Immunomodulating and Antitumor Activities of *Panellus serotinus* Polysaccharides

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This study was initiated in order to investigate the anticancer and immunomodulating activities of crude polysaccharides extracted in methanol, neutral saline, and hot water (hereinafter referred to as Fr. MeOH, Fr. NaCl, and Fr. HW, respectively) from the fruiting bodies of *Panellus serotinus*. Content of β-glucan and protein in Fr. MeOH, Fr. NaCl, and Fr. HW extracts of *P. serotinus* ranged from 22.92~28.52 g/100 g and 3.24~3.68 g/100 g, respectively. *In vitro* cytotoxicity tests, none of the various fractions of crude polysaccharides were cytotoxic against sarcoma 180, HT-29, NIH3T3, and RAW 264.7 cell lines at the tested concentration. Intraperitoneal injection with crude polysaccharides resulted in a life prolongation effect of 23.53~44.71% in mice previously inoculated with sarcoma 180. Treatment with Fr. HW resulted in an increase in the numbers of spleen cells by 1.3 fold at the concentration of 50 μg/mL compared with control. Treatment with Fr. NaCl resulted in improvement of the immuno-potentiating activity of B lymphocytes by increasing the alkaline phosphatase activity by 1.4 fold, compared with control, at the concentration of 200 μg/mL. Among the three fractions, maximum nitric oxide (13.48 μM) was recorded at 500 μg/mL in Fr. HW. Production of tumor necrosis factor alpha, interleukin-1β, and interleukin-6 was significantly higher, compared to the positive control, concanavalin A, at the tested concentration. Therefore, treatment with crude polysaccharides extracted from the fruiting body of *P. serotinus* could result in improvement of antitumor activity.

KEYWORDS : Antitumor activities, Crude polysaccharides, Immunomodulating, Mouse sarcoma 180, *Panellus serotinus*

Introduction

Panellus serotinus, known as late fall oyster mushroom, has been valued by humankind as an edible and therapeutic resource. A number of bioactive molecules, including antitumor substances, have been identified in many mushroom species. Polysaccharides, with antitumor and immunomodulating properties, are the best known and most potent mushroom derived substances [1, 2].

Polysaccharides are a structurally diverse group of biological macromolecules composed of repetitive structural features, which are polymers of monosaccharide residues joined to each other by glycosidic linkages [3]. Antitumor polysaccharides isolated from mushrooms are either water soluble β-glucans, β-glucans with heterosaccharide chains of xylose, mannose, galactose, and uronic acid or β-glucan protein complexes. As a general rule, immunopotential activity of the protein linked glucans is greater than that of the corresponding glucans [4]. The mode of action of β-glucan differs from that of conventional chemotherapeutic agents in that it is immunotherapeutic. Global awareness of cancer as the second largest cause of death in people of various ages and racial backgrounds has led to significant research efforts and clinical studies in the fight against the disease [2, 5].

Despite the therapeutic potential and the clinical importance of *P. serotinus*, no studies on its antitumor and immunomodulating activities have been reported. In the present study, crude polysaccharides were extracted from fruiting bodies of *P. serotinus* using methanol, neutral saline, and hot water for investigation of antitumor and immunomodulating activities. The *in vitro* cytotoxic activities of four cell lines and *in vivo* antitumor effects on sarcoma 180 tumor bearing mice were studied. In addition, proliferation of murine spleen cells, alkaline phosphatase (APase) activity, nitric oxide (NO), and cytokine production in murine peritoneal macrophages were also investigated.

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Materials and Methods

This study was conducted from November 2010 to December 2011 at the Animal House and Laboratory of Applied Microbiology, Division of Life Sciences. The experimental protocols were approved by the Animal Care Ethics Committee at the University of Incheon, Republic of Korea. All experimental procedures were performed in accordance with the guide for care and use of experimental animals.

Mushroom and extraction.   Fresh fruiting bodies of *P. serotinus* were collected from Deogyusan National Park, Korea. A pure culture was deposited in the Culture Collection and DNA Bank of Mushroom (CCDBM), Division of Life Sciences, University of Incheon, Korea, and an accession number was acquired, IUM-3346. Fresh fruiting bodies were dried with hot air at $40^{\circ}C$ for 48 hr and pulverized.

