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Antioxidant and Anti-hyperglycemic Activity of Polysaccharide Isolated from *Dendrobium chrysotoxum* Lindl

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Although polysaccharide is believed to play an important role in the medicinal effect of *Dendrobium chrysotoxum* Lindl (DCL), its role as an antioxidant and in anti-hyperglycemic induction was not reported. In this study, polysaccharide with molecular weight of approximately 150 kDa, herein named DCLP, was isolated from the stem of DCL, and its antioxidative, hypoglycemic and immune stimulating effects were evaluated using various *in vitro* and *in vivo* assay systems. DCLP inhibited hydroxyl radicals ($^{\cdot}OH$)-mediated deoxyribose degradation by scavenging hydroxyl radicals directly as well as by chelating iron ions. DCLP also showed dose-dependent scavenging activity on superoxide anions ($O_2^{\cdot -}$) and offered significant protection ($p < 0.001$) against glucose oxidase-mediated cytotoxicity in Jurkat cells. DCLP had immune stimulating effects, as evidenced by the DCLP-mediated increases in the level of DNA synthesis, viability, and cytokine secretion in mouse lymphocytes. Moreover, oral administration of DCLP produced a significant reduction in blood glucose level in alloxan-induced diabetic mice. These findings suggest that DCLP has a potential utility in treating patients who require enhanced antioxidation, immune function and/or hypoglycemic activity.

Keywords: Antioxidation, Anti-hyperglycemic, *Dendrobium chrysotoxum* Lindl, Immune stimulation, Polysaccharide, Traditional Chinese medicine

Introduction

Traditional oriental medicines have long been used to treat human diseases, particularly those associated with elderly patients. There has been increasing interest in examining the specific biological activity of traditional medicines, and numerous investigations support their antioxidant, immune stimulatory, antitumor, and anti-inflammatory activities (Satoh *et al*., 1996; Yamaguchi *et al*., 2000; Rao *et al*., 2006). Recent studies of traditional medicines have been carried out to identify their active ingredients and to test their efficacy in treating various diseases. As reported previously, several components isolated from traditional medicines have been proposed as useful therapeutic agents (Gong *et al*., 2004; Kim *et al*., 2005; Lee *et al*., 2005). Polysaccharides are one of the major active compounds responsible for the pharmacological activities contained in traditional herbal medicines (Li *et al*., 2001; Xie *et al*., 2004; Fang *et al*., 2005).

The traditional Chinese herbs, *Dendrobium,* have been used in the preparation of herbal medicines in many Oriental countries for a long time. Over 60 species of *Dendrobium* are found in China, and many studies have demonstrated their beneficial activities in antioxidant, immune stimulating and antitumor activities (Ye and Zhao, 2002). Of the *Dendrobium* medical plants, *Dendrobium chrysotoxum* Lindl (DCL) is the most commonly used herb in the Chinese medicine, Herba Dendrobii (stem of *Dendrobium*) (Li *et al*., 2005). Although polysaccharide is believed to play an important role in the medicinal effects of DCL, its role as an antioxidant and in anti-hyperglycemic and immune stimulation had not been examined. Therefore, this study isolated polysaccharide from the stem of DCL and examined its antioxidant property in an attempt to define the mechanism of its antioxidant action using cell-free and cell-mediated radical generating systems. This study also determined whether the DCL polysaccharide (DCLP) has immune stimulating activity. Finally, the effects of DCLP on blood glucose level were evaluated using normal and alloxan-induced diabetic mice.

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Materials and Methods

Chemicals, plastics, and mice. Unless otherwise specified, all chemicals used in this study were purchased from Sigma Chemical Co. and all the plastics were obtained from Falcon Labware (Becton-Dickinson). Male BALB/c mice (6 weeks old) were obtained from Orient Co. Mice were housed in automatically controlled conditions with a 12-h light/dark cycle, 22 ± 1°C, and 45-55% relative humidity. All mice had free access to standard rodent pellet food and water *ad libitum*, only except when fasted before experiments. Experiments were carried out according to the guidelines of the Animal Care Committees of Chonbuk National University.

