INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
18F–119/2018/20–10–2349–2356
DOI: 10.17957/IJAB/15.0814
http://www.fspublishers.org

Full Length Article

Antioxidant and Antitumor Activity of a Crude Glycosaminoglycan Extracted from *Sanguinolaria acuta*

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Abstract

Sanguinolaria acuta is a kind of delicious sea food in China and used as drugs. The antioxidant and antitumor activities of a crude glycosaminoglycan from *S. acuta* (SAG-1) were investigated. SAG-1 had the scavenging effect to free radicals. The IC_{50} value of diphenyl-picryl hydrazide (DPPH), hydroxyl radicals, and superoxide free radicals were 8.23, 12.2, and 13.2 mg/mL, respectively. The 10 mg/mL SAG-1 inhibition rate of lipid peroxidation of mice livers *in vitro* was 39.8%. *In vivo*, when compared with the normal mice group, D-galactose-induced aging mouse model group showed lower superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidases (GPX) activities in serum, liver, and kidney; and higher malondialdehyde (MDA) concentration (P<0.05). However, when 200 mg/kg·d SAG-1 was added to the aging mice, which showed an increased SOD and CAT activity in liver, kidney and GPX activity in serum, liver (P<0.05). The MDA concentration decreased significantly in the three tissues (P<0.05). The results showed that SAG-1 reduced the oxidative stress in aging mice and had the similar effect with Vc. *In vitro*, SAG-1 showed inhibitory effect on the proliferation of HeLa and CNE-2Z tumor cells. These findings indicate that the crude polysaccharide derived from *S. acuta* possesses antioxidative and antitumor activity, and may be used as a natural antioxidant.

Keywords: Oxidative stress; Antitumor activity; D-galactose induced aging model; Free radicals; Glycosaminoglycan from *Sanguinolaria acuta* (SAG-1)

Introduction

Many researches indicated that oxidative stress damage caused by reactive oxygen species (ROS) and oxygen free radicals can induce aging, cancer and cell death (Finkel *et al*., 2000; Seifried *et al*., 2007; Valko *et al*., 2007; Fuchs-Tarlovsky, 2013). Natural antioxidants which come from plant and animals can be used to resist free radical damage and prevent the occurrence and deterioration of diseases. Many polysaccharides obtained from marine materials have been proved to provide higher scavenging free radical activities that may supposed be used to prevent and cure diseases (Lin *et al*., 2009; Qiao *et al*., 2009; Zhu *et al*., 2010; Jiang *et al*., 2013; Fan *et al*., 2017).

Free radical theory is one popular mechanism of aging, which is based on the theory that the excessive production of free radicals during aerobic metabolism causes oxidative stress that destroys biomolecules in the body, and the structure and function of cells (Finkel and Holbrook, 2000). The aging mouse model induced by D-galactose has been extensively applied based on its physiological and genetic similarities to humans, and low-cost maintenance particularly in long-term studies (Boguski, 2002; Nadon, 2006; Tang and He, 2013; Govindan *et al*., 2016). Recently, the aging mouse model was applied to research the antioxidant activities *in vivo* of polysaccharides (Ke *et al*., 2009; Xu *et al*., 2011; Ding *et al*., 2016; Huo *et al*., 2016).

Mollusks are delicious food for the high-quality nutrition and used as traditional Chinese medicinal drugs for centuries. Now, the water-soluble polysaccharides from mollusks have been researched due to their wide range of bioactivities. Previous reports have indicated that in traditional Chinese medicine, mollusks such as *Mytilus coruscus, Mixylla rosacea, Hyriopsis cumingi, Cyclina sinensis, Haliotis Discus* were utilized to cure of inflammation, asthma, and dental ulcers (Pomin, 2009; Qiao *et al*., 2009; Saravanan and Shanmugam, 2010; Zhu *et al*., 2010; Jiang *et al*., 2011).

Sanguinolaria acuta is a kind of widely cultured shellfish in China. And it has abundant proteins, amino acids, and polysaccharides. However, compared to other bivalve species, there has less research about the *S. acuta* polysaccharides. This study researched the scavenging activity of free radicals and antitumor activity *in vitro* of a crude glycosaminoglycan from *S. acuta*, SAG-1. The aging

To cite this paper: Fan, X., X. Qin and C. Wu, 2018. Antioxidant and antitumor activity of a crude glycosaminoglycan extracted from *Sanguinolaria acuta*. *Int. J. Agric. Biol*., 20: 2349–2356

mouse model induced by D-galactose was applied to detect the antioxidation of SAG-1 *in vivo*. Three tumor cell lines were used to evaluate the antitumor activity of SAG-1 *in vitro*. The results of this study provide reference for the novel polysaccharide antioxidants and its utilization of *S. acuta*.

