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Polysaccharide Isolated from *Zizyphus jujuba* (紅棗 Hóng Zǎo) Inhibits Interleukin-2 Production in Jurkat T Cells

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ABSTRACT

Zizyphus jujuba (紅棗 Hóng Zǎo), a traditional Chinese herb widely used in many Asian countries, has been shown to possess vital biological activities such as anti-cancer activity. The objective of this study was to evaluate the immunomodulatory effect of deproteinated polysaccharide (DP) isolated from *Z. jujuba*. The DP isolated from *Z. jujuba* consisted of two polysaccharide fractions and their molecular weights (MWs) were found to be 143,108 and 67,633 Da, respectively. The DP could significantly decrease interleukin (IL)-2 production in phytohemagglutinin (PHA)-activated Jurkat T cells in a dose-dependent manner after 48 h of incubation, with the inhibition being 47.5%, 61.2%, and 81.7% for DP concentrations of 0.75, 1.75, and 2.5 mg/ml, respectively. Thus, our study showed that DP isolated from *Z. jujuba* may possess anti-inflammatory activity as it could significantly reduce IL-2 production in activated Jurkat T cells.

Key words: Immunomodulatory, Interleukin-2, Jurkat T cells, Polysaccharide, *Zizyphus jujuba*

INTRODUCTION

The activation and clonal growth of T cells is the central incident in the generation of immune responses.[1,2] A cascade of biochemical reactions and expression of genes can be initiated when T cells are activated by antigen or phytohemagglutinin (PHA).[2,3] However, the excessive or inappropriately prolonged immune response could paradoxically aggravate the injury or even cause death. Thus, caution should be exerted while using immunomodulatory medications. One of the immunomodulatory mechanisms is to regulate T lymphocyte activation and cytokine production.[2,4,5]

Zizyphus jujuba (紅棗 Hóng Zǎo), a traditional Chinese herb belonging to the genus *Zizyphus* (Rhamnaceae), is mainly found in the subtropical regions of Asia, such as China and Taiwan. *Z. jujuba* has been demonstrated to possess many biological activities including anti-Alzheimer's disease activity, alleviation of syndrome of scopolamine-induced amnesia, antiproliferation activity against melanoma cell, and anti-HIV activity,[6-8] all of which could be attributed to the presence of bioactive compounds such as triterpenoid acid, flavonoids, and polysaccharide. In several previous studies, polysaccharide from *Z. jujuba* has been shown to induce rat spleen cell proliferation and possess anti-hepatoma and melanoma cancer cell activity.[8-10] However, the immunomodulatory activity of polysaccharides from *Z. jujuba* has been explored less. The objective of this study was to evaluate the immunomodulatory effect of deproteinated polysaccharides (DP) isolated from *Z. jujuba*.

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MATERIALS AND METHODS

Materials

Z. jujuba fruits were purchased from a local farm in Gong-Guan, Taiwan and hot air dried at 50°C for 15 h. The Jurkat T cell line was provided by Department of Life Science, Fu Jen University. Cell culture medium RPMI-1640, fetal bovine serum (FBS), and trypsin-ethylenediaminetetraacetic acid (EDTA) were obtained from Gibco Laboratories (Grand Island, NY, USA). Phosphate-buffered saline (PBS) was purchased from Sigma Chemical. (St Louis, MO, USA). Interleukin (IL)-2 enzyme immunoassay kit (EIA kit; R and D systems, Minneapolis, MN, USA) was procured from Scientific Biotech Corp. (Taipei, Taiwan).

Instrumentation

The instruments used and their manufacturers are as follows: Laminar flow (model VCM-620; Jau-Hsin Co., Taipei, Taiwan); inverted microscope (TS100; Nikon, Tokyo, Japan); carbon dioxide incubator (Forma 3110; Thermo Scientific, Fremont, CA, USA); high-speed centrifuge (5810R; Eppendorf, Ramsey, MN, USA); and enzyme-linked immunosorbent assay (ELISA) reader (Multiskan EX; Thermo, Fremont, CA, USA).

