Eckol isolated from *Ecklonia cava* attenuates oxidative stress induced cell damage in lung fibroblast cells

Kyoung Ah Kang[a], Kyoung Hwa Lee[a], Sungwook Chae[a], Rui Zhang[a], Myung Sun Jung[a], Youngki Lee[b], So Young Kim[c], Hee Sun Kim[c], Hong Gu Joo[d], Jae Woo Park[e], Young Min Ham[f], Nam Ho Lee[f], Jin Won Hyun[a,*]

[a] *Department of Biochemistry, College of Medicine and Applied Radiological Science Research Institute, Cheju National University, Jeju-si 690-756, Republic of Korea*
[b] *Department of Histology, College of Medicine, Cheju National University, Jeju-si 690-756, Republic of Korea*
[c] *Department of Neuroscience, College of Medicine, Ewha Womans University, Seoul 110-783, Republic of Korea*
[d] *Department of Veterinary Pharmacology, Cheju National University, Jeju-si 690-756, Republic of Korea*
[e] *Department of Nuclear and Energy Engineering, Cheju National University, Jeju-si 690-756, Republic of Korea*
[f] *Department of Chemistry, College of Natural Sciences, Cheju National University, Jeju-si 690-756, Republic of Korea*

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Abstract **We have investigated the cytoprotective effect of eckol, which was isolated from *Ecklonia cava*, against oxidative stress induced cell damage in Chinese hamster lung fibroblast (V79-4) cells. Eckol was found to scavenge 1,1-diphenyl-2-picrylhydrazyl radical, hydrogen peroxide (H_2O_2), hydroxy radical, intracellular reactive oxygen species (ROS), and thus prevented lipid peroxidation. As a result, eckol reduced H_2O_2 induced cell death in V79-4 cells. In addition, eckol inhibited cell damage induced by serum starvation and radiation by scavenging ROS. Eckol was found to increase the activity of catalase and its protein expression. Further, molecular mechanistic study revealed that eckol increased phosphorylation of extracellular signal-regulated kinase and activity of nuclear factor κ B. Taken together, the results suggest that eckol protects V79-4 cells against oxidative damage by enhancing the cellular antioxidant activity and modulating cellular signal pathway.**

Keywords: Eckol; Oxidative stress; Apoptosis; Catalase; Signal pathway

1. Introduction

Balance between oxidants and antioxidants minimize molecular, cellular, and tissue damage. However, if the balance is upset in favor of the oxidants, oxidative stress occurs and often results in oxidative damage. Especially, 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 prime contributing factors for inflammation, aging, cancer, arteriosclerosis, hypertension and diabetes. For cytoprotection against ROS, cells have developed a variety of antioxidant defense mechanisms. These antioxidant defense systems include: (i) metal chelators such as ceruloplasmin and transferrin capable of preventing ROS formation by inhibiting metal catalyzed reactions such as Fenton reaction; (ii) low molecular weight antioxidant such as ascorbic acid, glutathione and tocopherols and (iii) ROS interacting enzymes such as superoxide dismutase, catalase and glutathione peroxidase [1].

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 [2,3], anti-plasmin inhibiting activity [4,5], antimutagenic activity [6,7], bactericidal activity [8], HIV-1 reverse transcriptase and protease inhibiting activity [9] and tyrosinase inhibitory activity [10]. Phlorotannin components, which is oligomeric polyphenol of phloroglucinol unit, are responsible for the biological activities of *Ecklonia* and phlorotannins such as eckol (a closed-chain trimer of phloroglucinol), 6,6′-bieckol (a hexamer), dieckol (a hexamer), phlorofucofuroeckol (a pentamer) were identified in *Ecklonia* species. During the investigation of antioxidative components in *E. cava*, we observed that eckol possessed very strong activity. Eckol, a trimeric compound of phloroglucinol with dibenzeno-1,4-dioxin skeleton, is one of the major phlorotannins isolated from *E. cava*.

In the present study, we have investigated the protective effect of eckol on cell damage induced by oxidative stress and the underlying possible mechanism of cytoprotection.

2. Materials and methods

2.1. Cell line and reagents

V79-4 cell line was derived from the American type culture collection. Eckol compound (Fig. 1) was obtained from Dr. Nam Ho Lee (Cheju National University, Jeju, Korea). The plasmid containing NF-κB binding site-luciferase construct was generous gifts of Dr. Young Joon Surh (Seoul National University, Seoul, Korea). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical and 2′,7′-dichlorodihydrofluorescein diacetate (DCF-DA) were purchased from Sigma Chemical

*Corresponding author. Fax: +82 064 726 4152.
E-mail address: jinwonh@cheju.ac.kr (J.W. Hyun).

Abbreviations: DPPH, 1,1-diphenyl-2-picrylhydrazyl; ROS, reactive oxygen species; ERK, extracellular signal-regulated kinase; ATZ, 3-amino-1,2,4-triazol; DCF-DA, 2′,7′-dichlorodihydrofluorescein diacetate

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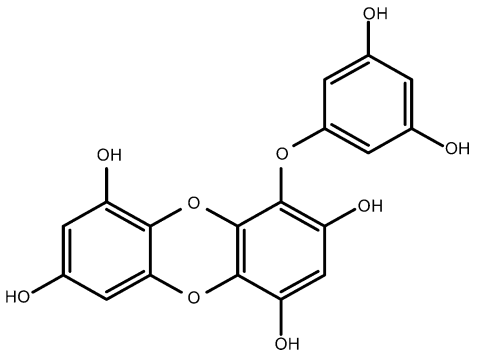

Fig. 1. Chemical structure of eckol.

Company, St. Louis, MO, USA. Primary rabbit polyclonal anti-ERK2, -phospho-ERK1/2, -IkBα and -NF-κB (p65) antibodies were purchased from Santa Cruz Biotechnology, Santa Cruz, CA, USA.