Isolation of polysaccharide. The dried sample of DCL was obtained from Shanghai Jiao Tong University after identification by botanists of this university. A voucher specimen (No. 2005-DCL) was also deposited at the herbarium of this university. Briefly, powdered DCL (100 g) was resuspended in absolute ethanol at 78°C for 1 h and then filtered through a filter paper (Whatman No. 3). The remainder was extracted three times with distilled water at 80°C. The collected water extract was evaporated and reduced to a 200 ml volume and then mixed with absolute ethanol (1 : 4, v/v) for 24 h to precipitate the polysaccharide compounds. After centrifugation, the precipitates were collected and washed with absolute ethanol and acetone, respectively, and then lyophilized to 16 g (16% of the initial amounts) to produce the dried polysaccharide sample of DCL (DCLP). DCLP was stored at −20°C and freshly dissolved in distilled water immediately before use.

SDS-PAGE and FT-IR analysis. SDS-PAGE was performed using a Mini-Protean II cell (Bio-Rad) with 10% polyacrylamide gels. DCLP was dissolved in sample buffer in the absence of 2-mercaptoethanol and analyzed without pre-boiling. The gels were stained with either with Coomassie brilliant blue (CBB) to detect proteins or with Schiff reagent to detect carbohydrates, as described elsewhere (Neville Jr and Glossmann, 1974). In addition, the molecular structure of DCLP was roughly determined by FT-IR (PerkinElmer, GX model).

Antioxidant assays using cell-free radical generating systems. A deoxyribose assay to determine the rate constant for the reactions between either antioxidants and hydroxyl radicals ($^{\bullet}OH$) (referred to a non-site-specific scavenging assay) or antioxidants and iron ions (referred as a site-specific scavenging assay) was conducted as described by Halliwell *et al.* (1987). In addition, the ability of DCLP to reduce the Fe^{3+}-EDTA complex to Fe^{2+}-EDTA complex, acting as pro-oxidant, was examined using the non-site-specific scavenging assay mixture without the addition of ascorbic acid (Kim *et al.*, 2005). DNA nicking assay was also performed using supercoiled pBR322 plasmid DNA prepared from DH5α using Wizard® Plus SV Minipreps (Promega, Madison, WI), as described elsewhere (Lee *et al.*, 2001). Finally, the scavenging activity on superoxide radical ($O_2^{\bullet -}$) was examined using a slight modification of the method described by Gotoh and Niki (1992).

Cell culture and treatment. Mouse splenocytes and Jurkat cells, or MCF-7 cells were cultured in RPMI-1640 or DMEM supplemented with 10% fetal bovine serum (FBS; HyClone, Logan, UT). When these cells had reached confluence, 1×10^5-10^6 cells per ml were resuspended in 100 μl media and spread onto 96-well flat-bottomed plates. Before the DCLP treatment, the cultures were switched to a new medium supplemented only with 0.5% FBS. The cells were then processed for an analysis of the antioxidant activity, DNA synthesis, cytotoxicity, and cytokine production.

Cellular assay for antioxidant activity and DNA synthesis. Jurkat and glucose/glucose oxidase (G/GO) were used as the model cell and radical generating systems, respectively. Briefly, Jurkat cells (1×10^5 cells/well) were placed onto 96-well plates and exposed to the G/GO system (10 mM D-glucose and 10 mU/ml glucose oxidase in RPMI 1640 medium with 0.5% FBS) with or without DCLP. At various time points, cell viability was measured using a MTT assay system as described previously (Lee *et al.*, 2006).

The level of DNA synthesis in the splenocytes, Jurkat, and MCF-7 cells incubated with different DCLP concentrations was measured by adding 1 μCi of [*methyl*-^{3}H] Thymidine deoxyribose (TdR; Amersham Pharmacia Biotech) to each well for 12-16 h during culture periods. Cells were collected with a cell harvester (Inotech Inc.), and the TdR content was determined using a liquid scintillation counter (Packard Instrument Co.).

Measurement of cytokine levels. The concentration of cytokines produced by lymphocytes after DCLP treatment was determined by ELISA. Briefly, the splenocytes cultured in 96-well tissue culture plates were exposed to different concentrations of DCLP for 72 h. The culture supernatants were then collected and the level of cytokines representing the stimulation of Th1-type (IFN-γ) and Th2-type (IL-4 and IL-6) T cells, and macrophages (and/or B cells) (TNF-α) was determined.

