Carbohydrate Polymers 118 (2015) 101–106

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Structure characterization and antitumor activity of a polysaccharide from the alkaline extract of king oyster mushroom

Xinkui Liu[a,*], Lin Wang[b], Chunmei Zhang[c], Hongmin Wang[d], Xiaohong Zhang[e], Yuexia Li[d]

[a] Department of Medical Records Management, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China
[b] Department of Radiotherapy, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China
[c] Nursing Department, Henan Provincial People's Hospital, Zhengzhou 450003, China
[d] Department of Respiratory, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China
[e] Department of Respiratory, Zhengzhou Central Hospital Affiliated to Zhengzhou University, Zhengzhou 450053, China

ARTICLE INFO

Article history:
Received 7 January 2013
Received in revised form 17 October 2014
Accepted 22 October 2014
Available online 30 October 2014

Keywords:
King oyster mushroom
Polysaccharide
Immunomodulatory
Antitumor

ABSTRACT

A water-soluble polysaccharide, designated as KOMAP, was isolated and purified from the alkaline extract of king oyster mushroom, which was composed of glucose (Glc), mannose (Man) and arabinose (Ara) in a molar ratio of 6.2:2.1:2.0. It had an average molecular weight of 2.1×10^4 Da. GC–MS analysis revealed that KOMAP was a linear structure of the polymer with a backbone composed of β-1,4-linked glucopyranosyl and β-1,3,6-linked mannopyranosyl units, which was terminated with α-1-linked arabinofuranosyl unit at C-6 position of β-1,3,6-linked mannopyranosyl residue along the main chain in the ratio of 3.1:1. The results in the animal experiment showed that 50, 100 and 200 mg/mL of KOMAP not only inhibited the tumor growth, but also increased relative thymus and spleen indices, LPS- or ConA-induced lymphocytes proliferation, and serum cytokine IL-2, TNF-α, and IFN-γ levels, as well as the activities of NK cells and CTLs in spleen of Renca tumor-bearing mice. In summary, our data indicate that the KOMAP exerts effective immunoregulatory and anti-tumor activities in vivo.

1. Introduction

During the past decades, polysaccharides and polysaccharide–protein complexes derived from natural sources, such as Traditional Chinese Medicine (TCM) and some medicinal mushrooms, have received much attention as a source of therapeutic agents for the prevention and cure of neoplastic diseases due to their immunomodulatory and antitumor effects (Mitchell, 2003). Several lines of direct evidence showed that the enhancement of host immune defense system by bioactive polymers has been suggested to be a safe means of inhibiting tumor growth without harming the host in contrast to existing cancer chemotherapy, radiotherapy and the majority of anti-tumor chemical compounds (Wang, Sun, Wu, Yang, & Tan, 2014; Yuan, Song, Li, Li, & Dai, 2006). These immunomodulating polysaccharides are regarded as biological response modifier (BRM) i.e. they cause no harm and place no additional stress on the body, but help the body to adopt to environmental and biological stress (Mizuno et al., 2000). From this standpoint, extensive study should be undertaken on polysaccharides isolated from natural sources with improving immunity potential.

Edible mushrooms contain physiologically beneficial bioactive compounds and can be good sources for the cancer treatment (Li et al., 2008; Sarangi, Ghosh, Bhutia, Mallick, & Maiti, 2006, Stajić, Vukojević, & Duletić-Lausević, 2009). As a type of edible mushroom, *Pleurotus eryngii*, commonly called the king oyster mushroom due to its good taste and nutritional value, has rapidly become a highly valued species among consumers in North America, Europe, and Asia (Royse, 1995). This mushroom contains many biologically active substances such as polysaccharides, lipids, peptide, sterols, and dietary fiber (Liu et al., 2010). Antitumor activity of the crude polysaccharides or purified polysaccharides from the water extract of king oyster mushroom have already been carried out by several works (Kim et al., 2004; Zhang, Cheung, Zhang, Chiu, & Ooi, 2004; Zhang, Zhang, Cheung, & Ooi, 2004; Yang et al., 2013). However, the chemical components and mechanisms of anti-tumor activity of purified polysaccharides from the alkaline extract of king oyster mushroom remain unclear. Therefore, the present paper was

* Corresponding author. Tel.: +86 037166295092
E-mail address: liuxinkui@21cn.com (X. Liu).

http://dx.doi.org/10.1016/j.carbpol.2014.10.058
0144-8617/

concerned with the isolation, chemical characterization and evaluation of the anti-tumor activity of the polysaccharide KOMAP from the alkaline extract of king oyster mushroom.