2.2. Radical scavenging activity

For detection of DPPH radical, eckol was added to a 1×10^{-4} M solution of DPPH and the reaction mixture was shaken vigorously. The amount of residual DPPH was determined at 520 nm. For detection of H_2O_2 amount, eckol and H_2O_2 were mixed with 0.1 M phosphate buffer (pH 5.0). Azino-di(3-ethyl-benzthiazoline-6-sulfonic acid)-peroxidase medium and peroxidase (1 U/ml) were added to the mixture and incubated at 37 °C for 10 min. The absorbance was determined at 405 nm. For detection of hydroxy radical, eckol was added to the reaction mixture (0.1 mM ferric chloride, 0.1 mM ascorbic acid, 0.1 mM EDTA, 1.0 mM H_2O_2 and 3 mM of deoxyribose in the 20 mM of phosphate buffer, pH 7.4). After incubation for 1 h, thiobarbituric acid was added, heated for 20 min and cooled. *n*-Butanol and pyridine mixture (15:1, v/v) was added and the absorbance of supernatant was measured at 532 nm.

2.3. Intracellular reactive oxygen species measurement and image analysis

It is reported that lung is an organ sensitive to oxidative stress [11]. To study the effect of eckol on oxidative stress, we used Chinese hamster lung fibroblasts (V79-4 cells). The V79-4 cells were treated with eckol and 30 min later, 1 mM H_2O_2 was added to the plate. After 30 min, DCF-DA solution was added and the fluorescence was detected at 485 nm excitation and at 535 nm. For image analysis for production of intracellular ROS, the cells were seeded in coverslip loaded 6 well plate and were treated with eckol. After 30 min, 1 mM H_2O_2 was added to the plate. After changing media, DCF-DA was added and incubated for 30 min at 37 °C. The image of stained cells was collected using the LSM 510 program on a Zeiss confocal microscope.

2.4. Lipid peroxidation inhibitory activity

H_2O_2 was added in eckol pretreated cells and the mixture was incubated for 1 h. The cells were homogenized in ice-cold 1.15% KCl and the cell lysates was mixed with 8.1% SDS, 20% acetic acid (adjusted to pH 3.5) and 0.8% thiobarbituric acid. The mixture was made up to a final volume of 4 ml with distilled water and heated. After cooling, *n*-butanol and pyridine mixture (15:1, v/v) was added and the absorbance was measured at 532 nm.

2.5. Cell viability

To determine the effect of eckol on the cell viability in H_2O_2 treatment, H_2O_2 was added in eckol pretreated cells and the mixture was incubated for 24 h. The [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium]bromide (MTT) solution was added and incubated for 4 h. The formazan crystals in each well were dissolved in 150 μl dimethylsulfoxide and the absorbance was measured at 540 nm. To determine the effect of eckol on the cell viability during serum starvation, cells were cultured in serum starved condition (0.1% fetal calf serum), and then treated with eckol for 1 h. The plate was incubated for further 6 h and the cell viability was measured using MTT test. To determine the effect of eckol on the cell viability in γ-ray radiation, the eckol pretreated cells were irradiated at 5 Gy and the plate was incubated for 24 h and the cell viability was measured using MTT test.

2.6. Flow cytometry analysis

The cells were harvested and fixed in 70% ethanol for 30 min at 4 °C. The cells were incubated for 30 min in the dark at 37 °C in PBS containing propidium iodide and 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.

2.7. Nuclear staining with Hoechst 33342

The cells were treated with Hoechst 33342 (a DNA specific fluorescent dye) 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.

2.8. Catalase activity

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 added to the lysates and protein content was determined. Fifty 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. The catalase 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.

2.9. Measurement of hydrogen peroxide

Level of H_2O_2 in medium is determined by PeroXOquant™ quantitative peroxide assay kit, which detects H_2O_2 based on oxidation of ferrous to ferric ion in the presence of xylenol orange.

2.10. Western blot

The total cell lysates were subjected to Western blot analysis using anti-ERK2, anti-phospho-ERK1/2 and anti-catalase antibodies. Cellular nuclear and cytosolic fractions were subjected to Western blot analysis to detect the expression of NF-κB (p65 subunit) and IκBα protein using anti-p65 and anti-IκBα antibodies, respectively. The bound primary antibodies were detected using secondary immunoglobulin G-horseradish peroxidase conjugates.

2.11. Immunofluorescent staining and confocal microscopy

Cells plated on coverslips were fixed within 4% paraformaldehyde for 30 min and permeabilized with 0.1% Triton X-100 in PBS for 2.5 min. Cells were treated with blocking medium (3% bovine serum albumin in PBS) for 1 h and incubated with NK-κB (p65 subunit) antibody diluted in blocking medium for 2 h. Immuno-reacted primary NK-κB antibody was detected by a 1:500 dilution of FITC-conjugated secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) for 1 h. After washing with PBS, stained cells were mounted onto microscope slide in the mounting medium with DAPI (Vector, Burlingame, CA, USA). Images were collected using the LSM 510 program on a Zeiss confocal microscope.

2.12. Electrophoretic mobility shift assay (EMSA)

Oligonucleotides containing NF-κB sequence (5′-AGT TGA GGG GAC TTT CCC AGGC-3′) were annealed, labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase, and used as probes. The probes (50 000 cpm) were incubated with the nuclear extracts at 4 °C for 30 min in a final volume of 20 μl containing 12.5% glycerol, 12.5 mM HEPES (pH 7.9), 4 mM Tris–HCl (pH 7.9), 60 mM KCl, 1 mM EDTA and 1 mM DTT with 1 μg of poly(dI–dC). Binding products were resolved on 5% polyacrylamide gel and the bands were visualized by autoradiography.

