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The Immunomodulating Effects of the Supplementation of *Paeonia Japonica* Extracts in Mice*

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ABSTRACT

Paeonia japonica var. pilosa N_{AKAI} (*PJ*; Baek-Jak-Yak) is a medicinal plant which has been widely used as a component of blood-building decoctions. This study was performed to investigate the immunomodulative effects of PJ in mice, using in vitro and in vivo experiments. The immunomodulative effects were studied in vitro by determining the proliferation of mice splenocytes and the production of three kinds of cytokines (IL-1β, IL-6, TNF-α) by mice peritoneal macrophages which were cultured with sequential fractions of PJ methanol extract (methanol, hexane, chloroform, ethylacetate, butanol and water). In an *in vivo* experiment using mice, different concentrations of PJ water extract were orally administrated every other day for two weeks. The production of cytokines (IL-1β, IL-6, TNF-α) secreted by activated macrophages, and the proliferation of mice splenocytes, were used as indices for immunocompetence. In vitro supplementation using a hexane fraction of PJ in the range of 1 to 100 μg/ml enhanced splenocyte proliferation by 1.8 to 12%, and by 10 - 15% using an aqueous fraction, compared to the control. IL-1β production was significantly increased with the supplementation of butanol, hexane and water extracts of PJ. Higher levels of IL-6 production were detected with supplementation of chloroform or water extracts. However, there were no significant differences in the production of TNF-α among the treated groups and the control. From the in vivo study, the highest proliferation of splenocytes was seen in the mice orally administrated with the PJ water extract at the concentration of 500 mg/kg body weight. In the case of cytokine production, IL-1β, IL-6, and TNF-α released by activated peritoneal macrophages were augmented by the oral administration of a PJ water extract. These results indicate that PJ may enhance the immune function by regulating splenocyte proliferation and cytokine production capacity in mice.

KEY WORDS: splenocyte proliferation, macrophage, cytokine, immunomodulative effect.

INTRODUCTION

Natural products are increasingly appreciated as leads for drug discovery and development. A number of investigators have studied various activities of natural products and have found that they have not only nutritional effects but also beneficial properties to cure various diseases and maintain good health.[1,2] Especially, those medicinal plants with long traditions have shown to promote various beneficial effects, such as enhancement of phagocytosis, cytokine induction, antibody production, induction of the mitogenic activity of spleen cells, anti-tumor effects,[3] and antioxidant effects. Recently, many investigations have initiated searches for immunomodulating substances from natural food sources. The immune system is a remarkably adaptive defense system that has evolved in vertebrates to protect them from invading pathogenic microorganisms and cancer. The immune system is able to generate an enormous variety of cells and molecules capable of specifically recognizing and eliminating an apparently limitless variety of foreign invaders. These cells and molecules act together against the foreign invaders by proliferation, differentiation and activation, and these alterations are dependent on environmental conditions.[4] Immunomodulators are substances that enhance immune responses by regulating one or more components of the immune system. Some investigators have reported that isolates from plants such as polysaccharides, lentinan, schizofilan, polysaccharide K (PSK) and ginsan, have immunoactivating properties.[5-11]

Paeonia japonica is a perennial which grows in light to medium shade, forming handsome 18" tall clumps of attractive foliage. Showy, single white flowers with abundant yellow stamens bloom in May. The fruits are bright red receptacles with dark blue seeds perched on top. In Chinese medicine, *PJ* was used for the treatment of hemorrhages in the retina of the eyes, infectious hepatitis and intestinal diseases, crab, diabetes, nephritis, hypertonia, and the treatment of Lepra. *PJ* extract improves the

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appetite, and helps with hyperacidity of the stomach. In the 1960s, the main component of *PJ*, paeoniflorin, was purified, and Egger reported anti-inflammatory and stomachache prevention effects of paeoniflorin.[12][13] There is also a report on the isolation of an antimicrobial compound from *PJ*.[14] Park suggested the potential of polysaccharide fractions of *PJ* as an immunostimulator.[15] However, very little research has been conducted on the immunomodulative effect of *PJ*. Therefore, this study was performed to evaluate the immunomodulative effect of *Paeonia japonica* extracts in mice.