Materials and Methods

Materials and Extract Preparation

S. acuta was purchased from the Zhanjiang Dongfeng aquatic product market (Zhanjiang, China). The shell was removed and the whole viscera was frozen at -18°C for later use. The whole viscera was hydrolyzed by an enzyme mixture of two proteases (1.5% papain: subtilisin at a ratio of 3:4). The hydrolysate was decolored with carbon and diatomaceous earth, and then precipitated by ethanol at a total content of 75% (v/v). The precipitate was washed with ethanol and alternating with acetone thrice. After the precipitate was decolored, proteins removed, subjected to ultrafiltration, and freeze-dried, crude glycosaminoglycan (SAG-1) was obtained. The yield of the crude polysaccharide was 1.48%. And in the nutritional component of SAG-1, total sugar was 63.15%, uronic acid 16.75%, glycosaminoglycan 40.05%, and sulfate 1.69%.

HeLa, CNE-2Z, and K562 cells were supplyed by the Shanghai Institute of Cell Biology (Shanghai, China).

Chemicals

D-galactose was analytic reagent(AR) purchased from the Beijing Chemical-Reagent Co. (Beijing, China). Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and malondialdehyde (MDA) testing commercial kits were obtained from the Nanjing Jiancheng Biotechnology Institute (Nanjing, China). a,a-Diphenyl-bpicrylhydrazyl (DPPH), pyrogallic acid and 1,10-phenanthroline were obtained from Sigma-Aldrich (St. Louis, MO, USA). RPMI-1640 culture medium, fetal bovine serum and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Gibco Co. (Domestic packing in China). Others are analytical reagents.

Experimental Animals and Feeding

SPF level Kunming mice, Male, weighing 20±2 g, and 4 weeks old, were obtained from the Guangdong Province Medical Experimental Animal Center, which was accredited by the CNAS National Laboratory in 2008. The mice were raised under the conditions with the 40%–60% humidity, 20°C–22°C. The mice were fed on a standard pellet diet (GB14924.3-2001, mouse feed standard, purchased from Guangdong Medical Experimental Animal Center, China). The pellet diet consisted of 8.22% water, 21.54% crude protein, 4.67% crude fat, 3.50% crude fiber, and 5.43% ash. Investigation on animals were conducted according the rule published by the US National Institutes of Health (Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, revised 1996). Guangdong Ocean University Ethics Committee supplied this research protocol.

Antioxidant Activity of SAG-1 *in vitro*

Scavenging activity of hydroxyl radical: According to the phenanthroline-Fe (ii) oxidation assay method (Zhong *et al.*, 2010), scavenging activity of hydroxyl radical was determinded. Samples (1 mL) with different concentrations, incubated with 0.75 mol/L phenanthroline (1 mL), 0.75 mmol/L $FeSO_4$ (1 mL), 0.01% H_2O_2 (1 mL), distilled water (2 mL) at 37°C and for 60 min. The OD_{536} was detected in Varioskan enzyme reader (ThermoFisher, USA). In A_p tube sample was instead by distilled water, in A_b tube H_2O_2 was replaced by distilled water. Ascorbic acid (Vc) replaced sample in positive control group. The scavenging ratio of hydroxyl radical was calculated by the formula:

$$E\% = (A_s - A_p)/(A_b - A_p) \times 100\%$$

Superoxide radical scavenging activity: Pyrogallol autoxidation method was used to detect the superoxide radical scavenging activity of SAG-1 (Zhong *et al.*, 2010). 15 μL SAG-1 with different concentrations were added into 250 μL, 0.05 mol/L Tris-HCl buffer solution (pH 8.2) for 10 min at 25°C, then add 25 μL pyrogallol solution to 1.25 mmol/L of water for 10 min, shaking quickly, measuring the absorbance at 325 nm every 30 s using a multifunctional enzyme standard. A total of 10 tests were performed for 5 min. The scavenging ability for inhibition of pyrogallol autoxidation was calculated by the formula:

$$E\% = (S_0 - S_1)/S_0 \times 100$$

Where S_0 was the slope of sample group, S_1 was the slope of blank control group. Vc replaced sample in positive control group.