One hundred grams of pulverized fruiting bodies of *P. serotinus* were extracted with 3,000 mL of 80% methanol and neutral saline (0.9% NaCl) with stirring at 150 rpm for 24 hr at $25^{\circ}C$ to obtain methanol and NaCl extracts. The mixture was filtered through two layers of Whatman No. 1 filter paper (Whatman, Maidstone, UK). The same quantity of sample was boiled at $100^{\circ}C$ for 3 hr with 3,000 mL deionized distilled water to obtain a hot water extract. The mixture was cooled at room temperature and filtered through a Whatman No. 1 filter paper. The residues of methanol, NaCl, and hot water extraction were then treated two more times in the same manner. All supernatants obtained of each extract were combined and mixed with four volumes of ethanol and allowed to stand overnight at $4^{\circ}C$. The precipitate was collected by centrifugation, dissolved in distilled water, dialyzed for 48 hr at $4^{\circ}C$, and lyophilized. This fraction was referred to as the methanol extract (Fr. MeOH), neutral saline extract (Fr. NaCl), and hot water extract (Fr. HW). The yields from the methanol, NaCl, and hot water extracts of *P. serotinus* were 20.54, 18.81, and 19.25% (w/w), respectively.

Animals.   Five-wk-old inbred male ICR mice (22 ± 3 g) were purchased from Central Lab. Animal Inc., Seoul, Korea. All mice were acclimated to the animal house for a period of one week. Mice were housed in an animal room at $23 \pm 2^{\circ}C$ under a 12-hr dark-light cycle (17:00~ 05:00) and a relative humidity of 50~60%. During the experimental period, mice received the standard basal diet, purchased from Central Lab Animal Inc. (Seoul, Korea).

Cell lines.   Mouse sarcoma 180, colon cancer (HT-29), mouse embryonic fibroblast cells (NIH3T3), and murine macrophage cell (RAW 264.7) lines were purchased from Korean Cell Line Bank of Seoul National University,

Seoul, Korea. HT-29, NIH3T3, and RAW 264.7 cell lines were cultured in Dulbecco's modified Eagle's medium supplemented with penicillin (100 U/mL), streptomycin (100 mg/mL), and 10% fetal bovine serum at $37^{\circ}C$ with 5% atmospheric CO_2 in a humidified incubator. Sarcoma 180 cells were maintained in ascitic form by serial transplantation every seven days in an ICR male mouse.

Determination of β-glucan and total protein.   A mushroom and yeast β-glucan assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) was used for quantitative determination of the β-glucan contents of three different fractions extracted from fruiting bodies of *P. serotinus*. In brief, for determination of total glucan (α- and β-), 5 mg of each fraction was suspended in 75 μL of concentrated HCl and incubated at $45^{\circ}C$ for 30 min; 500 μL distilled water was then added, and it was placed in a boiling water bath for 2 hr. The pH was neutralized with 500 μL of 2 N KOH, followed by centrifugation for 10 min at $1,500 \times g$. Fifty microliters of the supernatant was digested with an aliquot of exo-1,3-β-glucanase (20 U/ mL) plus β-glucosidase (4 U/mL) in 200 mM sodium acetate buffer (pH 5.0). The hydrolysates were incubated with 1.5 mL of glucose oxidase/peroxidase mixture (GO/ POD), followed by incubation at $40^{\circ}C$ for 1 hr. The absorbance of the solution was measured at 510 nm. For measurement of α-glucan, 5 mg of each fraction was suspended in 100 μL of 2 N KOH for 20 min and neutralized with 400 μL of 1.2 M sodium acetate buffer (pH 3.8). Then, the solution was centrifuged for 10 min at $1,500 \times g$ and aliquots of amyloglucosidase (1,630 U/mL) plus invertase (500 U/mL) were added to the 50 μL of supernatant, followed by incubation at $40^{\circ}C$ for 30 min. The solution was incubated with 1.5 mL of mixture of GO/POD at $40^{\circ}C$ for 20 min and absorbance was measured at 510 nm. β-Glucan was determined by subtracting α- glucan from total glucan content.

Protein content of each fraction was quantified by Bradford method [6], using bovine serum albumin (BSA) as a standard. Total protein content of the fractions is expressed as g of BSA equivalent per 100 g of dry weight.

Cytotoxicity.   A rapid colorimetric method previously described by Mosmann [7] was used in the 3-(4,5- dimethyl-1-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, for measurement of cell viability and proliferation. Briefly, for the MTT assay, 100 μL of HT- 29, NIH3T3, and RAW 264.7 cells (1×10^5 cells/well) were treated with 10, 100, 1,000, and 2,000 μg/mL concentrations of three different fractions (Fr. MeOH, Fr. NaCl, and Fr. HW) of *P. serotinus* and cultured for 24 hr in 96-well microplates at $37^{\circ}C$ with 5% atmospheric CO_2. Then, 10 μL of 5 mg/mL of MTT solution was added, followed by incubation at $37^{\circ}C$ with 5% atmospheric CO_2 for 4 hr

