Protective Effect of *Puerariae* Radix on Oxidative Stress Induced by Hydrogen Peroxide and Streptozotocin

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This study evaluated the protective effect of *Puerariae* radix against the oxidative stress induced by hydrogen peroxide (H_2O_2) and streptozotocin *in vitro* and *in vivo*, respectively. The ethanol extract scavenged intracellular reactive oxygen species (ROS), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, and prevented lipid peroxidation. This radical scavenging activity of the ethanol extract protected the cell viability of Chinese hamster lung fibroblast (V79-4) cells exposed to H_2O_2. Furthermore, this extract reduced the formation of apoptotic cells induced by H_2O_2, which was demonstrated by the decreased number of sub G_1 hypo-diploid cells and apoptotic cell body formation. The extract increased the activities of the cellular antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT). Administration of the extract to the streptozotocin induced diabetic rats decreased the blood glucose levels. The diabetic rats showed low activities of superoxide dismutase and catalase in the liver, and the ethanol extract increased the CAT activity. The increased level of lipid peroxidation in the diabetic rats reverted to near normal levels after being treated with the extract. This study showed that Puerariae radix was effective in the amelioration of diabetes, which may be a consequence of its antioxidant potential.

Key words *Puerariae* radix; antioxidant activity; reactive oxygen species

Enhanced oxidative stress and the changes in antioxidant capacity, which are observed in both clinical and experimental diabetes mellitus, are thought to contribute to the etiology of chronic diabetic complications.[1)] The implications of oxidative stress in the pathogenesis of diabetes is suggested not only by oxygen free radical generation due to non-enzymatic protein glycosylation and auto-oxidation of glucose, but also by changes in the tissue content and activity of the antioxidant defense systems.[2,3)] In addition, the increased oxidation of both lipids and proteins is associated with the development of diabetic complications. There is evidence suggesting that diabetes induces changes in the activities of the antioxidant enzymes in various tissues,[4)] and that their availability influences the susceptibility to various tissues to oxidative stress.[5,6)]

Puerariae radix is widely used in oriental medicine as an antipyretic and analgesic for treatment of the common cold. It is reported that saponins from the root of *Puerariae lobata* showed preventive effects on *in vitro* immunological injury of rat primary hepatocyte cultures.[7,8)] The *Puerariae* flos has been used traditionally to treat diabetes mellitus and to protect the liver. It is reported that kaikasaponin III from the flos of *Puerariae thunbergiana* showed hypoglycemic and hypolipidemic effects in streptozotocin induced diabetic rat.[7,9)]

This paper reports the protective effect of the ethanol extract from *Puerariae* radix on the oxidative damage induced by hydrogen peroxide (H_2O_2) and streptozotocin.

MATERIALS AND METHODS

Plant Material and Extraction The *Puerariae* radix material was purchased locally, and the specimens were stored for reference. The *Puerariae* radix was washed with distilled water several times and was extracted with ethanol at 65 °C for 3 h. The ethanol-soluble fraction was filtered and the supernatant was concentrated by rotary evaporation. Freeze-drying of the concentrates yielded a brown powder, which was dissolved in phosphate buffered saline (PBS) and saline for the *in vitro* and *in vivo* studies, respectively.

Chemicals The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, 2′,7′-dichlorodihydrofluorescein diacetate (DCF-DA), streptozotocin and Hoechst 33342 were purchased from Sigma Chemical Company, St. Louis, U.S.A., and thiobarbituric acid (TBA) from BDH Laboratories, England. The other chemicals and reagents used were of analytical grade. Tectoridin was supplied by Professor Dong Hyun Kim of the Department of Microbial Chemistry, College of Pharmacy, Kyung Hee University (Seoul, Korea). Primary sheep monoclonal superoxide dismutase, and -catalase antibodies were purchased from Biodesign International Company (Maine, U.S.A.).

Cell Culture It is reported that lung is an organ sensitive to oxidative stress.[10,11)] To study the effect of *Puerariae* radix on oxidative stress induced by hydrogen peroxide, we used Chinese hamster lung fibroblasts (V79-4 cells). V79-4 cells from the American Type Culture Collection were maintained at 37 °C in an incubator with a humidified atmosphere of 5% CO_2, and cultured in Dulbecco's modified Eagle's medium containing 10% heat-inactivated fetal calf serum, streptomycin (100 μg/ml) and penicillin (100 units/ml).