MATERIALS AND METHODS

1. Animals

Six-week-old ICR male mice (30 ± 2 g) were purchased from the KFDA (Korean Food and Drug Administration) and were kept under standardized animal house conditions (temperature 22 ± 2℃; photoperiod approximately 12 hours of light and 12 hours of dark daily; and relative humidity at 50 – 60%). Pelleted food and tap water were available ad libitum. Prior to the experiment, at least 7 days were allowed for the mice to become acclimatized to animal house conditions and daily handling.

2. Chemicals

RPMI medium 1640, Fetal bovine serum (FBS), and phosphate buffered saline (PBS) were purchased from GIBCO BRL (Grand Island, N.K., USA). Streptomycine-penicilline, lipopolysaccharide (LPS), sodium bicarbonate, ammonium chloride, trisma base, trisma hydrochloride, trypan blue solution, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrzolium bromide (MTT), and DMSO (Dimethylsulfoxide) were purchased from the Sigma Chemical Co., St. Louis, USA. Thioglycollate was obtained from DIFCO Lab. (Detroit, MI, USA). Cytokine kits were obtained from Intergen Co., USA. Solvents for extraction were extra pure grades.

3. Preparation of *PJ* extracts

Dried *Paeonia japonica* was purchased from the Kyungdong oriental market in Seoul and then powdered. Three hundred grams of *PJ* were extracted three consecutive decoctions from the same material in a boiling water bath with 600 ml of methanol or distilled water under reflux for 3 h. Some of the total methanol extracts were subjected to sequential fractionations with hexane, chloroform, ethylacetate, and then n-butanol (Fig. 1). The methanol, hexane, chloroform, ethylacetate, and n-butanol fractions, and an aqueous layer remaining after the n-butanol fractionation were independently evaporated under reduced pressure at 60℃, and completely dried by lyophilization. The dried total methanol extracts and each of the dried solvent fractions were dissolved in RPMI 1640 containing 10% FBS and less than 0.01% dimethyl sulfoxide as a final concentration, and used as samples for in vitro assay. The water extract was also evaporated and dried like the other fractions, and dissolved in filtered distilled water for in vivo assay.

4. *PJ* extract treatment

In the in vitro experiment, 6 fractions of *PJ* extracts – in concentrations of 1 μg/ml, 10 μg/ml, 50 μg/ml, 100 μg/ml, and 250 μg/ml – were added to the culture of splenocytes and peritoneal macrophages and cultured for 48 hours.

For the in vivo experiments, doses of *PJ* water extracts in concentrations of 0, 20, 100, 500 and 1000 mg/kg body weight were administered orally every other day for two weeks to five groups of mice, with 6 mice in each group. Mice were sacrificed on the 15th day after the beginning of the experiment, and splenocytes and peritoneal macrophages were collected for culture. Cells were cultured for 48 hours with or without mitogens (ConA or LPS).

5. Splenocyte preparation

Splenocytes were prepared by Mishell's method.[16] Spleen

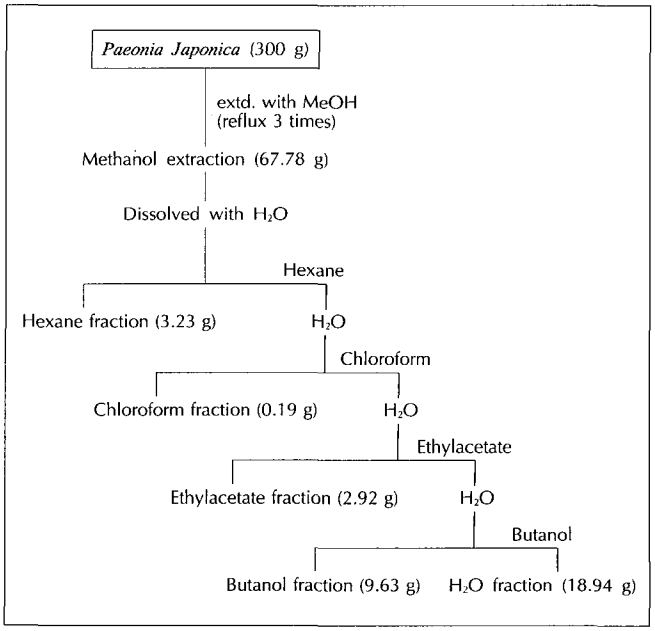

Fig. 1. Schematic diagram for solvent fractions from methanol extract of *Paonia japonica* var. pilosa NAKAI.

