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Triphlorethol-A from *Ecklonia cava* protects V79-4 lung fibroblast against hydrogen peroxide induced cell damage

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Abstract
In the present study, triphlorethol-A, a phlorotannin, was isolated from *Ecklonia cava* and its antioxidant properties were investigated. Triphlorethol-A was found to scavenge intracellular reactive oxygen species (ROS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, and thus prevented lipid peroxidation. The radical scavenging activity of triphlorethol-A protected the Chinese hamster lung fibroblast (V79-4) cells exposed to hydrogen peroxide (H_2O_2) against cell death, via the activation of ERK protein. Furthermore, triphlorethol-A reduced the apoptotic cells formation induced by H_2O_2. Triphlorethol-A increased the activities of cellular antioxidant enzymes like, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). Hence, from the present study, it is suggestive that triphlorethol-A protects V79-4 cells against H_2O_2 damage by enhancing the cellular antioxidative activity.

Keywords: *Triphlorethol-A, oxidative stress, apoptosis, antioxidant enzymes*

Introduction

Reactive oxygen species (ROS) are known to cause oxidative modification of DNA, proteins, lipids and small cellular molecules. ROS are associated with tissue damage and are the contributing factors for inflammation, aging, cancer, arteriosclerosis, hypertension and diabetes [1–7]. For cytoprotection against ROS, cells have developed a variety of antioxidant defense mechanisms. Enzymatic defense mechanisms involve superoxide dismutase (SOD), which catalyzes dismutation of superoxide anion to hydrogen peroxide, catalase (CAT), which converts hydrogen peroxide into molecular oxygen and water, and glutathionine peroxidase (GPx), which destroys toxic peroxides.

Ecklonia cava is a brown alga (Laminariaceae) that is abundant in the subtidal regions of Jeju Island in Korea. Recently, it has been reported that *Ecklonia* species exhibits radical scavenging activity [8,9], antiplasmin inhibiting activity [10–12], HIV-1 reverse transcriptase and protease inhibiting activity [13] and tyrosinase inhibitory activity [14]. Phlorotannins such as eckol (a phloroglucinol trimer, a closed-chain trimer), 6,6′-bieckol (a hexamer), dieckol (a hexamer) and phlorofucofuroeckol (a pentamer) were identified to be responsible for the biological activities in *Ecklonia*. During the investigation of antioxidative

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components in *E. cava*, we observed that triphloethol-A has very strong activities. Triphlorethol-A, an open-chain trimer of phloroglucinol, is one of phlorotannin components isolated from *E. cava* in this study, and has been previously isolated from *Ecklonia Kurome* [11].

In the present study, we have investigated the protective effect of triphlorethol-A on cell damage induced by hydrogen peroxide and the possible mechanism of cytoprotection.

Materials and methods

Preparation of triphlorethol-A

The dried *E. cava* (4 kg), collected from Jeju Island in Korea, was immersed in 80% methanol at room temperature for 2 days. The aqueous methanol was removed *in vacuo* to give a brown extract (1 kg), which was partitioned between ethyl acetate and water. The ethyl acetate fraction (230 g) was mixed with celite. The mixed celite was dried and packed into a glass column, and eluted in the order of hexane, methylene chloride, diethyl ether and methanol. The obtained diethyl ether fraction (14 g) was subjected to Sephadex LH-20 chromatography using $CHCl_3$—MeOH gradient solvent (2/1 → 0/1). The triphlorethol-A (220 mg) was obtained from these fractions and was identified according to the previously reported method (Figure 1) [15]. The purity of triphlorethol-A assessed by HPLC was >90%. Triphlorethol-A was freshly dissolved in DMSO; the final concentration of which did not exceed 0.1%.

Reagents

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, USA. PeroXOquant™ quantitative peroxide assay kit was purchased from Pierce, Rockford, IL, USA. The other chemicals and reagents were of analytical grade. Primary rabbit polyclonal anti-ERK 2 (42 kDa ERK) and -phospho-ERK1/2 (phosphorylated 44 kDa/42 kDa ERK) (Thr 202/Tyr 204) antibodies were purchased from Santa Cruz Biotechnology, Santa Cruz, CA, USA.

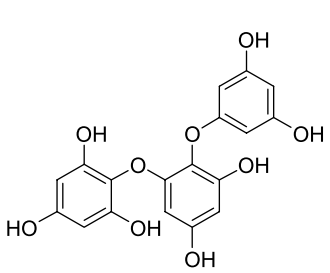

Figure 1. Chemical structure of triphlorethol-A.