Characterization of DCLP. The DCLP was treated with pronase E to degrade the protein, or with $NaIO_4$ to degrade the carbohydrate residues, as described elsewhere (Kim *et al.*, 2002). A DCLP sample (1 mg) was also incubated for 3 h at 37°C in 1 ml of the culture medium containing 1,000 units of polymyxin B (PMB) to determine if DCLP contained lipopolysaccharide (LPS)-like compounds. In addition, 10 mg of DCLP was dissolved in 1 ml distilled water and the tube was soaked in a boiling water bath for 20 min. The ability of the treated DCLP samples to stimulate lymphocyte proliferation was measured by a TdR uptake assay using mouse splenocytes.

Measurement of hypoglycemic activity in normal mice. Following an overnight fast, healthy mice (10 week-old) were divided into four groups ($n = 7$). All mice were then administrated orally and daily for one week with 0.5 ml of phosphate-buffered saline (PBS) supplemented without (negative control) and with tolbutamide (80 mg/kg body wt.) or DCLP (200 and 500 mg/kg body wt., respectively). Blood samples were drawn from the tail vein of the mice before the first administration and 1 h after the last administration of the samples. Blood glucose levels were determined using a commercially available quantification kit, AM201-K (Asan Pharmaceutical Co.).

Diabetes induction and DCLP treatment. In order to evaluate the effects of DCLP on the blood glucose levels in diabetic mice, the overnight fasted 10-week-old BALB/c mice were made diabetic with alloxan. Briefly, alloxan monohydrate was dissolved in cold distilled water just before intraperitoneal injection, and the mice received alloxan at a dose of 160 mg/kg body weight. Diabetes was confirmed by measuring the blood glucose level after 16 h of fasting at 7 days post injection, and the mice with blood glucose concentration above 300 mg/dl were used in the experiments. The diabetic mice were divided into four groups (7 per group) and were orally administrated with tolbutamide or DCLP everyday for one week, as described above.

Statistical analyses. All the data is expressed as a mean ± standard deviation (S.D.). A one-way ANOVA using SPSS ver. 10.0 software was used for multiple comparisons. A value of $p < 0.05$ was considered significant.

Results

Properties of DCLP. The nature of DCLP was determined by separating the sample by SDS-PAGE and staining the gels with either CBB or Schiff reagent. The band with a molecular weight of approximately 150 kDa was observed with Schiff reagent (Fig. 1A), while there was no a band stained with CBB (data not shown). This indicates that DCLP was not contaminated with any protein residues. Fig. 1B shows the FT-IR profile of DCLP. As shown in the figure, DCLP contained some special function groups, which means DCLP is a polysaccharide. For example, the peak at 3421 cm^{-1} was assigned to the O-H stretching vibration, the two peaks at 2932 cm^{-11} and 1380 cm^{-1} were the C-H stretching vibration, the peaks at 1737 cm^{-1} and 1250 cm^{-1} were the C=O and C-O stretching vibration, the peak at 1032 cm^{-1} was the C-O of the C-O-C stretching vibration, and the peaks at 875 cm^{-1} and 813 cm^{-1} were Mannose.

Scavenging effect of DCLP on hydroxyl radicals and superoxide anions. The scavenging effect of DCLP on the hydroxyl radicals generated by Fe^{3+} ions was measured by determining the extent of deoxyribose degradation, which is an indicator of the formation of thiobarbituric acid-malonaldehyde (TBA-MDA) adduct (Fig. 2A). The concentration-dependent inhibition of hydroxyl radical-induced deoxyribose degradation was observed in both the site-specific and non-site specific assays (Fig. 2A, top and middle panels). However, more antioxidant activity was observed in the site-specific assay than in the non-site-specific assay when the same concentration of DCLP was used. The ability of DCLP to reduce the Fe^{3+}-EDTA complex was also examined by measuring the level of deoxyribose degradation using the same reaction buffer without the addition of ascorbic acid. There was no increase in the formation of TBA-MDA by adding DCLP, while the addition of DCLP to the reaction buffer inhibited the TBA-MDA adduct in a dose-dependent manner (Fig. 2A, bottom panel).

