series of reactions, generate other oxygen free radicals. This causes further damage to the human body, promoting aging and causing many diseases [33, 34]. Thus, the timely removal of any superoxide anions that gather in the body is beneficial [35]. The results afforded for the removal of superoxide anions by SeGUP and GUP are illustrated in Fig. 5a. SeGUP and GUP exhibit different degrees of superoxide anion radical scavenging; moreover, the roles increase with an increase in polysaccharide concentration (0.1–3.2 mg/mL; SeGUP and GUP at 3.2 mg/mL for superoxide anion free radicals at a clearance rate of 70.00% and 56.17%, respectively).

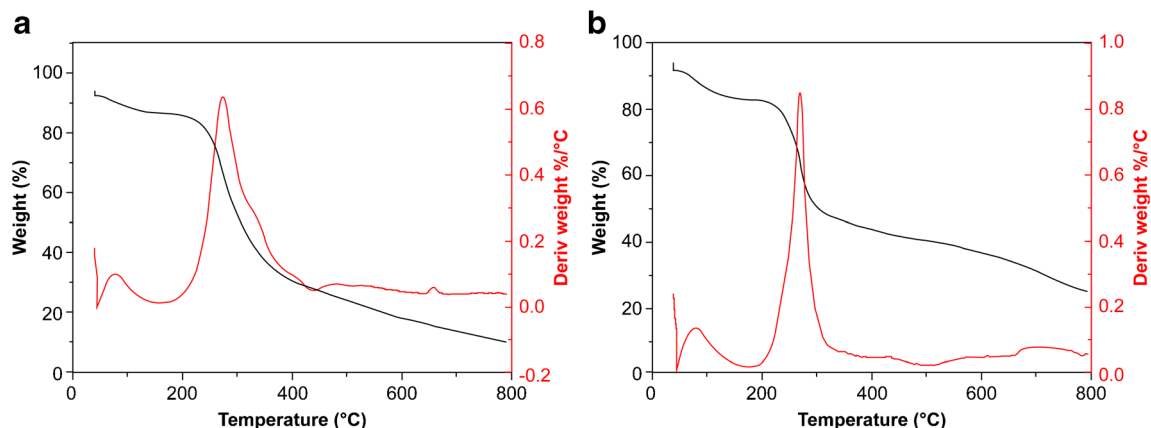


Fig. 4 Thermogravimetric analysis (TGA) curves of (a) *Glycyrrhiza uralensis* polysaccharide (GUP) and (b) selenized GUP (SeGUP)