2.13. Transient transfection and NF-κB luciferase assay

A day before transfection, cells were sub-cultured at a density of 1×10^6 cells in 60 mm dish to maintain approximately 60–80% confluency. The cells were transiently transfected with the plasmid harboring NF-κB promoter using the transfection reagent DOTAP according to the instructions given by the manufacturer (Roche, Mannheim, Germany). After overnight transfection, cells were treated with 30 μM of eckol for 24 h. Cell were then washed twice with PBS and lysed with reporter lysis buffer (Promega, Madison, WI, USA). After vortex-mixing and centrifugation at $12000 \times g$ for 1 min at 4 °C, the supernatant was stored −70 °C for the luciferase assay. After 20 μl of the cell extract was mixed with 100 μl of the luciferase assay reagent at room temperature, the mixture was placed in a luminometer to measure the light produced.

2.14. Statistical analysis

All the measurements were made in triplicate and all values were represented as mean ± S.E. The results were subjected to an analysis of the variance using the Tukey test to analyze the difference. $P < 0.05$ were considered significantly.

3. Results

3.1. Effect of eckol on radical scavenging and inhibition of lipid peroxidation

The radical scavenging effect of eckol on DPPH free radical, H_2O_2 and hydroxy radical scavenging activities was measured. Eckol exhibited quenching effects on these three ROS at dose-dependent manner (Fig. 2A–C). In addition, the radical scavenging effect of eckol on the intracellular ROS was measured. The intracellular ROS scavenging activity of eckol was 79% at 30 μM (Fig. 2D). As shown in Fig. 2E, the fluorescence intensity of DCF-DA staining was enhanced in H_2O_2-treated V79-4 cells. However, eckol reduced the red fluorescence intensity upon H_2O_2 treatment, thus reflecting a reduction in ROS generation. The ability of eckol 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 eckol. The inhibitory effect of eckol was 31% at 30 μM when compared to 4% inhibition in untreated group (Fig. 2F).

3.2. Effect of eckol on cell damage induced by oxidative stress

The protective effect of eckol on cell survival in H_2O_2-treated V79-4 cells was measured. Cells were treated with eckol at various concentrations for 1 h prior to the addition of H_2O_2. The cell viability was determined 24 h later by MTT assay. As shown in Fig. 3A, treatment with eckol induced a dose dependent increase in the cell survival rate. In order to study the cytoprotective effect of eckol 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 Fig. 3B showed that the control cells possessed intact nuclei, and the H_2O_2-treated cells showed significant nuclear fragmentation, a characteristic feature of apoptosis. However, when the cells were treated with eckol 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 eckol against apoptosis was also confirmed by flow cytometry. As shown in Fig. 3C, 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 1% of apoptotic sub-G_1 DNA content in untreated cells. Treatment with 30 μM of eckol decreased the apoptotic sub-G_1 DNA content to 34%. It is reported that serum starvation or irradiation produces a marked accumulation of ROS and results in cell death [12,13]. Hence, we examined whether eckol exhibits ROS scavenging effect and protective effect upon serum starvation or γ-radiation. The ROS scavenging effect by eckol was determined after 6 h of serum starvation or after 24 h of γ-radiation at 5 Gy. As shown in Fig. 3D, 30 μM of eckol exhibited 47% of ROS scavenging activity upon serum starvation and 43% upon γ-radiation. The cell survival was determined after 6 h of serum starvation or after 24 h of γ-radiation. As shown in Fig. 3E, eckol increased the cell survival rate to 21% upon serum starvation and 11% upon γ-radiation. These results suggest that eckol protects cell damage induced by oxidative stress.

3.3. Effect of eckol on catalase

In order to investigate whether the radical scavenging activity of eckol was mediated by the activity of antioxidant enzyme, the activity of catalase in eckol-treated V79-4 cells was measured. Eckol increased the activity of catalase at dose-dependent manner (Fig. 4A), showing 22, 28 and 41 U/mg protein at 0.3, 3 and 30 μM of eckol, respectively, as compared to 15 U/mg protein of the control. To confirm the activation of catalase by eckol in terms of protein expression, the Western blot analysis was performed. As shown in Fig. 4B, in the presence of eckol, the protein expression of catalase was increased. The 3-amino-1,2,4 triazol (ATZ) is known as a specific inhibitor of catalase [14]. To determine the effect of catalase inhibitor on eckol induced cytoprotection from H_2O_2 induced damage, V79-4 cells were pre-treated with 20 mM of ATZ for 1 h, followed by 30 min of incubation with eckol and exposure to 1 mM H_2O_2 for 24 h. As shown in Fig. 4C, ATZ treatment abolished the protective activity of eckol in H_2O_2 damaged cells. It is reported that most of the polyphenolic compounds interact with commonly used cell culture media to generate H_2O_2 [15]. The generated low level of H_2O_2 can trigger a rise in the levels of antioxidant enzymes. To confirm whether eckol generates H_2O_2 in the media, eckol was added to cell culture media at a final concentration of 30 μM and the amount of generated H_2O_2 was measured by the ferrous iron oxidation-xylenol orange assay. H_2O_2 was detected in minute levels in eckol-treated media (<3 μM of H_2O_2), suggesting that the antioxidant activities in eckol-treated cells were not increa-sed by H_2O_2 generated in eckol-treated media.

3.4. Effect of eckol on ERK protein and NF-κB transcription factor

It is reported that the expression and the activity of catalase are increased through NF-κB transcription factor and extracellular signal-regulated kinase (ERK) protein (an upstream of NF-κB) [16,17]. To better understand the protective mechanism of eckol 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 Fig. 5A, within 12 h eckol dramatically activated phosphorylated ERK. However, there was no change in the total ERK protein level. To determine the effect of ERK inhibitor on protection of eckol from H_2O_2-induced damage, V79-4 cells were pre-treated for 30 min with U0126 (10 nM), a specific inhibitor of ERK kinase,