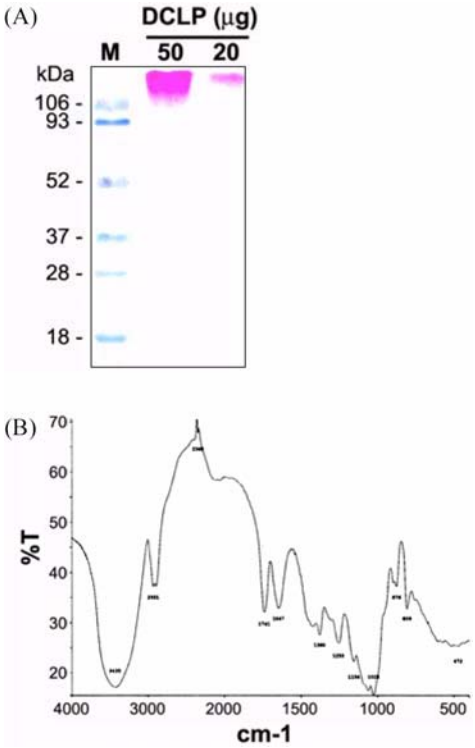

Fig. 1. SDS-PAGE and FT-IR analyses of *Dendrobium chrysotoxum* Lindl polysaccharides (DCLP). (A) DCLP was loaded at the indicated doses, electrophoresed, and stained with Schiff reagent. M represents a pre-stained molecular weight marker. (B) In addition, the DCLP was analyzed by FT-IR.

To further measure the scavenging effect of DCLP on Fe^{3+}-dependent hydroxyl radicals, this study investigated whether or not the sample reduced Fe^{3+}-dependent DNA nicking (Fig. 2B). When pBR322 plasmid DNA was dissolved in the reaction mixture, a time-dependent increase in the formation of single-stranded nicked DNA (Form II) was observed (data not shown). However, the addition of DCLP to the nicking reaction mixture inhibited Form II DNA formation thereby increasing the level of Form I DNA.

The scavenging effect of DCLP on superoxide anions was determined by monitoring the reduction of NBT induced by superoxide anions produced by the xanthine oxidase-mediated degradation of hypoxanthine. DCLP inhibited NBT reduction in a dose-dependent manner (Fig. 2C). The addition of 4 mg/ml DCLP to the reaction solution inhibited the production of superoxide anions by 50.5%. DCLP alone did not alter the absorbance of the reaction solution containing only NBT, suggesting that DCLP did not directly reduce NBT (data not shown). In addition, DCLP significantly inhibited the activity of DPPH radicals in a dose-dependent manner (data not shown).

Protective effect of DCLP on cytotoxicity induced by glucose oxidase-mediated oxidative stress. The antioxidant activity of DCLP was further examined using the G/GO

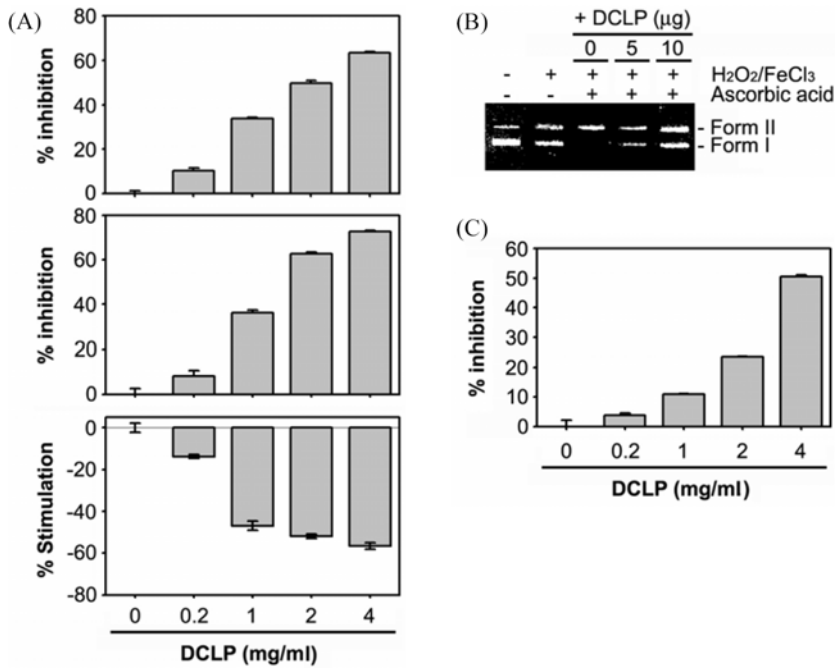

Fig. 2. Antioxidant potential of DCLP on cell-free radical generating systems. (A) Inhibitory effects of DCLP on deoxyribose degradation induced by hydroxyl radicals. The direct scavenging activity of hydroxyl radicals (top panel) and the chelating activity of iron ions by DCLP (middle panel) are expressed as the % inhibition. The ability of DCLP to reduce the Fe^{3+}-EDTA complex to Fe^{2+}-EDTA complex was also tested (bottom panel), and the data is expressed as the % stimulation. (B) Inhibitory effect of DCLP on DNA nicking caused by hydroxyl radicals. (C) Inhibitory effect of DCLP on NBT reduction induced by superoxide anions.

