

Research Article

Characterization of Polysaccharide by HPLC: Extraction and Anticancer Effects

Liming Gao,¹ Ya Di,¹ Jiandong Wu,¹ Ming Shi,² and Fulu Zheng¹

¹The First Hospital of Qinhuangdao, No. 258 Wenhua Road, Qinhuangdao 066004, China

²Department of Biological Engineering, College of Environment & Chemical Engineering, Yanshan University, No. 438 Hebei Street, Qinhuangdao 066004, China

Correspondence should be addressed to Liming Gao; gaolimingerhao@163.com

Received 5 July 2014; Accepted 19 July 2014; Published 3 September 2014

Academic Editor: Tifeng Jiao

Copyright © 2014 Liming Gao et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Cervical cancer is a serious health hazard for women's reproductive system cancer; the method of treatment for cervical cancer is still in surgery, chemotherapy, and radiotherapy as the basic means, but with many complications. The effects of natural medicines for cervical cancer are increasingly becoming the focus of people's attentions. By studying the polysaccharide of cervical cancer in mice, we found that shark cartilage polysaccharide can increase the serum levels of T-SOD and GSH and decrease MDA level significantly in the tumor mice. The distribution of the drug in the tissue was determined by HPLC method; the drug can be drawn in the liver and kidney the highest, followed by the spleen, lung, and brain levels being the lowest. Polysaccharide can inhibit tumor growth in the mice which may be connected with the enhanced immunity and the antioxidant capacity.

1. Introduction

Cervical cancer is a common cancer of the female reproductive system, which ranks second in the incidence of cancer in women worldwide, second only to breast cancer; it is a serious threat to women's health. According to the statistics worldwide, the number of new cases of cervical cancer each year reached 500,000, particularly common in developing countries; China's new cases are about 100,000, according for about 1/5 of the world total new case, and more than 30,000 women die of cervical cancer every year [1]. In recent years, the number of young patients with cervical cancer processes upward trend clearly, which deserves people's attention. The method of treatment for cervical cancer is still in surgery at present, chemotherapy, and radiotherapy as the basic means; the disadvantage is more complications. The treatment model has changed and the local control rate and survival have been improved. Now western medicine had been widely used, but it still has some shortcomings, such as severe toxicities, increased drug-induced disorders, the efficacy being not ideal, and the high drug prices, so that people look forward to natural medicine more. Because the toxicity of natural plant and animal is little, they are easily accepted by patients,

playing an important role in the anticancer drugs. Looking for anti-tumor drugs from natural plants and animals in order to improve the therapeutic effect is increasingly becoming the focus of attention [2, 3].

Polysaccharides are a class of natural macromolecular polymers in vivo generally. It is made of aloes or ketoses which connected together by glycoside bonding. Since the 1970s, with the development of polysaccharides special physiological function (transportation intercellular substance, regulation of immune function, cell-cell recognition, etc.), Polysaccharides, whose antitumor and immunomodulatory effects of polysaccharides draw more attention, caught more attention of drug workers; it has become one of the hot spots of cancer treatment [4–7].

At present, gained more and more novel structure and the unique role of bioactive polysaccharide from marine organisms has been expected to become a new drug resource of cancer treatment [8]. With further research, we have noted the so-called "sea scavenger" sharks which almost never suffer from cancer. After years of research, scientists found that shark cartilage polysaccharide, as an animal polysaccharide, has a variety of physiological effects: lowering blood pressure, antiradiation, anticoagulation of the blood,

antiviral, and antitumor [9–12]. For the study of the polysaccharide, predecessors mostly stay in the extraction process studied. In recent years there are only a few more reports in terms of functional studies. Currently, the country has listed health food products, shark cartilage capsules, made of shark cartilage. In other countries, the glycosaminoglycan chondroitin sulfate of shark's raw material products has been used in patients clinically [13, 14]. Research on shark cartilage mucopolysaccharide has caused widespread interest by scholars inside and outside.

The topic for the functional role of the polysaccharide to do further research and for the treatment of cervical cancer provides effective and less toxic side effects of natural medicine; the development of these drugs has a certain significance.

2. Experiments

2.1. Materials. In this experiment, we use the catshark cartilage as a raw material provided by Yanshan University Laboratory; the 8-week-old female Kunming mice were purchased from Beijing Military Medical Sciences weighing 20–24 g. U14 mouse cancer cell line was purchased from China Medical College Beijing tumor cell library.

2.2. The Extraction of Shark Cartilage Polysaccharides. In this study, shark cartilage was removed from sharks and taken into boiling water for 2.5 h to remove most of the bone marrow and adipose tissue, and then low-temperature drying, splintered and weight. Powder cartilage into 1000 mL 0.3 mol/L sodium hydroxide dilute alkali solution, followed by stirring at 65°C 3 hours, centrifuge, adjust the pH of the supernatant to 3, and centrifuge. We Sodium chloride solution was added slowly to the supernatant slowly until the precipitate to the most, the supernatant was centrifuged and the supernatant was concentrated by evaporation. The resultant product was washed by 60% and 80% ethanol solution.