under dark conditions. Following removal of the supernatant, purple formazan crystals produced were dissolved in 100 μL of dimethylsulfoxide, and quantified by measurement of optical density (OD) at 570 nm using a microplate reader. For the cytotoxicity assay of sarcoma 180, 50 μL of sarcoma 180 cells (2×10^5 cells/well) were treated with 10, 100, 1,000, and 2,000 μg/mL concentrations of three different extracts of *P. serotinus* and cultured for 24 hr in 96-well microplates at 37°C with 5% atmospheric CO_2. Then, 1 mg/mL of 2,3-bis(2-methoxyl-4nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) solution was mixed with 30 μL of 25 μM phenazine methosulfate, followed by incubation at 37°C with 5% atmospheric CO_2 for 2 hr under dark conditions. OD was then measured at 450 nm using a microplate reader. Viability was defined as the ratio (expressed as a percentage) of absorbance of treated cells to untreated cells that served as control.

***In vivo* assay of antitumor activity.**   Antitumor activities of three different extracts of *P. serotinus* were assayed against mouse sarcoma 180 cells (ascitic type, 5×10^5 cells) implanted in a six-wk-old ICR mouse. The test sample was dissolved in phosphate buffered saline (PBS, pH 7.4; Gibco BRL, Gaitherburg, MD, USA) and filtered through a 0.22 μm membrane filter (Millipore Co., Bedford, MA, USA), followed by intraperitoneal injection in mice for 10 consecutive days at a dose of 20 mg/kg, starting from 24 hr after tumor implantation. Antitumor activities of *P. serotinus* against sarcoma 180 tumor bearing ICR mice were evaluated according to the increase in life span (ILS). The method previously described by Geran *et al*. [8] was used for calculation of ILS.

$$ILS\ (\%) = [(T - C)/C)] \times 100$$

Where, T is the mean of survival day (MSD) of the treated groups and C is the MSD of the control group.

Proliferation of murine spleen cells.   The WST-1 assay was performed to test for proliferation of murine spleen cells [9]. Six-wk-old ICR male mice were sacrificed by cervical dislocation, followed by aseptic removal of the spleen and grinding of the spleen using a 100-mesh sieve (Bellco Glass Inc., Vineland, NJ, USA). Two volumes of lymphocyte separation medium (PAA Laboratory Gmbh, Pasching, Austria) were added to the extracted solution, which was then centrifuged for 20 min at 400 ×g. Monocyte cells of spleen were selectively separated and centrifuged three times for 5 min at 300 ×g. The spleen cells (2×10^5 cells/mL) were then added to RPMI 1640 medium supplemented with heat inactivated fetal bovine serum, followed by treatment with 50, 200, and 500 μg/mL concentrations of three different extracts of *P. serotinus* and incubated for 48 hr in 96-well microplates at 37°C with 5% atmospheric CO_2 under dark conditions. In the same manner,

lipopolysaccharide (LPS) used as a positive control was incubated with 5 and 10 μg/mL concentrations. Thereafter, 10 μL of a 5 mg/mL concentration of WST-1 assay solution was added to each well, followed by incubation for 4 hr at 37°C with 5% atmospheric CO_2 under dark conditions. OD was measured at 440 nm using a microplate reader.

APase activity in murine spleen cells.   A method previously described by Ohno *et al*. [10] was used for measurement of APase activity of murine spleen cells. Six-wk-old male ICR mice were sacrificed by cervical dislocation and cell suspensions of the spleen were prepared aseptically. Fifty, 100, and 200 μg/mL concentrations of three different extracts of *P. serotinus* were applied to 100 μL of spleen cells (1×10^6 cells/well), followed by incubation for 48 hr in 96-well microplates at 37°C with 5% atmospheric CO_2. LPS at 5 and 50 μg/mL was applied to 100 μL of spleen cells (1×10^6 cells/well), followed by incubation for 48 hr in 96-well microplates at 37°C with 5% atmospheric CO_2. Cell suspensions were collected and freeze-thawed, followed by addition of 50 mM of sodium carbonate buffer containing *p*-nitrophenyl-phosphate (0.1 mg/mL) and $MgCl_2$ (1 mM) to 10 μL of the cell lysate. The reaction mixture was incubated for 1 hr at 37°C with 5% atmospheric CO_2 and the reaction was terminated by addition of 50 μL of 0.3 N ice cold NaOH. Absorbance was measured at 405 nm. APase activity of spleen cells was expressed as the stimulation index (SI).

SI = mean OD in the treated group/mean OD in the control group.