Preparation of DP powder from *Z. jujuba*

A method that was described by Wang *et al*.[11] was modified. *Z. jujuba* powder (5 g) was mixed with deionized water (75 ml), followed by homogenizing the mixture for 1 min and shaking in a hot water bath at 90°C for 6 h. Then the solution was centrifuged (13,130 g, 30 min), supernatant collected, concentrated in the water bath at 40°C under vacuum, and diluted to 20 ml with deionized water. The crude extract (10 ml) was collected and mixed with 95% ethanol (30 ml) in a centrifuge tube, after which the solution was allowed to precipitate (4°C, 5 h) and was centrifuged at 13,130 g for 30 min. The supernatant was removed and the precipitate was freeze dried to obtain crude polysaccharide (CP) powder. Next, CP (0.2 g) was collected and mixed with 50 mM phosphate buffer solution (40 ml, pH 8), followed by shaking in a water bath (50°C, 1 h) to dissolve CP. Then 1 ml of 100 U/ml proteinase (Subtilisin A type VIII from *Bacillus L.*) was added and shaken in the water bath (60°C, 4 h) for protein digestion. Then the flask was kept in boiling water to terminate the reaction. After cooling to room temperature, the solution was centrifuged (13,130 g, 15 min) and the supernatant poured into a dialysis bag (Spectra/Por®6; SPECTRUM, Houston TX, USA) for dialysis (6 h) to remove the impurities with molecular weight (MW) <3500 Da. After dialysis, the solution in the bag was collected for concentration under vacuum, ethanol precipitation, and freeze drying to obtain DP. This preparation procedure could eliminate interference from protein and impurities with small MW. The amount of polysaccharide in DP was quantified using phenol–sulfuric acid method as described in a previous study by Chang *et al*.[12]

Cell culture

The method described by Gertscha *et al*.[13] was used for cell culture experiment. In brief, Jurkat T cells were cultured in RPMI 1640 medium containing 10% fetal bovine serum (FBS), 100 U/ml of penicillin, 100 mg/ml of streptomycin, and 2 mM l-glutamine. Then the cells were incubated at 37°C under an atmosphere of 90% humidity and 5% carbon dioxide.

Determination of cell viability

The method reported by Wu *et al*.[2] was used to determine cell viability. Approximately 2×10^4 Jurkat T cells were cultured in medium (control) or treated with various concentrations of DP (125-2500 μg/ml) for 48 h. Cell numbers including total, viable, and non-viable cells were counted under a microscope with a hemocytometer after staining with trypan blue. The percentage of viable cells was calculated using the equation:

Viability (%) = viable cell number/total cell number × 100.

Determination of IL-2 production

The method of Kuo *et al*.[14] was used to determine IL-2 production. Jurkat T cells (2×10^4 cells/well) were cultured without or with PHA (5 μg/ml) alone or in combination with various concentrations of DP (125-2500 μg/ml) for 48 h. The cell supernatants were collected and assayed for IL-2 concentration using the enzyme immunoassay kit (EIA kit; R and D systems).

Statistical analysis

Each experiment was carried out in triplicate and the data were subjected to analysis of variance and Duncan's multiple range test to find the significance on comparison of means ($P < 0.05$) by using the SAS software (Statistical Analysis System Institute Inc., Cary, North Carolina, USA).

RESULTS AND DISCUSSION

According to our previous study, the polysaccharide in DP from *Z. jujuba* consisted of two fractions, with the average MW being 143,108 and 67,633 Da, respectively. In addition, the monosaccharide composition of DP was shown to be rhamnose, arabinose, xylose, mannose, glucose, and galactose at a molar ratio of 2.2:7.8:1.2:0.2:1.4:3.8, respectively.[8] It is evident from Figure 1 that DP could significantly decrease IL-2 production in PHA-activated Jurkat T cells in a dose-dependent manner after 48 h of incubation, with the inhibition being 47.5%, 61.2%, and 81.7% for DP concentrations of 0.75, 1.75, and 2.5 mg/ml, respectively. Furthermore, the cell viability of Jurkat T cells was approximately 90% with a DP concentration of 2.5 mg/ml, demonstrating that the reduction of IL-2 was not caused by the cytotoxicity of DP [Figure 2].

In a recent study, a polysaccharide isolated from *Caltha palustris* was shown to inhibit IL-2 production in collagen-induced arthritis (CIA) mice.[15] Also, Liu and Lin[16] reported that strawberry and mulberry fruit polysaccharides were efficient in inhibiting proinflammatory cytokines including IL-1β and IL-6 in lipopolysaccharide (LPS)-stimulated macrophages. Also, a polysaccharide isolated from *Astragalus membranaceus* (黃耆 Huáng Qí) was found to be effective in reducing LPS-induced IL-1β production in THP-1 cells.[17] Similarly, Wu *et al*.[2] pointed out that a compound (tanshinlactone A) isolated

