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Antioxidant properties of 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose from *Elaeocarpus sylvestris* var. *ellipticus*

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ABSTRACT

The antioxidant potential of 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (PGG), isolated from *Elaeocarpus sylvestris* var. *ellipticus*, was investigated by various established systems based on cell-free and cell system experiments, such as radical detection, antioxidant enzyme assay, lipid peroxidation detection, and cell viability assay. PGG was found to quench the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and intracellular reactive oxygen species. PGG recovered the cellular antioxidant enzyme activities of superoxide dismutase, catalase, and glutathione peroxidase, which were reduced by H_2O_2 treatment, thereby resulting in the inhibition of lipid peroxidation. Cytoprotective effects of PGG were based on the results of DNA fragmentation, mitochondrial membrane potential ($\Delta\psi$), apoptotic body formation, and caspase-3 activity. The results suggest that PGG protects cells against H_2O_2-induced cell damage via antioxidant properties.

1. Introduction

Considerable interest has been focused on the identification of antioxidant compounds that are pharmacologically potent with low or no side effects for use in preventive medicine and the food industry. As plants are the source of antioxidants, which prevent the oxidative stress caused by the reduction process of oxygen, they represent a potential source of new compounds with antioxidant activities. Antioxidants help organisms deal with oxidative stress, caused by free radical-induced damage. Free radicals are chemical species which contain one or more unpaired electrons, which are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. Oxidative stress results from the exposure to reactive oxygen species (ROS), such as the superoxide anion and hydroxyl radical. It is known that high levels of ROS cause damage to cells, and are involved in human diseases, such as cancer, neurological degeneration, and arthritis, as well as accelerating the aging process. Therefore, the application of compounds with antioxidant properties may help prevent or alleviate many ROS-related diseases (Berg, Youdim, & Riederer, 2004).

Tannins are secondary metabolites widely distributed in the plant kingdom, and are commonly divided into condensed tannins and hydrolysable tannins (Cai, Luo, Sun, & Corke, 2004; Chung, Wong, Wei, Huang, & Lin, 1998). Some medicinal plants contain complex mixtures of both hydrolysable and condensed tannins. Condensed tannins are mainly the oligomers and polymers (e.g. monomer, dimer, and trimer) of flavan-3-ols (catechin derivatives), also known as proanthocyanidins. Several flavan-3-ols actually belong to the monomers of condensed tannins. The polymerised products of flavan-3,4-diols are considered to be another category of condensed tannins, also called leucoanthocyanidins. Hydrolysable tannins, including gallotannins and ellagitannins, possess a central core of polyhydric alcohol, including glucose, and hydroxyl groups, which are esterified either partially or wholly by gallic acid (gallotannins) or by hexahydroxydiphenic acid and other substituents, such as chebulic acid (ellagitannins) (Chung et al., 1998; Khanbabaee & Van Ree, 2001). When the glucose core is esterified with five or fewer galloyl groups, the resulting compounds are defined as gallotannin precursors. One well-known gallotannin precursors is 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (PGG), where five galloyl groups are

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attached to a glucose unit by ester bonds (Cammann, Denzel, Schilling, & Gross, 1989). PGG has phenol-glycoside linkages in its structure and the glycosylation process is a prominent modification reaction, which is often the final step in the biosynthesis of natural products, including phenolics, terpenoids, cyanohydrins, and alkaloids (Vogt & Jones, 2000). Glycosylation enables the storage of chemicals in high concentrations, various terpenoids and phenolics are stabilised by glycoconjugation (Crouzet & Chassagne, 1999). Thus, PGG with phenol-glycoside linkage can have chemical stability. Various plants species, including fruits, vegetables, and medicinal herbs, may contain a wide variety of free radical scavenging compounds, such as polyphenols. *Elaeocarpus sylvestris* var. *ellipticus* is a tropical and subtropical evergreen tree and shrub. It is distributed in the subtropical zone of Cheju in South Korea, in Southern China, and in Okinawa and Kyushu, Japan. PGG is a major chemical constituent of *E. sylvestris* var. *ellipticus*. PGG has been reported to possess several biological effects, such as anti-tumour, vasodilatory, anti-inflammatory, neuroprotective, hepatoprotective, and anti-angiogenic activities (Choi et al., 2002; Kang et al., 2005; Lee et al., 2004; Oh et al., 2001; Pae et al., 2006). In addition, from the same genus *Elaeocarpus*, ellagic acid derivatives, flavonoids, 4′-methylmyricetin, myricetin, myricetin rhamnoside, 4′-*O*-methylellagic acid 3-(2″,3″-di-*O*-acetyl)-α-L-rhamnoside and 4,4′-*O*-dimethylellagic acid 3-(2″,3″-di-*O*-acetyl) α-L-rhamnoside were isolated (Ito et al., 2002; Ray, Dutta, & Dasgupta, 1976). Among the chemical constituents, ellagic acid is known as a dimeric derivative of gallic acid and exists in the free form or bound as ellagitannins and gallotannins. These hydrolysable tannins, such as ellagic acid and gallic acid derivatives, have been approved for use as a food additive and antioxidant in some countries (Soong & Barlow, 2006). Thus PGG, a gallic acid derivative, should be evaluated for its antioxidant properties. The tannins are considered potential antioxidants, as their eventual oxidation, may lead to oligomerisation, *via* phenolic coupling and enlargement of the number of reactive sites (Bors & Michel, 2002).