NO production by RAW 264.7 macrophages.   A method described previously by Choi *et al*. [11] was used for assessment of NO production in the culture supernatants of RAW 264.7. Briefly, 100 μL of RAW 264.7 cells (1×10^5 cells/well) were treated with 50, 100, and 200 μg/mL concentrations of three different extracts of *P. serotinus* and incubated for 48 hr in 96-well microplates at 37°C with 5% atmospheric CO_2. LPS, the positive control, was applied to 100 μL of RAW 264.7 cells (1×10^5 cells/well) at concentrations of 1, 10, and 50 μg/mL, followed by culture for 48 hr in 96-well microplates at 37°C with 5% atmospheric CO_2. Then, an equal volume of Griess reagent (1% sulfanilamide, 0.1% naphthlethylenediamine dihydrochloride in 2.5% phosphoric acid) was mixed with the culture supernatants and allowed to stand for 10 min. OD was measured at 540 nm using a microplate reader. Nitrite concentration was calculated from a standard curve prepared with known concentrations of sodium nitrite.

Determination of cytokine production in murine peritoneal macrophages.   Six-wk-old male ICR mice were sacrificed by cervical dislocation, followed by

washing the peritoneal cavity with 5.0 mL of sterile cold PBS, and passed through a 100-mesh sieve for removal of debris. Then, the exudate cells were centrifuged 400 ×g for 30 min, followed by suspending pelleted cells in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, and 100 μg streptomycin at 37°C with 5% atmospheric CO_2 for 1 hr. Nonadherent cells were removed with warm PBS and adherent cells were then trypsinized and viable cell counts (Trypan blue test) were performed using a hemacytometer. Macrophages were cultured at a density of 5×10^5 cells/mL using three different concentrations (10, 100, and 1,000 μg/mL) of each extract of *P. serotinus* and incubated for 48 hr in 24-well microplates at 37°C with 5% atmospheric CO_2 under dark conditions. In the same manner, positive controls, LPS and concanavalin A (Con A), were incubated with different concentrations of 1, 5, and 10 μg/mL and supernatants were collected and used for ELISA cytokine assay. A commercially available ELISA kit (Koma Biotech, Seoul, Korea) for tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) was used according to the manufacturer's instructions for measurement of cytokine levels. Standard curves were used for calculation of cytokine concentration.

Statistical analysis. Data were expressed as mean ± SD. One-way analysis of variance, followed by Duncan's new multiple-range test, was performed for analysis of intergroup differences. SPSS ver. 11.5 (SPSS Inc., Chicago, IL, USA) was used for the analysis. A $p \leq 0.05$ was considered statistically significant.

Results and Discussion

**β-Glucan and protein content.** Measurements of β-glucan and protein contents of various fractions, extracted

Table 1. β-Glucan and protein contents of various fractions extracted from fruiting bodies of *Panellus serotinus*

Fractions	β-Glucan	Protein
Fr. MeOH	28.52 ± 2.61^a	3.24 ± 0.52
Fr. NaCl	22.92 ± 0.68^b	3.68 ± 0.87
Fr. HW	27.03 ± 1.27^a	3.43 ± 1.09