Cell culture

It is reported that lung is an organ sensitive to oxidative stress [16,17]. To study the effect of triphlorethol-A on oxidative stress induced by hydrogen peroxide, we used Chinese hamster lung fibroblasts (V79-4 cells). The 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).

Intracellular ROS measurement and image analysis

The DCF-DA method was used to detect the intracellular ROS level [18]. DCF-DA diffuses into cells, where it is hydrolyzed by intracellular esterase to polar 2′,7′-dichlorodihydrofluorescein. This non-fluorescent fluorescein analog gets trapped inside the cells and is oxidized by intracellular oxidants to a 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 triphlorethol-A 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. After addition of 25 μM of DCF-DA solution, the fluorescence of 2′,7′-dichlorofluorescein was detected at 485 nm excitation and at 535 nm emission using a PerkinElmer LS-5B spectrofluorometer.

For image analysis of production of intracellular ROS, the V79-4 cells were seeded in coverslip loaded with 6 well plate at 1×10^5 cells/ml. Sixteen hours after plating, the cells were treated with triphlorethol-A and 30 min later, 1 mM H_2O_2 was added to the plate. After changing media, 100 μM of DCF-DA was added in the well and was incubated for an additional 30 min at 37°C. After washing with PBS, stained cells were mounted onto microscope slide in the mounting medium (DAKO, Carpinteria, CA, USA). Images were collected using the LSM 510 program on a Zeiss confocal microscope.

DPPH radical scavenging activity

Various concentrations of triphlorethol-A were added to a 1×10^{-4} M solution of DPPH in methanol, and the reaction mixture was shaken vigorously. After 5 h, the amount of residual DPPH was determined at 520 nm using a spectrophotometer [19].

Measurement of hydrogen peroxide

Level of H_2O_2 is determined by PeroXOquant™ quantitative peroxide assay kits, which detect H_2O_2 based on oxidation of ferrous to ferric ion in the presence of xylenol orange [20].

Lipid peroxidation inhibitory activity

Lipid peroxidation was assayed by thiobarbituric acid reaction [21]. The V79-4 cells were seeded in a culture dish at 1×10^5 cells/ml. Sixteen hours after plating, the cells were treated with various concentrations of triphlorethol-A. One hour later, 1 mM H_2O_2 was added to the plate, and was incubated for further 1 h. The cells were then washed with cold PBS, scraped and homogenized in ice-cold 1.15% KCl. A measure of 100 μl of the cell lysates was mixed 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 made up 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 *n*-butanol and pyridine mixture (15:1, v/v) was added to each sample, and the mixture was shaken well. 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 triphlorethol-A 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 is based on the reduction of a tetrazolium salt by mitochondrial dehydrogenase in the viable cells [22]. 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 triphlorethol-A. An hour later, 1 mM H_2O_2 was added to the plate and was incubated at 37°C for an additional 24 h. A measure of 50 μl of the MTT stock solution (2 mg/ml) was then added to 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 dimethylsulfoxide and the A_{540} was read on a scanning multi-well spectrophotometer.

Flow cytometry analysis

Flow cytometry was performed to determine the apoptotic sub G_1 hypo-diploid cells [23]. 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 30 μM of triphlorethol-A. 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 (Becton Dickinson, Mountain View, CA, USA). The proportion of sub G_1 hypo-diploid cells was assessed by the histograms generated using the computer program, Cell Quest and Mod-Fit.

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 30 μM of triphlorethol-A 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 to examine the degree of nuclear condensation.

SOD activity

The V79-4 cells were seeded at 1×10^5 cells/ml, and 16 h after plating, the cells were treated with various concentrations of triphlorethol-A for 1 h. The harvested cells were suspended in 10 mM phosphate buffer (pH 7.5) and then lysed on ice by sonication twice for 15 s. Triton X-100 (1%) was then added to the lysates and was incubated for 10 min on ice. The lysates were centrifugated at 5000*g* for 30 min at 4°C to remove the cellular debris. The protein content of the supernatant was determined by Bradford method [24], with bovine serum albumin as the standard. The SOD activity was used to detect the inhibited level of auto-oxidation of epinephrine [25]. A measure of 50 μg of the protein was added to 50 mM phosphate buffer (pH 10.2) containing 0.1 mM EDTA and 0.4 mM epinephrine. Epinephrine rapidly undergoes auto-oxidation at pH 10 to produce adrenochrome, a pink colored product, which 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. The SOD activity was expressed as units/mg protein and one unit of enzyme activity was defined as the amount of enzyme required for 50% inhibition of auto-oxidation of epinephrine.