Herbal medicine has been used in Asia to improve the well-being of people for thousands of years, and is gaining interest in healthcare (Shi, Tong, Shen, & Li, 2008). As a herbal medicine, *E. sylvestris var. ellipticus* has been known to possess various biological activities, and gallic acid derivatives, such as PGG, has been used as food additives. To the best of our knowledge, there are few or no reports on the adverse effects of *E. sylvestris*.

As part of our ongoing research efforts to discover potential antioxidants through antioxidant screening, we examined PGG from *E. sylvestris* var. *ellipticus* to evaluate its antioxidant properties.

2. Materials and methods

2.1. Reagents

PGG (Fig. 1) was obtained from Professor Nam Ho Lee of Cheju National University, Republic of Korea. PGG was freshly dissolved in dimethyl sulfoxide (DMSO), yielding a final concentration, which did not exceed 0.1%. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, 2′,7′-dichlorodihydrofluorescein diacetate (DCF-DA), and Hoechst 33342 were purchased from Sigma Chemical Company (St. Louis, MO), and the thiobarbituric acid (TBA) was purchased from BDH Laboratories (Dorset, England). Anti-poly (ADP-ribosel) polymerase (Anti-PARP), and anti-caspase-3 antibodies were purchased from Cell Signaling Technology (Danvers, MA). 5,5′,6,6′-Tetrachloro-1, 1′,3,3′-tetraethylbenzimidazolcarbocyanine iodide (JC-1) was purchased from Molecular Probe (Leiden, The Netherlands). All other chemicals and reagents used were of analytical grade.

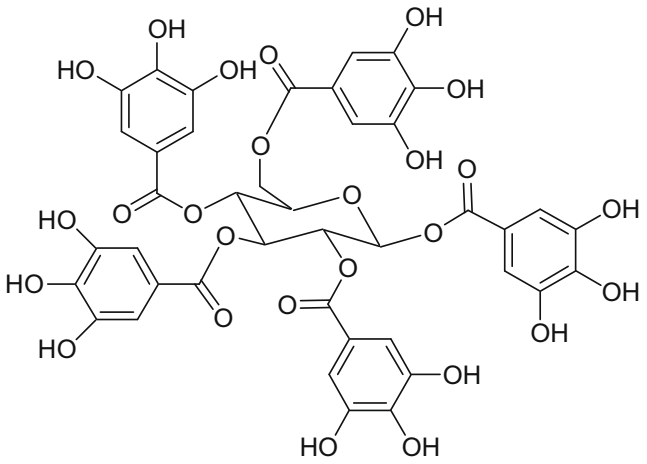

Fig. 1. Chemical structure of 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (PGG).

2.2. Cell culture

Previous reports have shown that the lung is an organ which is sensitive to oxidative stress (Murray et al., 2004; Pryor, Stone, Zang, & Bermudez, 1998). To study the effect of PGG on oxidative stress, we used Chinese hamster lung fibroblasts (V79-4 cells). The V79-4 cells were obtained from the American Type Culture Collection and maintained at 37 °C in an incubator at a humidified atmosphere of 5% CO_2 and cultured in Dulbecco's modified Eagle's medium containing 10% heat-inactivated foetal calf serum, streptomycin (100 μg/ml) and penicillin (100 units/ml).

2.3. DPPH radical scavenging activity

PGG at concentrations of 1, 10, and 50 μg/ml were added to a 1×10^{-4} M solution of DPPH in methanol, and the reaction mixture was shaken vigorously. After 30 min, the amount of DPPH remaining was determined at 520 nm (Soong & Barlow, 2006). The DPPH radical-scavenging activity (%) was calculated as

100 × [(optical density of DPPH radical treatment) − (optical density of PGG with DPPH radical treatment)]/(optical density of DPPH radical treatment).