Experimental Animals and Streptozotocin Induced Diabetes Male Sprague–Dawley rats (Life Science Co., Korea), weighing 200±20 g, were housed 6 rats per cage in a room maintained at 22±2 °C with an alternating 12 h light–dark cycle. The rats were allowed food pellets and tap water *ad libitum*, and were kept in these facilities for at least 2 d before the experiments. They were then divided into three groups of 6 rats each. The extract was dissolved in saline,

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and given orally by an injector for 12 d at a daily dose of 500 mg/kg. Diabetes in rats was induced by a single intravenous injection of streptozotocin at a dose of 150 mg/kg body weight in a 0.1 M citrate buffer pH 4.5. The control group was treated orally with saline for 12 d and the diabetic group was injected with streptozotocin (150 mg/kg). The diabetic group given the extract was injected with the extract (500 mg/kg) orally for 12 d after the streptozotocin (150 mg/kg) injection. At 12 d after the streptozotocin injection, the animals were sacrificed and the blood and liver were collected. The liver was isolated separately and homogenized with a glass/teflon homogenizer in 4 volumes of 0.1 M potassium phosphate buffer (pH 7.4). The homogenates were centrifuged at 15000×***g*** for 1 h and used as the enzymatic sources for the activity assay or the lipid sources for the peroxidation assay.

Determination of Blood Glucose Level The hyperglycemic incidence was monitored using the glucose oxidase method and is expressed in milligrams per deciliter (mg/dl).

Intracellular Reactive Oxygen Species (ROS) Measurement The DCF-DA method was used to detect the intracellular ROS.[12] DCF-DA diffuses into cells, where it is hydrolyzed by intracellular esterase to polar 2′,7′-dichlorodihydrofluorescein. This non-fluorescent fluorescein analog is trapped in the cells and is oxidized by intracellular oxidants to the highly fluorescent 2′,7′-dichlorofluorescein. The V79-4 cells were seeded in a 96 well plate at 1×10^5 cells/ml. Sixteen hours after plating, the cells were treated with various concentrations of the ethanol extract and 30 min later 1 mM H_2O_2 was added to the plate. The cells were incubated for an additional 30 min at 37 °C. Then, 50 μM of DCF-DA was added and the fluorescence of 2′,7′-dichlorofluorescein was detected at 485 nm excitation and at 535 nm emission, using a PerkinElmer LS-5B spectrofluorometer.

DPPH Radical Scavenging Activity Various concentrations of the ethanol extract were added to a 1×10^{-4} M solution of DPPH in methanol at room temperature, and the reaction mixture was shaken vigorously. After 5 h, the amount of DPPH remaining was determined using a spectrophotometer at 520 nm.[13]

Lipid Peroxidation Inhibitory Activity Lipid peroxidation was assayed by measuring the malondialdehyde (MDA) level.[14] The V79-4 cells were seeded in a culture dish at 1×10^5 cells/ml, and 16 h after plating, the cells were treated with various concentrations of the ethanol extract. One hour later, 1 mM H_2O_2 was added to the plate, which was incubated for a further 1 h. The cells were then washed with cold PBS, scraped and homogenized in ice-cold 1.15% KCl. One hundred microliters of the cell lysates was combined with 0.2 ml of 8.1% SDS, 1.5 ml of 20% acetic acid adjusted to pH 3.5 and 1.5 ml of 0.8% thiobarbituric acid (TBA). The mixture was adjusted with distilled water to a final volume of 4 ml and heated to 95 °C for 2 h. After cooling to room temperature, 5 ml of *n*-butanol and pyridine mixture (15 : 1, v/v) was added to each sample, and the mixture was shaken. After centrifugation at 1000×***g*** for 10 min, the supernatant fraction was isolated, and the absorbance was measured spectrophotometrically at 532 nm.

Cell Viability The effect of the ethanol extract on the viability of the V79-4 cells was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, which was based on the reduction of a tetrazolium salt by mitochondrial dehydrogenase in the viable cells.[15] The V79-4 cells were seeded in a 96 well plate at 1×10^5 cells/ml. Sixteen hours after plating, they were treated with various concentrations of the ethanol extract. One hour later, 1 mM H_2O_2 was added to the plate and incubated at 37 °C for an additional 24 h. Fifty microliters of the MTT stock solution (2 mg/ml) was then added to each well to a total reaction volume of 200 μl. After incubating for 4 h, the plate was centrifuged at 800×***g*** for 5 min and the supernatants were aspirated. The formazan crystals in each well were dissolved in 150 μl dimethylsulfoxide and the A_{540} was read on a scanning multi-well spectrophotometer.

Nuclear Staining with Hoechst 33342 The V79-4 cells were placed in a 24 well plate at 1×10^5 cells/ml. Sixteen hours after plating, the cells were treated with 100 μg/ml of the ethanol extract and after further incubation for 1 h, 1 mM H_2O_2 was added to the culture. After 24 h, 1.5 μl of Hoechst 33342 (stock 10 mg/ml), a DNA specific fluorescent dye, was added to each well (1.5 ml) and incubated for 10 min at 37 °C. The stained cells were then observed under a fluorescent microscope, which was equipped with a CoolSNAP-Pro color digital camera, in order to examine the degree of nuclear condensation.