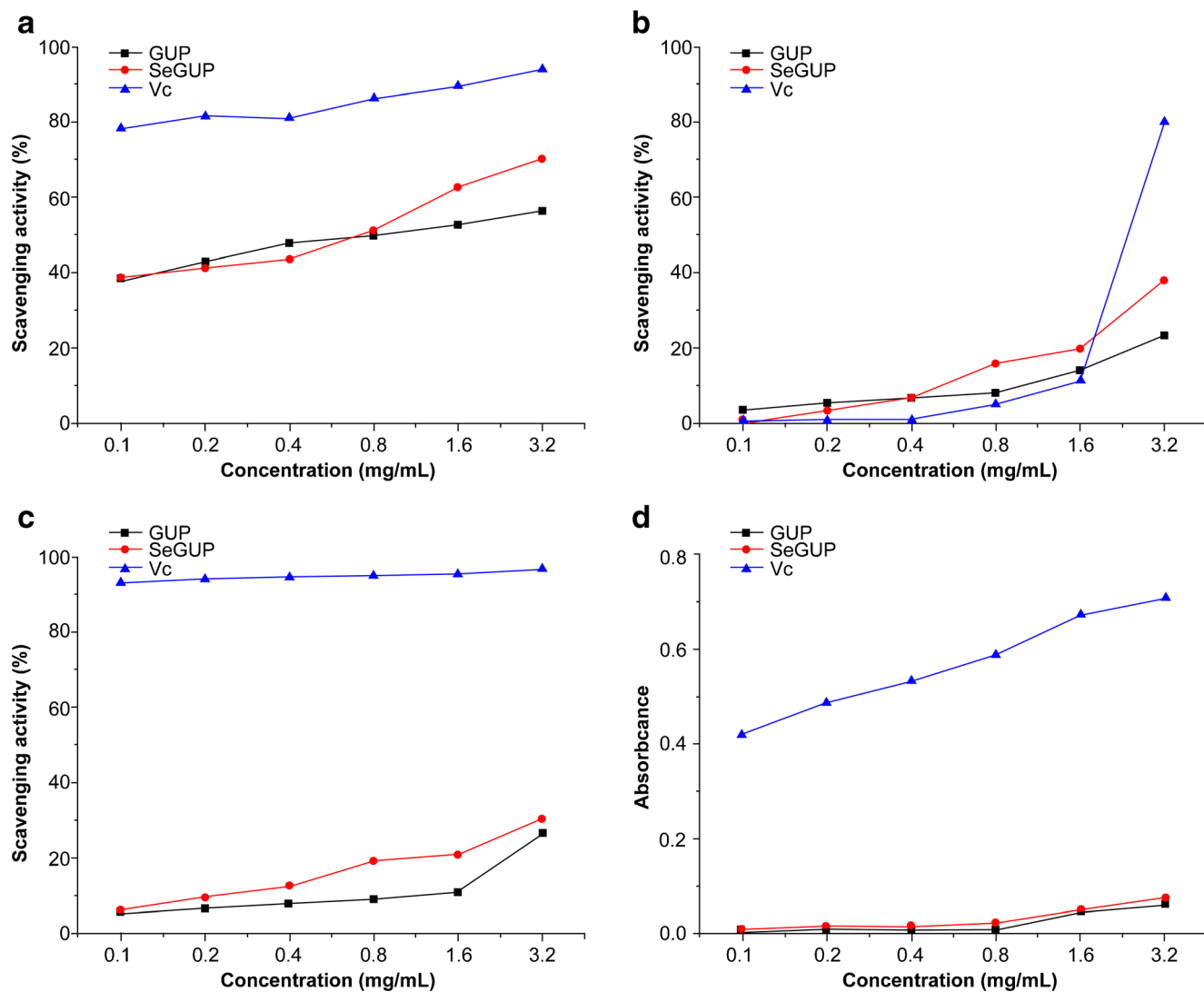


Fig. 5 Antioxidant effect of *Glycyrrhiza uralensis* polysaccharide (GUP) and selenized GUP (SeGUP): (a) superoxide radical scavenging activity, (b) hydroxyl radical scavenging activity, (c) 1,1-diphenyl-2-

picrylhydrazyl (DPPH) radical scavenging activity, and (d) reducing power; data are presented as mean values ($n = 3$)

SeGUP molecules comprise a unique O-Se-O moiety that undergoes a redox reaction with O_2^- ; therefore, its scavenging ability is better than that of GUP.

Hydroxyl radical assay

Hydroxyl radicals are currently known as the most active type of free radical reactive oxygen species. They react with almost all organic compounds present in cells to cause a series of chain reactions that destroy many compounds including lipids and proteins, thereby damaging cellular structure and function. These radicals can also damage biomembranes, leading to a variety of diseases. Therefore, the OH removal rate is an important indicator of antioxidant activity. The results of SeGUP and GUP hydroxyl radical scavenging are illustrated in Fig. 5b. As seen, SeGUP and GUP display different degrees

of scavenging on hydroxyl radicals, which are enhanced with increasing polysaccharide concentration (0.1–3.2 mg/mL). The SeGUP and GUP hydroxyl radical clearance rates at 3.2 mg/mL were determined as 38.02% and 23.44%, respectively. In this work, the selenium polysaccharides with the unique selenite structural groups exhibited strong nucleophilicity and formed complexes with metal ions; therefore, SeGUP displayed slightly better scavenging hydroxyl free radical activity than GUP.