2. Materials and methods

2.1. Materials and chemicals

King oyster mushroom was brought from one of the local markets in Zhengzhou city of China. DEAE-cellulose and Sephadex G-200 were purchased from Pharmacia Chemical Co. 3-(4, 5-dimethyl-2-thiazol)-2,5-diphenyl-2H-tetrazolium bromide (MTT); 1-phenyl-3-5-pyrazolone (PMP), Concanavalin A (Con A), lipopolysaccharide (LPS), T-series Dextran (T-2000, T-70, T-40, T-20, and T-10), the monosaccharide standards, trifluoroacetic acid (TFA) and bovine serum albumin (BSA) were purchased from Sigma Co. (St. Louis, MO, USA). Fetal bovine serum (FBS) and RPMI-1640 medium were purchased from Gibco Invitrogen Co. (Grand Island, NY, USA). Enzyme-linked immunosorbent assays (ELISA) for mouse interleukin-2 (IL-2), tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) were purchased from Pierce Biotechnology (Rockford, IL, USA). Commercial reagent kits for aspartate aminotransferace (AST), alanine aminotransferace (ALT), blood urea nitrogen (BUN), uric acid (UA) and creatinine (CRE) were purchased from Lanji Technology Development Co. (Shanghai, China). All the other chemicals used were of analytical grade.

2.2. Extraction, isolation and purification of polysaccharides

The dried king oyster mushroom (500 g) was ground to powders, and submitted to remove lipids by extracting 3 times with 10 vol of 95% EtOH under reflux at 75 °C for 3 h each time. The residue obtained was then extracted thrice with 15 vol of boiling water under reflux until no reaction of phenol–sulfuric acid and then dipped into 5% alkali solution for 24 h for three times. The extract was filtered through gauze and the suspension was then neutralized with hydrochloric acid (0.1 M) and concentrated to one-tenth of the volume with a rotary evaporator at 80 °C under vacuum. The proteins in extract were removed from the extract by using Sevag reagent (Staub, 1965). After the Sevag reagent was removed, the mixture was further precipitated with 4 vol of 95% EtOH at 4 °C for 24 h. After centrifugation, the resulting precipitate (crude polysaccharides, KOMCP) was washed sequentially with ethanol, acetone, ether, and vacuum-dried.

The crude polysaccharide KOMCP was applied to a DEAE-cellulose column (2.0 cm × 40 cm), eluted stepwise with distilled water, 0.5 and 1.0 M NaCl, respectively. The eluate (6 ml) was collected and monitored for carbohydrate content using phenol–sulfuric acid method at 490 nm (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). According to the elution profile, the eluates were combined, concentrated, dialyzed and lyophilized to give three fractions. The fraction containing polysaccharide eluted by distilled water solution was further applied to a column (2.5 cm × 100 cm) of Sephadex G-200 equilibrated with 0.1 M NaCl at a flow rate of 60 ml/h. After collection, the relevant fractions were concentrated, dialyzed and lyophilized to produce white power, of which the KOMAP was used in the subsequent studies.

2.3. General analysis of the polysaccharide

The total neutral sugar content and total uronic acid content use Glc and GalA as standard, respectively. The former was determined by the reaction with phenol in the presence of sulfuric acid at 486 nm (Dubois et al., 1956); while the latter was determined by photometry with m-hydroxybiphenyl at 523 nm (Blumenkrantz & Asboe-Hansen, 1973). Proteins were estimated by the Bradford assay (Bradford, 1976), using BSA as the standard.

The average molecular weight was determined by the High-performance gel-permeation chromatography (HPGPC), which was performed on a Waters HPLC system (Allances 2695, Waters, USA) with a column of TSK-GEL G3000 SWXL (7.8 mm × 300 mm), and a Waters 2410 differential refractometer. The mobile phase was 0.1 mol/l $NaNO_3$, and the flow rate was 1 ml/min. The sample (2 mg) was dissolved in the mobile phase (0.2 ml) and centrifuged. A 20 μl sample was injected in each run. The molecular weight was estimated by reference to the calibration curve made from a Dextran T-series standard of known molecular weight (T-2000, T-70, T-40, T-20, and T-10).