Flow Cytometry Analysis Flow cytometry was performed to determine the apoptotic sub G_1 hypo-diploid cells.[16] The V79-4 cells were placed in a 6 well plate at 1×10^5 cells/ml. Sixteen hours after plating, the cells were treated with 100 μg/ml of the ethanol extract. After a further incubation of 1 h, 1 mM H_2O_2 was added to the culture. After 24 h, the cells were harvested, and fixed in 1 ml of 70% ethanol for 30 min at 4 °C. The cells were washed twice with PBS, and then incubated for 30 min in the dark at 37 °C in 1 ml of PBS containing 100 μg propidium iodide and 100 μg RNase A. Flow cytometric analysis was performed using a FACSCalibur flow cytometer. The proportion of sub G_1 hypo-diploid cells was assessed by the histograms generated using the computer program, Cell Quest and Mod-Fit.

Superoxide Dismutase (SOD) Activity The V79-4 cells were seeded at 1×10^5 cells/ml. Sixteen hours after plating, the cells were treated with various concentrations of the ethanol extract for 1 h. The harvested cells were suspended in 10 mM phosphate buffer (pH 7.5) and lysed on ice by sonicating them twice for 15 s. One percentage of Triton X-100 was then added to the lysates and the mixture was incubated for 10 min on ice. The lysates were separated by centrifugation at 5000×***g*** for 10 min at 4 °C to remove the cellular debris. The protein content of the supernatant was determined using the Bradford method with bovine serum albumin as the standard.[17] The SOD activity was used to detect the level of epinephrine auto-oxidation inhibition.[18] Fifty micrograms of the protein was added to 500 mM of the phosphate buffer (pH 10.2) and 1 mM epinephrine. Epinephrine rapidly undergoes auto-oxidation at pH 10 to produce adrenochrome, which is a pink colored product that can be measured at 480 nm using a UV/VIS spectrophotometer in kinetic mode. SOD inhibits the auto-oxidation of epinephrine. The rate of inhibition was monitored at 480 nm and one unit of enzyme activity was defined as the amount of enzyme required to produce 50% inhibition. The SOD activity is expressed as units/mg protein.

Catalase (CAT) Activity Fifty micrograms of protein

was added to 50 mM of a phosphate buffer (pH 7) and 100 mM (v/v) H_2O_2; this mixture was then incubated for 2 min at 37 °C and the absorbance was monitored for 5 min at 240 nm. The change in absorbance is proportional to the breakdown of H_2O_2,[19] and one unit of enzyme activity was defined as the amount of enzyme required for the breakdown of 1 μM H_2O_2. The CAT activity is expressed as units/mg protein.

Western Blot The V79-4 cells were placed in a plate at 1×10^5 cells/ml. Sixteen hours after plating, the cells were treated with 50 μg/ml of extract. The cells were harvested at the indicated times, and washed twice with PBS. The harvested cells were then lysed on ice for 30 min in 100 μl of a lysis buffer [120 mM NaCl, 40 mM Tris (pH 8), 0.1% NP 40] and centrifuged at 13000×***g*** for 15 min. Supernatants were collected from the lysates and protein concentrations were determined. Aliquots of the lysates (40 mg of protein) were boiled for 5 min and electrophoresed in 10% SDS-polyacrylamide gel. Blots in the gels were transferred onto nitrocellulose membranes (Bio-Rad, Hercules, U.S.A.), which were then incubated with primary sheep monoclonal superoxide dismutase, and -catalase antibodies. The membranes were further incubated with rabbit anti-sheep immunoglobulin-G-horseradish peroxidase conjugates (Pierce, Rockland, U.S.A.), and then exposed to X-ray film. Protein bands were detected using an enhanced chemiluminescence Western blotting detection kit (Amersham, Little Chalfont, U.S.A.).

Statistical Analysis All the measurements were made in triplicate. The results were subjected to an analysis of the variance (ANOVA) using the Tukey test to analyze the difference. p values <0.05 were considered significant.