was aseptically removed from ICR mice sacrified by cervical dislocation. Using the flat end of a sterile syringe plunger, spleen was gently crushed in an ice-cold RPIM 1640. A subsequent cell suspension was put into a centrifuge tube through a 200-μm mesh nylon screen. The cell suspension was left aside for 10 min to precipitate the unbroken cell debris. The upper part of the cell suspension was carefully transferred to a new centrifugal tube and centrifuged at 400×g for 10 min. The resuspended pellet of spleen cells was again suspended in lysing buffer to remove red blood cells and then incubated for 5 min at room temperature with occasional shaking. A washing medium was added to fill the tubes, and the tubes were then centrifuged at 400×g for 10 min; the supernatant was then discarded. The pellet was washed again and resuspended to 5×10^6 cells/ml in RPMI 1640 culture medium containing 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin.

6. Spleen index

After the spleen was aseptically removed, it was weighed and the spleen index was calculated to standardize the differences in body weights of the mice.[17]

$$\text{Spleen index} = \frac{\text{weight of spleen (g)} \times 100}{\text{weight of mouse (g)}}$$

7. Splenocyte proliferation assay

Cell proliferations were determined by MTT colorimetric analysis, which is reported to be similar to the results of the ^{3}H-thymidine uptake assay (which is frequently used for cell cytotoxicity assays).[18-20] In the in vitro experiment, the same volumes of prepared splenocyte suspension of 5×10^6 cells/ml were plated in 96-well plates (Corning Glass, Corning, USA). Meanwhile, *PJ* extracts (at different final concentrations of 0, 1, 10, 50, 100, 250 μg/ml), ConA (5 μg/ml) or LPS (15 μg/ml) were also added. The cells in 96-well plates (5×10^6 cells/ml) were exposed to various concentrations of extracts and incubated at 37℃, and at 5% CO_2 in air, for 48 hrs. In the in vivo experiment, splenocytes of every group were separately suspended to 5×10^6 cells/ml and plated in 96-well plates. ConA (5 μg/ml) or LPS (15 μg/ml) was added as a positive control and incubated as in the in vitro experiment.

After 44 hr of incubation, 10 μl MTT solution (5 mg/ml of phosphate buffered saline) was added and further incubated for 4 hrs at 37℃. After aspirating the supernatant from the wells, 150 μl of DMSO (Dimethylsulfoxide; Sigma) was added for the dissolution of formazan crystals. The optical density (OD) of the samples was monitored by an ELISA reader at a wavelength of 540 nm. MTT (tetrazolium salt) is cleaved only by metabolically active cells and is reduced to a colored formazan, and the color (OD value) reflects cell viability quantitatively. Proliferation of splenocytes was calculated by the following equation.

$$\text{Proliferation (\%)} = (\text{OD of sample} / \text{OD of control 1}) \times 100$$

8. Primary culture of macrophages

Thioglycollate was intraperitoneally injected to ICR mice at a rate of 2 ml of 4% thioglycollate per 30 g of body weight. Macrophages were collected from peritoneal lavages of the mice 3 days after injection, washed twice with cold RPMI medium 1640 (L-glutamine, 25 mM HEPES buffer, sodium bicarbonate), and resuspended in RPMI containing 10% FBS. Macrophages in the suspension were stained with trypan blue, their numbers counted by using a microscope, and then diluted to 1×10^6 cells/ml with the same medium. The diluted macrophages were seeded into 24-well culture plates (Corning, USA, 1 ml/well) and incubated at 37℃ under 5% CO_2. After preincubation for 2 hrs, the medium was replaced with fresh medium to remove the non-adherent cells, and then various concentrations (1, 10, 100 μg/ml) of extracts were added. In the in vivo experiment, cells from every group were incubated only with or without mitogen.

9. Measurement of cytokine (IL-1β, IL-6, TNF-α) production in mouse peritoneal macrophages

Cells were incubated at 37℃ under 5% CO_2 conditions for 48 hrs, and then the supernatant medium was collected for the measurement of cytokines. Cytokine concentration was determined by using the ELISA method. Briefly, culture supernatant was added to 96-well ELISA plates pre-coated with goat anti-rabbit antibodies (Intergen Co., USA). It was then incubated with polyclonal anti-murine IL-1β, IL-6 or TNF-α antibody. After binding of biotinylate cytokine conjugate, streptavidin-conjugated alkaline phosphate and substrate solution were added. Optical density was measured at 540 nm in the ELISA plate reader within 5 minutes of adding the amplifier solution.