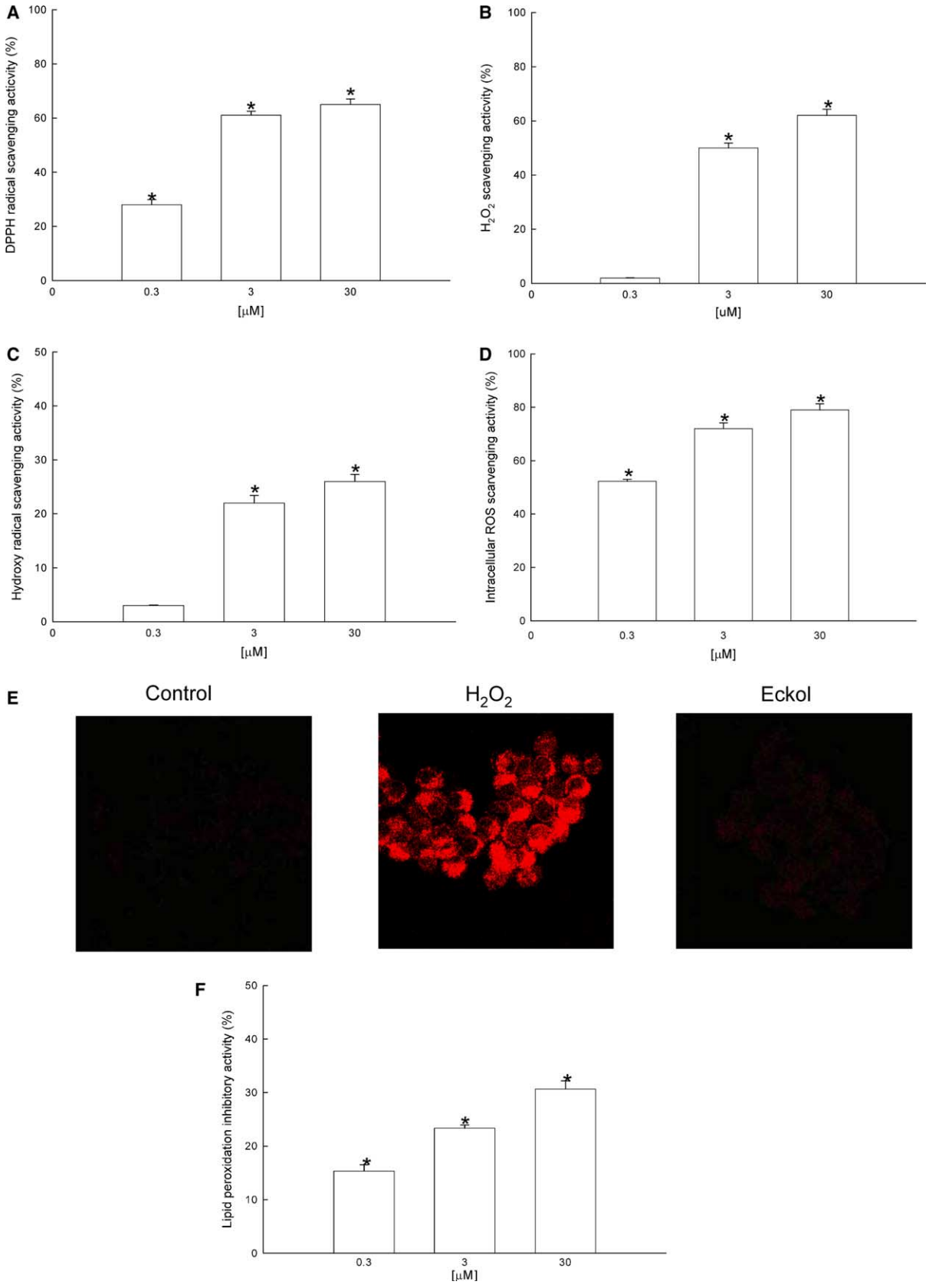

Fig. 2. Effect of eckol on scavenging ROS and inhibition of lipid peroxidation. The amount of DPPH radicals (A), H_2O_2 (B) and hydroxy radical (C) was determined spectrophotometrically. The intracellular ROS generated was detected by DCF-DA method (D) and by confocal microscopy (E). Representative confocal 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 eckol (original magnification × 400). (F) Lipid peroxidation was assayed by measuring the amount of TBARS. *Significantly different from control ($P < 0.05$).

followed by 30 min incubation with eckol and exposure to 1 mM H_2O_2 for 24 h. As shown in Fig. 5B, U0126 treatment abolished the protective activity of eckol in H_2O_2 damaged cells. Subsequently, we examined the effect of eckol on NF-κB, which is a downstream target of phospho-ERK pathway. As shown in Fig. 5C and D, eckol treatment increased the expression of NF-κB protein (p65 kDa) in nuclear fraction, showing decreased IκBα protein expression (p37 kDa) and accumulated NF-κB protein in the nucleus. NF-κB activation in eckol-treated cells was assessed by the EMSA with an oligonucleotide harboring a consensus NF-κB binding element. As shown in Fig. 5E, eckol-treated cells exhibited a high level of NF-κB activation. The transcriptional activity of NF-κB was also assessed using an NF-κB reporter plasmid containing the NF-κB binging DNA consensus site linked to a luciferase reporter gene. As shown in Fig. 5F, eckol was found to increase the transcriptional activity of NF-κB. To determine the effect of NF-κB inhibitor on protection of eckol from H_2O_2 induced damage, V79-4 cells were pre-treated for 30 min with lactacystin (10 μM), a NF-κB inhibitor via proteasome inhibition, followed by 30 min incubation with eckol and exposure to 1 mM H_2O_2 for 24 h. As shown in Fig. 5G, lactacystin