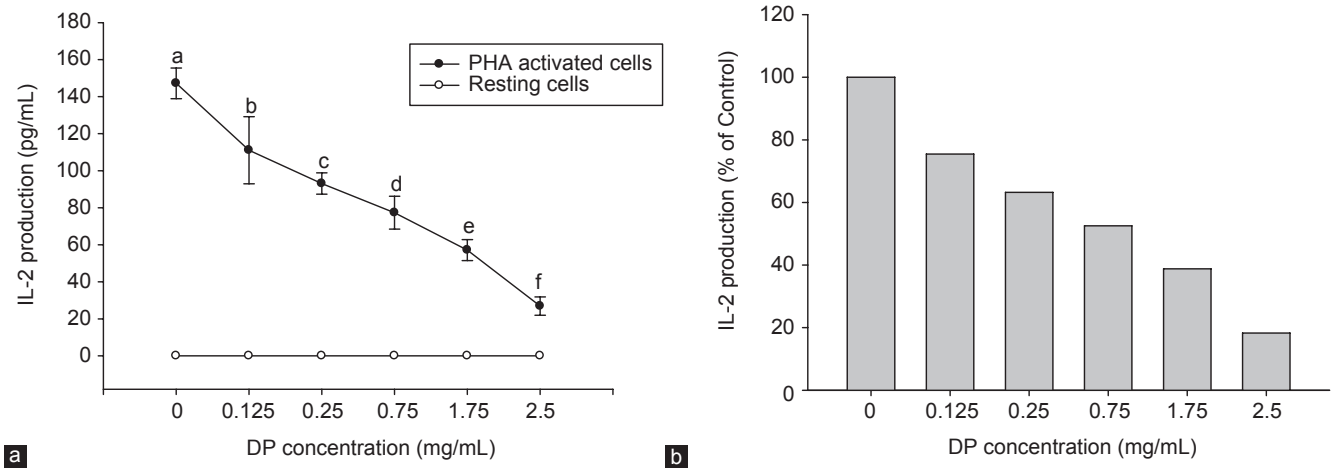

Figure 1. IL-2 production in Jurkat T cells treated with deproteinated polysaccharide (DP). Data presented are (a) IL-2 concentration and (b) IL-2 percentage of control. Jurkat cells (2×10^4/well) were cultured in medium or treated with 0.125-2.5 mg/ml of DP with or without PHA (5 μg/ml) for 48 h. Data with different letters (a-f) are significantly different at $P < 0.05$

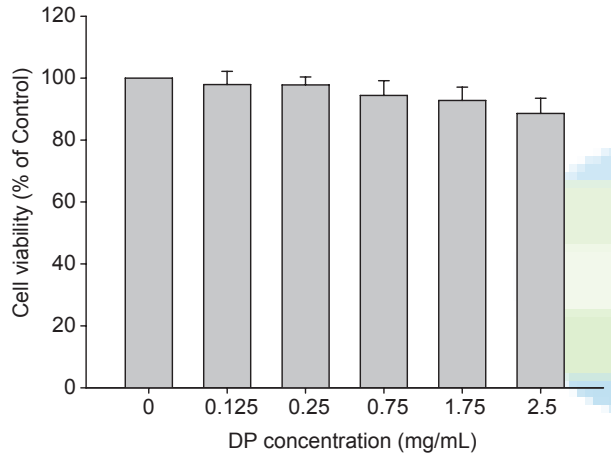

Figure 2. The viability of Jurkat T cells treated with deproteinated polysaccharide (DP). Jurkat cells (2×10^4/well) were cultured in medium or treated with 0.125-2.5 mg/ml of DP for 48 h. Numbers of cells (total, viable, and nonviable) were counted after trypan blue staining

from *Salvia miltiorrhiza* (丹參 Dān Shēn) could inhibit IL-2 gene expression through interruption of mitogen-activated protein kinase (MAPK) activation. Thus, the inhibitory effect of DP on IL-2 production observed in our study could be due to MAPK inactivation. Accordingly, polysaccharides, being reported as immune response stimulators, usually increase production of cytokines such as IL-2.[18,19] Our *in vitro* study further demonstrates that DP isolated from *Z. jujuba* could significantly reduce IL-2 production, possibly involving a central mechanism of several immunosuppressants as reported by Wu *et al*.[2] Thus, it can be summarized that the DP isolated from *Z. jujuba* might possess anti-inflammatory activity.

CONCLUSION

Two polysaccharides isolated from *Z. jujuba* could significantly inhibit IL-2 production in Jurkat T cells in a dose-dependent manner. This is only a preliminary study to demonstrate the anti-inflammatory effect of *Z. jujuba* polysaccharides on Jurkat T cells and further research is necessary to elucidate the detailed mechanism. In addition, various *in vivo* or genetic approaches should be conducted to verify the inhibitory effect of *Z. jujuba* polysaccharides on IL-2 production.

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