10. Statistics

All values are expressed as mean ± SD from 6 observations. The Student's t-tests for unpaired observations between the control and experimental samples were carried out for statistical evaluation of the differences; p values of 0.05 or less were considered as statistically significant.

RESULTS AND DISCUSSION

1. Mitogenic activity of PJ extracts on spleen cells

In the in vitro experiment, we examined the mitogenic activity of *PJ* on splenocyte proliferation in the presence of six extracts of *PJ* by MTT assay. In this assay, ConA (Concanavalin A), a common T cell mitogen, was added at the level of 5 μg/ml and LPS (Lipopolysaccharide), a B cell mitogen,[9] was added at 15 μg/ml, and these were used as the positive controls. The splenocyte proliferation with ConA or LPS was increased up to 48.19 ± 3.01% and 23.64 ± 2.11%. The butanol or water fractions of *PJ* enhanced splenocyte proliferation at most concentrations of the extracts examined. With 50 μg/ml of butanol extract, splenocyte proliferation was increased up to 10.65 ± 2.49%, and with 100 μg/ml of water extract, splenocyte proliferation was increased up to 15.16 ± 2.09% compared to the control, but these levels were lower than those of mitogen stimulated groups as shown in Fig. 2. Supplementation of high concentrations (250 μg/ml) of hexane, chloroform, and ethylacetate fractions suppressed splenocyte proliferation, but the butanol and water fraction supplementations enhanced splenocyte proliferation. Park reported that splenocyte proliferation was not affected by the supplementation of water extract of *PJ*, but that splenocyte proliferation was suppressed when the mitogens were treated.[21]

2. Cytokine production by macrophages treated with *PJ in vitro.*

Normal peritoneal macrophages collected from ICR mice were cultured with 0, 1, 10, and 100 μg/ml of *PJ* extracts for 48 hours. LPS (15 μg/ml) treatments were separately used for comparison purposes. Cytokine levels in the culture supernatant were measured by the ELISA method. Macrophages are activated in a non-specific manner when antigens of infectious agents invade the body, and may secrete various cytokines (IL-1, Il-6, IL-12, TNF, IFN, etc) and chemicals (NO, prostaglandins, etc) for communication to other immune cells, killing the invading pathogens and inducing fundamental host defense systems. Cytokines like IL-1, IL-2, IL-6 and TNF are frequently tested in relation to nutrition, and IL-1β, IL-6 and TNF-α are the main cytokines secreted by activated macrophages.[22-25] IL-1β is a representative cytokine which is secreted after activation of macrophages in the initial phase.[26] IL-1β concentration in the control group was 0.20 ± 0.08 ng/ml, while it was significantly increased to 1.98 ± 0.11 to 5.12 ± 0.90 ng/ml in the group of PJ butanol and water fractions. Except for the hexane fraction, every supplemented group released higher concentrations of IL-1β, and the addition of 10 μg/ml of hexane fraction enhanced IL-1β production to the

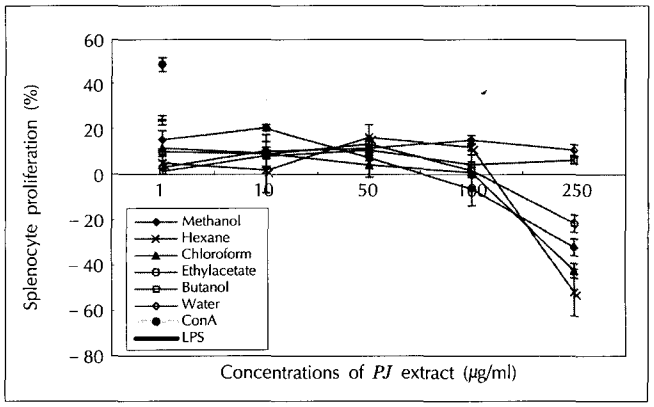

Fig. 2. Proliferation of mice splenocytes cultured with *Paeonia japonica* extracts in six fractions. Spleen cells (5 × 10^6 cells/well) were cultured with fraction of the extract on 96-well plate for 48 h. After culture, the degree of splenocyte proliferation was measured by MTT assay. The data present the mean values ± S.D. n = 3.

