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# Purification and characterization of an antitumor polysaccharide from *Portulaca oleracea* L.

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ABSTRACT

In the present study, we purified a unique polysaccharide component (POP) from *Portulaca oleracea* and found that it had pronounced anti-tumor effects in vivo model. Tumor weight, immune organ index and T lymphocyte subsets were employed to detect the immunoregulatory and antitumor effects of POP after administration. Hematological and biochemical analyses were also investigated in order to evaluate the toxicological aspects related to POP treatment. POP could significantly inhibit the growth of transplantable sarcoma 180 and potentiate the animal's immune responses including an increase in the number of white blood cell (WBC) and $CD4^+$ T-lymphocytes, as well as the ratio of $CD4^+/CD8^+$. Furthermore the serum aspartate transanimase (AST), alanine transaminase (ALT), urea nitrogen (BUN), and creatinine levels in S180-bearing mice were significantly reversed by POP. Considering all these results, it is suggested that the anti-tumor effect elicited by POP could be associated with its immunostimulating properties.



## 1. Introduction

Cancer is a leading cause of mortality worldwide. To date, chemotherapy is the main tool for cancer treatment, but chemotherapy alone does not achieve a satisfactory therapeutic result (Mushiake et al., 2005). A large number of chemical compounds have been identified as cytotoxic to cancer cells and normal cells, and attenuate the body's natural defenses (Salgaller & Lodge, 1998). In addition, evidence indicates that many tumors not only decrease the reactivity of circulating T cells (Poupot, Pont, & Jean-Jacques, 2005), but also induce T cell apoptosis as a mechanism of inhibiting anti-tumor activity (Hadden, 2003). To overcome this situation, it is essential to develop new anti-cancer compounds that protect cancer patients from the harmful side effects of chemotherapy without decreasing the therapy's efficacy against tumors.

Polysaccharides ranked the third among three kinds of macromolecules carrying essential biological information following nucleic acids and proteins. Recently, polysaccharide–protein complexes from natural sources such as bacteria, fungi, algae and plants, are found to be effective, non-toxic substances with wide variety of biological activities. Especially their immunomodulatory and anticancer effects have attracted lots of attention in the biochemical and medical areas (Leung, Liu, Koon, & Fung, 2006; Ooi & Liu, 2000). All these activities are due to their contribution to enhance immune function of the host organism. In many oriental countries, several immunomodulators composed of polysaccharides have been accepted such as lentinan, schizophyllan and krestin (Borchers, Stern, Hackman, Keen, & Gershwin, 1999; Liu, Ooi, & Fung, 1999).

*Portulaca oleracea* L., commonly known as purslane, which is a well-known traditional Chinese medicine (TCM) recorded in the Chinese Pharmacopoeia, is used as a diuretic, febrifuge, antiseptic, antispasmodic and vermifuge (Xiang et al., 2005). It has a wide range of pharmacological effects, such as antibacterial (Zhang, Ji, Qu, Xia, & Wang, 2002), hypolipidemic, anti-aging, anti-inflammatory (Chan et al., 2000), antioxidative (Dkhil, Abdel Moniem, Al-Quraishy, & Saleh, 2011), analgesic, and wound-healing activities (Rashed, Afifi, & Disi, 2003). Many studies have also shown that the major bioactive components of *P. oleracea* are polysaccharides, flavonoids, coumarins, monoterpene glycoside and alkaloids (Awad, 1994; Mohammad, Mohammad, & Farhad, 2004; Sakai, Inada, Okamoto, Shizuri, & Fukuyama, 1996). Until

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now, the majority of studies on this herb have focused on chemical compositions and bioactivities of small molecules. Although the antidiabetic, antiviral and antioxidant potential of various polysaccharide fractions from *P. oleracea* had been documented, there is no report on antitumor activities of polysaccharides from *P. oleracea*. In the present paper, we intend to examine the antitumor and immunomodulating activities of the polysacchrides isolated from *P. oleracea* and attempt to elucidate the mechanism behind it.

## 2. Materials and methods

### 2.1. Plant material

Aerial parts of *P. oleracea* were collected at a local farm in July in Hebei province and the material was identified by Botany Institute. The materials were shade-dried at room temperature. Other reagents were analytical grade as commercially available.