Polysaccharide samples (2 mg) were first methanolyzed with anhydrous methanol containing 2 M HCl at 80 °C for 16 h and then hydrolyzed with 2 M TFA at 120 °C for 1 h. The resulting hydrolysates were derivatized with PMP according to the method reported by Yang et al. (Yang, Zhao, Wang, Wang, & Mei, 2005) and analyzed on a DIKMA Inertsil ODS-3 column (4.6 mm × 150 mm) connected to a Shimadzu HPLC system (LC-10ATvp pump and SPD-10AVD UV-VIS detector) with UV absorbance detection at 245 nm. 20 μL of the PMP derivative was injected for each run and eluted with 80.0% PBS (0.1 M, pH 7.0) and 20.0% acetonitrile (v/v) at a flow rate of 1.0 mL/min.

2.4. Methylation analysis

The polysaccharide, KOMAP (20 mg), was methylated using according to Needs and Selvendran (1993). Complete methylation was confirmed by the disappearance of the OH band (3200–3700 cm^{-1}) in the IR spectrum. After completed methylation, the methylated products were hydrolyzed, then reduced and acetylated as described by Sweet, Shapiro, and Albersheim (1975). The partially methylated alditol acetates were then analyzed on a gas chromatography/mass (GC/MS) spectrometer (QP 2010, Shimadzu, Kyoto, Japan) with a SP-2330 capillary column (30 m × 0.25 mm, 0.2 mm film thickness) to analyze the glycosidic linkage. The temperature program was set as follows: the initial temperature of column was 160 °C, increased to 210 °C at 5 °C/min, held for 5 min at 210 °C, and then 210–250 °C at 5 °C/min.

2.5. Animals preparation and experiment design

Female specific pathogen-free (SPF) BALB/c mice (weighing from 18 to 20 grams) acquired from Experimental Animal Center, the Zhengzhou University (Zhengzhou, China), were kept for a minimum of one week prior to the study so that they were acclimatized. They were also free access to standard food and water and maintained in a SPF room with air-conditioning providing a 12/12 h light/dark cycle. Temperature inside the room was kept at 24 ± 1 °C, and humidity at 50 ± 10%. Animals were handled according to the rules and regulations of Institutional Animal Ethics Committee (IAEC) of Zhengzhou University.

The diluted Renca tumor cell suspension (2×10^5 cells) was subcutaneously injected into the armpit for 0.2 ml per mouse to prepare tumor-bearing mice. A total of 40 mice were allocated in random into four groups (10 mice in each group) 24 h after the tumor inoculation and treated with 50, 100 and 200 mg/kg of KOMAP for 10 consecutive days once daily by gavage, and another 10 tumor-bearing mice were administrated with sterile physiological saline intragastrically once daily (10 m/kg) served as normal control. One hour after the last drug administration, the rats were weighed and sacrificed by cervical dislocation. The tumor mass and their spleens and thymuses were instantly dissected, weighed and calculated according to the following formulae: Organ index (mg/10 g) = organ weight/body weight; the inhibitory rate

(%) = [(C − T)/C] × 100%, where C is the average tumor weight of the model control group; T is the average tumor weight of drug-treated groups.

2.6. Preparation of spleen lymphocytes

The spleens collected form the treated or untreated groups were aseptically chopped with two slides and filtered over a fine nylon mesh to produce single-cell suspensions. The cells were washed and lymphocytes were resuspended Tris–HCl-buffered NH_4Cl solution (0.15 M NH_4Cl, pH 7.2) to remove erythrocytes. Cells were finally maintained in RPMI 1640 medium supplemented with 100 IU/ml penicillin, 100 μg/ml streptomycin, and 10% FCS at 37 °C under humidified air with 5% CO_2 for further experiments.