CAT activity

A measure of 50 μg of protein was added to 50 mM phosphate buffer (pH 7) containing 100 mM (v/v) H_2O_2. The reaction mixture was incubated for 2 min at 37°C and the absorbance was monitored at 240 nm for 5 min. The change in absorbance with time was proportional to the breakdown of H_2O_2 [26]. The CAT activity was expressed as units/mg protein and one unit of enzyme activity was defined as the amount of enzyme required to breakdown of 1 μM H_2O_2.

Glutathione peroxidase (GPx) activity

A measure of 50 μg of the protein was added to 25 mM phosphate buffer (pH 7.5) containing 1 mM

EDTA, 1 mM NaN_3, 1 mM glutathione (GSH), 0.25 unit 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 [27]. The GPx activity was expressed as units/mg protein and one unit of enzyme activity was defined as the amount of enzyme required to oxidize 1 mM NADPH.

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 30 μM of triphlorethol-A. 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, USA), which were then incubated with primary rabbit monoclonal -ERK1/2 and -phospho ERK1/2. The membranes were further incubated with goat anti--rabbit immunoglobulin G-horseradish peroxidase conjugates (Pierce, Rockland, IL, USA), and then exposed to X-ray film. Protein bands were detected using an enhanced chemiluminescence Western blotting detection kit (Amersham, Little Chalfont, Buckinghamshire, UK).

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. $p < 0.05$ were considered significantly.

Results

Radical scavenging activity of triphlorethol-A

The radical scavenging effect of triphlorethol-A on the intracellular ROS and DPPH free radical scavenging activities was measured. The intracellular ROS scavenging activity of triphlorethol-A was 43, 69 and 76% at concentrations of 0.3, 3 and 30 μM, respectively (Figure 2A). In the presence of 2 mM of N-acetylcysteine (positive control), there was 85% of ROS inhibition (data not shown). As shown in Figure 2B, the fluorescent intensity of DCF-DA staining was enhanced in H_2O_2 treated V79-4 cells. However, triphlorethol-A reduced the red fluorescence intensity by H_2O_2 treatment, reflecting a reduction of ROS generation. The ROS scavenging activity of triphlorethol-A was found to be consistent with its DPPH radical scavenging activity (Figure 2C). The DPPH radical scavenging activity of triphlorethol-A was 29, 58 and 60% at concentration of 0.3, 3 and 30 μM, respectively, when compared to 92% inhibition in the presence of 2 mM N-acetylcystein.

Effect of triphlorethol-A on lipid peroxidation

The ability of triphlorethol-A to inhibit lipid peroxidation in H_2O_2 treated V79-4 cells was also investigated. The generation of thiobarbituric acid reactive substance (TBARS) was inhibited in the presence of triphlorethol-A. The inhibitory effect of triphlorethol-A was 15, 21 and 32% at concentration of 0.3, 3 and 30 μM, respectively, when compared to 11% inhibition in 0.1% DMSO treated group (Figure 3).

Effect of triphlorethol-A on cell damage induced by H_2O_2

The protective effect of triphlorethol-A on cell survival in H_2O_2 treated V79-4 cells was also measured. Cells were treated with triphlorethol-A at various concentrations for 1 h, prior to the addition to H_2O_2. The cell viability was determined 24 h later by MTT assay. As shown in Figure 4A, treatment with triphlorethol-A induced a dose dependent increase in the cell survival rate; 7% at 0.3 μM, 22% at 3 μM and 42% at 30 μM. In order to study the cytoprotective effect of triphlorethol-A on apoptosis induced by H_2O_2, nuclei of V79-4 cells were stained with Hoechst 33342 for microscopy and with propidium iodide for flow cytometric analysis. The microscopic pictures in Figure 4B showed that the control cells had intact nuclei, and the H_2O_2 treated cells showed significant nuclear fragmentation, characteristic of apoptosis. However, when the cells were treated with triphlorethol-A 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 triphlorethol-A against apoptosis was confirmed by flow cytometry. As shown in Figure 4C, an analysis of the DNA content in the H_2O_2 treated cells revealed an increase of 65% of apoptotic sub-G_1 DNA content, as compared to 2% of apoptotic sub-G_1 DNA content in untreated cells. Treatment with 30 μM of triphlorethol-A decreased the apoptotic sub-G_1 DNA content to 52%. These results suggest that triphlorethol-A protects cell viability by inhibiting H_2O_2 induced apoptosis.