### 2.2. Isolation and fractionation of the polysaccharide from P. oleracea

Dried aerial plant of *P. oleracea* (200 g) was defatted in a Soxhlet apparatus with 95% ethanol (boiling point: 60–80 °C) twice to remove some coloured materials, monosaccharides, oligosaccharides, and small molecule materials. The residue was then extracted with distilled water (8000 mL 3×) at 75 °C for three times and 3 h for each time. After centrifugation (1700 × *g* for 10 min), the supernatant was concentrated 10-fold, and poured into 3 vol. of 95% ethanol at 4 °C for 24 h to precipitate the crude polysaccharides. Then the crude polysaccharides were treated with Sevag reagent (1:4 n-butanol:chloroform, v/v, 400 mL) to remove proteins (Sevag, Lackman, & Smolens, 1938). After precipitation by ethanol and drying by solvent exchange, the deproteined polysaccharide fraction DCPOP (12.9 g) was obtained.

The DCPOP (4.6 g) was dissolved in distilled water (250 mL) and loaded on a DEAE-Cellulose column (2.0 cm × 40 cm, $Cl^-$) pre-equilibrated with distilled water. The column was eluted first with 3 column volumes of distilled water at a flow rate of 2 mL/min to obtain the neutral fraction (N-DCPOP), and then with stepwise gradient of aqueous NaCl solutions (0.2, 0.4 and 0.6 M) to obtain the charged fractions (A-DCPOPA and A-DCPOPB). The presence of carbohydrate was monitored spectrophotometrically by using the phenol–sulfuric acid reagent. Based on the results of this assay, the water-eluted fraction N-DCPOP was applied to a Sepharose CL-6B column (2.0 × 90 cm) and eluted with 0.15 M NaCl at 0.5 mL/min, yielding one purified polysaccharide (POP, 1.8 g, 39.13%, w/w, of the loaded crude polysaccharide).

### 2.3. Analysis of polysaccharide and protein contents

Gas chromatography (GC) was used for identification and quantification of the monosaccharides. The polysaccharide POP was hydrolyzed with 2 M TFA (2 mL) at 120 °C for 2 h. The hydrolyzed product was converted into the alditol acetates as described (Xiao, Huo, Jiang, & Yang, 2011) and analyzed by GC (Varian 3400, Hewlett–Packard Component, USA) with DM-2330 capillary column (30 m × 0.32 mm × 0.2 μm) and flame-ionization detector (FID). The temperature programme was set to increase from 120 to 250 °C with an increment of 5 °C/min and $N_2$ was the carrier gas. The standard monosaccharides were measured following the same procedure.

Total sugar content was determined by the phenol–sulfuric acid method using glucose as the standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Protein content was measured by the method of Bradford (1976). Uronic acid content was determined by the carbazole–sulfuric acid method (Bitter & Muir, 1962).

### 2.4. Mice and tumor cells

ICR mice (6–8 weeks old, $n = 50$) weighing 20.0 ± 2.0 g were purchased from the Animal Experimental Center of the Fourth Military Medical University (Xi'an, China) and were housed under specific pathogen-free conditions. The mice were housed with a 12 h light/dark cycle at room temperature and allowed free access to standard rodent food and water during the experiments. All animals were maintained for an acclimatization period of 7 days under laboratory conditions. All animal experiments were carried out with the approval and according to the guidelines of the Institutional Animal Ethics Committee.

Sarcoma 180 tumor cells, provided by the Fourth Military Medical University (Xi'an, China), were maintained by routine intraperitoneal inoculation of $1 \times 10^6$ viable cells in mice.

### 2.5. In vivo antitumor activity test

Assay of the antitumor activities of POP was done by the procedure of Misaki, Kakuta, Sakaki, Tanaka, and Miyaji (1981), with some modifications. Ascites of the tumor-bearing sarcoma 180 mouse drawn out under aseptic conditions were washed twice with phosphate-buffered saline (PBS), and then diluted 4-fold with PBS. 0.2 mL diluted solution ($5 \times 10^6$ cells) was used to conduct hypoderm inoculation on the axillary region of the right limb for per mouse. One day after inoculation, the mice were divided into 5 groups, each containing 10 mice. The tested polysaccharides dissolved in phosphate buffer saline (PBS) (pH 7.0) with low, middle, high doses (25, 50, 100 mg/kg, respectively) were given intragastrical administration once daily for 10 consecutive days. The positive control was given 5-FU (25 mg/kg), and the negative control was given PBS instead of the test solution.