DPPH radical-scavenging activity: The DPPH reaction system was tested as follow: In the test tube, 0. 5 mL 0. 6 mmol/l DPPH methanol solution and 1 mL of different concentration samples were added in turn, then 3.5 mL ethanol was added. The tube was incubated in dark at room temperature for 30 min, then was detected the absorbance of 517 nm wavelength. Vc replaced sample in positive control group. The scavenging ability was calculated by the formula:

$$\text{Scavenging activity } (\%) = [(A_0 - A_1)/A_0] \times 100\%$$

Where A_0 was the absorbance of the control and A_1 was the absorbance of sample or Vc.

Inhibited effect of Lipid Peroxidation *in vitro*

Thiobarbituric acid-reactive-substances (TBARS) assay was used to detect the lipid peroxidation inhibited effect in mouse liver homogenate. 1.0 mL sample solution with different concentration was added to 1.5 mL of 1% liver homogenate (w/v), then 0.05 mL of 0.5 mmol/L $FeCl_2$

and 0.5 mmol/L H_2O_2 were added in a tube. The tube was incubated for 60 min at 37℃ and then immediately add 20% (w/v) trichloroacetic acid 1.5 mL to precipitate protein, and centrifuged 3500 rpm for 10 min. The 3 mL supernatant was took out to a new tube, then add 0.8% (w/v) thiobarbituric acid (TBA) solution 1.5 mL in the new tube and heated for 15 min at 100°C, then centrifuged for 10 min at 4,000 rpm. The supernatant liquid was measured the absorbance at 532 nm. The inhibition effect on lipid peroxidation *in vitro* was calculated as formula:

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

Where A_0 is the absorbance of the control (water instead of sample), A_1 is the absorbance of the sample or Vc.

In vivo Antioxidant Activity of SAG-1 in Mice Aging Model reduced by D-galactose

Mice were housed under standard conditions. The mice were raised for one week acclimatization and divided into six groups randomly. Group NC (normal control group) were treated with saline by oral perfusion and intraperitoneal injection (i.p.), both are treated at a dose of 20 mL/kg body weight (BW) each day. The aging mouse model was induced by i.p. 5% (w/v) D-galactose dissolved in 0.9% (w/v) saline at a dose of 200 mg/kg BW each day for six weeks. From day 15, group AC (aging control group) were administered 0.3 mL saline by oral. Group PC (positive control group) received vitamin C at a dose of 10 mg/kg BW by oral. Group SAG-1H, M, and L (high-, medium-, and low-dosage group) were administered SAG-1 at a dose of 200 mg/kg, 100 mg/kg, and 50 mg/kg BW each day, respectively, by oral.

After 24 h of the final administration, blood, Liver and kidney samples were collected. Blood were centrifuged for 10 min at 4,000rpm, at 4°C, then separated the sera and stored at -80°C for further analysis. Liver and kidney samples were homogenized in cold saline and prepared 10% (w/v) of the tissue homogenates. The protein concentration was detected by the Lowry method. SOD GPX, CAT activities and MDA levels in the serum, liver, and kidney were analyzed by the assay kits following the test method specification.

Inhibitory Activity of SAG-1 on HeLa, CNE-2Z, and K562 Cell Proliferation

MTT method was used to determine the inhibition rates of SAG-1 on the proliferation of HeLa, CNE-2Z, and K562 cells (Liu *et al*., 2017). RPMI-1640 medium adding 10% (v/v) FBS, 100 unit/mL of penicillin and 100 unit/mL of streptomycin were added to inhibition of bacterial growth, was applied to foster the cancer cells. The cell suspension with a density of 2×10^4 cells/mL was added to a 96-well plate in the concentration of 100 μL/well and incubated at 37°C in 5% CO_2 incubator. Each concentration have three replicates. After 24 h, 20 μL of sample at different concentrations was added and blended with the cancer cells. After 44 h incubation, each well was added 20 μL MTT with the concentration 5 mg/mL, and the 96-well plate was shaked for 5 min at 800rpm in microplate oscillator, then incubated for 4 h in CO_2 incubator. In each well, 100 μL of 10% SDS solved in 0.01 M HCl was added and agitated to dissolve the formazan crystals. The OD_{570} value was detected in Varioskan enzyme reader. The inhibitory rate was calculated as follow:

$$\text{Inhibition rate (\%)} = [1 - (A_1/A_0)] \times 100$$

Where A_0 and A_1 are the OD_{570} value of control and sample, respectively.

