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Antioxidant activities of the oligosaccharides from the roots, flowers and leaves of *Panax ginseng* C.A. Meyer

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ABSTRACT

The chemical characterization and antioxidant activities of water-soluble ginseng oligosaccharides from roots (WGOS-R), flowers (WGOS-F) and leaves (WGOS-L) of *Panax ginseng* C.A. Meyer obtained by hot water extraction were investigated. The sugar content of WGOS-R, WGOS-F and WGOS-L were 95.87%, 87.07% and 83.09%, respectively. The ginsenosides and total phenols content decreased in the order of WGOS-L > WGOS-F > WGOS-R. WGOS-R comprised only Glc, WGOS-F and WGOS-L comprised Glucose (Glc) and Rhamnose (Rha) in a molar ratio of 6.0:1.0 and 7.0:1.0, respectively. *In vitro* antioxidant tests showed that WGOS-R exhibited higher antioxidant activity than WGOS-F and WGOS-L. *In vivo* antioxidant tests showed that WGOS-R significantly enhanced activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and total antioxidant capability (T-AOC) in the serum and liver and decreased malondialdehyde (MDA) level in the serum and liver.

1. Instruction

Reactive oxygen species (ROS) involve a series of free radicals such as superoxide anion radicals ($^1O_2$), hydroxylradical species ($^{\cdot}OH$), singletoxygen ($O^{\cdot}{}_2$) and hydrogen peroxide (H_2O_2) *etc.* These active oxygen compounds involving in many cellular metabolic and signaling process, also could damage cellular macromolecules causing lipid peroxidation and nucleic acid and protein alterations. Furthermore, more and more evidence indicated that ROS involved in many disease processes, such as aging, carcinogenesis, atherosclerosis, diabetes and rheumatoid arthritis (Finkel & Holbrook, 2000; Seifried, Anderson, Fisher, & Milner, 2007; Valko et al., 2007). Several lines of evidence from both epidemiological and experimental studies have found that antioxidants, including natural compounds from plants and synthetic compounds, play an important role in scavenging excessive free radicals, to delay or prevent oxidation and maintain the systematic health (Mohammed, 2002; Giles et al., 2003) However, the synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are suspected to be connected with liver damage and carcinogenesis (Grice, 1988; Witschi, 1986). Therefore, more and more interests are aroused to find natural antioxidants without toxicity.

Panax ginseng C.A. Meyer is one of the most precious and renowned drug in traditional Chinese medicinal plants, particularly in herbal oriental medicine for treatment of various diseases (Xiang, Shang, Gao, & Zhang, 2008). Evidence pointing out the medicinal efficacy of ginseng has been closely linked to its protective properties against free radical attack (Chen, 1996; Lee, Kim, & Chang, 1999; Maffei Facino, Carini, Aldini, Berti, & Rossoni, 1999). Many bioactive substances with antioxidant activities have been found in all parts of *P. ginseng*. Groups of scientists found that antioxidant activity of ginseng leaves mainly come from their abundant phenolics and flavonoids. Luo et al. demonstrated that a neutral polysaccharide from ginseng root exhibited equivalent antioxidant activity *in vitro* (Luo & Fang, 2008). So far, no investigation has been carried out on ginseng oligosaccharide that may account for antioxidant properties. Hence, oligosacchairdes of ginseng roots, flowers and leaves were prepared and evaluated for their *in vitro* and *in vivo* antioxidant activity.

2. Materials and methods

2.1. Materials

The cultured ginseng leaves, flowers and roots were collected from 5-year-old ginseng plants at Changbai Mountain,

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Jinlin Province, China in August 2010. The dried leaves, flowers and roots of ginseng were crushed and passed through 60 mesh sieve. The powders were stored in a freezer for further experiments. Sephadex G-25 was purchased from Pharmacia Fine Chemical AB, Uppsala, Sweden. Assay kits for superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), total antioxidant capability (T-AOC) and malondialdehyde (MDA) were the products of Nanjing Jiancheng Bioengineering Institute (Nanjing, China). D-Gal, hydrogen peroxide (H_2O_2), 1,1-diphenyl-2-picrylhydrazyl (DPPH), (1,1-diphenyl-2-picrylhydrazyl radical 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl) thiobarbituric acid (TBA), ethylenediaminetetraacetic acid (EDTA), ferrozine, trichloroacetic acid (TCA), deoxyribose (DR), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), Vitamin C (Vc) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of analytical grade.