RESULTS

Radical Scavenging Activity of the Ethanol Extract The antioxidant activities of the ethanol extract from *Puerariae* radix were evaluated by the intracellular ROS and DPPH free radical scavenging activities. The intracellular ROS scavenging activity of the ethanol extract was 22% at 1 μg/ml, 35% at 10 μg/ml, 54% at 50 μg/ml, and 63% at 100 μg/ml, respectively (Fig. 1A). The 2 mM of *N*-acetylcystein used as a positive control showed 86% ROS inhibition (data not shown). This ROS scavenging activity of the ethanol extract is consistent with its DPPH radical scavenging activity (Fig. 1B), showing 5% at 1 μg/ml, 6% at 10 μg/ml, 18% at 50 μg/ml, and 31% at 100 μg/ml compared with 92% of *N*-acetylcystein at 2 mM. However, the DPPH radical scavenging activity of the ethanol extract was low compared with its intracellular ROS scavenging activity. Tectoridin, an isoflavone compound in *Puerariae* species, showed intracellular ROS scavenging activity and DPPH radical scavenging activity. The intracellular ROS scavenging activity of tectoridin was 34% at 0.1 μg/ml, 45% at 1 μg/ml, and 56% at 10 μg/ml, respectively (Fig. 1C). DPPH radical scavenging activity of tectoridin showed 16% at 0.1 μg/ml, 19% at 1 μg/ml, and 29% at 10 μg/ml, respectively (Fig. 1D). Overall, these results suggest that the ethanol extract from *Puerariae* radix has an antioxidant effect.

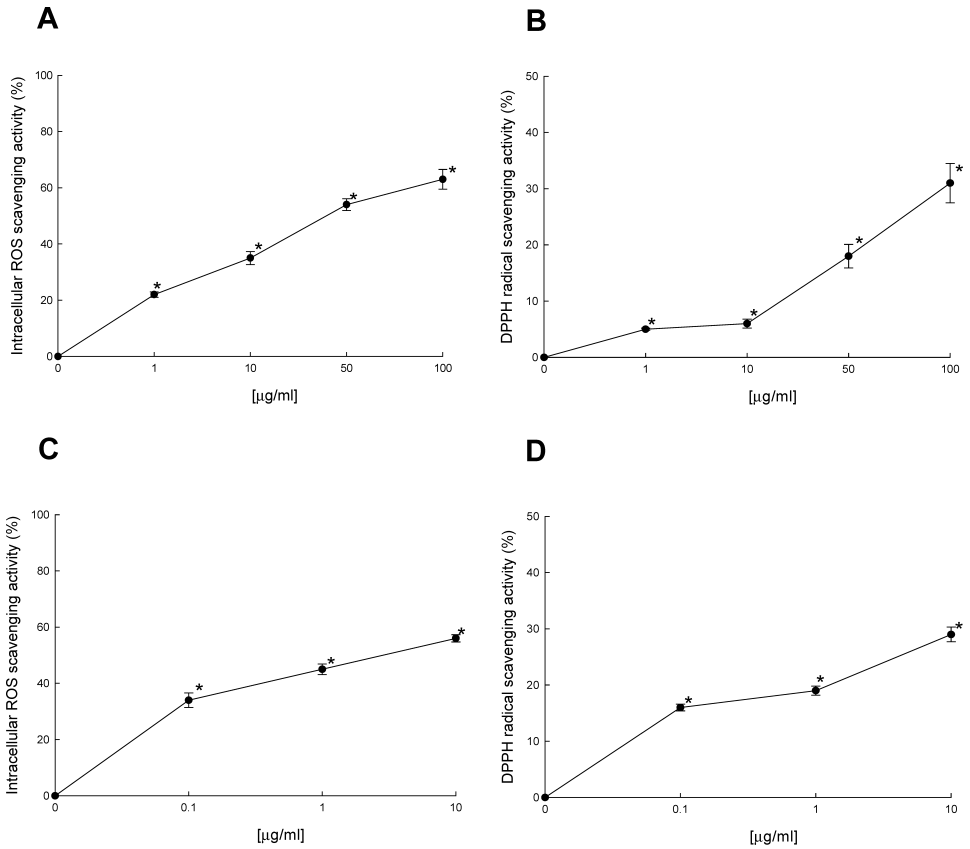

Fig. 1. Effect of the Ethanol Extract Isolated from *Puerariae* Radix and Tectoridin on Scavenging the Intracellular ROS and DPPH Radicals

The intracellular ROS scavenging effect by the ethanol extract isolated from *Puerariae* radix (A) and tectoridin (C) was detected using the DCF-DA method. The DPPH radicals scavenging effect by the ethanol extract isolated from *Puerariae* radix (B) and tectoridin (D) was determined using a spectrophotometer at 520 nm. Control refers to the cells untreated with the ethanol extract or tectoridin. Vertical error bars=S.E.; * Significantly different from control ($p<0.05$).

Effect of the Ethanol Extract on Lipid Peroxidation The ability of the ethanol extract to inhibit lipid peroxidation in H_2O_2-treated V79-4 cells was also tested. The generation of malondialdehyde (MDA) as well as its related substances that react with thiobarbituric acid (TBA) was inhibited by this extract in a dose dependent pattern. The extract showed 1.22 μM of MDA at 0 μg/ml, 1.05 μM at 1 μg/ml, 0.99 μM at 10 μg/ml, 0.90 μM at 50 μg/ml, and 0.75 μM at 100 μg/ml compared with 1.36 μM in H_2O_2 only treated group at 1 mM, and 0.99 μM in the untreated control group (Fig. 2).