Statistical Analysis

Data were expressed as the mean ±standard deviation (SD). One-way ANOVA and LSD test were used to evaluate data by the SPSS 17.0 software package. $P < 0.05$ was represented statistically significant.

Results

Scavenging Activity of SAG-1 for Free Radicals *in vitro*

DPPH free radicals are stable free radicals that have been utilized in evaluating the free-radical scavenging activities *in vitro* of antioxidants (Hu *et al.*, 2004). For SAG-1, the DPPH scavenging effects increased with higher concentrations. Vc had excellent scavenging activity which the half maximal inhibitory concentration (IC_{50} value) was 0.039 mg/mL (Fig. 1a). SAG-1 also showed scavenging activity, and the IC_{50} value was 8.23 mg/mL (Fig. 1b).

Hydroxyl free radicals (·OH) are highly reactive with a wide range of biomolecules, thus scavenging ·OH is an important antioxidant activity. Fenton reaction was often used for ·OH formation. Using this reaction, the IC_{50} value of SAG-1 was 12.2 mg/mL (Fig. 1d), whereas that of ascorbic acid was 0.38 mg/mL (Fig. 1c).

Among various ROS, superoxide free radical ($\cdot O_2$) is generated first. And$\cdot O_2$ may be decomposed to form singlet oxygen and OH, which are stronger ROS and induce lipid peroxidation. O_2 is also induce indirectly peroxidation of lipids to produce H_2O_2, which is precursor of OH. So, $\cdot O_2$ scavenging effect is important in antioxidant activity. Using this method, the IC_{50} value of SAG-1 was 13.2 mg/mL (Fig. 1f), while that of ascorbic acid was 0.24 mg/mL (Fig. 1e).

Inhibition of Lipid Peroxidation

$FeCl_2$-H_2O_2 system was often applied to induce lipid peroxidation of mouse liver (Liu *et al*., 2010). SAG-1 showed inhibitory effects of lipid peroxidation, which increased with higher sample concentrations. The highest

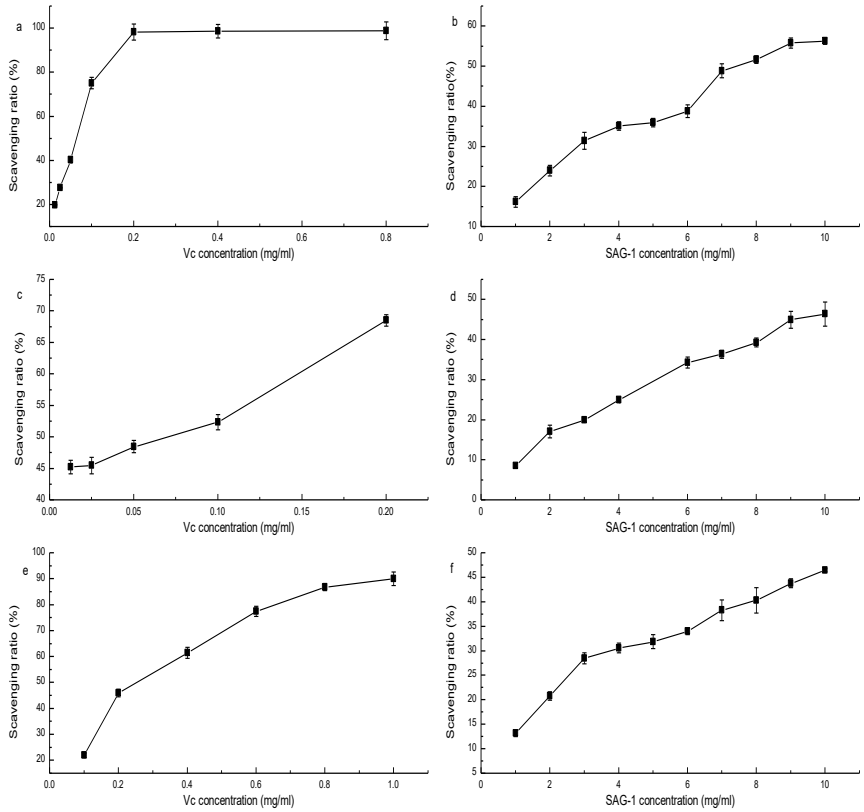

Fig. 1: Scavenging activity of V_C and SAG-1 for three free radicals, a and b are DPPH free radicals, c and d are hydroxyl free radicals, and e and f are superoxide free radicals