2.7. Measurement of lymphocyte transformation

Splenocytes were seeded in 96-well plates (1×10^7 cells/well) in the presence or absence of Con A (2.5 μg/mL) or LPS (5.0 μg/mL) for incubation at 37 °C in a humidified environment with 5% CO_2 for 44 h. Thereafter, 50 μl of MTT solution (2.0 mg/ml) were added to each well and continued for another 4 h incubation. Then the medium was discarded and 100 μl dimethylsulfoxide (DMSO) was then added to each well. 15 min later, the absorbance in each well was measured using an ELISA reader (Bio-Rad, USA) at 570 nm. The stimulation index (SI) was calculated according to the following formula: SI = the absorbance value for mitogen-cultures divided by the absorbance value for non-stimulated cultures.

2.8. Measurement of serum cytokines

Blood was collected and serum was immediately separated by centrifugation at 3000 rpm at room temperature for 10 min. Serum collected from tested mice were assayed for the level of IL-2, TNF-α, and IFN-γ using commercially available kits from R&D systems, with a solid-phase ELISA according to the instructions from the manufacturer. The absorbance was measured in an ELISA reader at 450 nm.

2.9. Assay for activities of natural killer (NK) cells and cytotoxic T lymphocytes (CTL)

2.9.1. Assay of NK activity

The standard ^{51}Cr-release assay was used to measure the activity of NK cell, which was described previously by Zeytin et al. (2008) with minor modification. In short, Yac-1 cells (target cells) were labeled with 250 μCi of $Na_2[^{51}Cr]O_4$ at 37 °C for 30 min. Thereafter, the NK cells (100 μl) collected from control or SMPA-treated groups were mixed with $Na_2[^{51}Cr]O_4$ labeled Yac-1 cells (100 μl) at a effector-to-target (E:T) ratio of 50:1 in 96-well plates. After incubation for 4 h at 37 °C in an atmosphere of 5% CO_2, the radioactivity of the gathered cell-free supernatants was counted using a Beckman LS 6500 gamma counter. Specific cellular cytotoxicity was calculated by the following formula: percentage of specific lysis = (experimental cpm − spontaneous cpm)/(maximum cpm − spontaneous cpm) × 100. Spontaneous cpm was the radioactivity from wells with target cells alone. Controls for spontaneous or maximal ^{51}Cr release were target cells in medium or in 1.5% Triton, respectively. All assays were done in triplicate.

2.9.2. Assay of CTL activity

CTLs were obtained from splenocytes of mice in each group and cultured together with $Na_2[^{51}Cr]O_4$ labeled Renca cells (1×10^4 cells/well) at a E:T ratio of 100:1 for 5 h in 96-well round-bottom plates in the presence of IL-2 (50 ng/mL). Target cell killing was determined by a Beckman LS 6500 gamma counter and percent cytotoxicity was calculated as described above.

Table 1
GC–MS analysis of polysaccharide.

Peak No.	Methylated sugar	Molar ratios	Linkage type
Residue A	2,3,6-Me_3-Glc*p*	3	1,4-linked-Glc*p*
Residue B	2,4-Me_3-Man*p*	1	1,3,6-linked-Man*p*
Residue c	2,3,5-Me_3-Ara	1	Terminal-Ara*f*

2.10. Biochemical parameters assay

Serum samples from the above treated groups were immediately stored at −80 °C until assayed. Biochemical indexes, including AST, ALT, BUN, UA and CRE, were also determined by the enzymatic method using commercial reagent kits according to the manufacturer's instructions.

2.11. Statistical analysis

All results were presented as mean ± S.D. One-way ANOVA was performed for data analysis by using SPSS and Dunnett's test. The results were considered statistically significant when having *P*-values less than 0.05.

3. Results and discussion

3.1. Isolation and chemical characterization of the polysaccharide

The crude polysaccharide KOMCP (46.8 g from 500.0 g) was isolated from the alkaline extract of king oyster mushroom with a yield of 9.36%. After fractionation on DEAE-cellulose column, three fractions were obtained. Among them, neutral fraction eluted with distilled water with relatively high content was repeatedly subjected to size-exclusion column chromatography on a Sephadex G-200 gel filtration column and eluted with 0.1 M NaCl at a flow rate of 1 mL/min, whereby one major polysaccharide peak was collected and then freeze-dried to yield KOMAP (4.25 g, 0.85% of starting material).