Effect of triphlorethol-A on ERK activation

The activation of extracellular signal regulated kinase (ERK) is known to induce cell proliferation [28].

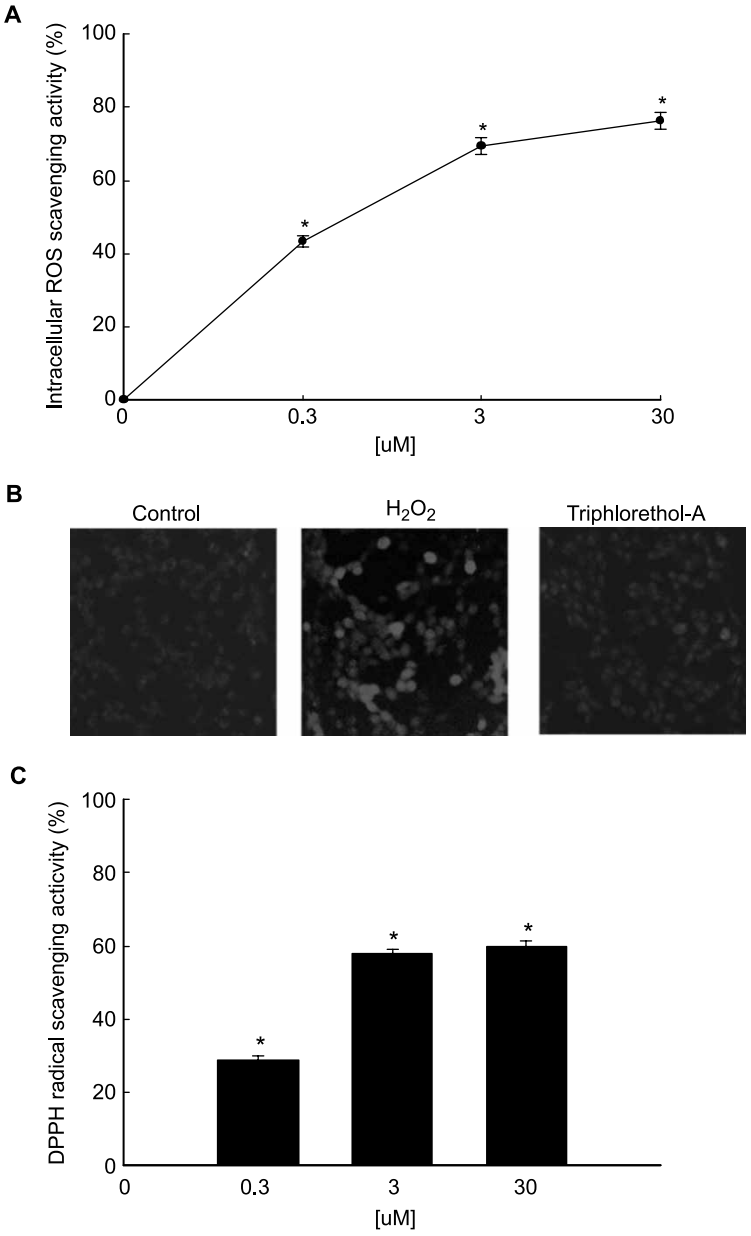

Figure 2. Effect of triphlorethol-A on scavenging intracellular ROS and DPPH radicals. The intracellular ROS generated was detected by DCF-DA method (A) and by confocal microscopy (B). Representative confocal images illustrate the increase in red fluorescence intensity of DCF produced by ROS in H_2O_2 treated V79-4 cells as compared to control and the lowered fluorescence intensity in H_2O_2 treated V79-4 cells in the presence of triphlorethol-A (original magnification $\times$ 400). (C) The amount of DPPH radicals was determined spectrophotometrically at 520 nm. *Significantly different from control ($p < 0.05$).

To better understand the protective mechanism of triphlorethol-A on V79-4 cells, we examined the activation of the ERK protein by western blot analysis with the phospho-ERK specific antibody. As shown in Figure 5A, within 6 h triphlorethol-A activated phosphorylated ERK dramatically. However, there was no change in the total ERK protein level. To determine the effect of ERK inhibitor on protection of triphlorethol-A from H_2O_2 induced damage, V79-4 cells were pre-treated for 30 min with U0126 (10 nM), specific inhibitor of ERK kinase, followed for 30 min with triphlorethol-A and exposed to 1 mM H_2O_2 for 24 h. As shown in Figure 5B, U0126 treatment abolished the protection activity of triphlorethol-A in H_2O_2 damaged cells.