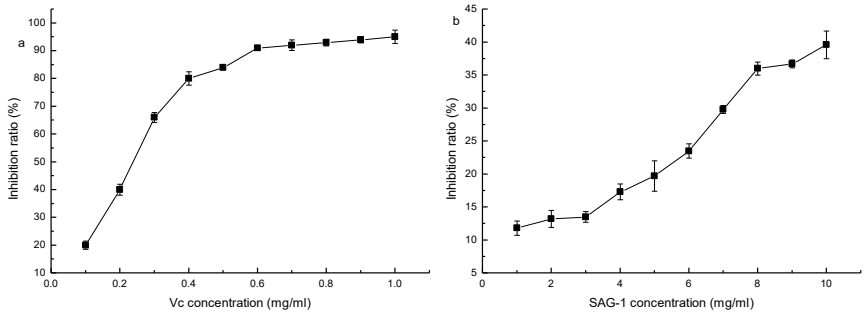

Fig. 2: Inhibition of lipid peroxidation by SAG-1. The absorbance values were converted to inhibition ratio (%) and the data were plotted as the mean of replicate inhibitory effects (%) ± 1 S.D. (n = 3) against SAG-1 concentration in mg SAG-1/mL reaction volume

inhibitory rate was 39.8% when the SAG-1 concentration was 10 mg/mL, which is lower than that of Vc (Fig. 2).

These findings indicate that SAG-1 has moderate inhibitory effects on lipid peroxidation compared to V_C. The inhibitory effects of antioxidants on lipid peroxidation may realte to their scavenging abilities for $OH^{\cdot}$ radicals and H_2O_2 that were generated by $FeCl_2$-H_2O_2 in this reaction system (Wang *et al.*, 2008). The lipid peroxidation inhibitory effects of polysaccharides had relation to their scavenging abilities of hydroxyl free radical and H_2O_2 (Liu *et al.*, 2007, 2010). In this research, the inhibitory effects of SAG-1 on lipid peroxidation may be attributable to the scavenging abilities for DPPH, OH, and superoxide radicals.

In vivo Antioxidant Activities of SAG-1

Aging mice model induced by D-galactose: D-Galactose are often induced aging mouse model which was used in the evalution of the antioxidant activity of SAG-1 *in vivo*. Table 1 shows that there were no significant differences in weight change among the experimental groups (p>0.05), indicating that the

Table 1: Weight change of the mice before and after the six-week experiment ($\bar{x} \pm s$, n=12)

Group	Dose (mg/kg·d)	Weight before (g)	Weight after 6w (g)	Increase in weight (g)
NC		32.32 ± 1.54	48.23 ± 4.23	15.92 ± 3.42
AC		30.47 ± 1.81	46.48 ± 2.43	16.02 ± 3.63
PC	10	30.55 ± 1.16	47.30 ± 2.72	16.75 ± 3.41
SAG-1L	50	31.97 ± 1.37	46.96 ± 1.91	14.99 ± 1.78
SAG-1M	100	29.65 ± 1.17	47.67 ± 2.59	16.02 ± 6.54
SAG-1H	200	29.70 ± 1.41	47.31 ± 3.22	17.61 ± 2.97

Table 2: Effect of SAG-1 on the activity of SOD, GPX, CAT and the levels of MDA in serum, liver, and kidney of D-gal-induced aging mice ($\bar{x} \pm s$, n = 12)