2.3. The Determination of the Polysaccharide. We use phenol-sulfuric acid method to determine polysaccharide extract. Principle of this method is the polysaccharide in concentrated sulfuric acid; dehydration or furfural and HMF can condense orange-red phenol compounds, within a certain range proportional to the depth of its color and sugar content, and in the 490 nm wavelength of maximum absorption peak, the UV spectrophotometry available in this wavelength. Phenol method can be used for determination of methylated sugar, pentose sugars, and polysaccharides. The method is simple and sensitive. The experiment is unaffected by the presence of proteins substantially, and the resulting color stability is over 160 min.

2.4. Preparation of U14 Cervical Tumor-Bearing Mouse Model. Take three normal mice by intraperitoneal injection method U14 tumor cell inoculation 0.2 mL cell suspension. The first eight days, put the mice to death, collect the ascites,

centrifuged, dilute the supernatant into density of 1.60×10^6 cells/mL, and inject 0.2 mL into mouse forelimb left armpit.

2.5. Experimental Groups and Drug Program. The inoculated mice were randomly divided into four groups of 8. The polysaccharide dissolved in distilled water to prepare a high-dose group (1000 mg/kg) and low-dose group (500 mg/kg); each group was fed 0.2 mL, negative control group was fed the same amount of distilled water, and positive control group was injected 0.2 mL CTX intraperitoneally (25 mg/kg), once a day. Administration continued for 14 days; first on day 15, all the mice were sacrificed.

2.6. Determination of Tumor Inhibition Rate. First on day 15 all the mice were sacrificed, and tumor of each group mice was taken and then weighed; the tumor inhibition rate is calculated according to the following formula:

$$\begin{aligned} & \text{Tumor inhibition rate (\%)} \\ &= \left[\left(\text{the average tumor weight of negative control group} \right. \right. \\ & \quad \left. \left. - \text{the average tumor weight of medication group} \right) \right. \\ & \quad \left. \times \left(\text{the average tumor weight} \right. \right. \\ & \quad \left. \left. \text{of negative control group} \right)^{-1} \right] \times 100\%. \end{aligned} \quad (1)$$

2.7. Determination of Thymus and Spleen Weight Index. The mice were taken off the neck to death; we remove the thymus and spleen and observe them by naked eye. In accordance with the “immune organ weights law” in “immunized animals screening procedures” on the tumor-bearing mouse thymus, the spleen and thymus index [15] was calculated according to formula 2:

$$\begin{aligned} & \text{Thymus (spleen) Index (mg/10 g)} \\ &= \left[\frac{\text{thymus (spleen) weight}}{\text{weight to tumor-bearing mice}} \right] \times 10. \end{aligned} \quad (2)$$

2.8. Serum and Tissue Antioxidant Parameters Were Measured. We prepare serum for the determination of serum SOD (superoxide dismutase), GSH (glutathione reductase), and MDA (malondialdehyde) level; we took liver tissue at the same time, plus saline formulated into 10% of the tissue homogenates, for the determination of their GSH, SOD, and MDA levels.

Determination of SOD. Serum total SOD activity is calculated as follows:

$$\begin{aligned} & \text{The total SOD activity} \\ &= (\text{Control tube absorbance} \\ & \quad - \text{Absorbance measurement tube}) \\ & \quad \times (\text{Control tube absorbance})^{-1} \\ & \quad \div 50\% \times \text{Dilution of the sample before the test.} \end{aligned} \quad (3)$$

The total tissue SOD activity is calculated using the following formula:

$$\begin{aligned}
 &\text{The total SOD activity} \\
 &= (\text{Control tube absorbance} \\
 &\quad - \text{Absorbance measurement tube}) \\
 &\quad \times (\text{Control tube absorbance})^{-1} \\
 &\quad \div 50\% \times \left(\frac{\text{Total reaction volume}}{\text{Sample volume}} \right) \\
 &\quad \div \text{Tissue protein content (mgprot/mL)}.
 \end{aligned} \tag{4}$$

Determination of Glutathione. Consider

$$\begin{aligned}
 &\text{GSH content in serum} \\
 &= (\text{OD value measured} - \text{blank OD value}) \\
 &\quad \times \text{standard concentration} \\
 &\quad \times (20 \times 10^{-3} \text{ mmol/L}) \\
 &\quad \times \text{GSH molecular weight (307)} \\
 &\quad \times \text{prior to the test sample dilution factor} \\
 &\quad \div (\text{OD value of the standard} - \text{blank OD value}),
 \end{aligned}$$