No uronic acid was detected in KOMAP by meta-hydroxydiphenyl colorimetric method. There was also no absence of protein and nucleic acid because of no absorption at 280 or 260 nm in the UV spectrum. KOMAP had total carbohydrate content of 94.5%, which was determined by the phenol–sulfuric method. On HPGPC, KOMAP showed a single and symmetrical sharp peak, indicating a typical indication of a homogeneous polysaccharide. Its molecular weight was estimated to be about 2.5×10^4 Da. GC analysis showed that KOMAP was mainly composed of glucose (Glc), mannose (Man) and arabinose (Ara) in a molar ratio of 6.2:2.1:2.0. The results from analysis of GC–MS in Table 1 indicated that β-1,4-linked glucopyranosyl and β-1,3,6-linked mannopyranosyl residues were major components of the backbone structure, which was terminated with α-1-linked arabinofuranosyl unit at C-6 position of β-1,3,6-linked mannopyranosyl residue along the main chain in the ratio of 3.1:1. This showed a good correlation between terminal and branched residues, and these molar ratios agreed with the overall monosaccharide composition described above.

3.2. Inhibition of polysaccharide on implanted Renca tumor growth in mice

To investigate whether polysaccharide KOMAP exerted an in vivo antitumor activity, Renca allograft tumors were excised from each group and weighed following KOMAP pretreatment at

Table 2
Inhibitory effect of polysaccharide on implanted Renca tumor growth in mice.

Groups	Dosage (mg/kg)	Tumor weight (g)	Inhibitory rate (%)
Control	–	2.02 ± 0.45	–
KOMAP	50	1.20 ± 0.20	40.59[a]
	100	1.11 ± 0.21	45.05[a]
	200	1.03 ± 0.19	49.01[b]

Values are shown as mean ± SD ($n = 10$). Significant differences compared to control group are designated as:
[a] $P < 0.05$.
[b] $P < 0.01$.

doses of 50, 100 and 200 mg/kg. As a result, the weight of the tumor was significantly suppressed in a dose-dependent manner for those three doses, while there was no delay observed for tumors growth in the control group. Compared to model control group, the tumor inhibitory rates of the KOMAP-treated groups were 40.59%, 45.05% and 49.01%, at the doses of 50, 100 and 200 mg/kg, respectively (Table 2). The present results showed that KOMAP inhibited the growth of implanted Renca tumor markedly.

3.3. Effect of polysaccharide on immune organ index and splenocyte proliferation of mice transplanted with Renca tumor

Many experiments have shown that it is by activating different immune responses in the host that a large number of polysaccharides from mushrooms generate antitumor effects (Ooi & Liu, 2000). To investigate whether humoral immunity or cell-mediated immunity can be raised by KOMAP, we measured the effect of KOMAP on organ indices and mitogen-stimulated splenocyte proliferation in mice transplanted with Renca tumor. As shown in Fig. 1A KOMAP administration increased the related indices of spleen and thymus in Renca-bearing mice in the increasing order of 50, 100 and 200 mg/kg ($P < 0.05$ or $P < 0.01$). In the meantime, splenocyte proliferation induced by Con A and LPS was pushed up significantly in