2.4. Intracellular ROS measurement

To detect intracellular ROS, the DCF-DA method was used. DCF-DA diffuses into cells, where it is hydrolysed by intracellular esterase to polar 2′,7′-dichlorodihydrofluorescein. This non-fluorescent fluorescein analogue is trapped in cells and can be oxidised to the highly fluorescent 2′,7′-dichlorofluorescein by intracellular oxidants (Rosenkranz et al., 1992). The V79-4 cells were seeded in a 96-well plate at 2×10^4 cells/well. Sixteen hours after plating, the cells were treated with PGG at concentrations of 1, 10, and 50 μg/ml. After 30 min, 1 mM H_2O_2 was added to the plate. The cells were incubated for an additional 30 min at 37 °C. After the addition of 25 μM DCF-DA solution for 10 min, the fluorescence of 2′,7′-dichlorofluorescein was detected using a Perkin–Elmer LS-5B spectrofluorometer. The intracellular ROS scavenging activity (%) was calculated as

100 × [(optical density of H_2O_2 treatment)−(optical density of PGG with H_2O_2 treatment)]/(optical density of H_2O_2 treatment).

The image analysis for the generation of intracellular ROS was achieved by seeding the cells on a cover-slip loaded six well plate at 2×10^5 cells/well. Sixteen hours after plating, the cells were treated with PGG at 10 μg/ml. After 30 min, 1 mM of H_2O_2 was added to the plate. After changing the media, 100 μM DCF-DA

was added to each well and was incubated for an additional 30 min at 37 °C. After washing with PBS, the stained cells were mounted onto a microscope slide in mounting medium (DAKO, Carpinteria, CA). The microscopic images were collected using the Laser Scanning Microscope 5 PASCAL program (Carl Zeiss, Jena, Germany) on a confocal microscope.

2.5. Superoxide dismutase (SOD) activity

The V79-4 cells were seeded in a culture dish at a concentration of 1×10^5 cells/ml, and 16 h after plating, were treated with PGG at 10 μg/ml. After 1 h, 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, and scraped. The harvested cells were suspended in 10 mM phosphate buffer (pH 7.5) and then lysed on ice by sonicating twice for 15 s. Triton X-100 (1%) was then added to the lysates and incubated for 10 min on ice. The lysates were clarified, by centrifugation at 5000*g* for 10 min at 4 °C, to remove cellular debris. The protein content of the supernatant was determined by the Bradford method, using bovine serum albumin as the standard. The SOD activity was used to detect the level of epinephrine auto-oxidation inhibition (Misra & Fridovich, 1972). Fifty micrograms of protein were added to 500 mM phosphate buffer (pH 10.2) and 1 mM epinephrine. Epinephrine rapidly undergoes auto-oxidation at pH 10 to produce adrenochrome, a pink-coloured product, which was assayed at 480 nm using a UV/Vis spectrophotometer in the kinetic mode. SOD inhibits the auto-oxidation of epinephrine. The rate of inhibition was monitored at 480 nm and the amount of enzyme required to produce 50% inhibition was defined as one unit of enzyme activity. The SOD activity was expressed as units/mg protein.

2.6. Catalase (CAT) activity

Fifty micrograms of protein were added to 50 mM phosphate buffer (pH 7.0) and 100 mM H_2O_2 and then this mixture was incubated for 2 min at 37 °C and the absorbance of the mixture were monitored for 5 min at 240 nm. The change in absorbance is proportional to the breakdown of H_2O_2 (Carrillo, Kanai, Nokubo, & Kitani, 1991). The CAT activity was expressed as units/mg protein.

2.7. Glutathione peroxide(GPx) activity

Fifty micrograms of the protein were added to 25 mM phosphate buffer (pH 7.5) containing 1 mM EDTA, 1 mM NaN_3, 1 mM glutathione (GSH), 0.25 units of glutathione reductase, and 0.1 mM NADPH. After incubation for 10 min at 37 °C, H_2O_2 was added to the reaction mixture at a final concentration of 1 mM. The absorbance was monitored at 340 nm for 5 min. The GPx activity was measured as the rate of NADPH oxidation by change in absorbance at 340 nm (Paglia & Valentine, 1967). The GPx activity was expressed as units/mg protein and one unit of enzyme activity was defined as the amount of enzyme required to oxidise 1 mmol NADPH.