Table 1. Proliferation of splenocyte cultured with six different fractions of PJ extracts

Conc. (μg/ml) / Fraction	Proliferation(%)[1] 1	10	50	100	250
Methanol	15.20 ± 4.02[2]	20.27 ± 1.31	7.25 ± 1.20	-6.82 ± 0.734	-31.95 ± 3.75
Hexane	4.99 ± 4.87	1.84 ± 9.86	16.23 ± 5.50	12.01 ± 1.63	-51.81 ± 10.38
Chloroform	11.48 ± 3.58	9.1 ± 1.34	4.21 ± 5.32	0.92 ± 2.25	-42.34 ± 3.16
Ethylacetate	9.96 ± 1.13	8.91 ± 5.37	13.27 ± 1.94	1.88 ± 2.51	-21.47 ± 3.87
Butanol	1.3 ± 1.30	8.08 ± 1.33	10.65 ± 2.49	4.4 ± 4.36	6.15 ± 1.43
Water	3.04 ± 2.96	10.16 ± 7.21	11.38 ± 1.62	15.16 ± 2.09	10.64 ± 2.44
ConA	48.19 ± 3.01				
LPS	23.64 ± 2.11				

1) Proliferation(%) = (mean of O.D. in test wells / mean of O.D. in control wells - 1) × 100

greatest extent. Koji reported that supplementation of celosian (from the seed of Celosia argentea, 10 μg/ml) significantly activated IL-1β production and that this activity was concentration-dependent.[27] Compared to the control level of 0.02 ng/ml, higher levels of IL-6 production were detected by supplementation of chloroform fractions at the concentrations of 1, 10, and 100 μg/ml (7.34 ± 2.49 ng/ml, 10.5 ± 0.18 ng/ml, 9.92 ± 0.17 ng/ml), of aqueous fractions at the concentration of 10 μg/ml (4.33 ± 1.38 ng/ml), and of ethylacetate fractions at the concentration of 1 μg/ml (3.45 ± 1.10 ng/ml). Lee reported that the addition of pine needle fractions enhanced IL-6 production, and that the chloroform fraction supplementation group was the most effective group.[28] In the present study, there were significant differences in the production of TNF-α among the treated groups and the control, and the supplementation of hexane, butanol, and the water fraction increased the production of TNF-α. Studies of the immunomodulating effects of polysaccharides extracted from Ganodema lucieum shows significant improvements in the production of TNF-α.[29] Koji reported that celosian significantly induced TNF-α production in mice, and suggested a possible immunoactivating function of celosian from this result.[27] Meanwhile, Quercetin, one of the bio-flavonoids which is contained in onion and garlic, suppressed TNF-α production by mice macrophages.[30] Our in vitro experiment showed that butanol and water fractions increased IL-1β production, and chloroform and water fractions increased IL-6 production (Fig. 3-5).

3. Oral administraion of PJ and splenocyte proliferation

Many studies using blood or tissue from experimental animals are currently in progress by researchers who are interested in the various effects of food intake on the body.[31][32] Regarding immune systems, the spleen is a major organ, and the size or the proliferation of splenocytes can be an indicator of immune function.[33][34] Oral administration of *PJ* water extracts in this study enhanced splenocyte proliferation in all administered groups in the range of 16 ± 0.15 to 37 ± 0.47%, as shown in Fig. 6. Mitogenic stimulation by ConA or LPS was not effective in increasing the splenocyte proliferation of *PJ* supplemented groups, except in the group supplemented with 100 mg/kg b.w. In a previous study on pine needles and Phlomis umbrosa, splenocyte proliferation administered with each of these plant extracts did not show any mitogenic effects of ConA or LPS in animals.[28][35]

4. Oral administraion of PJ and cytokine production

Immune responses are regulated by many cytokines secreted by T-cells and macrophages. Macrohages are known to play an important role in host defense mechanisms. When macrophages are stimulated with bacterial