Protective Effect of the Extract on Cell Survival The protective effect of the extract on cell survival in the H_2O_2-treated V79-4 cells was also measured. The cells were treated with the ethanol extract at various concentrations for 1 h prior to addition to H_2O_2. The cell survival was determined 24 h later using a MTT assay. As shown in Fig. 3, treatment with this extract induced a dose dependent increase in the cell survival: 4% at 1 μg/ml, 20% at 10 μg/ml, 25% at 50 μg/ml, and 35% at 100 μg/ml.

Reduction of H_2O_2-Induced Apoptosis by the Extract Treatment In order to analyze the protective effect of the ethanol extract on H_2O_2-induced apoptosis, the nuclei of the V79-4 cells were stained with Hoechst 33342 and propidium iodide dye for microscopic and flow cytometric detection, respectively. As shown in Fig. 4A, the control cells revealed intact nuclei, while the H_2O_2-treated cells showed significant nuclear fragmentation under a microscope, which is characteristic of apoptosis. However, a dramatic decrease in nuclear fragmentation was observed when the cells were treated with the ethanol extract for 1 h prior to the H_2O_2-treatment. In addition to the morphological evaluation, the protective effect of the extract was confirmed by flow cytometry. As shown in

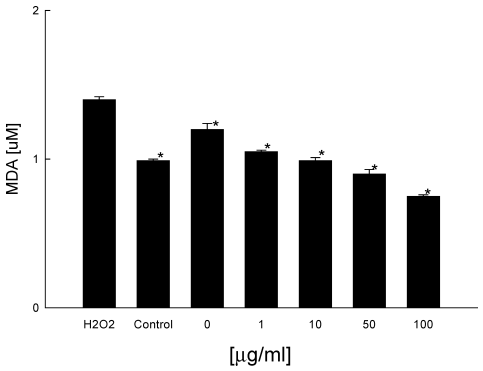

Fig. 2. Effect of the Ethanol Extract on Inhibition of Lipid Peroxidation

Lipid peroxidation was assayed by measuring the malondialdehyde formation. Vertical error bars=S.E.; * Significantly different from H_2O_2 group ($p<0.05$).

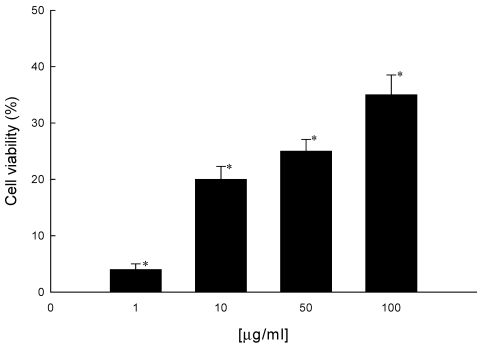

Fig. 3. Protective Effect of the Ethanol Extract on the H_2O_2 Induced Oxidative Damage of V79-4 Cells

The viability of V79-4 cells was determined by the MTT assay. Vertical error bars=S.E.; * Significantly different from the control ($p<0.05$).