2.2. Preparation for the oligosaccharides

Air-dried *P. ginseng* roots, flowers and leaves were crushed and extracted with 90% ethanol under reflux extraction at 40 °C to remove pigments and small lipophilic molecules. The residue was further extracted by water-saturated n-butanol for three times. After centrifugation (4000 rpm/min, 10 min, at 4 °C) and vacuum filtration, the precipitate was dried and further extracted thrice (90 min each) with distilled water at 70 °C. All the aqueous extracts were combined, concentrated under a reduced pressure and then lyophilized to yield the crude oligosaccharides. Then, the crude oligosaccharides were re-dissolved in distilled water and dialyzed against distilled water for 72 h in a dialysis sack (molecular weight cut-off of 3000 Da). The fraction out of dialysis sack (Mw <3000 Da) was collected and concentrated at 50 °C in vacuum and then lyophilized. Subsequently, the obtained oligosaccharides were applied to Sephadex G-25 column (2.5 cm × 90 cm) and equilibrated with degassed distilled water. The eluate was detected using phenol-sulphuric acid (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The appropriate fractions were combined, concentrated and then lyophilized to obtain the three oligosaccharides: WGOS-R (the oligosaccharide from ginseng roots), WGOS-F (the oligosaccharide from ginseng flowers), WGOS-L (the oligosaccharide from ginseng leaves).

2.3. Analysis of chemical composition

Total sugar content was determined by phenol–sulfuric acid method using glucose as standard (Dubois et al., 1956). The content of total ginsenosides was determined by the colorimetric method as Chen et al. described (Chen, Meng, Zhan, & Liu, 2009). The content of total ginsenosides was estimated by reference to a standard curve made from the ginsenoside standard (Re). The total phenols content in ginseng oligosaccharides was determined using the Folin–Ciocalteau assay (Sabir & Rocha, 2008). Gallic acid was used in a standard curve and the results were expressed in microgram of gallic acid equivalents per milliliter of dry extract.

2.4. Analysis of monosaccharide composition

The sample (2 mg) was hydrolyzed with 0.5 ml of 2 M trifluoroacetic acid (TFA) in an ampoule (2 ml). The ampoule was sealed under a nitrogen atmosphere and kept at 120 °C for 1 h, and the excess acid was completely removed by co-distillation with ethanol. After removing the acid, the products of hydrolysis were derivatized with 1-phenyl-3-methyl-5-pyrazolone (PMP) according to the method in the literature (Honda, Akao, Suzuki, Okuda, & Kakehi Kazuaki Nakamura, 1989). The monosaccharide derivatives were analyzed by Aglient RRLC 1200 SL system (Agilent Technologies, Wilmington, USA), equipping with a DIKMA Inertsil ODS-3 column (4.6 mm i.d. ×150 mm, 5 μm, Dikma, Japan), detected by UV–vis DAD detector and connected to a Chemstation system. The PMP derivative (20 μl) was injected, eluted with 82.0% phosphate-buffered saline (PBS, 0.1 M, pH 7.0) and 18.0% acetonitrile (v/v) at a flow rate of 1.0 mL/min at room temperature. The wavelength for UV detection was 245 nm.

2.5. Assay antioxidant activity in vitro

2.5.1. The DPPH scavenging activity

The DPPH quenching ability was measured by the method proposed by Konrath et al. (Yamaguchi, Takamura, Matoba, & Terao, 1998) with slight modifications. 1.5 mL freshly prepared DPPH solution (0.1 mM in methanol) was mixed with 4.5 mL of sample (0.1–0.6 mg/mL). The mixture was incubated at 25 °C for 30 min in the dark, and the absorbance of reaction liquid was measured at 517 nm. Vc was used as the positive control. The percentage scavenging radical was calculated using the following equation:

$$\text{Scavengingrate}(\%) = (A_0 - A_1)/A_0 \times 100$$

where A_0 is the absorbance of the control (water instead of sample), and A_1 is the absorbance of the sample.