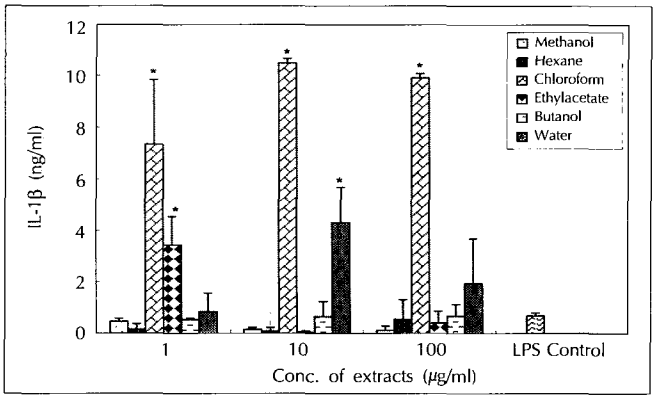

Fig. 4. IL-6 production by mice peritoneal macrophage cultured with six different fraction of *Paeonia japonica* extracts. The data present the mean values ± S.D. n = 3. *: Significantly different at p < 0.05.

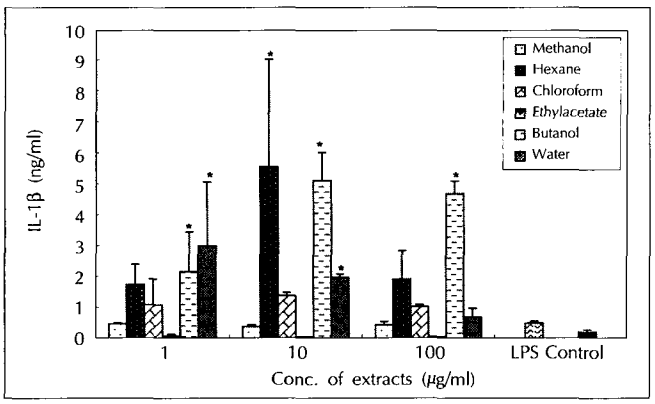

Fig. 3. IL-1β production by mice peritoneal macrophage cultured with six different fraction of *Paeonia japonica extracts. The data present the mean values ± S.D. n = 3.* *: Significantly different at p < 0.05.

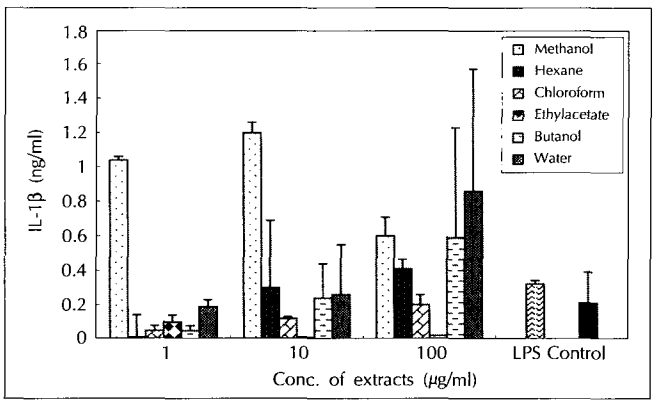

Fig. 5. TNF-α production by mice peritoneal macrophage cultured with six different fraction of *Paeonia japonica* extracts. The data present the mean values ± S.D. n = 3.

products, a variety of cytokines and chemicals are released to induce fundamental defense mechanisms. The activated macrophages can preferentially lyse tumor cells and also secrete the cytokine tumor necrosis factor which can kill tumor cells. Macrophages also show augmented phagocytosis against target cells, including tumor cells. Polysaccharides from plant materials have antitumor, immunomodulating, anti-inflammatory, hypoglycemic and antiviral activities.[36-39] Polysaccharides from water extracts of *PJ* were also reported to have immunomodulating effects.[15] In the present in vivo experiment, eight-week-old mice were fed ad libitum on a chow diet and the different concentrations of *PJ* extract were orally administrated every other day for 2 weeks. The production of cytokines (IL-1β, IL-6, and TNF-α) secreted by activated macrophage supernatant, cultured with/without LPS (lipopolysaccharide) or ConA (Conconavalin A), was used as an index for immunocompetence (Fig. 7-9). The animals in the *PJ* extract supplementation groups (500 mg/kg b.w. and 1000 mg/kg b.w.) showed enhanced levels of all three kinds of cytokine production without any mitogen stimulation, compared to those in control group. In LPS-stimulated animals, higher concentrations of IL-1β were detected in the 100 mg/kg b.w. supplementation group (758 ± 5.97 pg/ml, control: 244 ± 17.02 pg/ml) and the level of IL-6 production was enhanced in the 100 mg/kg b.w. supplementation group (758 ± 5.27 pg/ml, control: 245 ± 17.02 pg/ml); TNF-α production were similarly enhanced in the 500 mg/kg b.w. and 1000 mg/kg b.w. supplementation groups (1585 ± 77.78 pg/ml, 1573 ± 45.96 pg/ml, control: 1298 ± 10.61 pg/ml). Oral administration of many substances from plant ma-