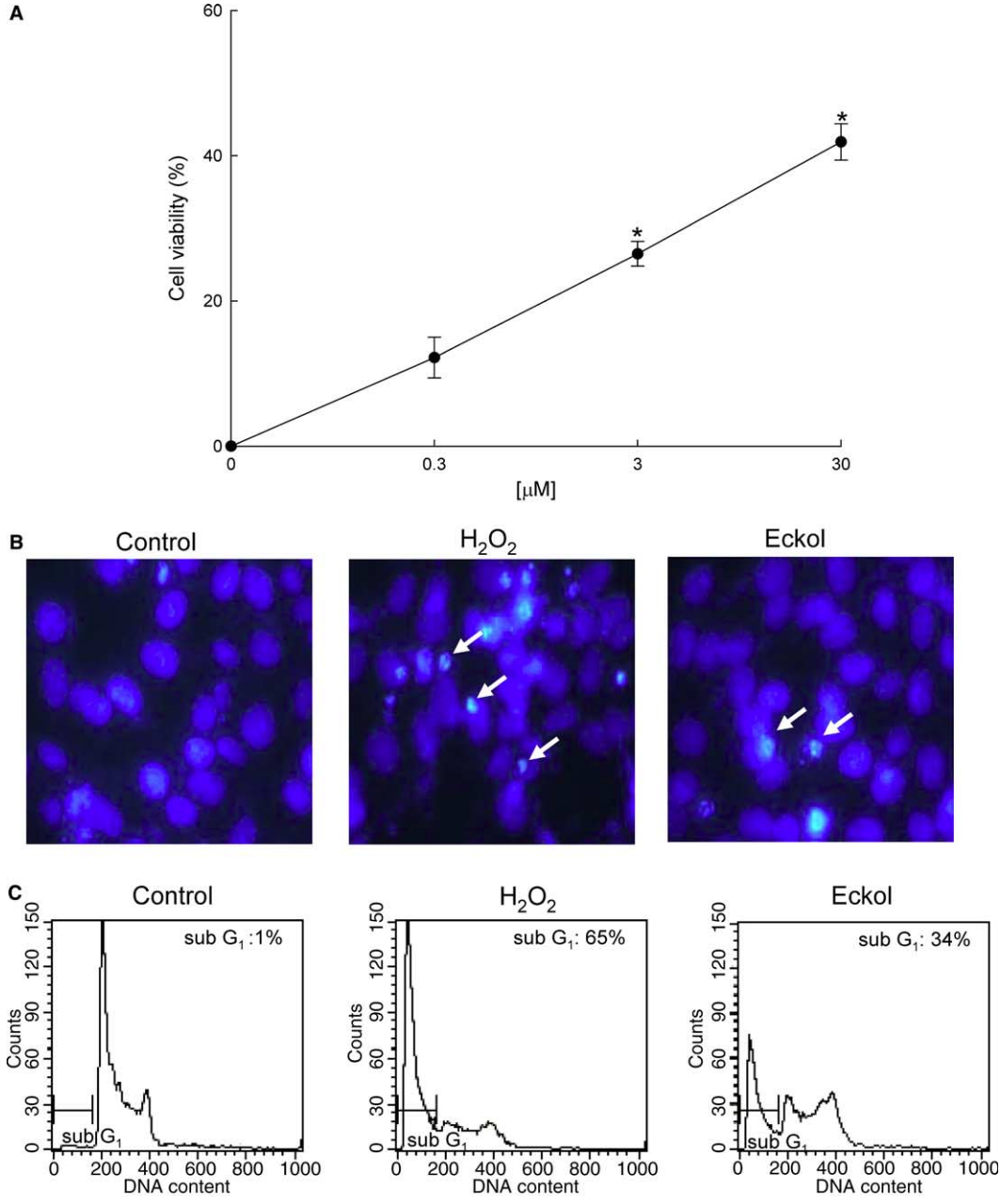

Fig. 3. Protective effect of eckol on oxidative stress damaged V79-4 cells. The viability of V79-4 cells damaged by H_2O_2 (A) was determined by MTT assay. *Significantly different from control ($p < 0.05$). Apoptotic body formation (B) was observed under a fluorescent microscope after Hoechst 33342 staining and is indicated by arrows. Apoptotic sub-G_1 DNA content (C) was detected by flow cytometry after propidium iodide staining. The intracellular reactive oxygen species (D) generated by serum starvation or radiation at 5 Gy was detected by DCF-DA method. The viability of V79-4 cells (E) upon serum starvation or radiation at 5 Gy was determined by MTT assay. The measurements were made in triplicate and values are expressed as means ± S.E. *Significantly different from serum starved cells ($P < 0.05$). **Significantly different from irradiated cells ($P < 0.05$).