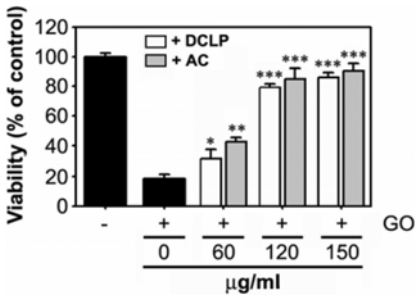

Fig. 3. Protective effects of DCLP on glucose oxidase (GO)-mediated oxidative stress. Jurkat cells were exposed to the G/GO system for 24 h in the presence of DCLP or acteoside, and cell viability was analyzed by the MTT assay. Acteoside was used as the control antioxidant. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ vs. GO treatment alone. AC, acteoside.

system, in which GO yields hydrogen peroxide via a catalytic reaction from its substrate, glucose (Fig. 3). DCLP significantly protected the Jurkat cells from G/GO-mediated cytotoxicity, and the protective activity was quite similar to that of acteoside, which used as the control antioxidant because its antioxidant potential was much higher than that of ascorbic acid (Kim *et al*., 2005). The addition of 60 and 120 μg/ml DCLP increased the viability by 30.9% ± 5.2 ($p < 0.05$) and 78.9% ± 2.7 ($p < 0.001$), respectively, compared with that of the G/GO treatment alone (18.3%).

Stimulating effects of DCLP on proliferation, viability, and cytokine secretion of mouse lymphocytes. The effect of DCLP on DNA synthesis in various types of cells was determined using a tritium incorporation assay. DCLP significantly increased the level of DNA synthesis in the splenocytes in that a treatment with 50 μg/ml DCLP increased the TdR uptake 6.72-fold ($p < 0.001$) compared with the untreated control (1,329 cpm) (Fig. 4A). However, this increase was not observed in the Jurkat cells and MCF-7 cells.

In order to determine if DCLP itself has a cytotoxic effect on lymphocytes, the splenocytes were treated with various DCLP concentrations and incubation times. When the cells were exposed to DCLP for 36 h, the level of MTT reduction increased significantly ($p < 0.05$) in all the treatments compared with the untreated control cells (Fig. 4B, upper panel). In addition, the MTT reducing activity of splenocytes was not decreased even when treated with 200 μg/ml DCLP for 48 h (Fig. 4B, below panel).

The splenocyte-specific stimulating activity of DCLP was further examined by measuring the level of cytokines produced by DCLP in the cells (Fig. 4C). A prominent increase in IFN-γ and IL-6 production was observed by treating them with DCLP. When the cells were exposed to 40 μg/ml DCLP for 72 h, 11.7 ng/ml IFN-γ and 34.2 ng/ml IL-6 were produced compared with 0.52 ng/ml IFN-γ and 0.7 ng/ml IL-6 produced in the untreated control cells, respectively. The DCLP treatment also increased the production of TNF-α compared