2.5.2. Assay of hydroxyl radical scavenging activity

The assay was performed as described by the method of Chung et al. with minor changes (Chung, Osawa, & Kawakishi, 1997). 0.1 ml solution of various concentrations of sample (0.25–15 mg/ml) was added to 1.2 ml of 10 mM phosphate buffer (PBS) at pH 7.4 containing 2.67 mM 2-deoxyribose and 0.13 mM EDTA. 0.2 ml of iron ammonium sulfate (0.4 mM) was added. Samples were kept in a water bath at 37 °C for 15 min; the reaction was started by adding 100 μl of ascorbic acid (1.0 mM) and 10 μl of H_2O_2 (0.1 M). Samples were maintained at 37 °C for 15 min, and then 2 ml of 1% TBA and 2 ml of 2% CCl_3COOH was added to the resulting mixture. Finally the mixture was heated in boiling water for 15 min and cooled by ice water. The absorbance was determined against a blank at 532 nm with spectrophotometer. Vc was used as the positive control. The scavenging percentage was calculated as following:

$$\text{Scavengingrate}(\%) = (A_0 - A_1)/A_0 \times 100$$

where A_0 is the absorbance of the control (water instead of sample), and A_1 is the absorbance of the sample.

2.5.3. Assay ferrous ion-chelating potential

The ferrous ion-chelating potential of sample was determined as in Dinis, Maderia, and Almeida (1994). The reaction mixture contained 1.0 ml of sample (0.5–10 mg/ml), 0.1 mL of $FeCl_2$ (2 mM) and 0.2 mL of ferrozine (5 mM). After incubating at 25 °C for 10 min, the absorbance was measured at 562 nm using EDTA as positive control. A lower absorbance indicates a higher chelating ability. The chelating ability was calculated using the following equation:

$$\text{Chelatingactivity}(\%) = (A_0 - A_1)/A_0 \times 100$$

where A_0 is the absorbance of the control (water instead of sample), and A_1 is the absorbance of the sample.

2.6. Assay of antioxidant activity in vivo

2.6.1. Animals and treatment

ICR male mice weighing 20.0 ± 2.0 g were obtained at 6–8 weeks of age. The mice were purchased from the Changchun Institute of Biological Products Co., Ltd. The mice were randomly housed by ten into cages and received standard mouse chow and water. The room conditions were maintained at room temperature with 12/12-h

Table 1
Content of sugar, ginsenosides and total phenols in WGOS-R, WGOS-F and WGOS-L.

Samples	Sugar (% of extract)	Ginsenosides (% of extract)	Total phenols (mg/100 g of extract)
WGOS-R	95.87	1.33	650
WGOS-F	87.07	2.48	1115
WGOS-L	83.29	4.62	1150

Values are means of three replicates ± SD.

light–dark cycle. Rodent laboratory mouse chow and water were provided *ad libitum*.

Seven days of acclimatization, the mice were randomly divided into six groups (10 mice in each group): normal control group, D-Gal model control group, Vc positive control group and WGOS-R treated groups. Ten normal mice served as normal control group and received intraperitoneal injection of saline solution and distilled water orally. D-gal aging mice was induced in D-gal model control group, positive control group and three WGOS-R treated groups by intraperitoneal injection of 1.35 g/kg body weight of D-gal once a day for 6 weeks. Simultaneously, mice in D-gal model control group were orally administrated with saline solution. Mice in the positive group were orally administrated with 100 mg/kg of Vc. The WGOS-R treated mice were orally administrated with a different dose of WGOS-R at 10, 100 and 200 mg/kg, respectively. All groups were performed once daily for 6 weeks.

2.6.2. Biochemical assay

At 24 h after last drug administration, all mice were weighted and sacrificed, and the blood were collected and centrifuged at 4000 × *g* at 4 °C for 10 min to separate the serums required. Followed this procedure, the livers were taken out from animals, washed and then homogenized immediately in ice-cold physiological saline. The homogenate was centrifuged at 4000 rpm/min at 4 °C for 10 min, and the supernatant was collected and for further analysis. The activities of superoxide dismutase (SOD), catalase (CAT), glutathione (GSH-Px), and malondialdehyde (MDA) were assayed using commercial reagent kits obtained from the Institute of Biological Engineering of Nanjing Jianchen (Nanjing, China) according to the commercial kit manufacturer's instructions.