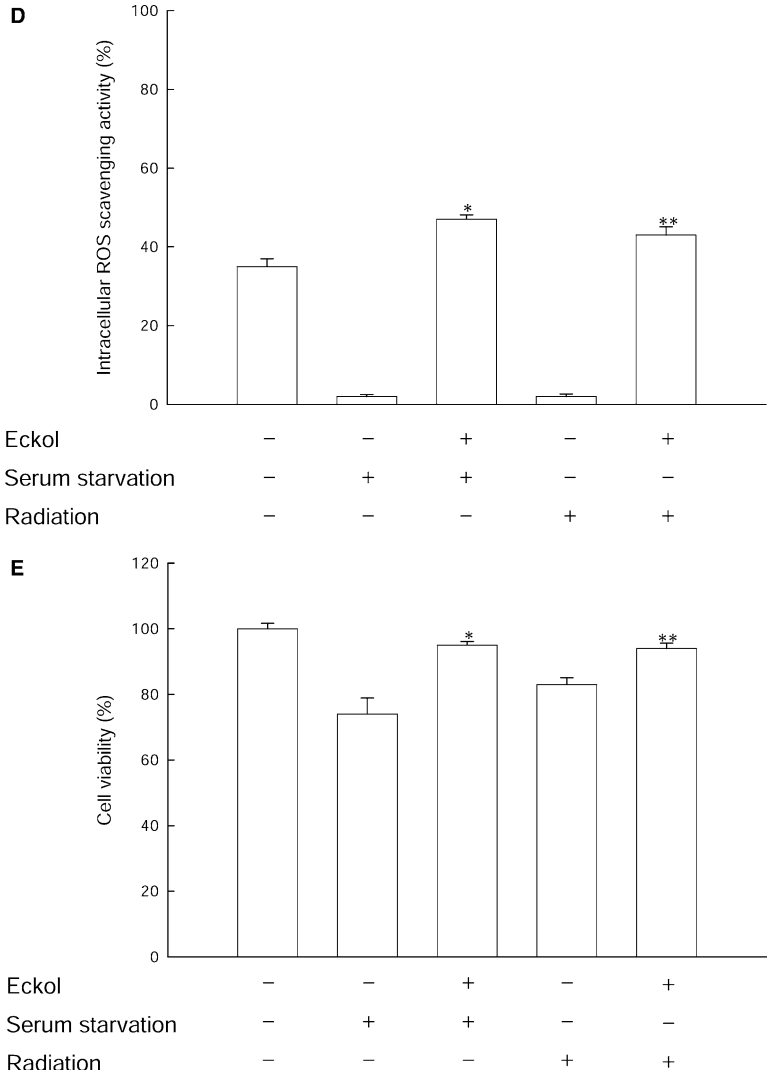

Fig. 3 (*continued*)

treatment abolished the protective activity of eckol in H_2O_2 dama-ged cells.

4. Discussion

Phlorotannin components, which are marine algal polyphenols and especially found in brown algae, are polymers of phloroglucinol [18]. They are commonly known to have defensive or protective functions against herbivores. Although some reports suggest that phlorotannins from algae exhibit antioxidant effect on free radicals [3,19,20], there are no reports on the cytoprotective effect against oxidative stress and the underlying mechanism of eckol. In our present study, it was observed that upon exposure to H_2O_2, eckol decreased the level of ROS. Eckol has a polyphenolic structure and polyphenols are electron-rich compounds, which are prone to enter into efficient electron-donation reactions and produce phenoxyl radical ($PhO^{\bullet}$) species as intermediates in the presence of oxidizing agents. Phenoxyl radicals are stabilized by resonance delocalization 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 [21]. This intrinsic stability of phenolic structures might be related to antioxidative activity of eckol. 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 eckol 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 (data not shown), indicating that the inhibition of ROS formation may be important for cytoprotection against oxidative damage. In addition, eckol showed cytoprotective effect against H_2O_2 exposed to murine macrophage (RAW 264.7) cells and Chinese hamster ovary (CHO) cells (data not shown).

Catalase is located at the peroxisome and converts hydrogen peroxide into molecular oxygen and water. Catalase plays important roles in cellular protection by oxidative stress induced cell damages [22]. Eckol was found to increase catalase

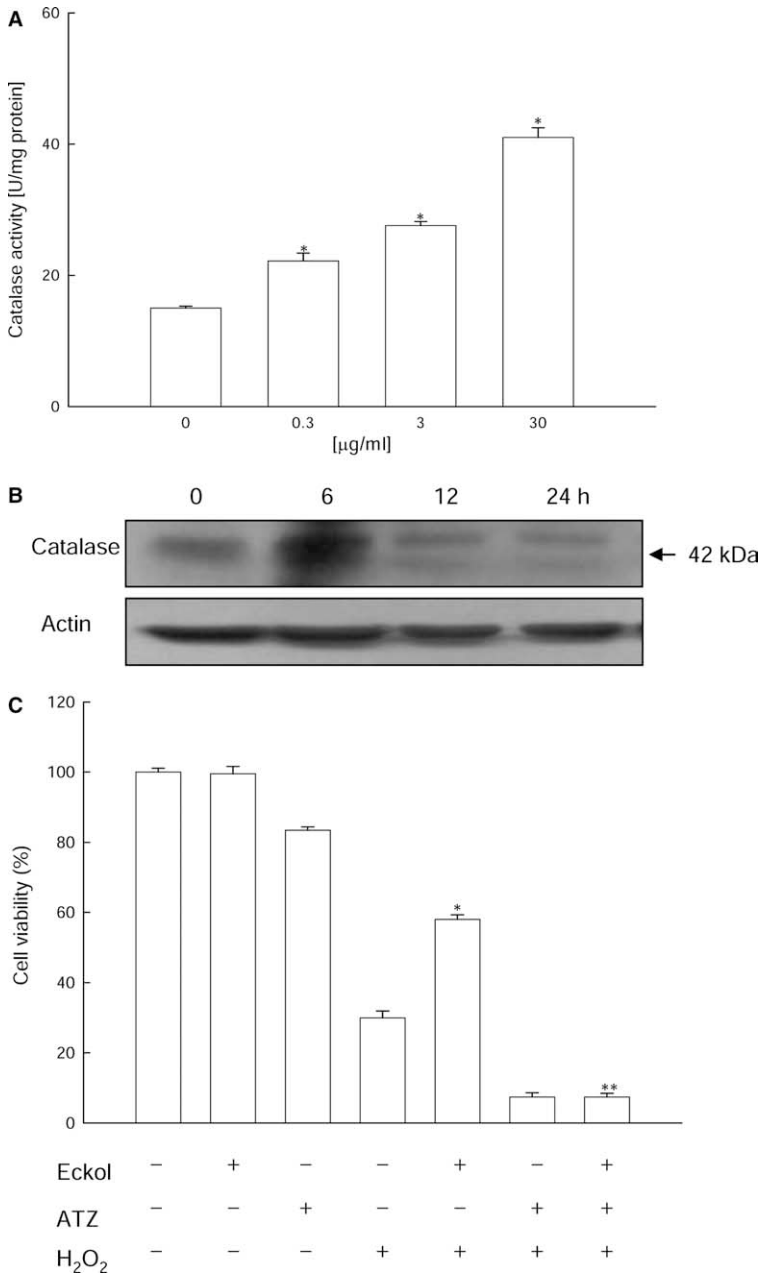

Fig. 4. Effect of eckol on catalase. The enzyme activities (A) are expressed as average enzyme unit per mg protein ±S.E. *significantly different from control ($P < 0.05$). Cell lysates were electrophoresed and the expression of catalase (B) was detected by its specific antibody. After treatment of ATZ, eckol or/and H_2O_2, the viability of V79-4 cells (C) was determined by MTT assay. *Significantly different from H_2O_2-treated cells ($P < 0.05$), **Significantly different from eckol plus H_2O_2 treated cells ($P < 0.05$).