On the 11th day, the surviving mice were sacrificed by cervical dislocation and their blood was collected for hematological and biochemical assay before execution. After the mice were weighed, their tumors, spleen and thymus were collected and weighed. Then the inhibiting ratio of the tumor and the organ indices were calculated by the following formulas: the inhibiting ratio (%) = $[(A - B)/A] \times 100\%$, where $A$ is the average tumor weights of the negative control, $B$ is the average tumor weights of treated groups; the organ index (thymus and spleen) = organ weight/body weight. This study was repeated three times, and the results were consistent.

### 2.6. Measurement of lymphocyte transformation

Spleens were aseptically removed from sacrificed mice with scissors and forceps in cold phosphate buffered saline (PBS) and gently homogenized with a loose teflon pestle. Contaminating red blood cells were removed by treatment with ammonium chloride (0.8%, w/v). After two washes in RPMI-1640 medium, the cells were resuspended in RPMI 1640 medium containing 10% FBS. The splenocytes proliferation was assessed by using MTT-based colorimetric assay as previously described (Shuai et al., 2010). Briefly, a total of $2 \times 10^6$ cells in 200 μL RPMI 1640 medium containing 10% FBS were stimulated with or without mitogen (2.5 μg/mL Con A or 10 μg/mL LPS) in a 96-well flat-bottom microtiter plate. After incubation at 37 °C in a humid atmosphere with 5% $CO_2$ for 48 h, 50 μl of MTT solution (1 mg/mL) was added to each well and incubated for another 6 h. The plate was centrifuged on 1500 rpm for 5 min and the supernatant was discard, and then 150 μl/well $Me_2SO$ was added to dissolve formazan crystals. Finally, the plate was analyzed

at 490 nm using a microplate reader (Thermo Labsytems, Helsinki, Finland). The experiment was performed in triplicate.

### 2.7. Flow cytometric analysis of $CD4^+$ and $CD8^+$ expressions on peripheral blood T-lymphocyte

On the last day of experiment, heparinized peripheral blood was collected from all groups immediately after the mice were executed, and diluted (1:1) with PBS. The isolated peripheral blood mononuclear cells (PBMC) from the blood were stained with fluorescein isothiocyanate (FITC)-conjugated anti-mouse $CD4^+$ and phycoerythrin (PE)-conjugated anti-mouse $CD8^+$ antibodies. And then the percentage of $CD4^+$T or $CD8^+$T cell in PMBC were measured by flow cytometry (Epics AlttraII, Beckman Coulter, USA) using Expo 32 ADC Analysis Software.

### 2.8. Hematological and biochemical assay

The blood samples were obtained from the tumor-bearing mice for the determination of hematological and biochemical parameters. Blood was collected in heparinized tubes, and the number of white blood cells (WBC) was counted microscopically by a hematocytometer. The serum aspartate transanimase (AST), alanine transaminase (ALT), urea nitrogen (BUN), and creatinine levels were determined by the enzymatic method using commercial reagent kits (Lanji, Shanghai, China), according to the manufacturer's instructions.

### 2.9. Statistical analysis

The data were expressed as mean ± S.D. and examined for their statistical significance of difference with Standard's *t*-test. *P* values less than 0.05 were considered statistically significant.

## 3. Results and discussion

### 3.1. Isolation, purification and composition of the polysaccharide

The water-soluble crude polysaccharides (DCPOP), with a yield of 6.45% of dried raw material, were obtained from *P. oleracea* by defatting 95% ethanol, hot water extraction, ethanol precipitation, deprotein by Sevag method, dialysis against water and drying by solvent exchange. Crude polysaccharides DCPOP were further fractionated by DEAE-Cellulose anion-exchange and Sepharose CL-6B gel-permeation chromatography. The elution profile of the DCPOP on the DEAE-cellulose anion-exchange chromatography column was illustrated in Fig. 1A. The first peak, eluted with distilled water, was ascribed to a neutral N-DCPOP fraction, and the other two peaks eluted with 0.2 → 0.6 M NaCl, were the acidic polysaccharide fractions (A-DCPOPA and A-DCPOPB). The neutral fraction N-DCPOP were collected and applied to the Sepharose CL-6B gel-permeation chromatography column, resulting in the elution profile in Fig. 1B. The eluate by 0.15 M NaCl was collected and dialysed against distilled water, and then lyophilized to give one main fraction POP.