2.7. Statistical analysis

All values are expressed as the mean ± SD. Comparison between any two groups was evaluated using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons tests. Differences was considered to be statistically significant if $P < 0.05$.

3. Result and discussion

3.1. Characteristic of ginseng oligosaccharides

The yields of ginseng oligosaccharides were determined as WGOS-R (21.66%) > WGOS-F (19.94%) > WGOS-L (18.08%). According to the result of phenol–sulfuric method, WGOS-R contained higher amounts of sugar (95.87%) than WGOS-F (87.07%) and WGOS-L (83.09%). The total phenols content was relatively lower in WGOS-R (650 mg/100 g) than WGOS-F (1115 mg/100 g) and WGOS-L (1150 mg/100 g). The content of ginsenoside in WGOS-R, WGOS-F and WGOS-L was 1.33%, 2.58%, and 3.27%, respectively (Table 1). HPLC analysis showed that both WGOS-F and WGOS-L were composed of Glc and Rha in a molar ratio of 6.0:1.0 (Fig. 1B) and 7.0:1.0 (Fig. 1C), respectively. WGOS-R was only composed of Glc (Fig. 1A.).

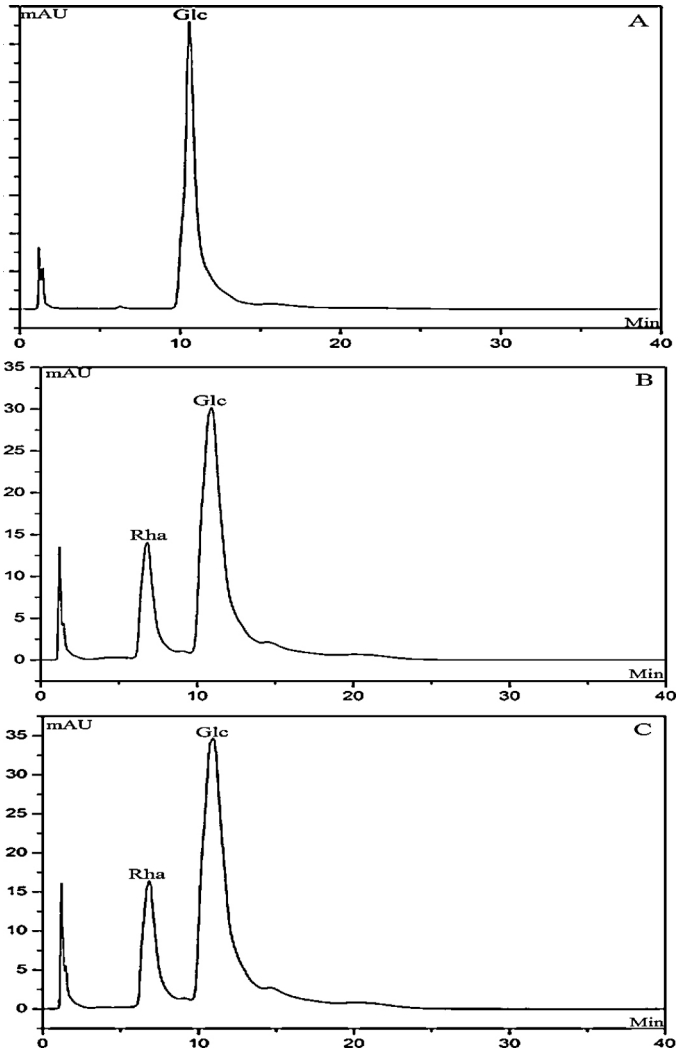

Fig. 1. HPLC analysis of monosaccharide composition of WGOS-R (A), WGOS-F (B) and WGOS-L (C). The three oligosaccharides were hydrolyzed, dried, PMP-labeled and analyzed by HPLC as described in the experimental methods.