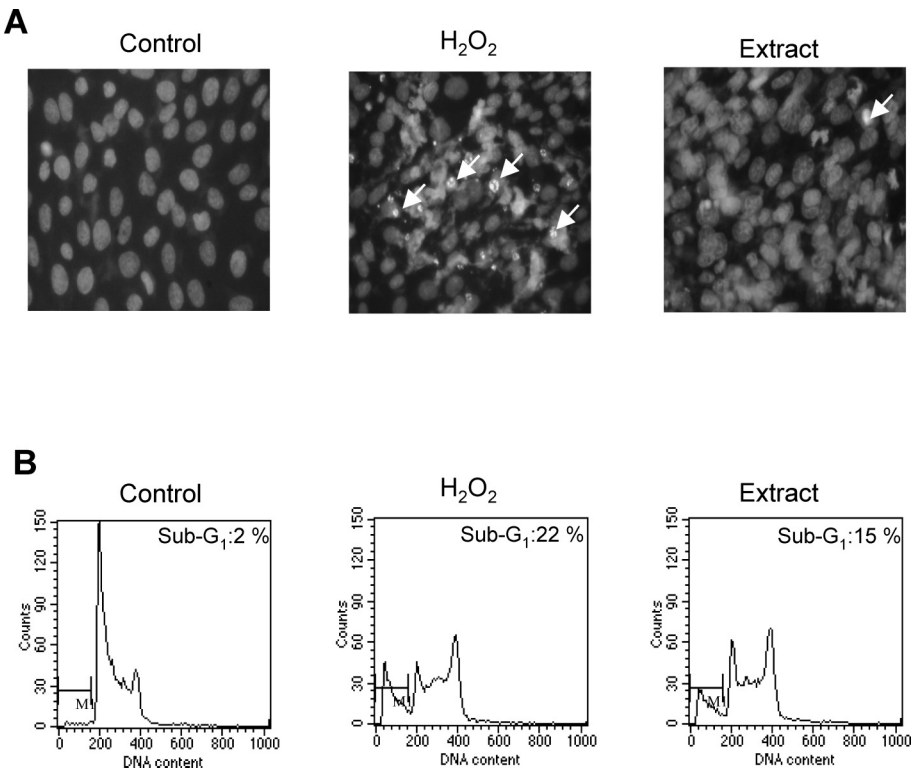

Fig. 4. Apoptotic Body Formation (A) and Apoptotic Sub-G_1 DNA Content (B) of the V79-4 Cells Treated with H_2O_2 and the Ethanol Extract

Apoptotic body formation was observed under a fluorescent microscope after Hoechst 33342 staining and apoptotic sub-G_1 DNA content was detected by flow cytometry after propidium iodide staining. The apoptotic bodies are indicated by arrows.

Fig. 4B, an analysis of the DNA content in the H_2O_2 only treated cells revealed an increase (22%) in the proportion of cells with an apoptotic sub-G_1 DNA content. Treatment with 100 μg/ml of the extract decreased the apoptotic sub-G_1 DNA content to 15%. *N*-acetylcystein at 2 mM, which was used as the positive control, decreased the apoptotic sub-G_1 DNA content to 4%. These results suggest that this extract protects the cell viability by preventing H_2O_2-induced apoptosis.

Effect of the Extract on Superoxide Dismutase (SOD) and Catalase (CAT) The SOD and CAT activities in the ethanol extract treated V79-4 cells were measured to investigate whether or not the antioxidant activity of this extract is mediated by the increased activities of the antioxidant enzymes. The ethanol extract increased the activities of these two enzymes (Figs. 5A, B): for the SOD activity, 16 U/mg protein at 1 μg/ml, 21 U/mg protein at 10 μg/ml, 39 U/mg protein at 50 μg/ml, and 47 U/mg protein at 100 μg/ml, compared with the 10 U/mg protein of the control; for the CAT activity, 29 U/mg protein at 1 μg/ml, 31 U/mg protein at 10 μg/ml, 39 U/mg protein at 50 μg/ml, and 45 U/mg protein at 100 μg/ml, compared with the 12 U/mg protein of the control. These enzyme activities in the extract treated cells were increased in a time dependent pattern (Figs. 5C, D). However, the protein expressions of the two enzymes by the extract were not induced in a time dependent pattern (Fig. 5E).

Effects of the Extract on Blood Glucose Level and Oxidative Stress in Streptozotocin Induced Diabetic Rats It was reported that oxidative stress plays an important role in the experimental diabetes induced by streptozotocin.[18] This study also investigated the effect of the ethanol extract on hyperglycemia and hepatic oxidative stress in the streptozotocin induced diabetic rats. The blood glucose level was significantly higher in the rats from the first day of the streptozotocin injection, and reached 3—4 fold that of the control group. The blood glucose level in the extract (500 mg/kg) treated diabetic rats was lower than that in the diabetic group, as shown in Fig. 6A. Streptozotocin increased the level of he-