Effect of triphlorethol-A on the intracellular antioxidant systems

In order to investigate whether the radical scavenging activity of triphlorethol-A was mediated by the activities of antioxidant enzymes, the SOD, CAT and GPx activities in triphlorethol-A treated V79-4 cells were measured. Triphlorethol-A increased the activities of these three enzymes (Figure 6A); in the case of SOD activity, at concentration of 0.3, 3 and 30 μM

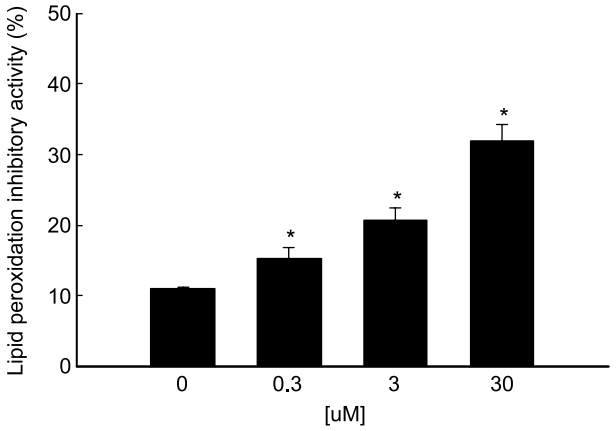

Figure 3. Effect of triphlorethol-A on inhibition of lipid peroxidation. Lipid peroxidation was assayed by measuring the amount of TBARS. *Significantly different from control ($p < 0.05$).

of triphlorethol-A, the activity was 16, 18 and 21, as compared to 11 U/mg protein of the control; in the case of CAT activity, it was 15, 23 and 31 U/mg protein at concentration of 0.3, 3 and 30 μM, as compared to 15 U/mg protein of the control; in the case of GPx activity, it was 12, 14 and 17 U/mg protein at concentration of 0.3, 3 and 30 μM, as compared to 7 U/mg protein of the control. The 3-amino-1,2,4 triazol (ATZ) is known as a specific inhibitor of CAT [29]. To determine the effect of CAT inhibitor on protection of triphlorethol-A from H_2O_2 induced damage, V79-4 cells were pre-treated with 20 mM of ATZ for 1 h, followed for 30 min with triphlorethol-A and exposed to 1 mM H_2O_2 for 24 h. As shown in Figure 6B, ATZ treatment abolished the protection activity of triphlorethol-A in H_2O_2 damaged cells. It is reported that most polyphenolic compounds interact