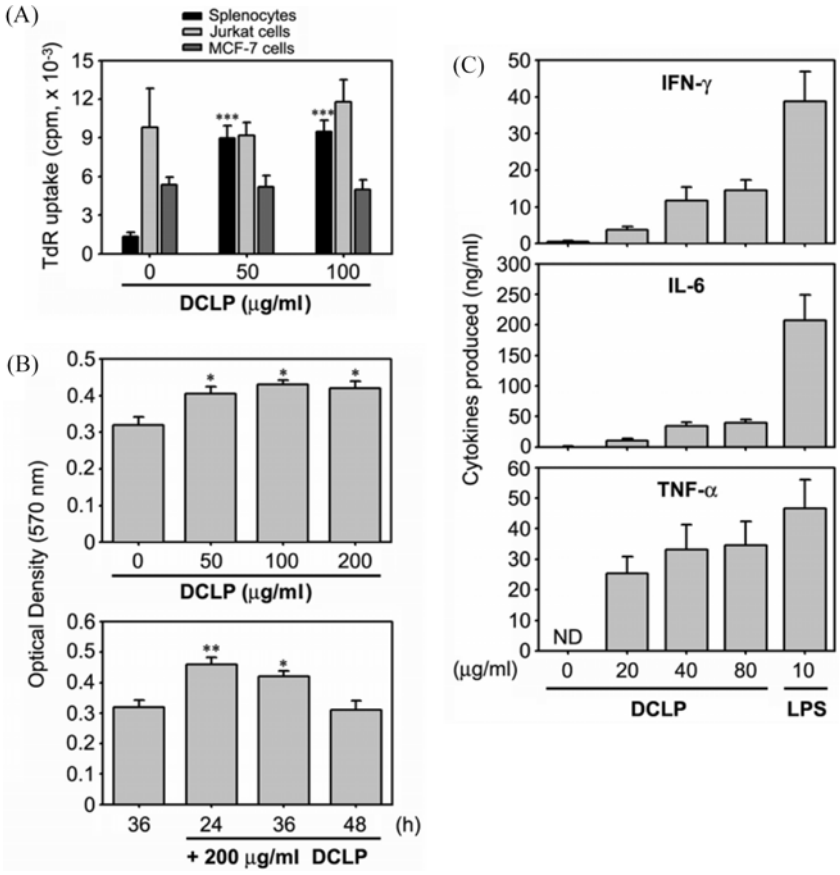

Fig. 4. Immune stimulating effects of DCLP. (A) Effects of DCLP on cell proliferation in various types of cells. The cells were incubated with the indicated concentrations of DCLP and exposed to 1 μCi/ml [*methyl*-^{3}H] TdR for the last 12 h of the 48 h culture period. ***$p < 0.001$ vs. control. (B) Effects of DCLP on MTT-reducing activity of splenocytes. Splenocytes were incubated with the indicated concentrations of DCLP for 36 h (upper panel) or the indicated times with 200 mg/ml DCLP (below panel) and processed for MTT staining. *$p < 0.05$ and **$p < 0.01$ vs. control. (C) Effects of DCLP on cytokine production by mouse splenocytes. The cells were exposed to the indicated amounts of DCLP or lipopolysaccharide (LPS) for 72 h and the levels of IFN-γ (top panel), IL-6 (middle panel), and TNF-α (bottom panel) were analyzed by ELISA. LPS was used as the positive control. ND, not detected.

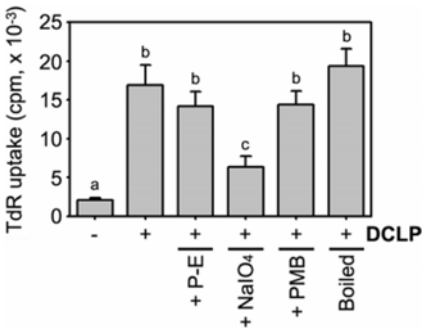

Fig. 5. Effects of pronase E, $NaIO_4$, polymyxin B, and heating on the splenocyte-stimulating activity of DCLP. The ability of the treated DCLP samples to stimulate lymphocyte proliferation was measured by a TdR uptake assay. Here, 50 mg/ml of the DCLP samples was added to the splenocytes cultures. The superscript letters indicate the significant differences ($p < 0.05$) between the experimental and control values. P-E, pronase E; PMB, polymyxin B.

with the control, such that the level of TNF-α production was similar to that obtained after treating the splenocytes with LPS (46.7 ng/ml). However, no change in the level of IL-4 was detected after stimulating the splenocytes with DCLP (data not shown).

Characterization of DCLP on lymphocyte stimulation. In order to further clarify whether or not the splenocyte-stimulating activity of DCLP was due to its carbohydrate residue, the sample was treated with either pronase E, $NaIO_4$, or PMB, and their ability to stimulate the splenocytes was examined. As shown in Fig. 5, the treatment of DCLP with $NaIO_4$ but not pronase E inhibited the stimulating activity of the sample. PMB, which is an inhibitor of the LPS-like mitogen-mediated response, did not reduce the DCLP activity. In contrast, the same PMB treatment significantly inhibited the LPS-mediated increase in TdR uptake by splenocytes (data not shown). Finally, DCLP was boiled for 20 min and its ability to stimulate lymphocyte proliferation was measured.