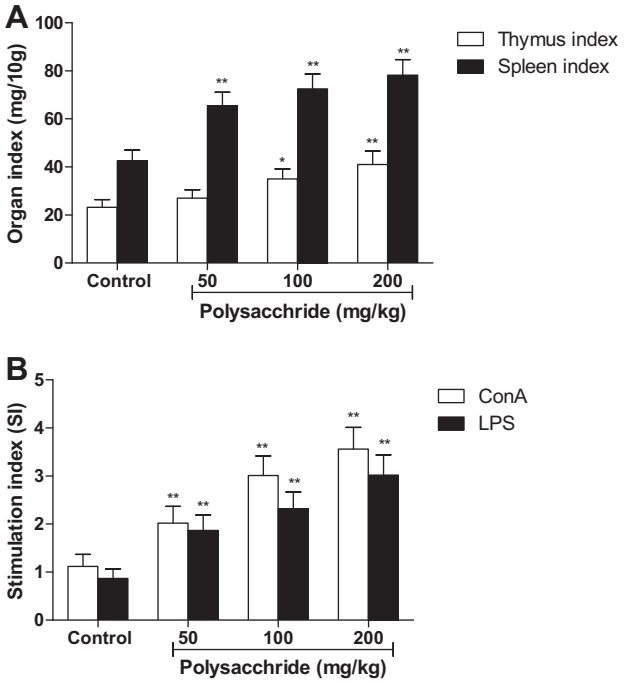

Fig. 1. Effect of polysaccharide on (A) immune organ index and (B) splenocyte proliferation of mice transplanted with Renca tumor. Values are shown as mean ± SD ($n = 10$). Significant differences compared to control group are designated as $^{*}P < 0.05$, and $^{**}P < 0.01$.

Table 3
Effect of polysaccharide on serum levels of IL-2, TNF-a, and IFN-γ of mice transplanted with Renca tumor.

Groups	Dosage (mg/kg)	IL-2 (pg/ml)	TNF-α (pg/ml)	IFN-γ (pg/ml)
Control	–	10.42 ± 3.34	18.31 ± 3.81	12.20 ± 3.07
KOMAP	50	15.55 ± 5.41[a]	38.25 ± 4.53[b]	53.21 ± 7.85[c]
	100	23.20 ± 6.50[b]	52.72 ± 7.99[c]	65.02 ± 8.20[c]
	200	28.11 ± 6.87[b]	65.32 ± 8.04[c]	80.07 ± 9.01[c]

Values are shown as mean ± SD ($n = 10$). Significant differences compared to control group are designated as:
[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$.

KOMAP-treated mice compared with that in control mice, with a P-value of less than 0.01 (Fig. 1B).

3.4. Effect of polysaccharide on serum levels of IL-2, TNF-α, and IFN-γ of mice transplanted with Renca tumor

As cytokines have key importance in developing immune response (Asano, Kaneda, Hiragushi, Tsuchida, & Higashino, 1997; Yang, Li, Xue, Wang, & Liu, 2014), the effect of KOMAP on the production of serum cytokines IL-2, TNF-α, and IFN-γ in Renca-bearing mice was investigated through ELISA. As seen in Table 3, the expression levels of IL-2, TNF-α, and IFN-γ in serum were significantly higher in the KOMAP-treated groups than those in the tumor model group administrated with PBS ($P < 0.05$, $P < 0.01$ or $P < 0.001$), which implied that IL-2, TNF-α, and IFN-γ may be involved in the immune response. These increases may also explain the antitumor properties of KOMAP.

3.5. Effect of polysaccharide on the activities of NK cells and CTL of mice transplanted with Renca tumor

Increasing evidence has indicated that both CTLs and NK cells are effector cells. The former plays a major role in cancer immunity while the latter attacks tumor cells (Sunila, Hamsa, & Kuttan, 2011). Therefore the effects of KOMAP on the cytotoxic activities of NK cells and CTLs were measured, with the results shown in Fig. 2. NK cells and CTL activities in mice with KOMAP treatment were remarkably higher than those in the model control group. These results suggest that NK cells and CTLs help to increase the antitumor effects of KOMAP in mice transplanted with Renca tumor.

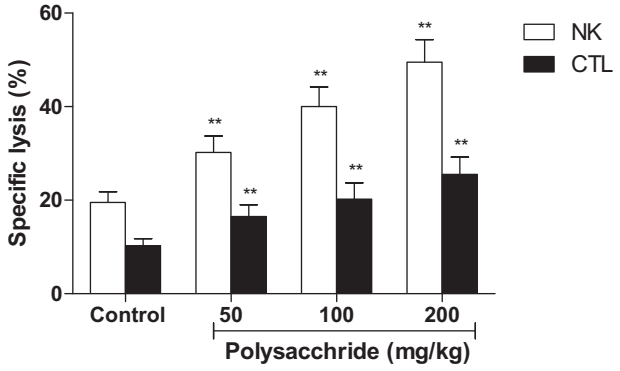

Fig. 2. Effect of polysaccharide on the activities of NK cells and CTL of mice transplanted with Renca tumor. Values are shown as mean ± SD ($n = 10$). Significant differences compared to control group are designated as $^{**}P < 0.01$.

Table 4
Effect of polysaccharide on the hepatic and renal function indexes of mice transplanted with Renca tumor.