2.8. Lipid peroxidation detection

Lipid peroxidation was assayed by the measurement of related substances that react with thiobarbituric acid (TBARS) (Ohkawa, Ohishi, & Yagi, 1979). The V79-4 cells were seeded in a culture dish at a concentration of 1×10^5 cells/ml, and 16 h after plating, were treated with PGG at 10 μg/ml. After 1 h, 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 homogenised in ice-cold 1.15% KCl. About 100 μl of cell lysates were 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 to a final volume of 4 ml with distilled water and heated to 95 °C for 2 h. After cooling to room temperature, 5 ml of a mixture of *n*-butanol and pyridine (15:1, v/v) were added to each sample, and the mixture was shaken vigorously. After centrifugation at 1000*g* for 10 min, the supernatant fraction was isolated, and the absorbance measured at 532 nm.

2.9. Cell viability

The effect of PGG on the viability of the V79-4 cells was determined using the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium] bromide (MTT) assay (Carmichael, Degraff, Gazdar, Minna, & Mitchell, 1987). Fifty microlitres of the MTT stock solution (2 mg/ml) was added into each well, to attain 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 of dimethyl sulfoxide and read at A_{540} on a scanning multi-well spectrophotometer.

2.10. Nuclear staining with Hoechst 33342

The V79-4 cells were placed in a 24-well plate at a concentration of 1×10^5 cells/ml, and 16 h after plating, were treated with PGG at 10 μg/ml 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, equipped with a CoolSNAP-Pro colour digital camera, to examine the degree of nuclear condensation.

2.11. DNA fragmentation

Cellular DNA fragmentation was assessed by analysis of the cytoplasmic histone-associated DNA fragmentation using a kit from Roche Diagnostics, according to the manufacturer's instructions.

2.12. Measurement of mitochondrial membrane potential ($\Delta\psi$)

Mitochondrial membrane potential ($\Delta\psi$) was determined by the mitochondria specific dye, JC-1 (Cossarizza, Baccarani-Contri, Kalashnikova, & Franceschi, 1993). The cells were harvested, washed and suspended in PBS containing JC-1 (10 μg/ml). After 15 min of incubation at 37 °C, the cells were washed, suspended in PBS and analysed by flow cytometry.

2.13. 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 10 μg/ml of PGG. 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 lysis buffer [120 mM NaCl, 40 mM Tris (pH 8), 0.1% NP 40] and centrifuged at 13,000*g* for 15 min. Supernatants were collected from the lysates and protein concentrations were determined. Aliquots of the lysates (40 μg 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, CA), which were then incubated with primary antibody. The membranes were further incubated with secondary immunoglobulin G-horseradish peroxidase conjugates (Pierce Biotechnology, Rockford, IL), and then exposed to X-ray film. Protein bands were detected using an enhanced chemiluminescence Western blotting detection kit (Amersham, Little Chalfont, UK).