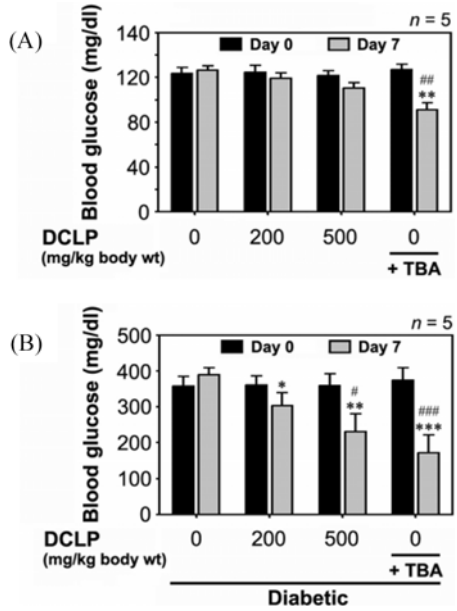

Fig. 6. Hypoglycemic effect of DCLP in normal and diabetic mice. Healthy normal (A) and alloxan-induced diabetic mice (B) were administrated with the DCLP or tolbutamide, and blood samples were collected from the mice before the first administration and 1 h after the last administration of the samples and were processed for the analysis of glucose concentrations, as described in the Materials and methods section. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ vs. control. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ vs. day 0. TBA, tolbutamide.

The splenocyte-stimulating activity of DCLP was not affected by boiling. However, concanavalin A activity was inhibited significantly by the treatment (data not shown).

Anti-hyperglycemic effect of DCLP on blood glucose level in normal and alloxan-induced diabetic mice. It has been shown that polysaccharides from traditional herbal medicine have an anti-hyperglycemic activity in streptozotocin- or alloxan-induced diabetic animals (Xie *et al*., 2004; Li *et al*., 2006). Therefore, this study was also designed to determine whether DCLP have an anti-diabetic activity in alloxan-induced hyperglycemic mice. As shown in Fig. 6A, there was no significant difference between the basal blood glucose level in both the control and experimental groups. On day 7, however, tolbutamide-treated mice had significantly lower blood glucose concentrations (91.2 ± 13.6 mg/dl; $p < 0.01$), as compared to the PBS-treated control mice (126.4 ± 8.5 mg/dl).

Alloxan-treated mice had dramatically high basal blood glucose level (approximately 360 mg/dl). In contrast, DCLP treatment decreased significantly blood glucose levels such that seven days administration of DCLP at doses of 200 and 500 mg/kg body weight reduced the levels up to 303.6 ± 35.8 ($p < 0.05$) and 231.7 ± 49.4 mg/dl ($p < 0.01$), respectively, as compared to the alloxan treatment alone (389.2 ± 19.4 mg/dl) (Fig. 6B). Tolbutamide also had a significant anti-hyperglycemic activity ($p < 0.001$). However, body weight of the mice was not changed by treating DCLP itself during the experimental periods (data not shown).

Discussion

For a long time, plant-derived antioxidants have been used to reduce the level of oxidative stress within the human body (Rice-Evans *et al*., 1995; van Poppel and van den Berg, 1997). Although macromolecules such as proteins and polysaccharides have been shown to be the major components associated with the immune stimulating activity of traditional herbal plants, recent studies have also demonstrated their potential, particularly their antioxidant (Ghosh *et al*., 2006). The polysaccharide isolated from cultured *Cordyceps*, which is one of the most valued traditional Chinese medicines, has strong antioxidant activity against superoxide radicals and protects hydrogen peroxide-induced cytotoxicity of PC12 cells (Li *et al*., 2003). In addition, the polysaccharide present in traditional Chinese medicine *Ganoderma lucidum,* which is used for the immune dysfunction related to illness, reduces the formation of DPPH radicals (Xie *et al*., 2006). This means that polysaccharide also has a potential antioxidant role.

Hydroxyl radicals are the most reactive of all the reduced forms of dioxygen and are believed to initiate cell damage both *in vitro* and *in vivo* (Rollet-Labelle *et al*., 1998). Thus, we initially used the Fe^{3+}-dependent system to examine the ability of DCLP to scavenge hydroxyl radicals generated by iron. In the system, ascorbic acid acts as an electron donor rather than an antioxidant on account of its high redox activity and thus facilitates the production of hydroxyl radicals via the reduction of Fe^{3+}-EDTA complex (Aruoma *et al*., 1997). DCLP inhibited deoxyribose degradation more by chelating iron ions than by scavenging hydroxyl radicals directly. The scavenging activity of DCLP was also confirmed using a DNA nicking assay, which indicates that DCLP effectively mitigates the oxidative stresses on susceptible biomolecules, such as DNA. In addition, the ability of DCLP to reduce the Fe^{3+}-EDTA complex was not observed. This suggests that DCLP is a potential antioxidant but does not possess the redox potential to act as an electron donor that generates hydroxyl radicals in the deoxyribose assay (Laughton *et al*., 1989). Therefore, it is believed that the antioxidant property of DCLP is different from those of ascorbic acid and acteoside, which are powerful antioxidants with oxidoreductive potential (Kim *et al*., 2005).