3.2. Antioxidant antivities in vitro of ginseng oligosaccharides

3.2.1. DPPH radical scavenging activity

The antioxidant properties of ginseng oligosaccharides were investigated by DPPH assay. DPPH, a stable N-centered free radial, has been well used to employ the ability of free-radical scavenging properties or hydrogen donation of compounds and medicine materials (Hatano et al., 1989). As shown in Fig. 2, DPPH scavenging activities of the three oligosaccharides increased with increasing concentrations. At the concentration of 0.5 mg/ml, the DPPH radical scavenging rate of WGOS-R, WGOS-F and WGOS-L was 83.09%, 56.76% and 50.79%, respectively, with IC_{50} value of 0.069 mg/ml, 0.149 mg/ml and 0.273 mg/ml, respectively. Among the three oligosaccharides, WGOS-R possessed stronger scavenging activity than that of WGOS-F and WGOS-L. This was probably due to the difference in the monosaccharide composition between them. In addition, it should be noted that the total phenolic content in them was decreased in the order of WGOS-R < WGOS-F < WGOS-L, suggesting that the DPPH radical scavenging activities of ginseng oligosaccharides were probably owing to their carboxyl group in hexuronic acid, instead of phenolic compounds (Wang, Mao, & Wei, 2012).

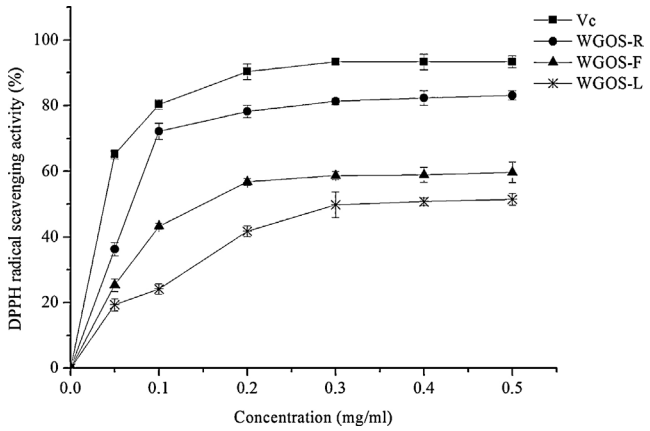

Fig. 2. DPPH radical scavenging activity of WGOS-R, WGOS-F and WGOS-L. Each value represents the mean ± SD ($n = 3$).

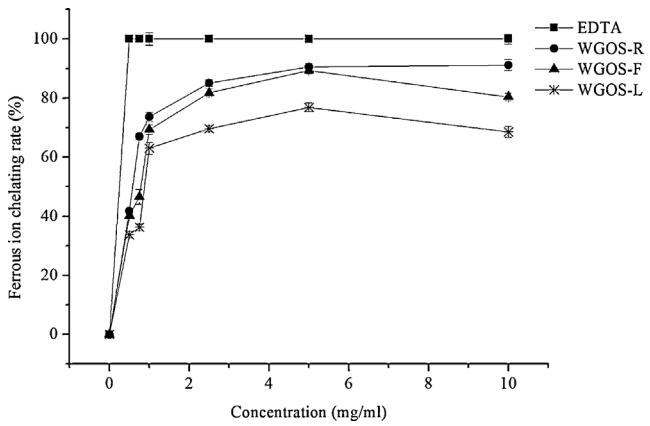

Fig. 4. Fe^{2+} chelating activity of WGOS-R, WGOS-F and WGOS-L. Each value represents the mean ± SD ($n = 3$).