Liver GSH content

$$\begin{aligned}
 &= (\text{OD value measured} - \text{blank OD value}) \\
 &\quad \times \text{standard concentration} (20 \times 10^{-3} \text{ mmol/L}) \\
 &\quad \times \text{GSH molecular weight (307)} \\
 &\quad \times \text{dilution of the test sample} \\
 &\quad \div (\text{OD value of the standard} - \text{blank OD}) \\
 &\quad \div \text{protein concentration}.
 \end{aligned}$$

Determination of Malondialdehyde. Consider

$$\begin{aligned}
 &\text{Serum (plasma) MDA content (nmol/mL)} \\
 &= (\text{measured OD values} - \text{control OD value}) \\
 &\quad \times \text{standard concentration} \\
 &\quad \times \text{dilution of the sample prior to testing} \\
 &\quad \div (\text{standard OD value} - \text{blank OD value}), \\
 &\text{Liver tissue MDA content (nmol/mgprot)} \\
 &= (\text{measured OD values} - \text{control OD value}) \\
 &\quad \times \text{concentration of the sample standard} \\
 &\quad \div \text{protein concentration (mgprot/mL)} \\
 &\quad \div (\text{standard OD value} - \text{blank OD value}).
 \end{aligned} \tag{6}$$

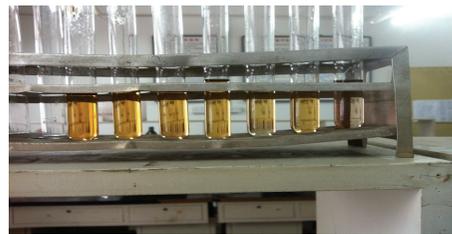


FIGURE 1: Each concentration of the standard solution.

Determination of Protein Content. The formula is as follows:

$$\begin{aligned}
 &\text{Protein concentration (g/L)} \\
 &= (\text{measured OD value} - \text{blank OD value}) \\
 &\quad \times \text{standard concentration (g/L)} \\
 &\quad \div (\text{standard OD value} - \text{blank OD value}).
 \end{aligned} \tag{7}$$

2.9. HPLC Determination of the Distribution of the Drug in Tissues. Two hours after the last administration, the heart, liver, spleen, lung, kidney, and other tissues of the mice are completely removed, weighed, and ground, mixed with 10% saline, centrifuged for 10 min, adding methanol into supernatant and ultrasonic shock for 20 min. Mix well, centrifuge for 10 min, and filter the supernatant with 0.45 μm membrane.

The column was Edipse XDB-C18 ($4.6 \times 250 \text{ mm}$, $5 \mu\text{m}$). A mobile phase of acetonitrile and mobile phase B were 0.1% phosphate buffered saline (pH = 3). Ratio of the two mobile phases is A : B = 15 : 85, with a gradient elution procedure to adjust the ratio of the two-phase flow; column temperature was room temperature; flow rate was 0.5 mL/min; injection volume was 20.0 μL ; detection wavelength was 200 nm; and each run time was 5 min.

3. Result

3.1. Shark Cartilage Extract Polysaccharides. After pretreatment for shark cartilage, we say its weight: 62.7 g. After digestion of dilute alkali solution, a salt solution to dislodge the protein, ethanol precipitation, and a series of operations, we eventually dry the precipitate to obtain 1.98 g of the polysaccharide crude extract.

3.2. Determination of Shark Polysaccharide. Prepared according to the method of the standard, the concentration of glucose standard solution was obtained the color shown in Figure 1.

In the 490 nm wavelength measured absorbance standard concentrations are shown in Table 1.

Standard curve using Excel software, standard glucose levels ($\mu\text{g/mL}$) for the x -axis and y -axis plotted as absorbance are obtained using the standard curve regression equation: $Y = 0.0151X + 0.0364$, and the correlation coefficient $r^2 = 0.9933$. The linear range is 10–60 $\mu\text{g/mL}$. The resulting standard curve is as shown in Figure 2. In accordance with

TABLE 1: Glucose standard solution absorbance.

Glucose concentration absorbance ($\mu\text{g/mL}$)	10	20	30	40	50	60
First	0.221	0.347	0.515	0.608	0.782	0.945
Second	0.221	0.346	0.516	0.608	0.781	0.945
Third	0.222	0.347	0.516	0.608	0.782	0.944

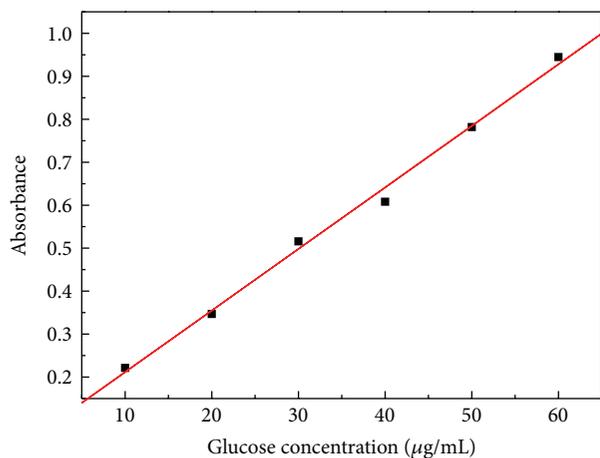


FIGURE 2: Glucose standard curve.

the standard curve regression equation, the sugar content can be drawn from the test corresponding absorbance. Calculated by the test solution to be tested for the average absorbance of 0.407, as the Y values into the regression equation, derived X value 24.54 $\mu\text{g/mL}$, the final content of polysaccharide extract obtained was 81.8%, the total extraction rate of 2.58%.