The POP showed a single and symmetrically sharp peak on HPGPC (data not shown) at a retention time of around 25 min, which corresponded to an average molecular weight of 24.6 kDa. POP contained 92% carbohydrate and was free of proteins according to the result of a UV scan at 200–400 nm (no absorption at 280 nm) and the Bradford method. According to GC analysis, POP was composed of mannose, arabinose, glucose and galactose, with molar ratios of 2.1:5.2:2.1:11.2, strongly indicating that the polysaccharide was heterogeneous.

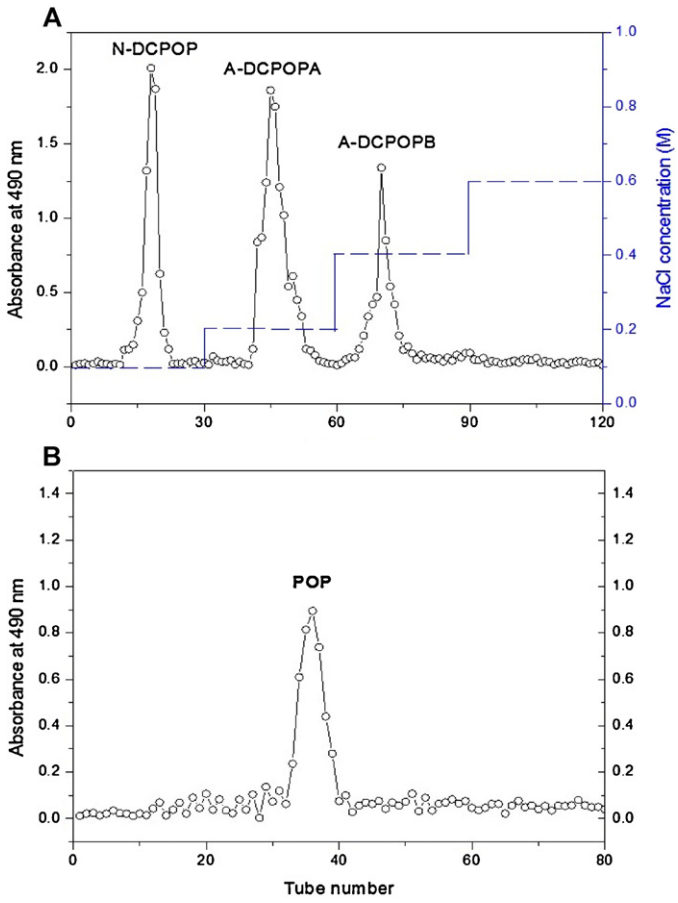


**Fig. 1.** (A) Elution profiles of crude polysaccharides DCPOP on DEAE-Cellulose anion-exchange chromatography column (2.0 cm × 40 cm, $Cl^-$) with stepwise gradient of aqueous NaCl elute (0, 0.1, 0.3, 0.5, and 1 M). (B) Elution profiles of fraction N-DCPOP on Sepharose CL-6B gel-permeation chromatography column (2.0 cm × 90 cm) with 0.15 M NaCl elute.

### 3.2. In vivo antitumor assay

As shown in Table 1, oral administration of POP could significantly inhibit the growth of mouse transplantable sarcoma 180, with the inhibitory rate of 29.94% for 25 mg/kg, 44.07% for 50 mg/kg and 37.29% for 100 mg/kg, respectively. The tumor inhibitory rate in the group treated with POP at the 50 mg/kg was comparable to that of the 5-FU (46.89%). More importantly, POP could significantly increase the weight of the immune organs in mice ($P < 0.05$ or $P < 0.01$) compared to the negative group, especially at the dose of 50 mg/kg. However, the spleen indexes in 5-FU-treated group was lower than that of the negative control ($P < 0.01$), indicating the suppressing effect of 5-FU on immune system as reported previously. Taken together with these data, the results implied POP not only inhibit the growth of transplantable tumor in vivo, but also improved the immune functions of tumor-bearing mice.