activity, suggesting that the scavenging of ROS may be related to the increase in antioxidant enzyme activity. Therefore, the effects of eckol on cell viability might involve dual actions: direct action on oxygen radical scavenging, as shown by DPPH radical, H_2O_2, OH radical scavenging, and indirect action through induction of anti-oxidative enzymes. Many studies are demonstrating the antioxidant activity of polyphenols, in terms of scavenging activity of hydroxyl, superoxide anion and DPPH radical and H_2O_2. Most of the polyphenols shown to have strong antioxidative activity have intrinsic virtue of the hydrogen donating capacity of their phenolic groups [23]. Hence, the scavenging effect of antioxidants on radical is thought to be due to their hydrogen donating ability and electron delocalization. From the results we obtained, eckol might have relatively lower ability as hydrogen donor than other polyphenols, for dibenzo-1,4-dioxin moiety in eckol lack conjugation between rings, restricting its radical delocalization. However, eckol increased antioxidant enzyme activity, which might be the uniqueness of eckol unlike other polyphenols. Catalase regulates the cell growth via activation of ERK pathway, leading to the acceleration of the cell growth inhibited by oxidative stress [24]. The phosphorylation of ERK can phosphorylate cytoplasmic and nuclear targets and participates in a wide range of cellular programs including

proliferation, differentiation and movement [25,26]. The level of phosphorylated ERK in eckol-treated cells was induced, and upon treatment with U0126 (a specific inhibitor of ERK kinase), the protective activity of eckol in H_2O_2 damaged cells was abolished, which suggests that the protective effect of eckol on cells may also be involved in activating ERK pathway. Recent reports demonstrated that the ERK signaling cascade has been implicated in NF-κB activation through phosphorylation of inhibitory IκBα [27]. The association between the ERK signaling cascade and NF-κB activation is also supported by the finding that the ERK-regulated kinase p90RSK phosphorylates and thereby inactivates IκBα in response to mitogenic stimuli [28]. Our present study revealed that the transcription activity of NF-κB in nucleus is elevated in eckol-treated cells and lactacystin, NF-κB inhibitor, abolished the protective activity of eckol against H_2O_2 damaged cells, supporting the involvement of NF-κB in cell survival by eckol. The molecular mechanisms underlying the anti-apoptotic effect/cell protective effect of NF-κB against oxidative stress induced cell damage contributed to induction of catalase, and blocking the activation of NF-κB led to downregulation of catalase [16]. Based on our results, we suggest that enhanced NF-κB via phospho-ERK activated by eckol provides augmentation of catalase capacity, resulting in cell protective effect against oxidative stress. In addition, Akt, also known as PKB, is activated via PI3K-dependent signaling pathway and has received considerable attention as an important antiapoptotic protein through which various survival signals suppress cell death [29]. Recent studies have demonstrated that Akt enhances the degradation of IκBα and to cooperate with other factors, thereby inducing the promoter activity of NF-κB [30]. In addition, PC12 cells overexpressing bcl-2 exhibited activated NF-κB via Akt activation and enhanced the catalase activity and result in cytoprotective effect against oxidative stress [16], suggesting that Akt is also responsible for protection of cells from oxidative stress. There-