3.2.2. Hydroxyl radicals scavenging activity

The hydroxyl radical is accepted to be the most reactive and poisonous free radical, and induce severe damage to adjacent biomolecules as an active initiator for lipids peroxidation (Chance, Sies, & Boveris, 1979; Ke et al., 2009). An accepted reaction system containing Fe^{3+}-EDTA-H_2O_2-deoxyribose in the aqueous phase was used to generate ·OH and measure inhibitory activity of ginseng oligosaccharides, and the results were summarized in Fig. 3. The three oligosaccharides were able to scavenge hydroxyl radical. At the concentrations ranging from 0.25 to 15 mg/ml, the hydroxyl radical scavenging ability of WGOS-R increased markedly and dose-dependently, and reached 76.85% at 15 mg/ml. Compared with WGOS-R, WGOS-F and WGOS-L exhibited a good scavenging activity at a low concentration (<10 mg/ml), but with the concentration increasing, the scavenging effect will not increase any more. The IC_{50} values of WGOS-R, WGOS-F and WGOS-L were 0.882 mg/ml, 0.961 and 3.33 mg/ml, respectively. In addition, both the IC_{50} values of WGOS-R and WGOS-F of hydroxyl radicals were lower than that of Vc (1.13 mg/ml).

3.2.3. Fe^{2+} chelating activity of ginseng oligosaccharides

Iron (Fe) and copper (Cu) ions are important elements for the human body, whereas these metal ions have potentially dangerous. Fe^{2+}, with high reactivity, can stimulate lipid peroxidation and accelerate lipid peroxidation, thereby driving the chain reaction of lipid peroxidation (Benedet & Shibamoto, 2008). So the Fe^{2+} chelating activity is considered as an important antioxidant property of materials. In this paper, the Fe^{2+} chelating ability was determined by the reduction of absorbance at 562 nm, the red color is quantitatively formed by the reaction of ferrozine with Fe^{2+}. As shown in Fig. 4, WGOS-R exhibited higher chelating activity than WGOS-F and WGOS-L. At the concentrations ranging from 0.5 to 10 mg/mL, the Fe^{2+} chelating activity of WGOS-R was measured as 41.70–91.19%, with IC_{50} value of 0.565 mg/mL. The Fe^{2+} chelating activity of WGOS-F and WGOS-L was 80.36% and 68.53% at 5 mg/mL, with the IC_{50} values of 0.774 and 0.867 mg/mL, respectively. The results indicated that WGOS-R and WGOS-F possessed a much stronger ferrous chelating activity than WGOS-L. However, the metal chelating activity of the three oligosaccharides was significantly lower than that of EDTA, which had the strongest chelating capacity, and achieved 100% at concentration of 0.5 mg/ml. The bioactivities of oligosaccharides are closed related to their molecular weight, monosaccharide composition, configuration of glycosidic linkages, position of glycosidic linkages, as well as the degree of substitution on alcohol or phenolic hydroxyl groups (Bohn & BeMiller, 1995). Thus, the structural features and structure–function relationships involved in Fe^{2+} chelating activities of ginseng oligosaccharides needed to be further studies.

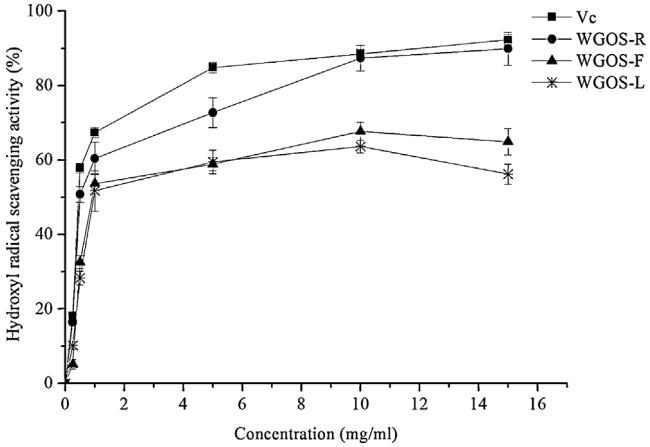

Fig. 3. Hydroxyl radical scavenging activity of WGOS-R, WGOS-F and WGOS-L. Each value represents the mean ± SD ($n = 3$).