### 3.3. Effects of POP on spontaneous and mitogen-induced splenocyte proliferation in mice

Spleen is one of the major immune organs in which T-lymphocyte and B-lymphocyte coexist to induce cell and humoral immune response, respectively. It is well known that splenocyte proliferation is an indicator of immunomodulation. Since splenocyte proliferation is related to immunity improvement of T-lymphocyte or B-lymphocyte (Yang, Jia, Meng, Wu, & Mei, 2006). Therefore the effect of POP on spontaneous and

**Table 1**
Effects of POP on tumor growth, spleen index and thymus index in tumor-bearing mice.

| Groups | Treatment (mg/kg) | Spleen index (mg/g) | Thymus index (mg/g) | Tumor weight (g) | Inhibitory rate (%) |
|---|---|---|---|---|---|
| Control | Vehicle | 6.85 ± 0.74 | 2.12 ± 0.41 | 1.77 ± 0.15 | – |
| 5-FU | 25 | 4.21 ± 0.99[b] | 2.21 ± 0.39 | 0.94 ± 0.12[c] | 46.89 |
| POP | 25 | 8.64 ± 1.02[b] | 2.56 ± 0.41[a] | 1.24 ± 0.13[a] | 29.94 |
| | 50 | 9.43 ± 1.45[b] | 2.98 ± 0.63[b] | 0.99 ± 0.14[c] | 44.07 |
| | 100 | 8.34 ± 1.21[b] | 2.63 ± 0.34[a] | 1.11 ± 0.12[c] | 37.29 |

S180-bearing mice were treated with 5-FU (25 mg/kg) and POP at doses of 25, 50, 100 mg/kg for 10 days once daily. Negative control group received the same volume of normal saline. Thymus/spleen index, tumor weight and the inhibitory rate were determined on day 11. Values are mean ± S.D. of 10 mice.
[a] Significantly different from negative control group at $P < 0.05$.
[b] Significantly different from negative control group at $P < 0.01$.
[c] Significantly different from negative control group at $P < 0.001$.

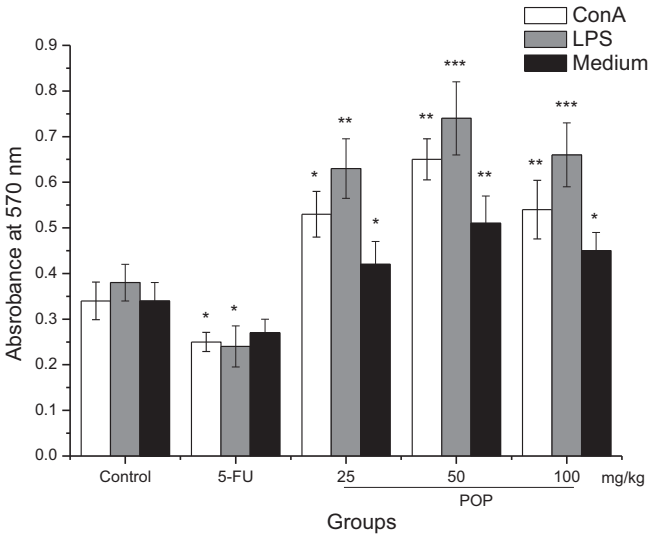


**Fig. 2.** Effects of POP on spleen lymphocyte proliferation in S180-bearing mice. S180-bearing mice were treated with 5-FU (25 mg/kg) and POP at doses of 25, 50, 100 mg/kg for 10 days once daily. Negative control group received the same volume of normal saline. The lymphocyte proliferation was determined by MTT assay. Values are mean ± S.D of 10 mice. *Significantly different from negative control group at $P < 0.05$. **Significantly different from negative control group at $P < 0.01$. ***Significantly different from negative control group at $P < 0.001$.