Groups	Dosage (mg/kg)	AST (U/L)	ALT (U/L)	BUN (mmol/L)	CRE (mmol/L)	UA (mmol/L)
Control	–	208.54 ± 30.21	89.24 ± 10.12	10.23 ± 1.87	28.56 ± 4.21	23.54 ± 4.17
KOMAP	50	199.04 ± 21.21	85.5 5 ± 8.98	9.82 ± 1.54	27.01 ± 3.79	22.84 ± 3.54
	100	188.21 ± 19.54[a]	79.73 ± 7.86[a]	9.13 ± 1.40	26.62 ± 3.10	21.25 ± 3.04
	200	170.21 ± 15.48[a]	75.68 ± 6.67[a]	7.82 ± 0.83[a]	23.55 ± 2.82[a]	18.72 ± 2.84[a]

Values are shown as mean ± SD (n = 10). Significant differences compared to control group are designated as:
[a] $P < 0.05$.

3.6. Effect of polysaccharide on the hepatic and renal function of mice transplanted with Renca tumor

As ideal therapeutic agents, they contain not only the ability of repressing tumor growth but also the minimum toxicity to their normal organs, such as live and kidney. ALT and AST are considered to be sensitive hallmarks of hepatocellular damage and the values provide a quantitative evaluation for the degree of liver damage (Al-Habori, Al-Aghbari, Al-Mamary, & Baker, 2002). BUN, UA and CRE levels are biomarkers or indicators of renal injury, and its high content often indicates the malfunctions of kidney (Fu et al., 2012). To examine the potential toxicological effects of KOMAP administration on the host, we evaluated the serum levels of AST, ALT, BUN, UA and CRE in mice bearing Renca tumor. As shown in Table 4, the increased levels of serum ALT, AST, BUN, UA, and CRE in Renca tumor transplanted mice were obviously attenuated after KOMAP treatment, especially at the high dose ($P < 0.05$), supporting the fact that KOMAP exhibits very few toxicological effects on liver and kidney function in Renca tumor-bearing mice.

4. Conclusions

Cancer is a leading cause of mortality worldwide and chemoprevention has been a promising means of reducing the occurrence of cancer by delaying the process of carcinogenesis (Jahan, Chaudhary, & Goyal, 2009; Kaufman & Earnshaw, 2000). The great majority of chemical compounds identified as cytotoxic to cancer cells are also toxic to normal cells, including many kinds of immune cells in the host (Borchers, Stern, Hackman, Keen, & Gershwin, 1999; Ehrke, 2003). It is well known that specific immunity and non-specific immunity play important roles in antitumor defense (Xu, Yao, Sun, & Wu, 2009). The low immune function of an organism may not only result in the generation and development of a tumor, but also be one of the most important factors that prevent the tumor patients' recovery (Borchers et al., 1999). Emerging evidence suggested that numerous polysaccharides isolated from natural source, with fewer side effects, have been proven to have anti-tumor activity through enhancing the immune response (Cho & Leung, 2007; Schepetkin & Quinn, 2006). Therefore, the discovery and identification of new antitumor substances with little toxicity to host, which can potentiate the host immune function has become an important goal of research in immunopharmacology and oncotherapy (Yuan et al., 2006). Consistent with this viewpoint, the present experiments showed that a polysaccharide KOMAP from the alkaline extract of the king oyster mushroom not only significantly and dose-dependently inhibit the growth of implanted Renca tumor in mice at the doses of 50, 100 and 200 mg/kg, but also improve the immune function by increasing relative spleen and thymus weight, promoting Con A- and LPS-induced splenocyte proliferation, elevating the activities of NK cell and CTL in spleen, and increasing the concentration of IL-2, TNF-α, and IFN-γ in the serum of Renca tumor-bearing mice. Besides, KOMAP administration does not induce any toxicity to their normal organs, such as liver and kidney. To conclude, all the data above implied that KOMAP deserves further study to develop its potential as a nutraceutical agent for cancer therapy or an immunomodulatory adjuvant for chemotherapy.

Acknowledgement

This work was supported by the Youth Innovation Fund Project of the First Affiliated Hospital of Zhengzhou University (Zhengzhou, China).

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