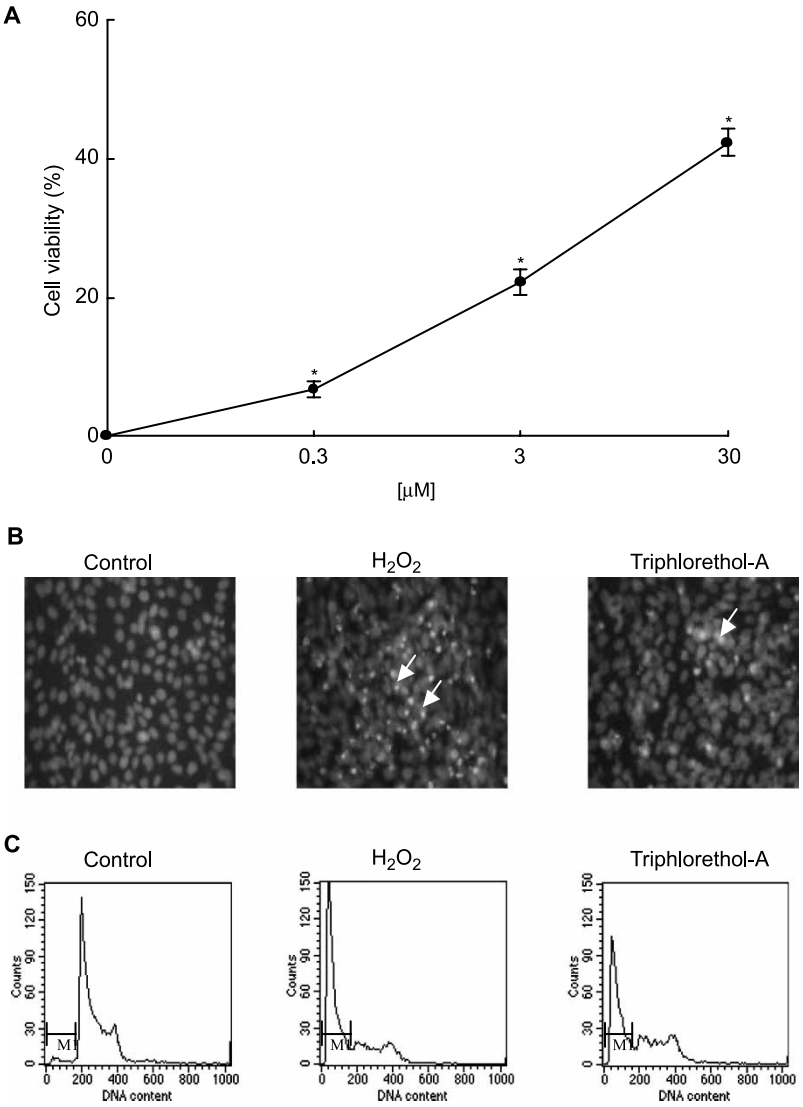

Figure 4. Protective effect of triphlorethol-A on H_2O_2 induced oxidative damage of V79-4 cells. (A) The viability of V79-4 cells was determined by MTT assay. (B) Apoptotic body formation was observed under a fluorescent microscope after Hoechst 33342 staining and apoptotic bodies are indicated by arrows. (C) Apoptotic sub-G_1 DNA content was detected by flow cytometry after propidium iodide staining.

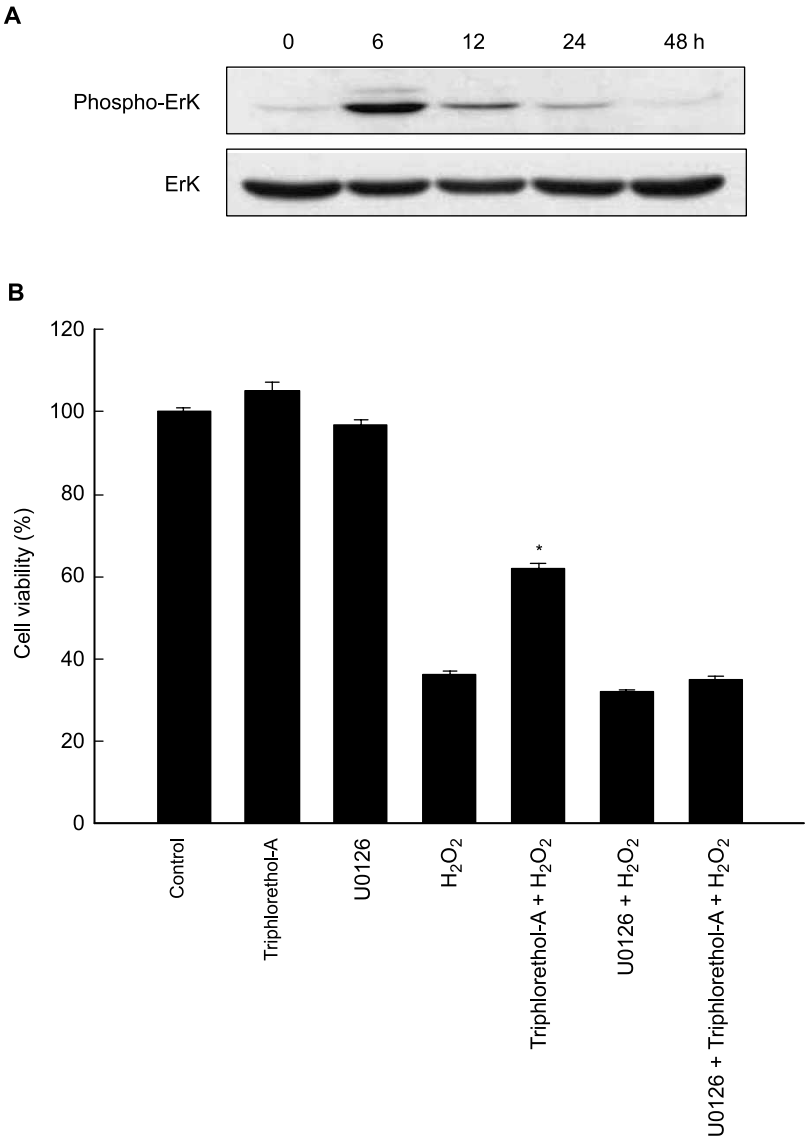

Figure 5. Effect of triphlorethol-A on ERK activation. (A) Cell lysates were electrophoresed and proteins of ERK1/2 and phospho-ERK1/2 were detected by their respective antibodies. (B) After treatment of U0126, triphlorethol-A or/and H_2O_2, the viability of V79-4 cells was determined by MTT assay. *Significantly different from H_2O_2 treated cells ($p < 0.05$).