3.3. Antioxidant activities in vivo of WGOS-R

3.3.1. Effect of WGOS-R in the activities of antioxidant enzymes in serum and liver of D-gal induced aging mice

Aging is the result of metabolism disorder. One of the aging theories is the free radical damage hypothesis proposed by Harman (1956). It states that endogenous oxygen radicals were formed as part of the normal metabolic processes and resulted in a pattern of increasing damage (Ho, Liu, & Wu, 2003). However, healthy body systems protect themselves against free radicals damage by antioxidant enzymes including SOD, CAT, GSH-Px, *etc.* SOD, a scavenger of superoxide radicals, protects against oxygen free radical by catalyzing the elimination of superoxide radical, which damages the membrane and biological structures. GSH-Px and CAT, the two antioxidant enzymes, play major roles in the decomposition of H_2O_2 to H_2O and O_2. Meanwhile, GSH-Px catalyze glutathione (GSH) into oxidized glutathione (GSSG), thereby reducing the toxic peroxide into non-toxitoc hydroxyl compounds, and protecting the cell membrane structure and function from damage caused by peroxides. In the present study, changes in the activities of antioxidant enzymes in mice were investigated. Effects of WGOS-R and Vc on activities of SOD, CAT and GSH-Px in serum and liver of aging mice were shown in Tables 2 and 3. D-gal injection caused a significant decrease ($P < 0.05$) in serum and liver antioxidant enzymes level in treated mice when compared with normal

Table 2
Effect of WGOS-R on activities of SOD (U/ml), CAT (U/ml), T-AOC (U/ml), GSH-Px (U/ml) and levels of MDA (nmol/ml) in serum of D-gal induces aging mice.

Group	SOD	CAT	GSH-Px	T-AOC	MDA
Normal control group	402.55 ± 52.93	174.53 ± 10.34	441.67 ± 19.59[a]	7.26 ± 0.63	37.36 ± 4.03
Model control group	306.79 ± 26.14[a]	143.78 ± 11.63[a]	357.14 ± 44.93[a]	5.43 ± 0.99[a]	42.10 ± 1.83[a]
Positive control group	375.16 ± 79.55[b]	229.18 ± 25.07[c]	591.07 ± 69.64[c]	7.52 ± 0.57[b]	32.29 ± 1.84[b]
WGOS-R (10 mg/kg)	438.19 ± 50.08[b]	197.11 ± 23.94[b]	654.76 ± 52.50[c]	12.31 ± 2.00[c]	31.13 ± 4.20[a]
WGOS-R (100 mg/kg)	392.88 ± 40.81[b]	171.70 ± 24.58[b]	447.02 ± 32.75[b]	7.99 ± 1.02[b]	34.95 ± 5.20[b]
WGOS-R (200 mg/kg)	346.50 ± 24.83	177.02 ± 12.57[b]	530.95 ± 68.19[b]	7.98 ± 1.18	33.22 ± 3.65[b]

Values were expressed as mean ± SD ($n = 10$).
[a] $P < 0.05$ *vs.* normal control group.
[b] $P < 0.05$ *vs.* D-gal group.
[c] $P < 0.01$ *vs.* D-gal group.

Table 3
Effect of WGOS-R on activities of SOD (U/mg protein), CAT (U/mg protein), T-AOC (U/mg protein), GSH-Px (U/mg protein) and levels of MDA (nmol/mg protein) in liver of D-gal induces aging mice.

Group	SOD	CAT	GSH-Px	T-AOC	MDA
Normal control group	154.71 ± 4.55	31.80 ± 1.97	3937.35 ± 269.73[a]	1.44 ± 0.09	1.26 ± 0.21
Model control group	145.55 ± 2.43[a]	24.68 ± 7.08[a]	2917.47 ± 357.81	0.80 ± 0.14[a]	2.23 ± 0.16[a]
Positive control group	150.72 ± 4.41[c]	28.67 ± 5.00[b]	3381.05 ± 660.46	1.56 ± 0.05[b]	1.72 ± 0.33[c]
WGOS-R (10 mg/kg)	165.49 ± 10.69[c]	41.94 ± 9.18[b]	4052.14 ± 662.19[c]	1.90 ± 0.01[c]	1.66 ± 0.02[c]
WGOS-R (100 mg/kg)	154.11 ± 2.93[c]	30.25 ± 1.46[b]	3770.46 ± 295.58 [b]	1.76 ± 0.11[c]	1.76 ± 0.12[c]
WGOS-R (200 mg/kg)	151.01 ± 2.98[c]	30.01 ± 1.35[b]	3662.29 ± 595.34 [b]	1.59 ± 0.12[c]	1.75 ± 0.04[c]