DPPH assay

DPPH is a very stable nitrogen-centered free radical. Its stability is mainly attributed to resonance of its three benzene rings and spatial barriers so that the unpaired electrons at the center of the nitrogen atoms cannot undergo proper electronic

Table 1 Results of the acute toxicity test with SeGUP

Group	Number of mice	Dose (mg/kg)	Number of dead mice	Mortality (p)	Survival rate (q)
Control	10	0	0	0	1
1	10	100	0	0	1
2	10	150.87	1	0.1	0.9
3	10	228.95	3	0.3	0.7
4	10	347.42	5	0.5	0.5
5	10	527.23	8	0.8	0.2
6	10	800	10	1	0

pairing [36]. As a stable free radical, DPPH can capture other free radicals and is widely used in the quantitative determination of biological samples and food antioxidant capacities. The results of DPPH removal by SeGUP and GUP are presented in Fig. 5c. SeGUP and GUP exhibit different inhibitory effects on DPPH; however, all values increase with an increase in polysaccharide concentration (0.1–3.2 mg/mL). At 3.2 mg/mL, the DPPH radical scavenging rates of SeGUP and GUP reached 30.31% and 26.5%, respectively.

Reducing power assay

The reducing power of antioxidants and their antioxidant properties are related. An antioxidant scavenges free radicals via electron-withdrawing carboxyl groups by its own reduction [37]. In general, antioxidant activity becomes stronger as the strength of the antioxidant reducing power increases [38]. The results of reducing power of SeGUP and GUP are presented in Fig. 5d. Both polysaccharides exhibit some reducing power; moreover, all values increase with an increase in polysaccharide concentration (0.1–3.2 mg/mL). The SeGUP and GUP reducing power reached 0.074 and 0.062, respectively, at 3.2 mg/mL. These results suggest that selenium modification affects the antioxidant activity of GUP *in vitro*.

Table 2 Effect of SeGUP and GUP on GSH-Px, SOD and MDA activity in the blood serum of mice ($x \pm s$, $n = 10$)

Group	Dose (mg/kg)	GSH-Px (U/mL)	SOD (U/mL)	MDA (nmol/mL)
NC	0	222.15 ± 3.19	77.713 ± 2.139	20.71 ± 2.77
VC	25	240.25 ± 4.88**	98.851 ± 0.725	13.85 ± 2.25**
SeGUP-L	100	216.78 ± 4.71	90.241 ± 2.616	15.39 ± 3.50**
SeGUP-M	200	224.52 ± 4.68	92.392 ± 3.128	15.23 ± 3.41**
SeGUP-H	300	237.20 ± 3.62**	111.75 ± 6.232**	13.53 ± 2.23**
GUP-L	100	225.47 ± 2.36	91.624 ± 6.087	17.62 ± 2.27*
GUP-M	200	221.33 ± 2.41	96.485 ± 4.182	17.25 ± 3.49*
GUP-H	300	231.24 ± 2.93*	107.0872.549*	15.32 ± 2.12**

SeGUP-L: SeGUP-Low-dose group, SeGUP-M: SeGUP-Medium-dose group, SeGUP-H: SeGUP-High-dose group, GUP-L: GUP-Low-dose group, GUP-M: GUP-Medium-dose group, GUP-H: GUP-High-dose group

Data are expressed as means ± SD ($n = 10$) and evaluated by GraphPad Prism6

Differences were considered to be statistically significant if $P < 0.05$

* $P < 0.05$ compared with the control group (NC)

** $P < 0.01$ compared with the control group (NC)

Acute toxicity

The experimental results of the acute toxicity tests are shown in Table 1. As seen, the mice in the control group showed no abnormal reaction. During the acute toxicity test, the mice from different groups died at different time intervals. Particularly, the highest dose group (800 mg/kg) died within 12 h after administration. According to the modified Karber's method [39], the LD50 is 321.14 mg/kg and its 95% confident limit is 258.68–398.67 mg/kg. As a result, the concentrations of GUP and SeGUP in the low-dose group, medium-dose group, and high-dose group were determined as 100, 200, and 300 mg/kg, respectively.