mitogen-stimulated splenocyte proliferation in tumor-bearing mice was investigated using the T-cell mitogen, concanavalin A (ConA), or the B-cell mitogen, LPS. As shown in Fig. 2, a moderate increase of splenocyte proliferation was observed following the polysaccharide POP treatment in the absence of mitogens, which indicates that POP was directly of great benefit to splenocytes proliferation by itself. In the presence of mitogens (Con A or LPS), POP also dramatically increased splenocyte proliferation, suggesting that it had considerable comitogenic activity. While Con A- and LPS-stimulated splenocyte proliferations in the 5-FU-treated group were significantly lower than those of the model control ($P < 0.05$). The present results indicated that POP had directly mitogenic or comitogenic effect on mouse splenocytes and could stimulate proliferation of spleen lymphocyte, strengthen immunological response and improve immunity.

### 3.4. Effects of POP on peripheral blood T-lymphocyte subpopulations of tumor-bearing mice.

T cells could differentiate into two different subsets ($CD4^+$ T and $CD8^+$ T cells) according to their specific surface molecule, who are capable of inducing the death of tumor cells and played pivotal roles in immunomodulation (Gerloni & Zanetti, 2005). As we know, helper T cells are called as CD4+ T cells, which can excrete cytokines and express CD4 molecules on their surface, and cytotoxic T cells, also known as CD8+ T cells, express CD8 on their surface. In order to find out the mechanism by which POP take effect on the immune cells, the $CD4^+$ and $CD8^+$ expressions on peripheral blood T-lymphocyte were tested by flow cytometric analysis. As the results, the frequency of $CD4^+$ T cells and the ratio of $CD4^+/CD8^+$ in peripheral blood of tumor-bearing mice were elevated following treatment with POP ($P < 0.05$ or $P < 0.01$), while that of $CD8^+$T cell was significantly decreased by POP, especially at the dose of 50 mg/kg (Table 2). In addition, 5-FU treatment caused a reduction in the number of $CD4^+$ and $CD8^+$ T-lymphocyte subpopulation of peripheral blood. These results suggested that POP treatment could activate helper T cells to exert their immunomodulatory effects in tumor-bearing mice.

### 3.5. Effects of POP on hematological and biochemical parameters of tumor-bearing mice

As shown in Table 3, oral administration of POP over a period of 10 days could significantly increase the percentage of WBC in peripheral blood ($P < 0.05$, $P < 0.01$ or $P < 0.001$) compared with negative control group, especially at the 50 mg/kg dose, while very significant inhibition on the WBC count was observed by 5-FU (25 mg/kg).

**Table 2**
Effects of POP on the percentage of peripheral blood T-lymphocyte subpopulations of tumor-bearing mice.

| Groups | Treatment (mg/kg) | $CD4^+$ (%) | $CD8^+$ (%) | $CD4^+/CD8^+$ ratio |
|---|---|---|---|---|
| Control | Vehicle | 22.43 ± 2.87 | 27.72 ± 1.89 | 0.81 |
| 5-FU | 25 | 20.23 ± 2.05 | 13.64 ± 1.19[b] | 1.48 |
| POP | 25 | 30.42 ± 6.27[a] | 15.42 ± 1.68[b] | 1.97 |
| | 50 | 37.85 ± 4.415[b] | 9.54 ± 1.64[b] | 3.97 |
| | 100 | 32.49 ± 5.09[a] | 14.58 ± 1.53[b] | 2.23 |

S180-bearing mice were treated with 5-FU (25 mg/kg) and POP at doses of 25, 50, 100 mg/kg for 10 days once daily. Negative control group received the same volume of normal saline. Peripheral blood T-lymphocyte subpopulations ($CD4^+$ and $CD8^+$) of tumor-bearing mice were determined by Flow cytometric analysis. Values are mean ± S.D. of 10 mice.
[a] Significantly different from negative control group at $P < 0.05$.
[b] Significantly different from negative control group at $P < 0.01$.