Values were expressed as mean ± SD ($n = 10$).
[a] $P < 0.05$ *vs.* normal control group.
[b] $P < 0.05$ *vs.* D-gal group.
[c] $P < 0.01$ *vs.* D-gal group.

mice (Table 2). Both in serum and liver, WGOS-R at the dose of 10–200 mg/kg and Vc administration could increase the activities of CAT and GSH-Px obviously ($P < 0.05$ or $P < 0.01$) as compared to aging mice. Similarly, treatment with 10–100 mg/kg of WGOS-R also increased SOD activity significantly, and all the maximal effect occurred at 10 mg/kg. As reported, the improved effects on antioxidant enzymes of some polysaccharides were partially due to their immune activities (Liu et al., 2010; Yuan et al., 2009). Our previous studied had demonstrated that WGOS-R could stimulate macrophages and spleen proliferation, suggesting the enhanced activity of antioxidant enzymes may be related to its immune enhancement (Jiao et al., 2012; Wan, Jiao, Yang, & Shu, 2011).

Lipid peroxidation, a process provoked by free radicals, generates many aldehyde products, among which MDA is regarded as the main product of the endogenous lipid peroxidation and has been used as markers of oxidative stress (Urso & Clarkson, 2003). In this study, the MDA levels in mice serum and liver were measured using the thiobarbituric acid method. It is based on the formation of the red adduct in acidic medium between thiobarbituric acid and malondialdehyde, a colorless product of the lipid peroxidation, measured at 532 nm. Tables 2 and 3 showed the experiment results for MDA assay. After the administration of D-gal at a dose of 1.35 g/kg body weight once daily for 6 weeks, an obvious increase in MDA production was observed compared with that of normal control ($P < 0.05$). In contrast, this increase was reversed by treatment of WGOS-R at dose of 10, 100 and 200 mg/kg and Vc. The findings suggested that WGOS-R could significantly improve the MDA levels in the D-gal-induced mice.

T-AOC represents the non-enzymatic antioxidant defense system. Therefore, measure of serum and tissues T-AOC may reflect the influence of samples to non-enzymatic antioxidant defense system in aging mice. As shown in Tables 2 and 3, T-AOC levels were decreased remarkably in the D-gal-treated mice both in serum and liver compared with the normal mice ($P < 0.05$). This decrease was recovered by treatment of WGOS-R at doses of 10–200 mg/kg and Vc at 100 mg/kg, respectively. Notably, WGOS-R, WGOS-F and WGOS-L showed much lower antioxidant activity than Vc *in vitro*, but WGOS-R (10, 100 and 200 mg/kg) exhibited higher T-AOC levels than Vc in antioxidant *in vivo* assay at all tested doses. And this result can be corroborated by many other investigations (Anderson & Phillips, 1999; Fardet, Rock, & Rémésy, 2008; Liu et al., 2010). The paradoxical results might be because of the different influencing factors of antioxidant effects between *in vitro* and *in vivo* assays. Numerous factors may influence the antioxidant action of the compound *in vivo*, such as digestibility, bioavailability and metabolism (Fardet et al., 2008). In addition, WGOS-R modulated the T-AOC of serum and liver, meaning that WGOS-R not only promoted the enzymatic antioxidant defense system but also enhanced the non-enzymatic antioxidant defense system.

This study firstly represents the antioxidant activity of the oligosaccharides isolated from *P. ginseng* roots, flowers and leaves. On the basis of the results above, it can be clearly concluded that WGOS-R had stronger antioxidant activity than WGOS-F and WGOS-L *in vitro*. However, the content of ginsenosides and total phenolic in WGOS-L was higher than that of WGOS-R and WGOS-F, indicating oligosaccharide in them played key role in their antioxidant property. Furthermore, administration of WGOS-R could significantly enhance the activities of antioxidant enzymes (SOD, CAT and GSH-Px) and total antioxidant capability (T-AOC) in the serum and liver, and decrease malondialdehyde (MDA) production in serum and liver of aging mice. These results revealed that WGOS-R could be considered as a potential and natural antioxidant.

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