Antioxidant activities of SeGUP and GUP *in vivo*

As shown in Tables 2 and 3, the SeGUP and GUP groups (at doses of 100, 200, and 300 mg/kg) showed a significant ($P < 0.05$) increase in enzymatic activities (GSH-Px and SOD) both in blood serum and liver compared with the normal control mice. The SeGUP and GUP groups at these aforementioned doses exhibited significantly ($P < 0.05$) decreased MDA levels in the serum and livers. The enzymes (GSH-Px, SOD, and MDA) protect against oxidative stress and tissue

Table 3 Effect of SeGUP and GUP on GSH-Px, SOD and MDA activity in the liver tissue of mice ($\bar{x} \pm s$, $n = 10$)

Group	Dose (mg/kg)	GSH-Px (U/mL)	SOD (U/mL)	MDA (nmol/mL)
NC	0	144.15 ± 7.53	213.27 ± 2.05	29.071 ± 2.147
VC	25	236.25 ± 3.06	226.56 ± 3.85	19.785 ± 2.25
SeGUP-L	100	217.63 ± 4.23	205.75 ± 5.47	21.723 ± 2.141**
SeGUP-M	200	228.47 ± 2.62	209.42 ± 5.53	20.204 ± 3.192**
SeGUP-H	300	288.15 ± 2.84*	246.71 ± 2.68*	19.881 ± 3.074**
GUP-L	100	141.08 ± 2.57	204.66 ± 2.38	29.612 ± 2.207
GUP-M	200	178.11 ± 2.89	206.36 ± 3.72	28.439 ± 2.150
GUP-H	300	274.02 ± 2.77*	241.68 ± 1.92*	23.533 ± 2.023*

SeGUP-L: SeGUP-Low-dose group, SeGUP-M: SeGUP-Medium-dose group, SeGUP-H: SeGUP-High-dose group, GUP-L: GUP-Low-dose group, GUP-M: GUP-Medium-dose group, GUP-H: GUP-High-dose group

Data are expressed as means ± SD ($n = 10$) and evaluated by GraphPad Prism6

Differences were considered to be statistically significant if $P < 0.05$

* $P < 0.05$ compared with the control group (NC)

** $P < 0.01$, compared with the control group (NC)

damage by converting reactive oxygen species into non-toxic compounds [40]. GSH-Px decomposes hydrogen peroxide into water and oxygen, preventing the formation of hydroxyl radicals [41]. As a free radical scavenger, SOD plays an important role in anti-oxidative damage [42]. MDA is a metabolite of lipid peroxidation, and its content in serum or tissue can reflect the extent of lipid peroxidation damage and the level of oxidation [43]. In our experiments, we found that GUP and SeGUP were both good antioxidants and that they played an important role in protecting biological systems.

Conclusion

In this work, SeGUP was produced by the $\text{HNO}_3\text{-Na}_2\text{SeO}_3$ method with *Glycyrrhiza uralensis* polysaccharide (GUP) as a raw material. The selenium content reached 1.339 mg/g, while the Mw decreased significantly to 0.58×10^4 Da. FT-IR spectroscopy demonstrated that SeGUP, which was successfully produced by selenium modification, not only retained the basic polysaccharide structure but also exhibited a selenite characteristic peak. Compared to those of the unmodified GUP, the SeGUP thermal stability and particle size changed significantly. *In vitro* and *in vivo* antioxidant tests indicated that SeGUP displayed good antioxidant activity. The results also revealed that synthetic SeGUP was an organic selenium polysaccharide with good antioxidant ability. Thus, this compound shows great potential for application in green selenium health products and biological antioxidants. Further research on the chemical structure and anti-inflammatory mechanism of SeGUP will be carried out in the future.

Abbreviations: DPPH, 1,1-Diphenyl-2-picrylhydrazyl; FT-IR, Fourier transform-infrared; GUP, *Glycyrrhiza uralensis* polysaccharide; GSH-Px, Glutathione peroxidase; HPGFC, High-performance gel filtration chromatography; ICP-OES,

Inductively coupled plasma-optical emission spectrometer; IR, Infrared; MDA, Malondialdehyde; Mw, Molecular weight; NADH, β -Nicotinamide adenine dinucleotide; NBT, Nitroblue tetrazolium chloride; PMS, Phenazine methosulfate; RID, Refractive Index Detector; Se, Selenium; SeGUP, Selenized *Glycyrrhiza uralensis* polysaccharide; SOD, Superoxide dismutase; TG, Thermogravimetric; TGA, Thermogravimetric analysis.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All experiments related with animals were approved by the Animal Ethics Committee of Shihezi University (Approval No. AECSU2013–17).

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