**Table 3**
Effects of POP on hematological and biochemical parameters of tumor-bearing mice.

| Groups | Treatment (mg/kg) | WBC ($\times 10^6$/mL) | AST (U/L) | ALT (U/L) | BUN (mmol/L) | Creatinine (mmol/L) |
|---|---|---|---|---|---|---|
| Control | Vehicle | 5.23 ± 0.87 | 118.58 ± 11.80 | 62.13 ± 7.73 | 7.54 ± 0.95 | 68.67 ± 7.22 |
| 5-FU | 25 | 4.74 ± 0.91[a] | 189.54 ± 21.04[c] | 87.34 ± 10.14[b] | 12.49 ± 1.18[c] | 81.82 ± 9.10[c] |
| POP | 25 | 5.58 ± 1.34[a] | 111.87 ± 15.47 | 59.04 ± 7.42 | 7.18 ± 0.59 | 57.25 ± 6.06[a] |
| | 50 | 6.73 ± 1.77[c] | 84.48 ± 12.04[b] | 43.20 ± 7.28[b] | 5.46 ± 0.70[b] | 39.63 ± 3.85[c] |
| | 100 | 5.89 ± 1.42[b] | 100.23 ± 11.48[a] | 54.51 ± 7.19[a] | 6.89 ± 0.47[a] | 50.14 ± 7.56[c] |

S180-bearing mice were treated with 5-FU (25 mg/kg) and POP at doses of 25, 50, 100 mg/kg for 10 days once daily. Negative control group received the same volume of normal saline. The blood samples were obtained from the tumor-bearing mice for the determination of hematological and biochemical parameters using commercial reagent kits. Values are mean ± S.D. of 10 mice.

[a] Significantly different from negative control group at $P < 0.05$.
[b] Significantly different from negative control group at $P < 0.01$.
[c] Significantly different from negative control group at $P < 0.001$.

The effects of POP on the serum AST, ALT, BUN and creatinine levels in S180-bearing mice were shown in Table 3. POP significantly decreased the serum AST, ALT, BUN and creatinine levels in S180-bearing mice compared with control group ($P < 0.05$, $P < 0.01$ or $P < 0.001$), while the serum AST, ALT, BUN and creatinine levels in tumor-bearing mice were markedly increased on day 10 after 5-FU treatment ($P < 0.05$, $P < 0.01$ or $P < 0.001$), suggesting its damaging effects to liver or kidney.

## 4. Conclusion

In the present study, a polysaccharide (POP) from *P. oleracea* was obtained by hot water extraction and then purified by DEAE-Cellulose anion-exchange and Sepharose CL-6B gel-permeation chromatography. The POP was a homogeneous polysaccharide with a molecular weight of 24.6 kDa, as determined by HPGPC. GC analysis showed that POP was composed of mannose, arabinose, glucose and galactose in the molar ratios of 2.1:5.2:2.1:11.2. The antitumor and immunoregulatory activities of POP were evaluated in an animal model transplanted with sacroma 180. In the present study, the POP we used could inhibit the in vivo growth of sarcoma 180 through oral administration in mice and improve the status of immune organ in tumor-bearing mice, such as spleen and thymus. It is well recognized that antitumor effects of polysaccharides may be through the indirect pathway by activating the immune responses in the host. For this reason, we also examined the potentiality of POP on the host immune defense system of tumor-bearing mice. Splenocytes from POP-treated mice displayed a significantly higher proliferation with or without mitogen (ConA and LPS), indicating its positive regulation on both T and B lymphocyte proliferation. Furthermore, POP treatment caused a significant increment in the percentage of CD4+ T lymphocyte and a decrease in the percentage of CD8+ T lymphocyte of tumor-bearing mice peripheral blood, respectively. Accordingly the ratio of CD4+/CD8+ T lymphocyte was significantly increased by the treatment of POP, supporting the fact that helper T cells (Th) was activated by POP. In addition, oral administration of POP could significantly increase the peripheral blood WBC count and decrease the serum AST, ALT, BUN and creatinine levels of tumor-bearing mice. These were consistent with the previous documents that the antitumor activities of polysaccharides were most attractive due to their low toxicity to normal cells and the apparent lack of side effects in clinical patients (Fukushima, 1989). Considering the results in the present study, we confirmed that POP from *P. oleracea* can be developed individually as potent biological response modifiers for cancer treatment and also as standard anticancer drugs. Further studies on the mechanism by which this complex induces these effects and additional clinical usefulness in therapies of cancer are needed.

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