with commonly used cell culture media to generate H_2O_2 [30]. This generated low level of H_2O_2 can trigger the rise in antioxidant enzymes. Triphlorethol-A was added to cell culture media at a final concentration of 30 μM and amount of H_2O_2 was measured 1 h later by the ferrous iron oxidation-xylenol orange (FOX) assay. As shown in Table I, H_2O_2 was little detected in triphlorethol-A treated media (< 4.3 μM H_2O_2), suggesting the antioxidant activities in triphlorethol-A treated cells were not increased by H_2O_2 generated in triphlorethol-A treated media.

Discussion

Phlorotannins, which are marine algal polyphenols and are also known as brown algae, are polymers of phloroglucinol [31]. Although some reports suggest that phlorotannins from algae exhibit the antioxidant effect on free radicals [9,32,33], there are no reports on the antioxdant activity of triphlorethol-A, isolated from *E. cava*. In our present study, it was observed that upon exposure to H_2O_2, triphlorethol-A decreased intracellular ROS generation and DPPH radical level. Triphloroethol-A has a polyphenol structure. Polyphenols are electron-rich compounds and prone to enter into efficient electron-donation reactions with oxidizing agents to produce phenoxyl radical (PhO·) species as intermediates. Phenoxyl radicals are stabilized by resonance delocaliztion of the unpaired electron to the *ortho* and *para* positions of the ring. In addition to the resonance stability, phenoxyl radicals can also be stabilized by hydrogen bonding with an adjacent hydroxyl group. Phenoxyl radicals also undergo dimerization ("phenol coupling") to produce new C—C or C—O linkage [34]. These