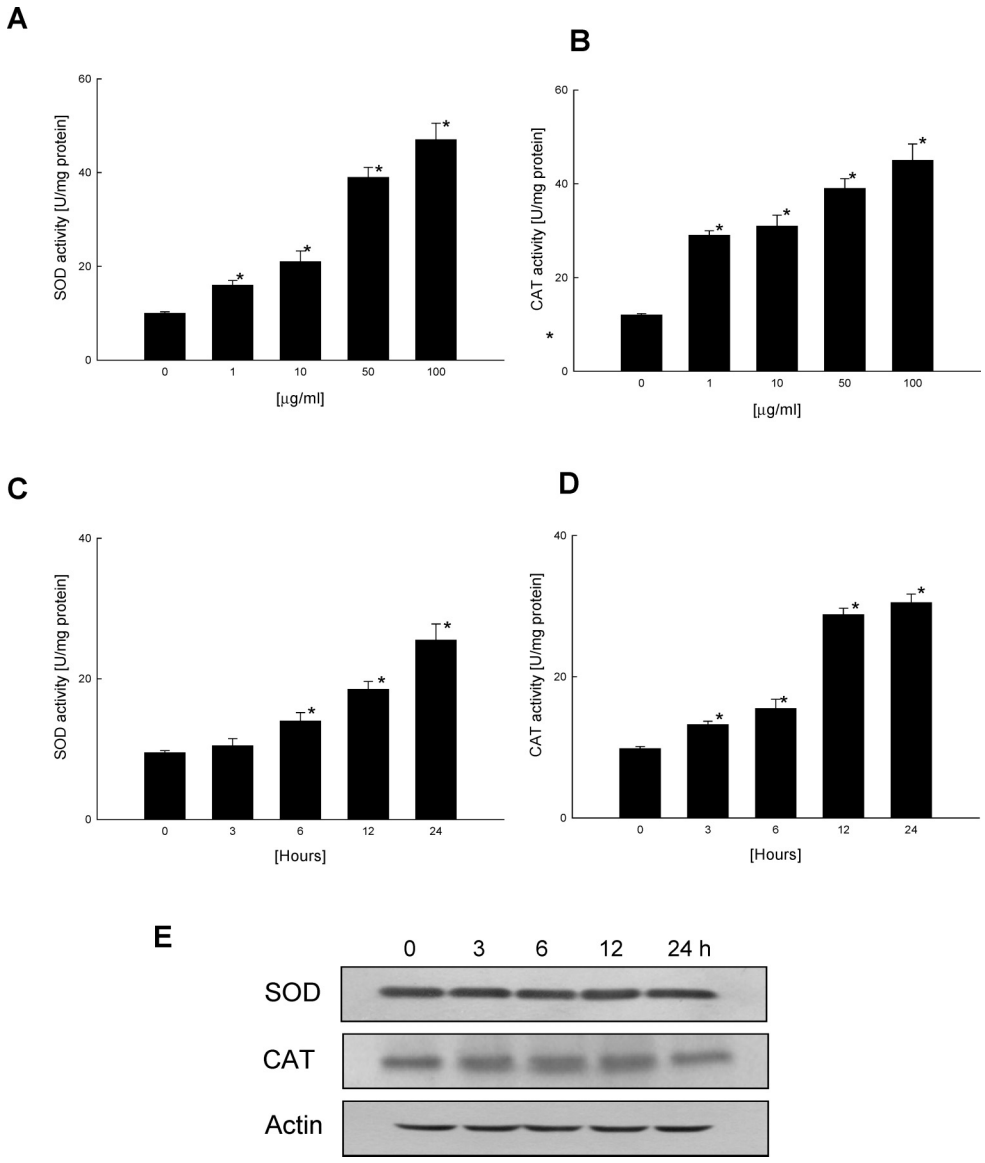

Fig. 5. Effects of the Ethanol Extract on Antioxidant Enzyme Activity and Protein Expression in the V79-4 Cells

SOD and CAT by extract in the V79-4 cells showed the activity at dose (A, B) and at time (C, D), respectively, and their protein expressions at an indicated time (E). The data represent three experiments and are expressed as the average enzyme unit per mg protein±S.E.; Control refers to the cells untreated with ethanol extract. * Significantly different from control ($p<0.05$).