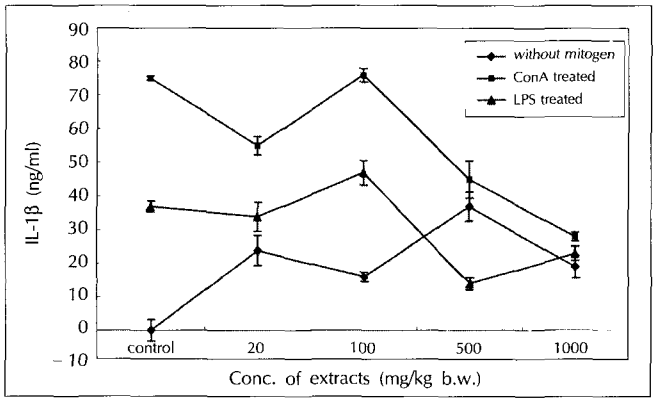

Fig. 6. Increase in proliferaiton of splenocytes of mice orally administered with different levels of *Paeonia japonica* water extract treated with or without mitogen. Spleen cells (5×10^6 cells/well) were cultured with or without mitogen on 96-well plate for 48 h. After culture, the degree of splenocyte proliferation was measured by MTT assay. The data present the mean values ± S.D. of 6 mice.

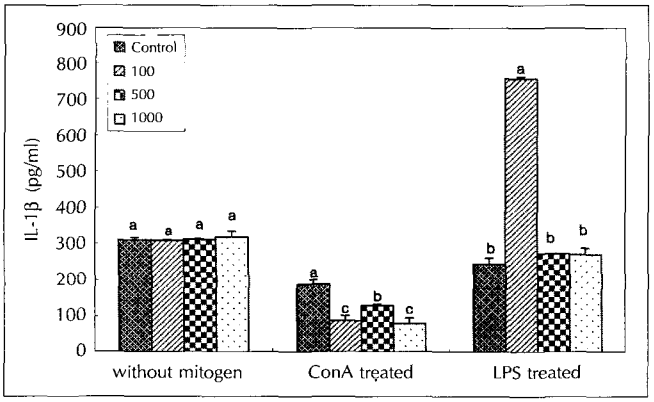

Fig. 8. IL-6 production by activated peritoneal macrophage of mice orally administered with different levels of *Paeonia japonica* water extract treated with or without mitogen. The data present the mean values ± S.D. n = 6 with different letters within every mitogen groups are significantly different from each other at α = 0.05 as determined by Duncan's multiple range test (a > b > c).

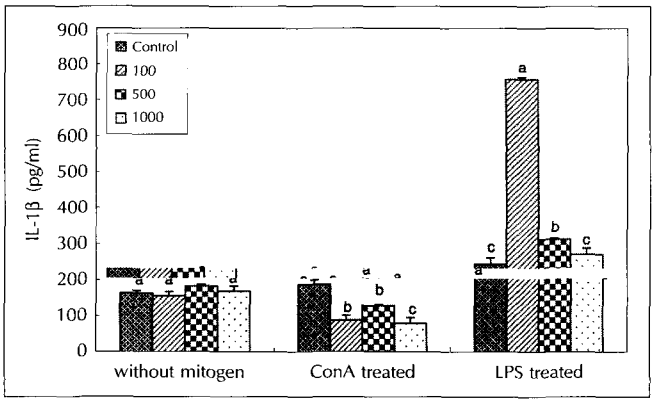

Fig. 7. IL-1β production by activated peritoneal macrophage of mice orally administered with different levels of *Paeonia japonica* water extract treated with or without mitogen. The data present the mean values ± S.D. n = 6 with different letters within every mitogen groups are significantly different from each other at α = 0.05 as determined by Duncan's multiple range test (a > b > c).