3.3. Animals Generally Observed. After administration of the first four days, all mice grew visually observable mass, and mice are in good spirit and move freely, and no deaths occurred. After 14 days of continuous administration of each treatment group mice were having sparse dull hair, were lying curled with back arched, and were action-insensitive, apathetic, unresponsive, and so on, and because of the increasing of tumor weight, tumor-bearing mice significantly increased body weight. Negative control group and treated group were compared with the above symptoms of weight. Mental state of positive control group of mice is better, the action is more flexible, and weight gain is not obvious (shown in Table 2 and Figure 3).

3.4. The Polysaccharide Solid Tumor Inhibition Rate. The polysaccharide in mice results in tumor inhibition rates are shown in Table 3. High and low dose polysaccharides have anti-tumor effect; tumor inhibition rates were 34.73% and 65.81%, respectively, and the positive control group CTX tumor inhibition rate is 63.71%. Regarding weight compared with the negative control group tumors, average tumor weight difference between the polysaccharide low-dose group and positive control group was significant ($P < 0.01$), and

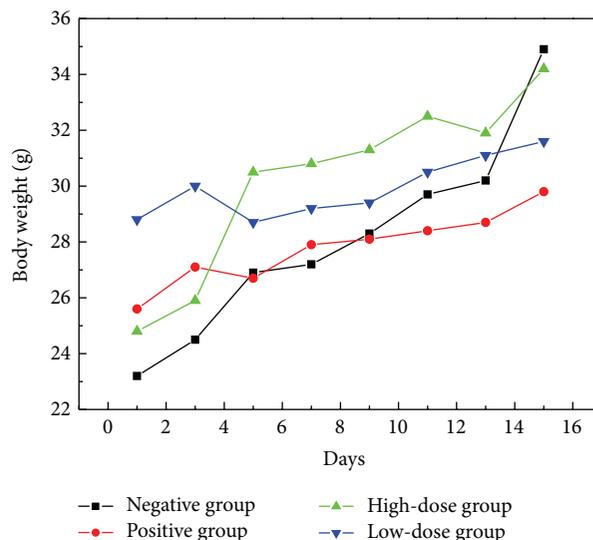


FIGURE 3: The average weight of the mice in each group situation.

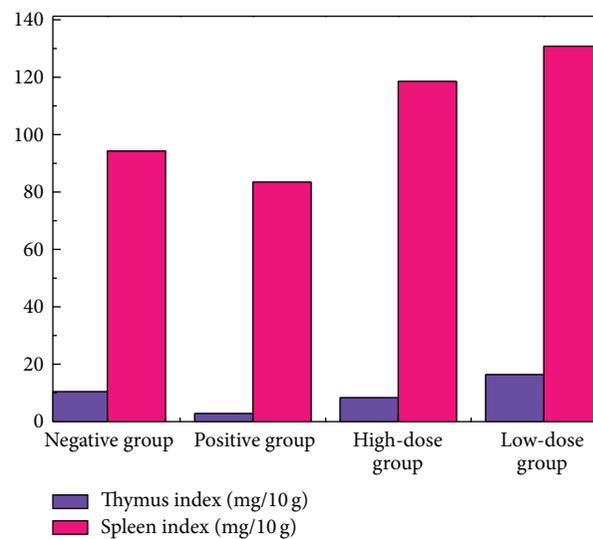


FIGURE 4: Thymus and spleen index of mice.

the average tumor weight difference between the high-dose group was significant ($P < 0.05$). The average tumor weight of the low-dose group was compared with the CTX-positive control group, and the difference was not significant ($P > 0.05$); the high-dose group was compared with the control group, CTX-positive, and the difference was significant ($P < 0.05$); results suggest that low doses of shark cartilage have significant inhibition of solid tumor polysaccharide with mice.

3.5. Determination of Mouse Thymus (Spleen) Index. Each group of mice thymus (spleen) index as shown in Table 4 and Figure 4, the body of thymus and spleen was reduced in positive group mice was with significantly reduced volume of thymus and spleen, thymus color was gray, leaf was unclear, and spleen is pale red. The thymus index and spleen index of

TABLE 2: The average weight of each group of mice (g).