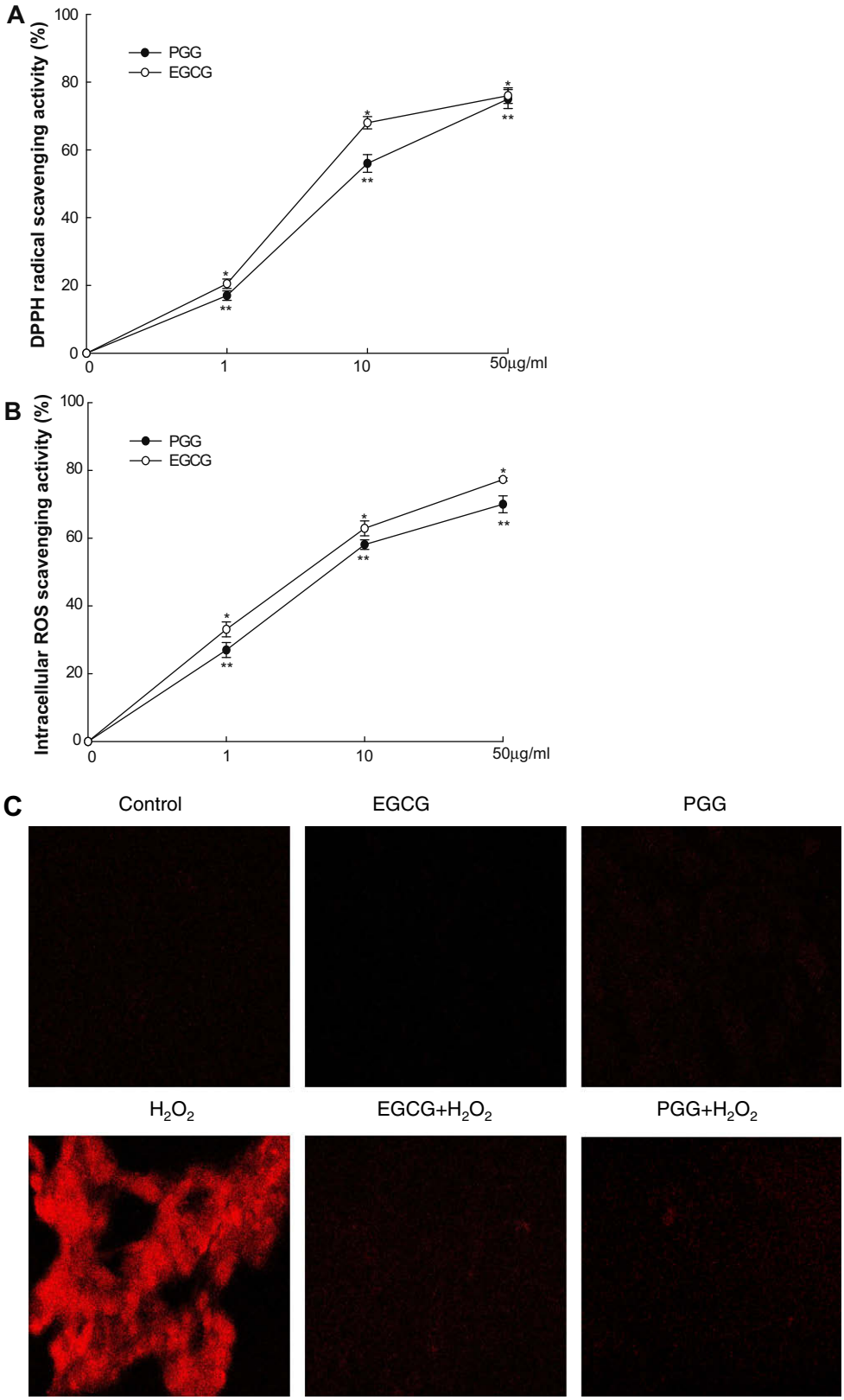

Fig. 2. Effect of PGG on the scavenging of: (A) DPPH radical, and (B) intracellular ROS. Measurements were made in triplicate and values are expressed as means ± standard error. *Significantly different from control ($p < 0.05$). (C) The representative confocal images illustrate the increase in red fluorescence intensity of DCF produced by ROS in H_2O_2-treated cells compared to the control and the lowered fluorescence intensity in H_2O_2-treated cells with PGG or EGCG (original magnification 400×).

2.14. Statistical analysis

All the measurements were made in triplicate. The results were subjected to an analysis of variance using the Tukey test to analyse the differences. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Radical scavenging activity of PGG

The radical-scavenging effects of PGG on the DPPH radical and intracellular ROS were measured. The DPPH radical-scavenging activity was 17% at 1 μg/ml, 56% at 10 μg/ml, and 75% at 50 μg/ml. The DPPH radical-scavenging activity of epigallocatechin gallate (EGCG), a major green-tea polyphenol compound used as a positive control, showed 21% at 1 μg/ml, 68% at 10 μg/ml, and 76% at 50 μg/ml (Fig. 2A). The intracellular ROS-scavenging activity of PGG was 27% at 1 μg/ml, 58% at 10 μg/ml, and 70% at 50 μg/ml; The intracellular ROS scavenging activity of EGCG was 33% at 1 μg/ml, 63% at 10 μg/ml, and 77% at 50 μg/ml (Fig. 2B). The 50 μg/ml sample of PGG did not significantly increase inhibition very much, when compared with 10 μg/ml of PGG. Hence, we concluded that 10 μg/ml was as the optimal dose for further study.

In addition, the fluorescence intensity of DCF-DA staining was measured using a confocal microscope. This analysis revealed that both PGG and EGCG as a positive control at 10 μg/ml reduced the red fluorescence intensity with H_2O_2 treatment as shown in Fig. 2C, thus reflecting a reduction in ROS generation. These results indicate a reduction of ROS by PGG treatment, and suggest that PGG possesses antioxidant properties.