Superoxide anions are the most common free radicals *in vivo* and are generated in a variety of biological systems by either auto-oxidation processes or by enzymes. The concentration of superoxide anions increases under oxidative stress and related situations (von Harsdorf *et al*., 1999; Matés *et al*., 2002). Moreover, superoxide anions produce other types of cell-damaging free radicals and oxidizing agents, and

indirectly initiate lipid peroxidation (Yen and Heieh, 1997; Liu and Ng, 2000). In the NBT system, xanthine oxidase, which is one of the main enzymatic sources of ROS *in vivo*, generates superoxide radicals, which then reduces NBT to yield blue formazan (Gotoh and Niki, 1992). The NBT reduction was actively inhibited by DCLP, which suggests that the DCLP is a potential scavenger of superoxide anions and hydroxyl radicals.

In order to further evaluate the antioxidant property of DCLP in living cells, the degree to which DCLP protects against radical-induced cell injury was tested in Jurkat cells using a MTT assay system. DCLP significantly prevented the hydroxyl radical-mediated cell death. Considering the fact that hydrogen peroxide induces apoptosis through the generation of hydroxyl radicals, it is suggested that DCLP can inhibit ROS-mediated apoptosis in cells. It is also believed that DCLP inhibits the GO-mediated cell death by chelating intracellular metal ions rather than by scavenging superoxide anions directly. This is because more hydroxyl radicals are generated through the Fenton reaction in the G/GO system than through the Haber-Weiss reaction (Lee *et al*., 2006).

Several active compounds regulate the induction of immune response. In particular, polysaccharides can act as an immune stimulator (Fang *et al*., 1985). However, there are reports showing that polysaccharides have dual functions to act as both an inhibitor and stimulator of proliferation (Li *et al*., 2006; Xie *et al*., 2006). This contrary action of polysaccharide is believed to be derived from their structures and chemical properties, and types of cells studied. In this study, DCLP activated the proliferation of splenocytes. This was found to be a cell-specific response, as evidenced by the lack of proliferation observed in Jurkat and MCF-7 cells. In addition, treating the splenocytes with DCLP increased the level of IFN-γ, IL-6 and TNF-α, thereby indicating that DCLP induces the direct stimulation of B lymphocytes and/or macrophages as well as T lymphocytes. Moreover, this study showed that the DCLP did not contain LPS-like compounds or lectin compounds, and the stimulating activity of the sample was attributed to the carbohydrate residues contained in DCLP. This finding is in good agreement with the results from a previous study (Kim *et al*., 2002).

Diabetes is one of the major health problems in the population worldwide. Many studies have been focused on the development of a new anti-diabetic agent from dietary and medicinal plants. In particular, polysaccharides were found to have a significant anti-hyperglycemic activity (Kiho *et al*., 1999; Xie *et al*., 2004). This study also showed that DCLP treatment significantly reduced the levels of blood glucose in alloxan-induced diabetic mice. Alloxan induces type I diabetes mellitus by disrupting the balance between cellular antioxidant defenses and free radical formation as well as by partially destructing β-cells in the pancreas (Abdel-Barry *et al*., 1997; Szkudelski *et al*., 1998). This postulates that polysaccharides having high antioxidant potential may have a role to prevent the development of diabetes (Li *et al*., 2006). Therefore, this study suggests that DCLP may play a beneficial role in treating diabetic patients, and its role is closely related to the antioxidant potential. However, the precise mechanism by which DCLP reduces blood glucose levels in diabetic mice will require further detailed study.

In conclusion, oxidative stress may play a role in aging as well as in several degenerative diseases. The finding that DCLP with a molecular mass of approximately 150 kDa has potent antioxidant, immune stimulating, and anti-hyperglycemic properties highlights the potential use of DCLP as a therapeutic agent for diabetic patients. Studies aimed at identifying the physicochemical property of DCLP, further elucidating the intracellular signaling mechanism of DCLP and determining its pharmacological abilities *in vivo* are currently underway.

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