Days/group	1	3	5	7	9	11	13	15
Negative group	23.2	24.5	26.9	27.2	28.3	29.7	30.2	34.9
Positive group	25.6	27.1	26.7	27.9	28.1	28.4	28.7	29.8
High-dose group	24.8	25.9	30.5	30.8	31.3	32.5	31.9	34.2
Low-dose group	28.8	30.0	28.7	29.2	29.4	30.5	31.1	31.6

TABLE 3: Inhibition rate of each group of mice ($\pm S$).

Group	Dose (mg/kg)	Starting weight (g)	Final weight (g)	Tumor weight (g)	Inhibition rate (%)
Negative group	—	23.24 \pm 0.81	34.94 \pm 1.21	3.896 \pm 0.46	0
Positive group	25	25.66 \pm 1.12	29.82 \pm 1.43	1.474 \pm 0.10**	63.71
High-dose group	1000	24.82 \pm 1.58	34.23 \pm 0.84	2.543 \pm 0.52**	34.73
Low-dose group	500	28.84 \pm 1.26	31.64 \pm 1.12	1.332 \pm 0.23**	65.81

TABLE 4: Mice in each group thymus (spleen) index ($\pm S$).

Group	Dose (mg/kg)	The final number of animals	Thymus index (mg/10 g body weight)	Spleen index (mg/10 g body weight)
Negative group	—	4	10.43 \pm 1.36	94.26 \pm 3.84
Positive group	25	6	2.81 \pm 0.74 [#]	83.45 \pm 2.76
High-dose group	1000	4	8.32 \pm 0.58*	118.54 \pm 10.85 [#]
Low-dose group	500	5	16.38 \pm 1.64 ^{***}	130.78 \pm 13.24 ^{***}

TABLE 5: The polysaccharide impact ($\bar{X} \pm S$) for mouse serum antioxidant capacity.

Group	Dose (mg/kg)	SOD (U/mL)	GSH (mg/L)	MDA (nmol/mL)
Negative group	NS	89.84 \pm 8.87	3.43 \pm 0.68	4.26 \pm 0.78
Positive group	25	124.36 \pm 9.96	2.91 \pm 0.49	8.45 \pm 0.69
High-dose group	1000	136.42 \pm 10.52 ^{***}	4.32 \pm 0.98*	7.54 \pm 1.05*
Low-dose group	500	168.43 \pm 9.48 ^{***}	6.78 \pm 0.63 ^{***}	3.78 \pm 0.86 [#]

Note: compared with the positive control group, * $P < 0.05$, ** $P < 0.01$; compared with negative control group, [#] $P < 0.05$, ^{##} $P < 0.01$.

the polysaccharide of both high- and low-dose groups were higher than the positive control group, and the thymus was observed to have more complete appearance and white color, the color of the normal spleen. The polysaccharide low-dose group and spleen index and thymus index have increased compared to CTX-positive and CTX-negative groups; the statistical analysis showed that the polysaccharide low-dose group and the positive control group showed significant difference ($P < 0.01$); shark cartilage polysaccharide of high-dose group compared with the positive control group showed significant differences ($P < 0.05$); high- and low-dose group and negative control group showed low doses of the negative control group, and the difference was significant ($P < 0.05$) compared with high-dose group and negative control groups, but the difference did not reach a significant level ($P > 0.05$), and negative control groups and the positive control group were significantly difference ($P < 0.05$). This indicates that CTX will not be able to promote the growth of immune organs but will be destroyed, inhibiting the development of the thymus and spleen tissue, and high- and low-dose groups can promote the growth of the thymus and spleen, and thymus tissue of the high-dose group developed a certain promoting effect, but the effect is not obvious, to promote the development of the role of spleen tissue, and the

effect of low-dose group was significant, suggesting that the polysaccharide has better effect than CTX.

3.6. *Effects of Shark Cartilage Polysaccharides on Serum Antioxidant Capacity in Mice.* As shown in Table 5, the high- and low-dose group could significantly improve tumor-bearing mouse serum SOD activity, compared with the negative control group, and the difference was significant ($P < 0.01$); and CTX-positive group was significantly higher ($P < 0.05$), whereas serum CTX levels of SOD-positive group compared with the control group, although a certain degree rises, but the difference between the two has not yet reached a significant level ($P > 0.05$), indicating that the polysaccharide can increase SOD activity in tumor-bearing mice, better than CTX. Meanwhile, the low-dose group had significantly higher serum GSH content compared with the negative group ($P < 0.05$), while the high-dose group showed a rising trend, though, but has not yet reached a significant level ($P > 0.05$); with CTX group phase ratio, serum GSH content of low-dose group increased significantly ($P < 0.01$), but with no significant difference (P between the high-dose group and CTX-positive group > 0.05). Those high doses of the polysaccharide treatment can significantly increase serum GSH levels in tumor-bearing mice, and CTX has similar

TABLE 6: The polysaccharide impact for mouse liver tissue antioxidant capacity.