Values are expressed as mean ± SD (n = 3).
Values in the second column that do not share a common superscript are significantly different at $p \leq 0.05$.
Fr. MeOH, fractions extracted with 80% methanol; Fr. NaCl, fractions extracted with 0.9% NaCl solution; Fr. HW, fractions extracted with hot water.
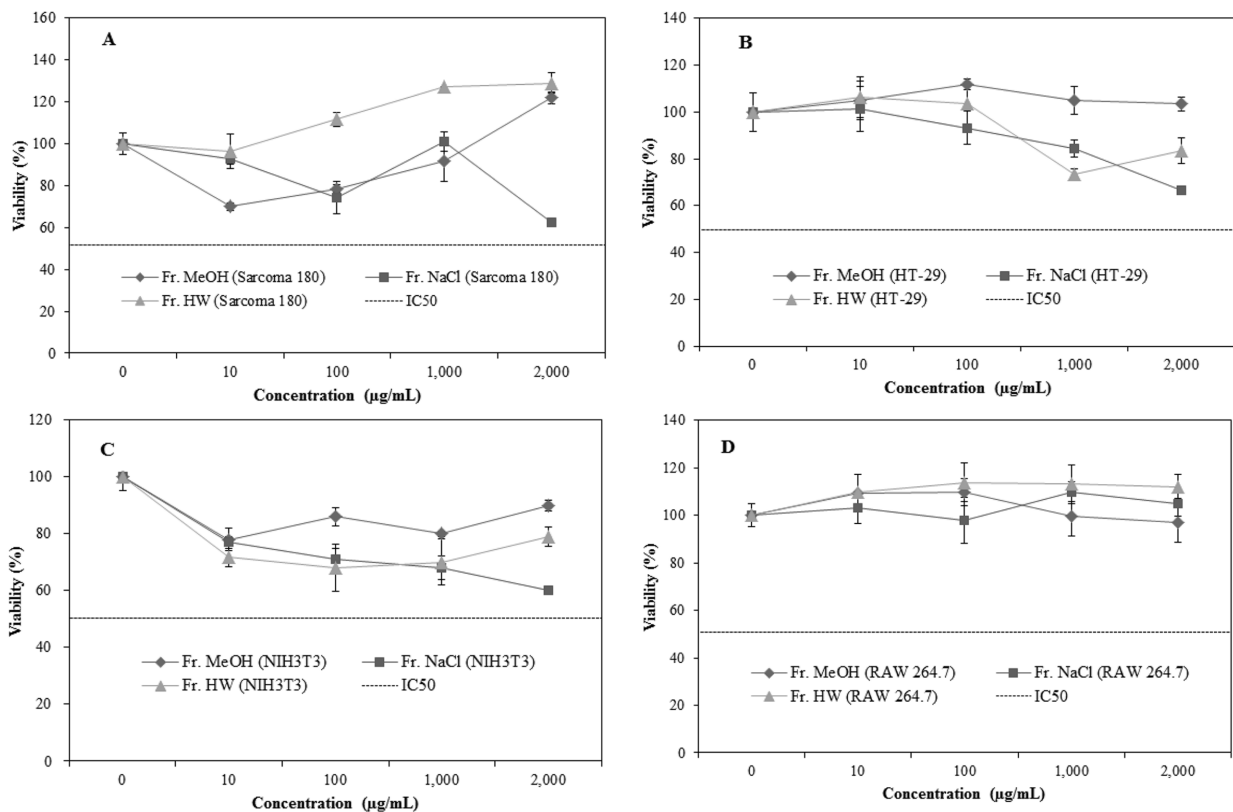
Fig. 1. *In vitro* cytotoxicity activity against sarcoma 180 (A), HT-29 (B), NIH3T3 (C), and RAW 264.7 (D) of different concentrations of various fractions extracted from fruiting bodies of *Panellus serotinus*. Values are expressed as means ± SD (n = 5). Fr. MeOH, fractions extracted with 80% methanol; Fr. NaCl, fractions extracted with 0.9% NaCl solution; Fr. HW, fractions extracted with hot water.

from fruiting bodies of *P. serotinus*, were performed (Table 1). The highest amount of β-glucan was recorded in Fr. MeOH (28.52 g/100 g), followed by Fr. HW (27.03 g/100 g) and Fr. NaCl (22.92 g/100 g), respectively. Similar protein levels were recorded in Fr. FeOH, NaCl, and Fr. HW fractions of *P. serotinus*.

The immunomodulating effects of β-glucans during development of immune reactions are well established. Due to their immunomodulatory and antitumor effects, β-glucans and β-glucan-protein complexes isolated from mushrooms have been used as a source of therapeutic agents [12]. Several investigators have isolated and purified immunomodulating polysaccharides from mushrooms as a biological response modifier [13]. The anti-tumor activities of polysaccharides are mainly the result of their immunopotentiation effects [14, 15].

***In vitro* assay of cytotoxicity.**　　The results of the *in vitro* cytotoxicity activities of three different fractions of *P. serotinus* against sarcoma 180, HT-29, NIH3T3, and RAW 264.7 cell lines are shown in Fig. 1. At 10~2,000 μg/mL, cell viability of Fr. MeOH, Fr. NaCl, and Fr. HW fractions against sarcoma 180, HT-29, NIH3T3, and RAW 264.7 cell lines ranged from 70~122, 74~101, and 96~129%, respectively (Fig. 1A), 103~112, 67~101, and 73~106%, respectively (Fig. 1B), 78~90, 60~77, and 68~79%, respectively (Fig. 1C), and 97~110, 98~110, and 110~114%, respectively (Fig. 1D).

The results indicated that Fr. MeOH, Fr. NaCl, and Fr. HW fractions extracted from fruiting bodies of *P. serotinus* had no significant cytotoxic effects on four cell lines at the tested concentrations. *In vitro* cytotoxicity assays can be used for general screening of chemicals for prediction of toxicity [16]. In our earlier study, hot water extract from *Elfvingia applanata* did not inhibit proliferation of HT-29, Hep G2, TR, and sarcoma 180 cancer cells [17]. In another study, Lee *et al.* [18] reported that a hot-water extract of *Inonotus obliquus* exerted little inhibitory activity against proliferation of human colon cancer cells, in good agreement with our results.