	NC	AC	PC (10 mg/kg·d)	SAG-1L (50 mg/kg·d)	SAG-1M (100 mg/kg·d)	SAG-1H (200 mg/kg·d)
Serum						
SOD (U/mL)	186.21 ± 5.32	166.97± 4.44[a]	179.55 ± 1.02[c]	170.95 ± 13.74	171.61 ± 18.04	174.36 ± 11.09
GPX (U/mL)	336.67 ± 8.62	234.67± 15.01[b]	308.66± 15.20[d]	291.33 ± 5.13[c]	302.67 ± 3.05 [d]	329.33 ± 10.01[d]
CAT (U/mL)	6.56 ± 1.45	3.12 ± 0.71[a]	5.31 ± 0.74[d]	4.75 ± 0.77[c]	4.90 ± 0.14[d]	5.61 ± 0.39[d]
MDA (U/mL)	5.23 ± 0.44	7.02 ± 1.32[a]	5.40 ± 0.41[c]	6.14 ± 1.20	5.38 ± 1.02[c]	5.28 ± 0.78[c]
Liver						
SOD (U/mL)	161.30 ± 15.06	119.08 ± 9. 51[a]	132.29 ± 9.20	137.08 ± 13.89	148.76 ± 8.37[c]	142.94 ± 3.35 [c]
GPX (U/mL)	260.60 ± 24.21	162.20 ± 22.03[b]	255.68 ± 14.14[d]	168.45 ± 16.48	239.21 ± 23.75 [c]	254.27 ± 26.69
CAT (U/mL)	34.07 ± 3.11	24.40 ± 3.09[b]	33.76 ± 5.33 [d]	25.99 ± 3.09	26.68 ± 1.94	31.19 ± 4.10[d]
MDA (U/mL)	4.19 ± 0.96	5.23 ± 0.32[a]	4.42 ± 0.88 [c]	4.81 ± 0.98	4.86 ± 1.07	4.55 ± 0.67 [c]
Kidney						
SOD (U/mL)	167.49 ± 6.73	136.40 ± 9.51[b]	165.11 ± 14.29	155.88 ± 6.02 [d]	160.34 ± 14.80 [d]	161.37 ± 8.03 [d]
GPX (U/mL)	73.46 ± 5.81	54.74 ± 7.91b	70.91 ± 9.95d	65.49 ± 11.5	68.98 ± 8.80c	69.45 ± 2.59c
CAT (U/mL)	28.26 ± 0.30	22.46 ± 0.52[b]	26.44 ± 2.37[c]	23.24 ± 1.45	23.99 ± 1.60	26.24 ± 1.50[c]
MDA (U/mL)	2.90 ± 0.62	4.19 ± 0.49[b]	3.10 ± 1.33[c]	3.51 ± 1.60	3.08 ± 0.86[d]	2.59 ± 0.28[d]

The data are expressed the mean ± SD, n = 12 for each group. [a]$P<0.05$ compared to the normal control group (NC); [b]$P<0.01$ compared to the normal control group (NC); [c]$P<0.05$ compared to the aged control group (AC); [d]$P<0.01$ compared to the aged control group (AC)

injection of D-glucose and SAG-1 did not affect the growth, diet of mice. Furthermore, AC group showed a marked increasing in MDA levels and decrease in SOD, GPX, and CAT activity in sera, livers, and kidneys when compared to the NC group ($p<0.05$ or $p<0.01$) (Table 2).

The decreased of antioxidant enzymes acitivities and MDA production indicated D-Galactose injection induced the aging mouse, which were consistent with the reports from Huo and Zhong (Zhong *et al.*, 2010; Huo *et al.*, 2016).

Effect of SAG-1 on the antioxidant enzyme activities in serum, liver, and kidney of aging mice modle: The effects of SAG-1 and Vc on of SOD, GPX, and CAT activities in serum, liver, and kidney of mice were shown in Table 2. In serum, compared to the AC group, the GPX and CAT activities had significant increase in the SAG-1L, M, H groups ($p<0.05$ or $p<0.01$), whereas SOD activity increased but had no significant ($p>0.05$). The PC group also showed a significant increase in the three antioxidant enzymes activities ($p<0.05$ or $p<0.01$).

In the liver, SAG-1H group that treated with 200 mg/kg SAG-1 showed marked increase in the SOD, GPX, and CAT activities compared to AC group ($p<0.05$ or $p<0.01$); the 100 mg/mL SAG-1 group (SAG-1M) showed an increase in SOD and GPX activity ($p<0.05$), and the low dose group (SAG-1L, 50 mg/kg SAG-1) showed no significant change. The PC group had a highly significant increase in GPX and CAT activity ($p<0.01$).

In the kidney, the SAG-1L, M, and H groups showed a significant increase in SOD activity in aging mice ($p<0.01$). GPX activity in the SAG-1H, M groups remarkably increased ($P<0.05$). CAT activity in the SAG-1H group increased significantly ($p<0.05$). PC group had a marked increase in GPX and CAT activity of aging mice ($p<0.05$), which is similar to that in the liver.