3.2. Effect of PGG on SOD, CAT, and GPx

To investigate whether the radical-scavenging activity of PGG was mediated by the activity of an antioxidant enzyme, the activities of SOD, CAT, and GPx in PGG-treated V79-4 cells were measured. PGG was found to increase the activities of all three enzymes. SOD activity was 24.2 U/mg protein at 10 μg/ml of PGG, compared to 18.1 U/mg protein of the control (Table 1). The exposure of cells to H_2O_2 decreased the SOD activity to 6.1 U/mg protein, however, treatment with PGG resulted in an increase to 10.6 U/mg protein. In the case of CAT activity, PGG revealed a similar pattern to the SOD activity. The activity of CAT was increased with PGG treatment, with 54.4 U/mg protein at 10 μg/ml of PGG, compared to 35.1 U/mg protein for the control (Table 1). H_2O_2-treated V79-4 cells reduced CAT activity to 17.4 U/mg protein; however, treatment with PGG resulted in an increase to 28.7 U/mg protein. Finally, GPx activity was 15.5 U/mg protein at 10 μg/ml of PGG as compared to 11.2 U/mg protein for the control (Table 1). The H_2O_2-treated V79-4 cells decreased the GPx activity to 2.5 U/mg protein; however combination of PGG and H_2O_2 restored GPx activity to 5.3 U/mg, thus suggesting that PGG increased the activities of antioxidant enzymes such as SOD, CAT, and GPx.

Table 1
The effects of PGG on SOD, CAT and GPx activity in V79-4 cells.

	Control	PGG	H_2O_2	PGG + H_2O_2
SOD	18.1 ± 0.9	24.2 ± 0.7	6.1 ± 0.2	10.6 ± 0.5*
CAT	35.1 ± 2.3	54.4 ± 1.7	17.4 ± 1.2	28.7 ± 2.1*
GPx	11.2 ± 0.5	15.5 ± 0.4	2.5 ± 0.3	5.3 ± 0.4*

The data represent three experiments and are expressed as average enzyme units per mg protein.
* Significantly different from H_2O_2 treatment ($p < 0.05$).

3.3. Effect of PGG on lipid peroxidation induced by H_2O_2

The ability of PGG to inhibit membrane lipid peroxidation in H_2O_2-treated cells was investigated. H_2O_2-induced damage to cell membranes, one of the most important lesions, is responsible for the loss of cell viability. As shown in Fig. 3, the exposure of V79-4 cells to H_2O_2 showed an increase in the lipid peroxidation, which was monitored by the generation of TBARS. However, treatment with PGG was found to inhibit the H_2O_2-induced peroxidation of lipids compared to H_2O_2 treatment alone. The result indicates that PGG possesses inhibitory property against H_2O_2-induced membrane lipid damage and in turn provides cytoprotection.

3.4. The effect of PGG on cell viability treated with H_2O_2

The protective effect of PGG on cell survival in H_2O_2-treated cells was evaluated. Cells were treated with PGG at 10 μg/ml for 1 h, prior to the addition of H_2O_2. Cell viability was determined 24 h later by the MTT assay. As shown in Fig. 4, treatment with PGG increased cell survival to 66%, compared to 54% for H_2O_2 treatment, PGG at 10 μg/ml did not show cytotoxicity (cell viability of 107% compared to the control). Thus, PGG showed a cytoprotective effect on H_2O_2-induced cell damage.

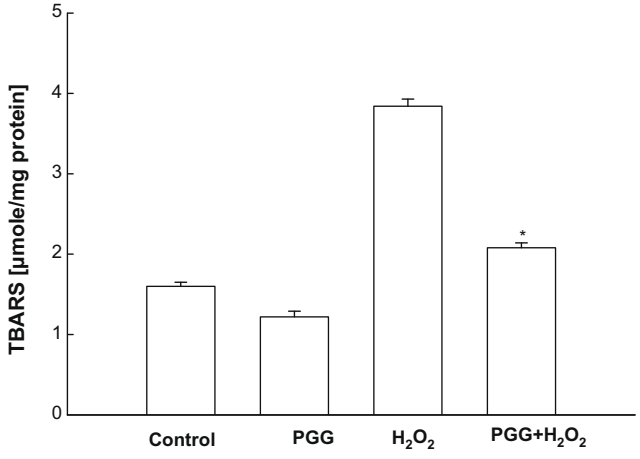

Fig. 3. The effect of PGG on lipid peroxidation. Lipid peroxidation was assayed by measuring the amount of TBARS formation. *Significantly different from H_2O_2-treated cells ($p < 0.05$).