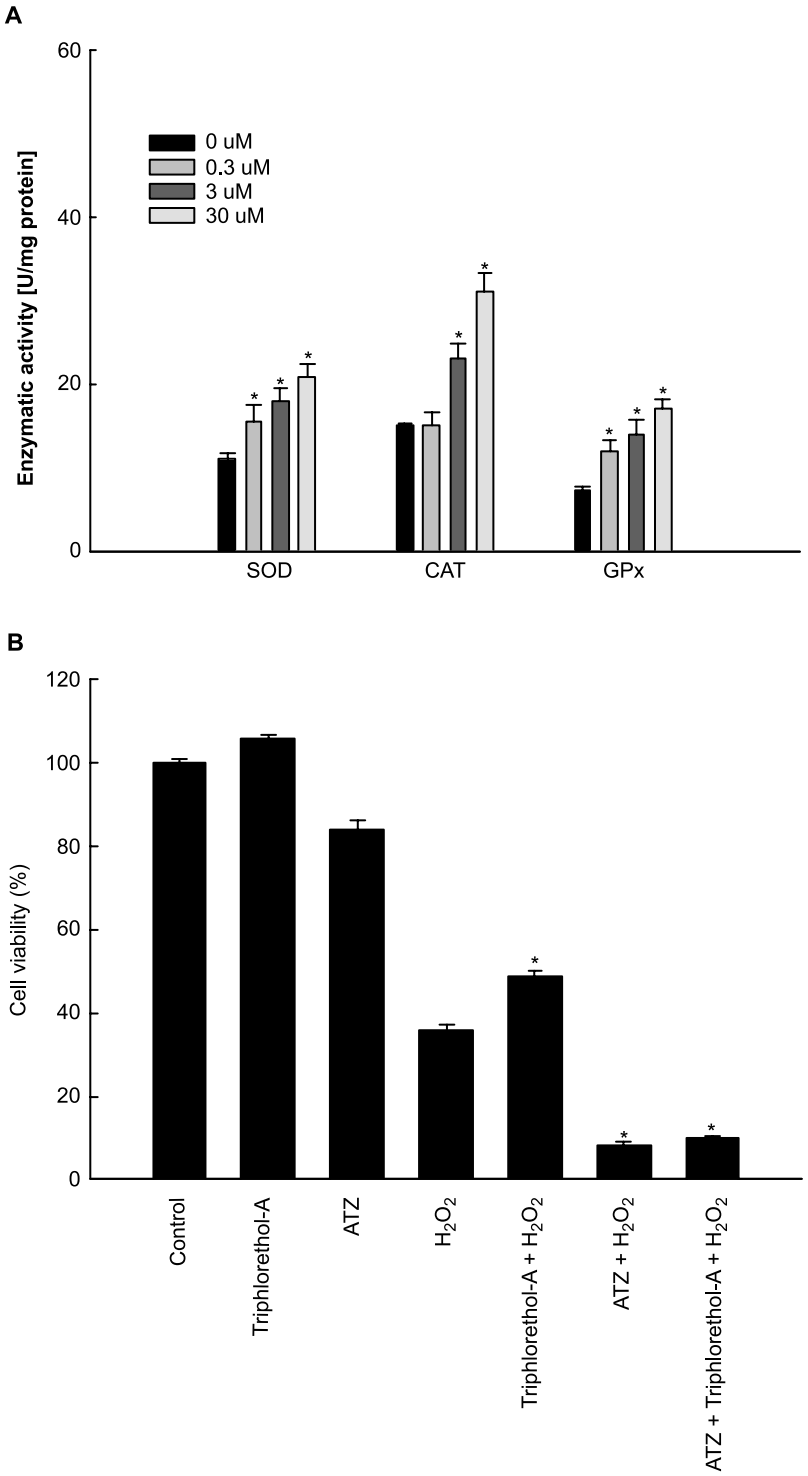

Figure 6. Effect of triphlorethol-A on the activities of antioxidative enzymes. (A) The data represents three experiments and are expressed as average enzyme unit per mg protein ± S.E. *Significantly different from control ($p < 0.05$). (B) After treatment of ATZ, triphlorethol-A or/and H_2O_2, the viability of V79-4 cells was determined by MTT assay. *Significantly different from H_2O_2 treated cells ($p < 0.05$).

intrinsic stability of phenolic structures might be related to antioxidative activity of triphloroethol A. The cells exposed to H_2O_2 exhibited distinct morphological features of apoptosis, such as nuclear fragmentation and an increase in sub G_1 DNA content. However, cells that were pretreated with triphlorethol-A had significantly reduced percentage of apoptotic cells, as shown by morphological changes and reduction in sub G_1 DNA content. Our results are also consistent with the antioxidant activity of N-acetylcysteine, which also prevents H_2O_2 induced apoptosis, indicating that the inhibition of ROS formation may be important for cytoprotection against oxidative damage. In many cell types, ERK

Table I. Generation of H_2O_2 in cell-culture media.

	$[H_2O_2]$ present in DMEM media (μM)
None	1.5 ± 0.1
DMSO*	1.7 ± 0.5
Triphlorethol-A	4.3 ± 1.1

Triphlorethol-A at a final concentration of 30 μM was added to DMEM culture media and incubated at room temperature for 1 h. H_2O_2 was then measured by the FOX assay. Data are means ± SD. * DMSO at the same concentration used to dissolve triphlorethol-A.

pathway is induced by a variety of extracellular stimuli [35]. The phosphorylation of ERK can phosphorylate cytoplasmic and nuclear targets and participates in a wide range of cellular programs including proliferation, differentiation, and movement [36–38]. The level of phosphorylated ERK in triphlorethol-A treated V79-4 cells was induced, and treatment of U0126, specific inhibitor of ERK kinase, suppressed the protection activity of triphlorethol-A in H_2O_2 damaged cells, suggesting that the protective effect of triphlorethol-A on cells may also be involved in activating ERK pathway. Antioxidant enzymes, like SOD, CAT and GPx, play significant roles in effective augmentation of antioxidant defense mechanisms in cells. SOD converts superoxide radicals to hydrogen peroxide and subsequently to water by the activity of CAT and GPx. Triphlorethol-A increased SOD, CAT and GPx activities, suggesting that the scavenging of ROS may be related to the increased antioxidant activity. Therefore, the effects of triphlorethol-A on cell viability might involve dual actions: Direct action on oxygen radical scavenging, as shown by DPPH radical scavenging, and indirect action through induction of anti-oxidative enzymes.

In conclusion, triphlorethol-A, a phlorotannin, exerted intracellular ROS and DPPH radical scavenging activity, promoted cell viability via activation of ERK protein, inhibited H_2O_2 induced apoptosis, and enhanced the effects of antioxidant enzymes.

Acknowledgements

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