Effect of SAG-1 on MDA concentratration in serum, liver, and kidney of aging mice induced by D-galactose: Lipid peroxidation is an oxidative metamorphic reaction. Free radicals and some small molecules produced by lipid peroxidation can damage the function of many fine cells, such as changing the permeability and fluidity of cell membrane. It also causes damage to protein and DNA (causing a variety of base damage and the formation of various fluorescent products), and selective damage to DNA molecule guanine base, which is closely related to the occurrence and development of many diseases. Therefore, the inhibition of lipid oxidation becomes very important (Frolich *et al.*, 2010). As a product of lipid peroxidation, MDA was often used as the index of lipid peroxidation in body. The decrease of MDA concentration indicates that the level of lipid peroxidation decreases and the oxidative stress decreases (Finkel *et al.*, 2000). The MDA levels in the SAG-1H (200 mg/kg group) and PC group (V_C) were significantly decreased in the tissues (serum, liver, and kidney). The results suggested that the administration of SAG-1 and V_C could inhibite the lipid peroxidation in aging

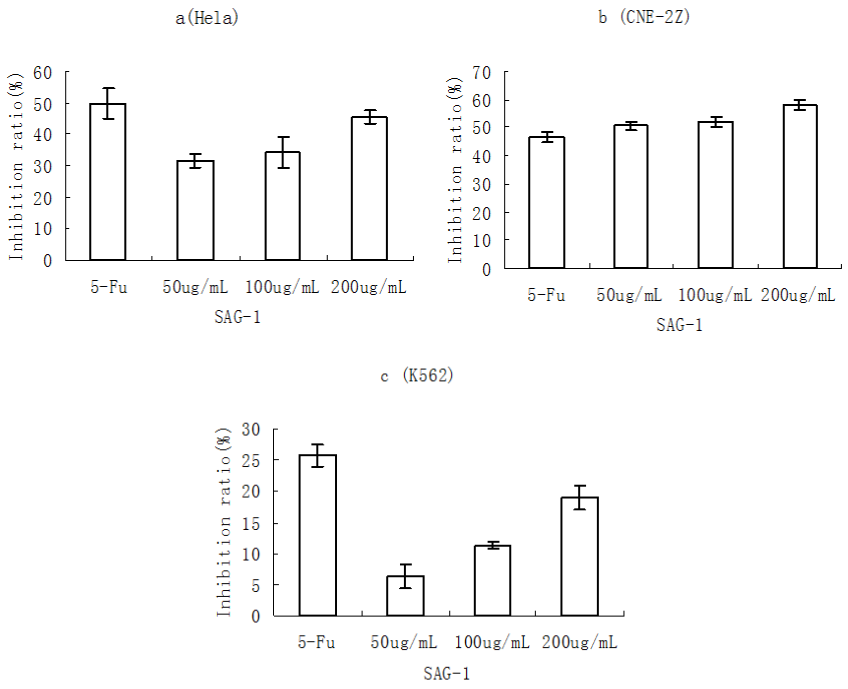

Fig. 3: Inhibitory effect of SAG-1 on the proliferation of HeLa (a), CNE-2Z (b), and K562 (c) tumor cells

mice effectively. *In vitro* detections, the inhibition of lipid peroxidation of SAG-1 was much lower than that of V_C, but *in vivo*, the inhibition of SAG-1was similar or stronger than that of V_C. These findings of SAG-1 were in accordance with that of previous studies (Fardet *et al.*, 2008; Liu *et al.*, 2010). The discrepancies of inhibitory effects between *in vitro* and *in vivo* are possibly due to the differences in their responses to abiotic and biotic factors.

Inhibitory Effect of SAG-1 on Tumor Cell Proliferation

The inhibitory effects of SAG-1 on the HeLa, CNE-2Z, and K562 tumor cells proliferation were indicated in Fig. 3. SAG-1 had significant inhibition in the proliferation of tumor cells in a dose-effective ralationship. The inhibition rates of 200 μg/mL SAG-1 on HeLa, CNE-2Z, and K562 tumor cells were 45.61, 58.14 and 18.98%, respectively. For the CNE-2Z cells, the inhibitory effect of SAG-1 was similar or higher to that of 5-fluorouracil (5-FU), which is a typical antitumor drug.

The level of oxidative tress is strongly related with the malignant proliferation and transformation of tumor cells. Compounds which can decrease the level of oxidative stress in tumor cells can inhibit tumor cell proliferation and transformation (Leng *et al.*, 2005; Jiang *et al.*, 2011). SAG-1 has the anticancer activity *in vitro*, which might be correlated with its free radicals scavenging activity *in vitro* and decreasing oxidative stress in vivo. The relationship of antioxidative and anticancer activity can be further researched.