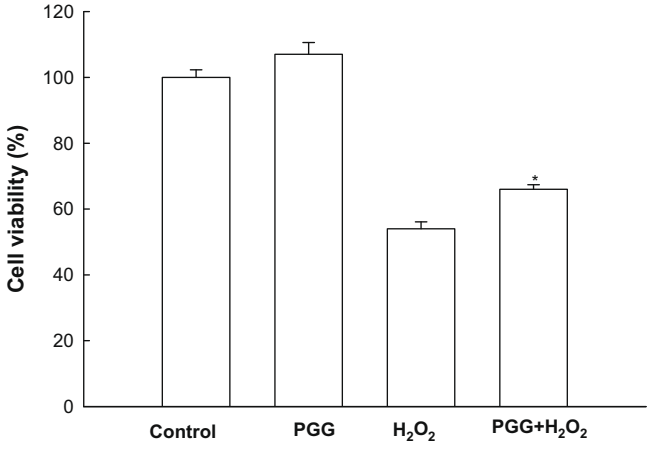

Fig. 4. The effect of PGG on H_2O_2-induced cell death. The viability of V79-4 cells was determined by MTT assay. *Significantly different from H_2O_2-treated cells ($p < 0.05$).

3.5. The effect of PGG on H_2O_2-induced apoptosis

To study the cytoprotective effect of PGG on apoptosis induced by H_2O_2, the nuclei of V79-4 cells were stained with Hoechst 33342 for microscopic examination. The microscopic pictures in Fig. 5A indicated that the control cells had intact nuclei, and that the H_2O_2-treated cells showed significant nuclear fragmentation, characteristic feature of apoptosis. However, when the cells were treated with PGG at 10 μg/ml for 1 h, prior to H_2O_2 treatment, a dramatic decrease in nuclear fragmentation was observed. In addition to the morphological evaluation, the protective effect of PGG against apoptosis was also confirmed by measuring DNA fragmentation, mitochondrial membrane potential ($\Delta\psi$), and apoptosis-related proteins expression. Treatment of cells with H_2O_2 increased the levels of cytoplasmic histone-associated DNA fragmentations, compared to control groups, however, treatment with 10 μg/ml of PGG was found to decrease the level of DNA fragmentation (Fig. 5B). During the apoptotic process, the opening of the mitochondrial membrane pore occurs (Zamzami et al., 1995), and induces the loss of $\Delta\psi$ (Cai, Yang, & Jones, 1998; Zamzami et al., 1996). The H_2O_2-treated cells resulted in the loss of $\Delta\psi$, as substantiated by an increase in fluorescence (FL-1) with the JC-1 dye (Fig. 5C). However, PGG treatment at 10 μg/ml blocked the loss of $\Delta\psi$ in H_2O_2-treated cells. Finally, we examined the caspase-3 activity by Western blot since it is known as the major effecter caspase of the apoptotic process. PGG was found to inhibit the H_2O_2-