***In vivo* assay of antitumor activity.**　　Antitumor activities of Fr. MeOH, Fr. NaCl, and Fr. HW fractions of *P. serotinus* were tested against sarcoma 180 tumor bearing mice. According to the results, the highest ILS was recorded in Fr. MeOH (44.71%), followed by Fr. NaCl (43.53%), and Fr. HW (23.53%), respectively compared to the control group (Fig. 2). According to the results, the mean life span of the group treated with Fr. MeOH showed a significant increase and fruiting bodies of *P. serotinus* might contain effective antitumor substances against sarcoma 180.

Shim *et al.* [19] reported that treatment of *Paecilomyces sinclairii* with methanol extract resulted in inhibited growth
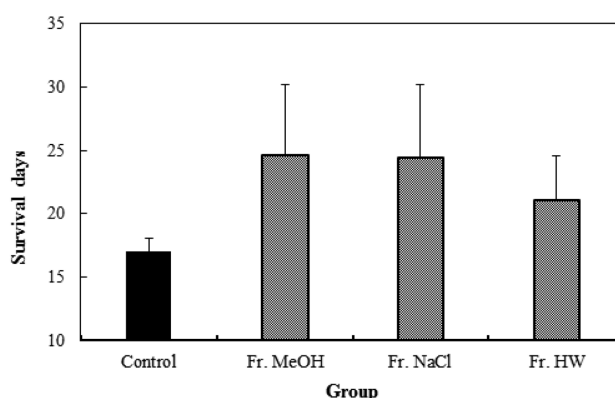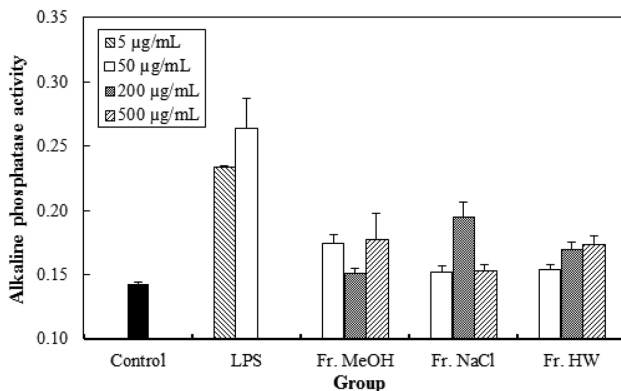

Fig. 2. Effect of various fractions isolated from *Panellus serotinus* on the life span of ICR mice inoculated with sarcoma 180. Values are expressed as means ± SD (n = 10). Fr. MeOH, fractions extracted with 80% methanol; Fr. NaCl, fractions extracted with 0.9% NaCl solution; Fr. HW, fractions extracted with hot water.

of sarcoma 180 tumor cells and prolongation of the life span of mice by 32.3%. In general, the criteria for judging the antitumor effect of any substance include prolongation of the life span by more than 25% [20]. This observation is consistent with our observations; the mean life span of the group treated with Fr. NaCl showed a significant increase. It might be concluded that polysaccharides of *P. serotinus* have a strong anticancer effect.

Proliferation of murine spleen cells.　　The effect of Fr. MeOH, Fr. NaCl, and Fr. HW fractions of *P. serotinus* on proliferation of murine cells is shown in Fig. 3. According to the results, Fr. HW exhibited significantly excellent
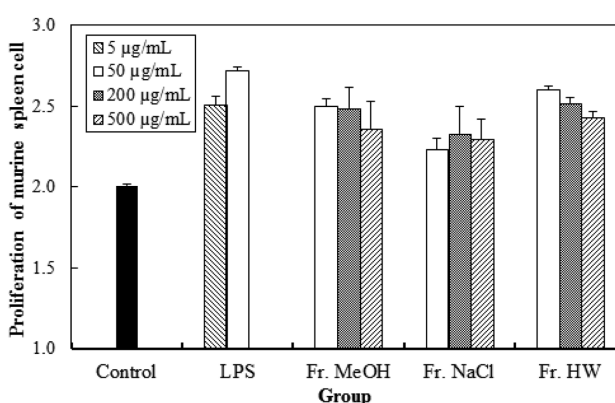
Fig. 3. Effect of various fractions extracted from fruiting bodies of *Panellus serotinus* on proliferation of murine spleen cells. Values are expressed as means ± SD (n = 5). LPS, Lipopolysaccharide was used as a positive control; Fr. MeOH, fractions extracted with 80% methanol; Fr. NaCl, fractions extracted with 0.9% NaCl solution; Fr. HW, fractions extracted with hot water.