Discussion

The free radical scavenging activity of polysaccharides have been investigated in various marine organisms. *In vitro* SAG-1 showed lower scavenging activity to free radical than Vc and appeared to be similar to polysaccharides from *Cyclina sinensis* (Jiang *et al.*, 2011), *Hyriopsis cumingii* (Qiao *et al.*, 2009), *Holothuria fuscogliva* (Li *et al.*, 2017). These results showed that the polysaccharide has the antioxidant activity *in vitro* but the bioactivity was affected by the composition in extracted substances. So the SAG-1 should be purified and researched for its activity.

Free radicals are regarded as products of aerobic metabolism in the human body. Previous studies have reported that natural polysaccharide extracted from marine animals have free radical scavenging activities (Zhu *et al.*, 2010; Jiang *et al.*, 2011, 2013; Liu *et al.*, 2012). Many structure features including the composition and type of major monosaccharide, degree of sulfation, molecular weight, and glycosidic branching affected the antioxidant activity of polysaccharides (Zhang *et al.*, 2011). The underlying mechanism may be related with the supply of hydrogen. The findings of this study indicate that the free radical scavenging effect of SAG-1 may be related with the presence of sulfate groups. And the antioxidant activity of SAG-1 may be a function of several factors.

D-galactose can be metabolized by two enzymes, D-galactokinase and galactose-1-phosphate uridyltransferase, in normal body. Excessive D-galactose can be catalyzed

into galactitol by aldose reductase, which cannot be metabolized and will accumulate in cells in turn. Therefore, the excessive D-galactose lead to stress and generate ROS (Hsieh *et al.*, 2009). And the accumulation of glycation end-products (AGE) in body, which produced by reaction of D-galactose and free amino acids in proteins or peptides, can also accelerate aging. In this study, 200 mg/kg body weight/day D-gal was injected in mice for 6 weeks.

Many researches implicated that aging is related with the decrease of antioxidant level in animals. In the aging process, the body's antioxidant level decreased, leading to an increase in lipid peroxidation (Schuessel *et al.*, 2006). SOD, GPX and CAT are the major antioxidant enzymes and usually utilized as the biomarkers of oxidative stress (Aliahmat *et al.*, 2012). The SAG-1 can significantly enhance the antioxidant enzymes activity in aging mice and reduce the MDA content. The results showed SAG-1 has obvious antagonistic effect on oxidative stress in aging mice induced by D-galactose. So SAG-1 was a food antioxidant *in vitro* and in vivo. The further mechanism of SAG-1 needs another investigation.

Excess intracellular ROS induce genomic instability, modify gene expression and participate in signaling pathways to induce the development of cancer (Toyokuni, 2016). In the cancer cells ROS were demand high concentrations to maintain their high proliferation rate (Jiang *et al.*, 2011; Sosa *et al.*, 2013). SAG-1 showed inhibition of the proliferation of cancer cells, which may be related to the decrease of free radicals content in cancer cells. And further experimental results are required to confirm.

Sanguinolaria acuta is a kind of delicious marine food. Many papers indicated that antioxidant reduce adverse effect and toxicities of cancer chemotherapy. It can be used as an auxiliary product of chemotherapeutic drugs to improve the success rate of cancer treatment (Fuchs-Tarlovsky, 2013). SAG-1 may be used as a material for chemotherapy assisted food.

Conclusion

The antioxidant and anntitumor activities of crude glycosaminoglycan from *Sanguinolaria acuta* (SAG-1) were evaluated in this paper. SAG-1 showed the scavenging effect of free radicals *in vitro*. The scavenging rate for hydroxyl radicals, superoxide radicals, and diphenyl-picryl hydrazide (DPPH) free radicals was 47.1%, 44.5% and 57.5%, respectively. The inhibition rate of lipid peroxidation of mice livers *in vitro* was 39.8%. In vivo antioxidant testing, the application of 200 mg/kg SAG-1 resulted in a marked increased in SOD, GPX, and CAT activity, and decreased MDA levels in serum, liver, and kidney of aging mice induced by D-Galactose. In the detection of anticancer activity, the SAG-1 showed inhibition effect of the HeLa and CNE-2Z cells proliferation. The inhibition rates of 200 μg/mL SAG-1 on HeLa and CNE-2Z tumor cells were 45.61, 58.14 which were similar with that of 5-Fu. These findings indicate that SAG-1 may be a novel source of natural antioxidants that may be potentially utilized in health foods and therapeutics.

Acknowledgments

This work was financially supported by special funds for the construction of modern agricultural industrial technology system in China (No. CARS-49).

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(Received 04 June 2018; Accepted 09 July 2018)