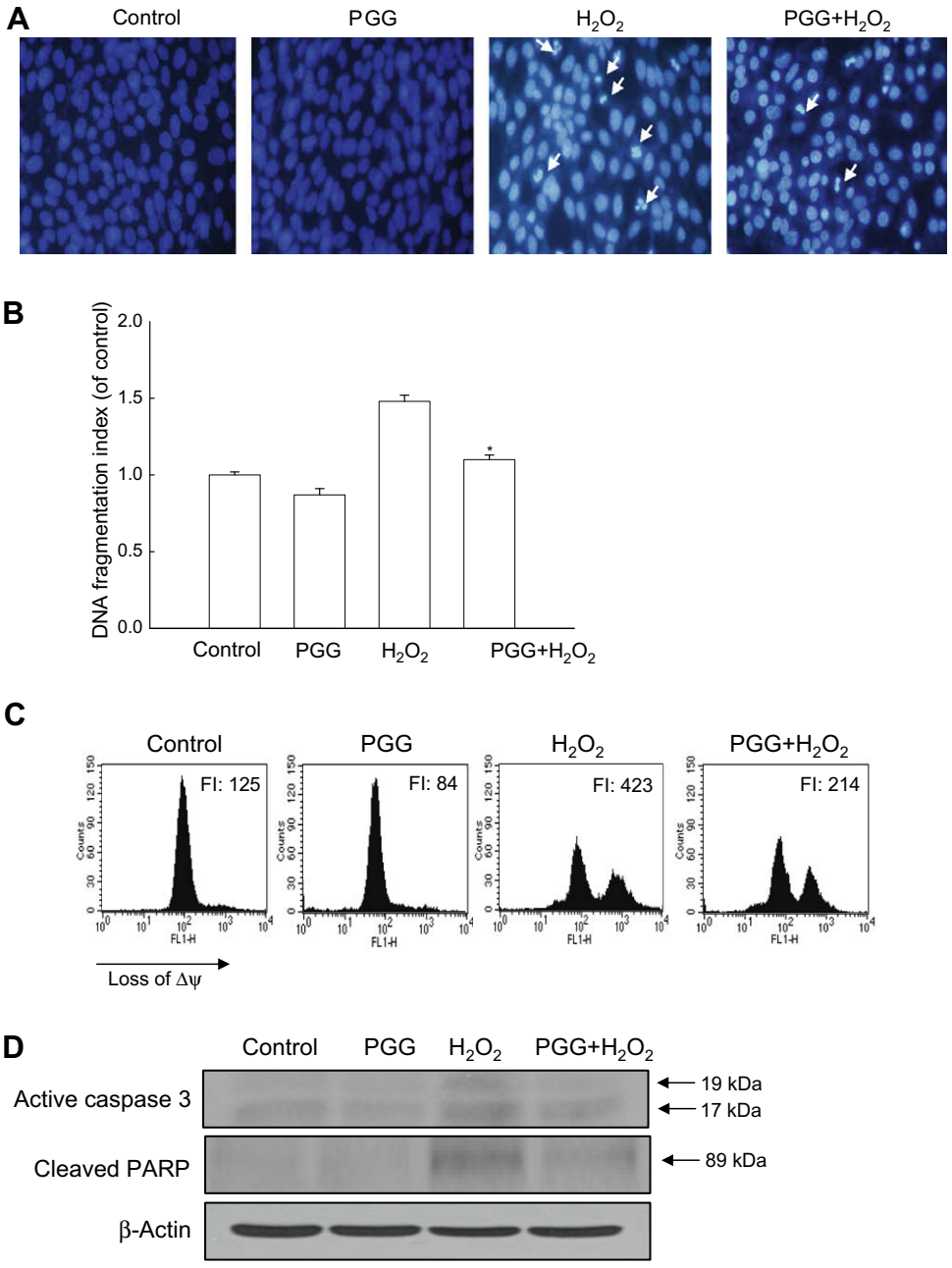

Fig. 5. The effect of PGG on H_2O_2-induced apoptosis. Apoptotic body formation was observed under fluorescent microscope after Hoechst 33342 staining and apoptotic bodies are indicated by arrows (A). DNA fragmentation was quantified by ELISA kit (B). *Significantly different from H_2O_2 treatment ($p < 0.05$). The mitochondrial membrane potential ($\Delta\psi$) was analysed using flow cytometry after staining cells with JC-1 (C). FI indicates the fluorescence intensity of JC-1. Western blot analysis was performed using anti-caspase-3 and PARP antibodies (D).

induced active form of caspase-3 (17 kDa and 19 kDa), which is further demonstrated by the cleavage of poly ADP-ribosyl polymerase (PARP) (89 kDa) (Fig. 5D). The results suggest that PGG protects from cell death by inhibiting apoptosis induced by H_2O_2 treatment.

4. Discussion

The present study demonstrated that PGG (gallic acid derivative) exerts anti-apoptotic activity on V79-4 cells, through antioxidant properties. PGG increased the intracellular ROS and DPPH radical-scavenging activities and then enhanced antioxidant enzyme activity of V79-4 cells exposed to H_2O_2. These antioxidant properties were proven to be related with a cytoprotective effect. The cells exposed to H_2O_2 exhibited distinct features of apoptosis, such as apoptotic body, loss of mitochondrial membrane potential ($\Delta\psi$), DNA fragmentation, and activation of caspase-3. However, cells that were pretreated with PGG had significantly reduced the apoptotic phenomenon shown in Figs. 5A–D. This antioxidant activity was related to both the direct scavenging of ROS and the indirect endogenous defence systems such as SOD, CAT, and GPx.

In general, the radical-scavenging activity of polyphenols depends on the molecular structure and the substitution pattern of the hydroxyl groups, the availability of phenolic hydrogens, and the possibility of stabilisation of the resulting phenoxyl radicals via hydrogen donation or by expanded electron delocalisation (Benkovic et al., 2008). The structural requirement considered to be essential for effective radical-scavenging criteria is the presence of an *ortho*-dihydroxy group (catechol structure), possessing electron-donating properties and being a radical target. As seen from its chemical structure (Fig. 1), PGG has five galloyl groups which are an additional catechol structure with *ortho*-dihydroxy groups.

Thus, it can be stated that PGG has an intrinsic feature of antioxidant activity, because it has structural advantage of various catechol moieties. Also, it has been shown that a galloyl group could considerably strengthen antioxidant capacity (Cai, Mei, Jie, Luo, & Corke, 2006). The results presented in this report indicate that PGG efficiently attenuated oxidative stress-induced cell damage through antioxidant properties, thus suggesting that *Elaeocarpus sylvestris* var. *ellipticus* may be a promising source of PGG as a functional food material with antioxidant properties.

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