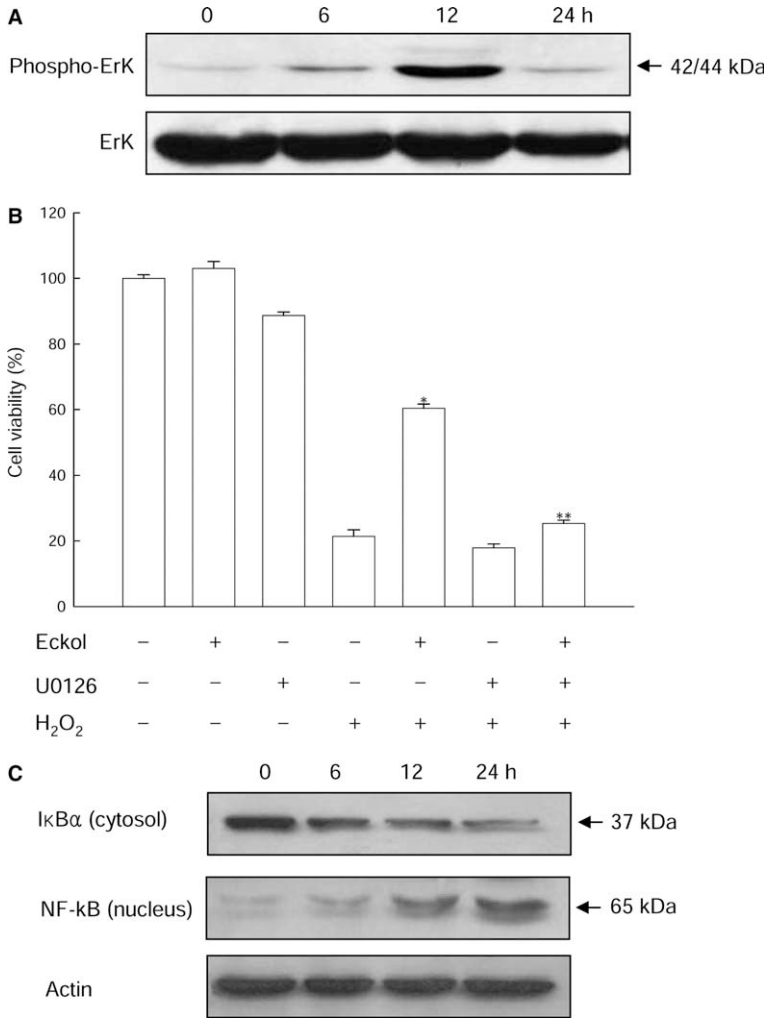

Fig. 5. Effect of eckol on ERK protein and NF-κB transcription factor. Cell lysates were electrophoresed and proteins of ERK2 and phospho-ERK1/2 (A) were detected by their respective specific antibodies. After treatment with U0126, eckol or/and H_2O_2, the viability of V79-4 cells (B) was determined by MTT assay. *Significantly different from H_2O_2-treated cells ($P < 0.05$), **Significantly different from eckol plus H_2O_2-treated cells ($P < 0.05$). Cytoplasmic IκBα and nuclear NF-κB (p65) from eckol-treated cells (C) were detected with antibodies specific for IκBα and NF-κB (p65), respectively. NF-κB (p65) translocated into the nucleus (D) from eckol-treated cells was detected using immunocytochemical analysis. DNA binding activity of NF-κB (E) from eckol-treated cells was detected using EMSA. Transcriptional activity of NF-κB (F) in eckol treated cells was assessed using plasmid containing NF-κB binding site-luciferase construct. *Significantly different from control ($P < 0.05$). After treatment with lactacystin, eckol and H_2O_2, the viability of V79-4 cells (G) was determined by MTT assay. *Significantly different from eckol plus H_2O_2-treated cells ($P < 0.05$).

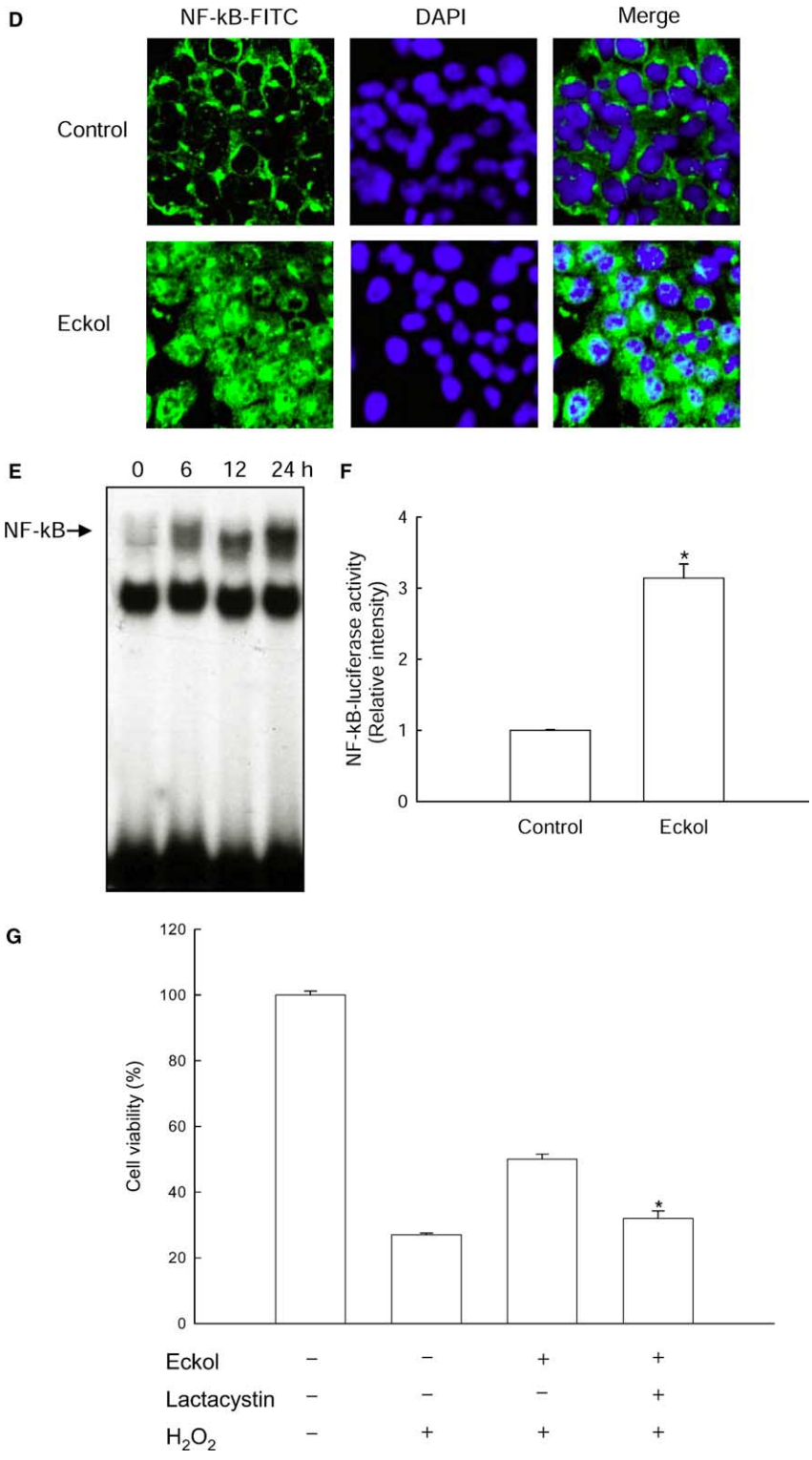

Fig. 5 (*continued*)

fore, further studies are needed to demonstrate that eckol may have possible alternate mechanism to promote catalase production.

Polyphenols are the most abundant antioxidant in our food diet. Dietary intake of polyphenols for prevention of disease related with oxidative damage is very important for maintaining healthy life. There are several reports regarding bioavailability of polyphenols from edible plants [31–33]. Seaweeds have been used as food stuff in the Asian diet for centuries. And they have some of valuable components for food application and medicinal use [34]. However, there is no report on the bioavailability of seaweed chemical components in dietary intake compared to plant foods. Evaluation of the bioavailability and metabolism of eckol is remained for further research.

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