Group	Dose (mg/kg)	SOD (mg/prot)	GSH (mg/prot)	MDA (nmol/mgprot)
Negative group	NS	189.21 ± 4.28	10.43 ± 0.36	17.26 ± 1.54
Positive group	25	223.23 ± 10.47 [#]	2.81 ± 0.74	10.43 ± 1.62
High-dose group	1000	242.54 ± 14.68 [#]	8.32 ± 0.58 ^{*##}	8.54 ± 0.85 ^{*##}
Low-dose group	500	284.68 ± 17.32 ^{*#}	15.32 ± 0.84 ^{*##}	4.76 ± 0.74 ^{*##}

Note: compared with the positive control group, * $P < 0.05$, ** $P < 0.01$; compared with negative control group, # $P < 0.05$, ## $P < 0.01$.

effect, but the effect of low-dose treatment is superior to the polysaccharide CTX-positive group. MDA activity was measured in serum tumor-bearing mice results which show that, compared with the negative control group, low-dose group had significantly lower serum MDA activity in mice ($P < 0.05$), while the CTX group of tumor-bearing mice did not reduce the activity of serum MDA effect ($P > 0.05$). Meanwhile, in low- and high-dose groups compared with the CTX group, serum MDA was significantly lower ($P < 0.05$). Therefore, polysaccharide can reduce the content of serum MDA.

3.7. Effects of the Polysaccharide on Liver Tissue Antioxidant Capacity. Each group of mice administered continuously for 14 days was killed, the liver tissues were measured for SOD, GSH, and MDA activity, and the results are shown in Table 6. From the table, compared to the negative control group, high- and low-doses of SOD activity group and CTX group were significantly increased ($P < 0.05$); differences were compared with the positive control group; the high-dose group and the positive group are not significant ($P > 0.05$), low-dose group and CTX-positive group were significantly increased compared to the drop ($P < 0.05$). For the determination of GSH, compared with the negative control group, low-dose group GSH levels were significantly higher ($P < 0.05$); compared with CTX, high- and low-dose groups were significantly different in the level of activity ($P < 0.01$). Compared with negative group, the content of MDA in high and low dose group mice liver were lower, the differences were highly significant ($P < 0.01$); compared with the CTX group, the high- and low-dose groups were significantly lower than CTX treatment group ($P < 0.05$). Hypoxia is one of the physiological abnormalities characteristic of solid tumors, and these results suggest that the polysaccharide, maybe by improving the organization's ability to achieve the antioxidant defenses, improves the hypoxic state.

3.8. HPLC Determination of the Distribution of the Drug in Tissues

3.8.1. Chondroitin Sulfate Standard Curve and Regression Equation. According to the standard method of preparation, the concentration of the prepared solution of the test sample to the standard chromatograms obtained from the map read out the corresponding peak area (Table 7) to obtain a standard curve (Figure 6 peak area under the concentration) and the linear regression equation. Figure 5 is chromatogram of standard 0.4 mg/mL

TABLE 7: Absorption peak area of each concentration of standard.

Standard concentration $\mu\text{g/mL}$	0.1	0.2	0.4	0.6	0.8
Peak area mAU * S	50.1421	63.0977	92.743	115.428	139.675

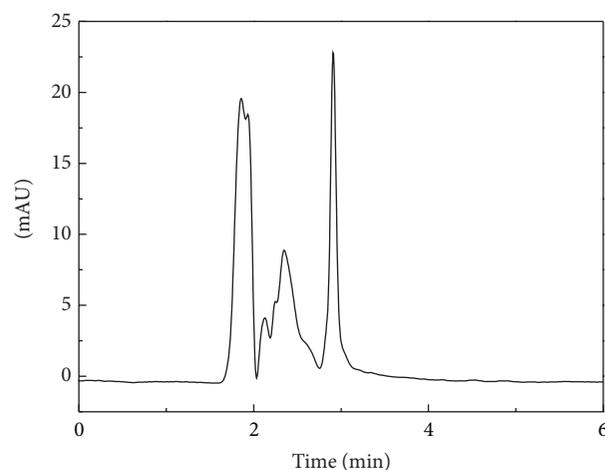


FIGURE 5: Chromatogram of standard 0.4 mg/mL.

Retention time is 3.594; DAD1 A; Sig is 200, 4; Ref = 360, 100. Residual standard error is 1.93706. Regression equation is $y = 125.39383x + 40.14115$, where x is the content of chondroitin sulfate and y is the peak area; dependency is 0.99891. Chondroitin sulfate at 0.1–0.9 $\mu\text{g/mL}$ is in good linear relationship.