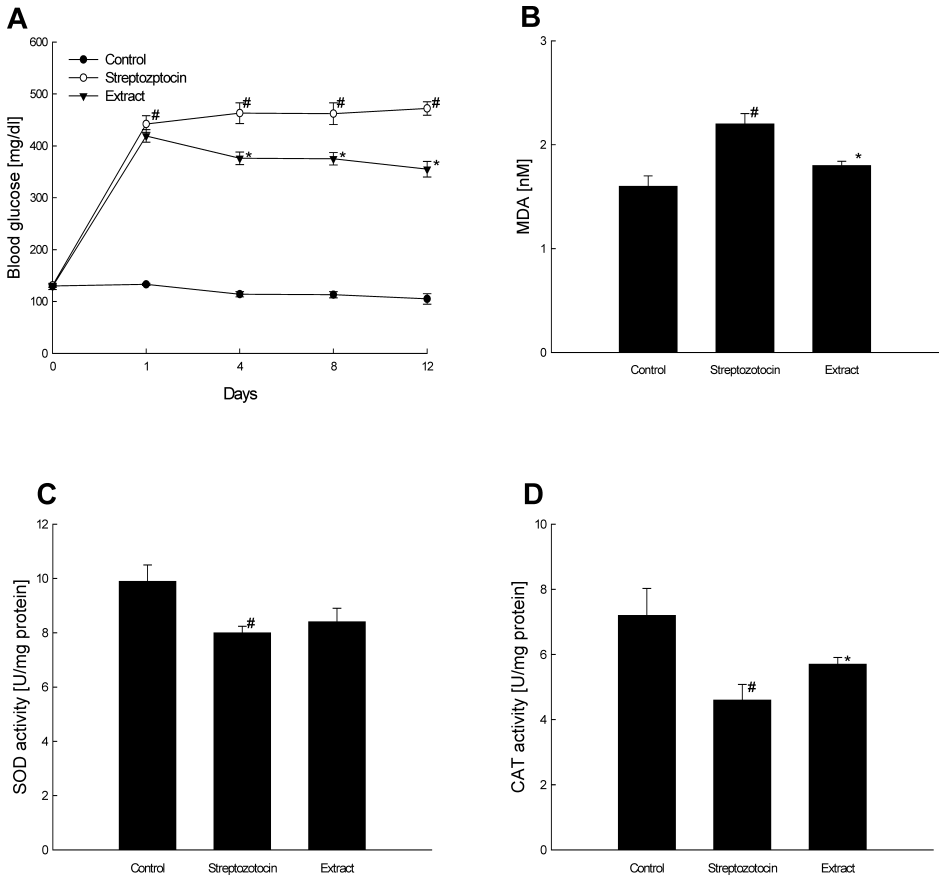

Fig. 6. Effects of the Ethanol Extract on the Blood Glucose Level and Oxidative Stress in the Streptozotocin Induced Diabetic Rats

The ethanol extract was administered orally for 12 d after the streptozotocin injection, as described in Materials and Methods. Twelve days after the streptozotocin injection, the blood glucose level (A), hepatic MDA produced by lipid peroxidation (B), the hepatic SOD and CAT activities were detected (C and D). *,# Significantly different from streptozotocin and control, respectively ($p<0.05$).

patic lipid peroxidation in diabetic rats. However, the ethanol extract suppressed the streptozotocin induced lipid peroxidation in the rats as shown in Fig. 6B. This study also examined the alteration in the activities of SOD and CAT because these enzymes play a major role in protecting the cells and tissue from oxidative stress. Indeed, streptozotocin administration decreased the activities of hepatic SOD and CAT. Treatment with the ethanol extract prevented the CAT alterations, but not the SOD activity, as shown in Figs. 6C and D.

DISCUSSION

The ethanol extract from *Puerariae* radix increased the intracellular ROS and DPPH radical scavenging activities and was manifested by the enhanced viability of V79-4 cells exposed to H_2O_2. The protective effect of this extract on H_2O_2-induced apoptosis was observed under a microscope and with a flow cytometer. The cells exposed to H_2O_2 exhibited the distinct morphological features of apoptosis, such as nuclear fragmentation and an increase in the sub G_1-hypodiploid cells. However, the cells pretreated with the ethanol extract had a significantly lower number of apoptotic cells, as shown by the morphology and sub G_1 DNA content. The ethanol extract of *Puerariae* radix increased the SOD and CAT activity, which indicates that this extract can effectively scavenge O_2^- and H_2O_2. The effects of the ethanol extract on the cell viability might involve dual actions: the direct action of oxygen radical scavenging, as shown by the DPPH radical scavenging by the ethanol extract (Fig. 1B), and the indirect action *via* the induction of the anti-oxidative enzyme of SOD and CAT by the ethanol extract (Figs. 5A, B). The structural damage of the tissues or complications in diabetes mellitus might be due to oxidative stress; such stress may also play an important role in the development of streptozotocin-induced diabetes.[20] Some antioxidants have been suggested to have beneficial effects in the treatment of oxidative stress-disease.[21—23] In this respect, the possible anti-diabetic effect of *Puerariae* radix was examined. As shown in these experiments, the orally administered ethanol extract of *Puerariae* radix prevented the increase in the blood-glucose levels in the established diabetic state. The protective effect of the ethanol extract on streptozotocin cytotoxicity was examined in the liver of rats exposed to streptozotocin. The liver is an organ of central metabolic importance and is known to undergo oxygen free radicals mediated injury in diabetes mellitus.[24] The increased MDA in the progression of diabetes may have a role in the tissue damage associated with diabetes. The level of lipid peroxidation was significantly higher in the liver of the streptozotocin treated rats, and treatment with the ethanol extract apparently attenuated the MDA level. This result shows that the ethanol extract of *Puerariae* radix might protect the tissue components against the cytotoxic action of streptozotocin. The increased production of oxygen free radicals in diabetes is suggested by glycation as

well as by decreased availability of the enzymatic and non-enzymatic antioxidants.[25] It has been reported that diabetes induces alterations in the activities of the antioxidant enzymes in various tissues.[4] This finding was also demonstrated in the present animal study using streptozotocin. The streptozotocin treatment showed low activities of superoxide dismutase and catalase in the liver, and the ethanol extract increased the CAT activity but not SOD. However, the ethanol extract increased the SOD and CAT activities in the cells exposed to H_2O_2. This might reflect an idiosyncrasy of the *in vitro* system used in this study. Treatment of the ethanol extract *in vivo* system lowered the blood glucose level, but its effect was slight. One cause we think possible is use of a crude ethanol extract. In further study, we will try to find active compounds from *Puerariae* species, which are effective in the amelioration of diabetes mellitus. In conclusion, the ethanol extract of *Puerariae* radix may attenuate the development of streptozotocin induced diabetes in rats as a consequence of its antioxidant effect.

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