Fig. 4. Effect of various fractions extracted from fruiting bodies of *Panellus serotinus* on alkaline phosphatase activity in murine spleen cells. Values are expressed as means ± SD (n = 5). LPS, Lipopolysaccharide was used as a positive control; Fr. MeOH, fractions extracted with 80% methanol; Fr. NaCl, fractions extracted with 0.9% NaCl solution; Fr. HW, fractions extracted with hot water.

Fig. 5. Effect of various fractions extracted from fruiting bodies of *Panellus serotinus* on nitric oxide production in RAW 264.7. Values expressed as means ± SD (n = 5). LPS, Lipopolysaccharide was used as a positive control; Fr. MeOH, fractions extracted with 80% methanol; Fr. NaCl, fractions extracted with 0.9% NaCl solution; Fr. HW, fractions extracted with hot water.
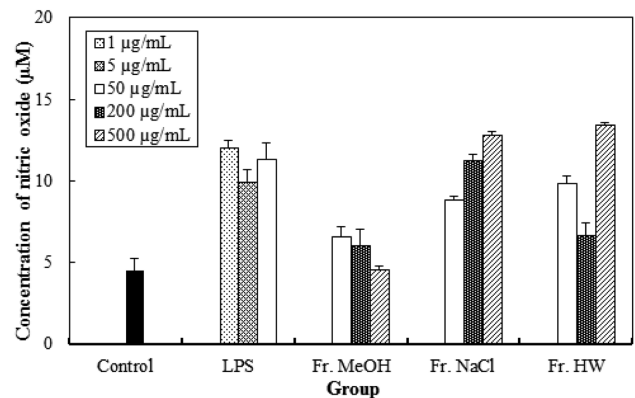
activities, while good and moderate effects were observed for Fr. MeOH and Fr. NaCl, respectively, compared to control. However, at the concentration of 50 μg/mL, LPS also exhibited excellent activities.

The results suggested that β-glucan of *P. serotinus* can improve the immune response of the host by stimulating proliferation of immune-organ, murine spleen cells. Li *et al*. [21] reported that treatment with proteoglycan extracted from crude liquid culture medium and mycelia of *Phellinus nigricans* resulted in stimulated proliferation of lymphocytes of spleen cells and increased production of TNF-α. Murine spleen cells are the main residence of various immune cells and are also important for host immune response.

APase activity in murine spleen cells. Stimulation of splenic lymphocytes with LPS, Fr. MeOH, Fr. NaCl, and Fr. HW at 50 μg/mL resulted in an increase in APase activities of 1.63-, 1.22-, 1.06-, and 1.08-fold, respectively, compared to control (Fig. 4). APase activity in murine spleen cells showed a significant increase in Fr. NaCl at 200 μg/mL, compared with positive control.

Cha *et al*. [22] reported an increase in APase activity of 1.2~1.6-fold upon stimulation with crude polysaccharides of *Agaricus brasiliensis* at concentrations of 50~200 μg/mL. Therefore, it is concluded that treatment with Fr. NaCl could result in improved immunostimulating activity of the host via increasing APase activity.

NO production by RAW 264.7 macrophages. NO production activity in the culture supernatants of RAW 264.7 macrophages with various concentrations (50, 200, and 500 μg/mL) of Fr. MeOH, Fr. NaCl, and Fr. HW

fractions of *P. serotinus* ranged from 4.61~6.59, 8.89~12.85, and 6.68~13.48 μM, respectively. In the control group, 4.47 μM of NO was released, while 12.04, 9.97, and 11.37 μM of NO were produced by treatment with LPS at the concentrations of 1, 5, and 50 μg/mL (Fig. 5).

The results demonstrated that stimulation with β-glucan of *P. serotinus* can result in an increase in production of NO and improvement of the immune response in ICR mice. Our results were similar to those of Kim *et al*. [23], who observed that RAW 264.7 macrophages stimulated by polysaccharides extracted from *Phellinus linteus* showed an increase in production of NO in a dose-dependent manner. Ooi and Liu [12] reported that polysaccharides extracted from mushrooms exert anti-tumor effects through activation of different immune responses in the host rather than by direct killing of tumor cells.

Cytokine production in murine peritoneal macrophages. The results on production of cytokines of three different fractions of *P. serotinus* are shown in Fig. 6. At 10~1,000 μg/mL of Fr. MeOH, Fr. NaCl, and Fr. HW, production of TNF-α, IL-1β, and IL-6 ranged from 60.04~89.45, 179.38~208.31, and 75.36~130.50 pg/mL, respectively (Fig. 6A), 32.68~59.71, 78.00~103.20, and 81.45~155.60 pg/mL, respectively (Fig. 6B), and 148.08~191.63, 202.78~254.78, and 214.93~239.92 pg/mL, respectively (Fig. 6C). The results indicated that production of TNF-α, IL-1β, and IL-6 was significantly higher, compared to control, and IL-6 production was excellent, compared to TNF-α, IL-1β, and Con A at the tested concentrations of the various fractions of *P. serotinus*.