3.8.2. The Concentration of Drug in the Tissue Sample. Take the heart, liver, spleen, lung, and kidney of high- and low-dose group; after treating each tissue sample, we can obtain drug content shown in Table 8. From Figure 8 in each group it is observed that in liver and kidney tissue drug content is generally higher than in other tissues, followed by the content of the heart, spleen, and lung relatively little content. The result shows that drug distribution in mice was wide range. Figure 7 is samples of liver tissue of a low-dose of the chromatogram.

4. Discussions

In this study, shark cartilage polysaccharides was extracted with dilute and alcohol precipitation method, purified by phenol-sulfuric acid method, but the total withdrawal rate

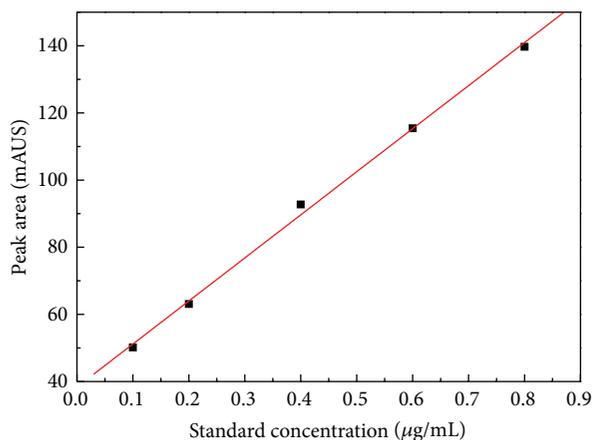


FIGURE 6: Chondroitin sulfate standard curves.

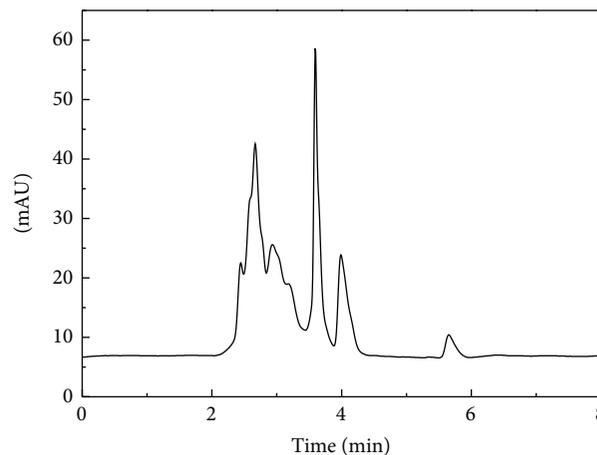


FIGURE 7: Samples of liver tissue of a low dose of the chromatogram.

TABLE 8: Distribution of drugs in different tissues.

Classes	Drug content of the low-dose group (µg/mL)	Drug content of the high-dose group (µg/mL)
Heart	0.33	0.62
Liver	0.88	0.73
Spleen	0.26	0.16
Lungs	0.19	0.10
Kidney	0.84	0.90

is low, and extraction conditions remain to be further optimized; overall for shark cartilage polysaccharide extraction method and content of appraisal provide research basis.

The body ability of antioxidant defense system strength and weakness are closely related to health, and the antioxidant effect of the defense system is mainly to eliminate free radicals and reactive oxygen species in order to prevent the occurrence of lipid peroxide, decomposition of peroxide, and peroxide blocking chain. The body produced oxygen free radicals by enzymes and nonenzyme system and formed lipid peroxides. Due to the excess of oxygen free radicals and lipid peroxides, DNA is damaged and mutations occur, leading to cell damage, which are closely associated with tumor formation. Such MDA is formed in the process of lipid peroxides, MDA is a kind of mutagen and genetic agent, and the development of human tumor probably has to do with it. Our study found that treatment group compared with negative control group and CTX-positive control group can significantly reduce the MDA in the serum in mice and that shark cartilage polysaccharide could inhibit the generation of MDA in the serum and inhibit portability tumor development. The body's antioxidant system mainly consists of antioxidant enzymes and antioxidants. SOD can clear the super oxide anion radicals in the body protecting cells from injury. It plays a key role at body's anti-oxidant. Thus, SOD activity is closely related to tumor and inflammation diseases. Positive compared with negative control group, CTX group, mice thymus index, spleen index, SOD content in serum and tissue, and GSH levels were significantly lower; this is

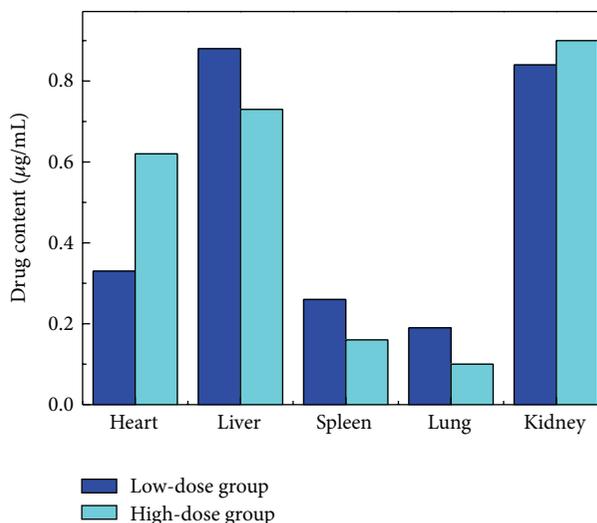


FIGURE 8: High- and low-dose group of organizations in the distribution of the drug content.