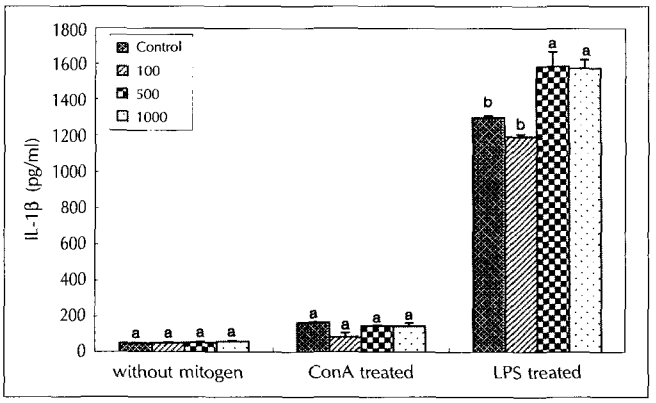

Fig. 9. TNF-α production by activated peritoneal macrophage of mice orally administered with different levels of *Paeonia japonica* water extract treated with or without mitogen. The data present the mean values ± S.D. n = 6 with different letters within every mitogen groups are significantly different from each other at α = 0.05 as determined by Duncan's multiple range test (a > b > c).

terials, including Xanthii strumariu, Bezoar Bovis, Shi-karon, pine needles, and C. cristata, were also reported to enhance cytokine production by mice macrophages.[28,40,41] These results may support the possibility of polysaccharides from plant material acting as immunomodulators, including *PJ* water extracts.

CONCLUSION

This study was performed to evaluate the potential of *Paeonia japonica* as an immunomodulator. In an in vitro experiment, the production capacity of cytokines (IL-1β, IL-6, and TNF-α) was used as an immunocompetence index: the cytokines were secreted by activated mouse macrophages (1×10^6 cells/ml), which were cultured with three concentrations (1, 10, and 100 μg/ml) of methanol extracts of *PJ* and 5 fractions (hexane, chloroform, ethylacetate, butanol, and water) obtained from methanol extract. Mouse splenocyte proliferation in the presence of various extracts from *PJ* was also examined by MTT assay. The production of IL-1β was significantly enhanced with the presence of butanol extracts in all three, and hexane and water extracts at the concentration of 10 μg/ml. Compared to the control, higher levels of IL-6 production were detected by supplementation with a chloroform fraction at concentrations of 1, 10, and 100 μg/ml, and with aqueous fractions at concentrations of 1 and 10 μg/ml. However, there were no significant differences in the production of TNF-α between treated groups and the control. Observations of mouse splencocyte proliferation indicated that supplementation using a hexane fraction and an aqueous fraction of *Paeonia japonica*, in the range of 1 – 100 μg/ml, enhanced proliferation at the level of 1.8 – 16% and 10 – 15%, respectively, compared to that of the control.

In the in vivo study, mice were orally administered for two weeks with water extracts of PJ, and this enhanced the immune function in similar fashion to the in vitro experiment. Splenocyte proliferation was increased in every supplemented group, especially in the 500 mg/kg b.w. supplemented group. The animals in *PJ* extract supplementation groups (at levels of 500 mg/kg b.w. and 1000 mg/kg b.w.) showed higher levels of production of all three cytokines (without any mitogen stimulation), compared to those in control group. In the LPS-stimulated animals, higher concentrations of IL-1β were detected in the 100 mg/kg b.w. supplementation group, and the level of IL-6 production was enhanced at the 100 mg/kg b.w. supplementation group; TNF-α production was also enhanced at the 500 mg/kg b.w. and 1000 mg/kg b.w. supplementation levels. Therefore, it could be suggested that *Paeonia japonica* water extract may regulate immune functions by enhancing cytokine production by activated peritoneal macrophage in vivo. Also, the activation of mouse peritoneal macrophage with LPS appeared to be effective in increasing the production of cytokine. The results of this study suggest that PJ may contain immunomodulative agents, which are soluble in the polar solvents. Further investigations are needed to identify the stimulative components, the mechanism by which the immunomodulating activity may exert its effect, and the clinical effects of PJ supplementation.

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