TNF-α, Il-1β, and IL-6 are important regulators of host defense against tumor cells [24].
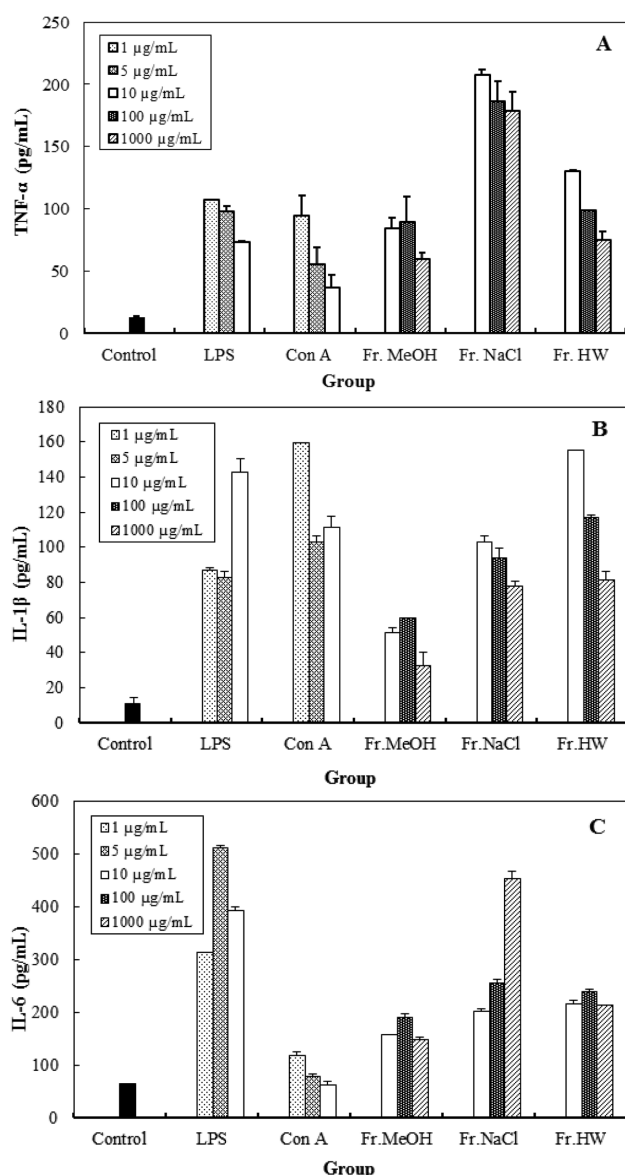
Fig. 6. Effect of different concentrations of various fractions extracted from fruiting bodies of *Panellus serotinus* on tumor necrosis factor α (TNF-α) (A), interleukin-1β (IL-1β) (B), and interleukin-6 (IL-6) (C) production. Values are expressed as means ± SD (n = 5). LPS, lipopolysaccharide; Con A, Concanavalin were used as positive controls; Fr. MeOH, fractions extracted with 80% methanol; Fr. NaCl, fractions extracted with 0.9% NaCl solution; Fr. HW, fractions extracted with hot water.

Therefore, the observed increase in production of cytokines would suggest an enhanced ability of the host to combat the growth of tumors. Macrophages can be activated by β-glucans and other cell mediators to kill tumor cells by production of TNF-α. The bioactivities of polysaccharides and polysaccharide-protein complexes are dependent on binding on the surface receptor of immune cells. These receptors, which are known as pattern recognition molecules, can recognize foreign ligands during initial phases of the immune response [25]. Specifically, macrophages might bind polysaccharides via toll-like receptor 4, CD14, complement receptor 3, scavenger receptor, dectin-1, and mannose receptor [26]. Our results are in agreement with those reported by Ooi and Liu [12], who showed that polysaccharides from mushrooms exert antitumor effects via activation of different immune responses in the host rather than by directly attacking cancer cells. Indeed, in our study, we show that mushroom polysaccharides trigger production of varying levels of TNF-α, IL-β, and IL-6 by macrophages. Even among fractions and different concentrations of polysaccharide, the levels of cytokine release are different. These differences may reflect the structural and conformational variations as well as bioavailability of polysaccharides from these extracts.

Acknowledgements

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