mainly because of CTX being a kind of immunosuppressant and it caused, by inducing cell apoptosis, thymus and spleen shrinks, thus leading to the decrease of antioxidant defense system of organization. While, the thymus and spleen index SOD, MDA level of high and low dose group have been increased, and tip shark cartilage polysaccharide probably inhibits lymphocyte apoptosis of immune organ and enhances the antioxidant capacity of organizations to inhibit tumor development. Shark cartilage polysaccharide probably inhibits lymphocyte apoptosis of immune organ and enhances the antioxidant capacity of organizations to inhibit tumor development.

5. Conclusions

Shark cartilage polysaccharide in mice tissues distribution is broad, most abundant in the liver and kidney, which may be related to the metabolism of the liver and kidney as the main

metabolic organs. Visibly, shark cartilage polysaccharide could inhibit tumor growth in mice solid tumor and improve the index of thymus and spleen in mice, showing that it can promote the growth of immune organs and increase the body's immune function and the antioxidant defense system ability, thus suppressing tumor growth.

Conflict of Interests

The authors declare that they do not have any direct financial relation with the commercial identities mentioned in this paper that might lead to a conflict of interests for any of the authors.

References

- [1] Y. Xu, Z. Ouyang, P. Liu, and C. Chen, "Magnetic resonance imaging in uterine fibroid embolization," *Chinese Journal of Gynecology and Obstetrics*, vol. 10, no. 4, pp. 23–25, 2011.
- [2] X. Zhuan and X. Gu, "Epidemiological investigation of cervical cancer risk factors," *Chinese Maternal and Child Health*, vol. 23, pp. 4053–4056, 2008.
- [3] L. Li and C. Li, "Prevention and screening of cervical cancer," *Chinese Journal of Practical Gynecology*, vol. 19, p. 151, 2003.
- [4] Z. Xiong and D. Chen, "Advances in tumor mechanism," *Chinese Medicine*, vol. 7, no. 12, pp. 112–114, 2001.
- [5] M. Yang, "Research on polysaccharide antitumor mechanism of action," *Zhejiang Journal of Medicine*, vol. 12, no. 6, pp. 65–68, 2002.
- [6] S. Sipka, G. Ábel, J. Csongor, G. Chihara, and J. Facht, "Effect of lentinan on the chemiluminescence produced by human neutrophils and the murine macrophage cell line C4M ϕ ," *International Journal of Immunopharmacology*, vol. 7, no. 5, pp. 747–751, 1985.
- [7] R. Goldman, "Characteristics of the β -glucan receptor of murine macrophages," *Experimental Cell Research*, vol. 174, no. 2, pp. 481–490, 1988.
- [8] L. Zhao, "Li Ming Chun study mechanisms of tumor progression marine organisms Polysaccharide," *Practical Journal of Medicine*, vol. 9, no. 27, pp. 845–846, 2010.
- [9] X. Hao, S. Ye, and Z. Wu, "The polysaccharide anticoagulant effect," *China Marine Drugs*, vol. 44, no. 4, pp. 17–22, 1992.
- [10] D. Fu, Z. He, and L. Li, "Antithrombotic effect of shark cartilage glycosaminoglycan study," *China Marine Drugs*, vol. 43, no. 3, 1992.
- [11] F. Jia, Z. He, and L. Wang, "Cartilage angiogenesis anticancer mechanism of inhibition of the active ingredient," *Chinese Journal of Biochemical Pharmaceutics*, vol. 18, no. 2, pp. 68–71, 1997.
- [12] M. Zhang, L. Chen, and X. Mai, "Anticancer effect of shark cartilage progress," *Tianjin Pharmacy*, vol. 15, no. 1, pp. 44–46, 2003.
- [13] D. Gingras, A. Renaud, N. Mousseau et al., "Sharkearila-geextractsasantiangiogenieangents: smartofbitterI115," *Caneer-metastasis*, vol. 19, no. 1, 2000.
- [14] H. Wu, B. Yuan, and B. Jiao, "Pharmacological and clinical research progress shark cartilage preparations," *China Marine Drugs*, vol. 4, no. 8, pp. 51–55, 2001.
- [15] Y. Chang, "Schisandra polysaccharide of H22, S180 experimental study inhibition of tumor-bearing mice," *TCM information*, vol. 4, no. 5, pp. 18–20, 2002.



Hindawi

Submit your manuscripts at
<http://www.hindawi.com>

