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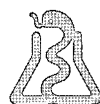

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Antioxidant and Cytotoxicity Effects of Luteolin

Jinny Park[1], Kyoung Ah Kang[2], Rui Zhang[2], Mei Jing Piao[2], Soyoon Park[3], Ju Sun Kim[4], Sam Sik Kang[4] and Jin Won Hyun[2]

[1]*Division of Hematology/Oncology, Internal Medicine, Gachon University Gil Medical Center, Incheon 405-760*
[2]*Department of Biochemistry, College of Medicine, Cheju National University, Jeju-si 690-756*
[3]*Department of Pathology, College of Medicine, Cheju National University*
[4]*Natural Products Research Institute and College of Pharmacy, Seoul National University, Seoul 110-460, Korea*

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ABSTRACT. Flavonoids are polyphenolic compounds that are ubiquitous in plants. They have been shown to possess a variety of biological activities, such as antioxidant and anticancer. Reactive oxygen species (ROS) lead to damages of cellular molecules and it is the one of various factors to induce cancer. The one of flavonoids, Luteolin, was found to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and intracellular reactive oxygen species (ROS). Moreover luteolin showed protection on hamster lung fibroblast cells (V79-4 cell) damage induced by H_2O_2. And then it was investigated whether it may show cytotoxicity effect against various cancer cells by MTT. Luteolin at 10 μg/ml showed the cell viability of 63.2%, 34.7%, 18.4% and 71.4% against NCI-H460, HeLa, U937 and MCF-7, respectively. As a result, luteolin shows more sensitive to U937 cells among the tested cancer cell lines. In summary, luteolin has antioxidant and cytotoxicity effect on various cancer cell lines.

Keywords: Oxidative stress, Reactive oxygen species, Cytotoxicity

INTRODUCTION

Oxidative stress has been reported as the cause of several age related and chronic diseases such as cancer, diabetes, neurodegenerative and cardiovascular diseases (Tiwari, 2004; Cui *et al.*, 2004; Ceriello and Motz, 2004; Klaunig and Kamendulis, 2004; Willcox *et al.*, 2004; Ballinger, 2005; Gibson and Huang, 2005). Although the available data are still limited, epidemiological studies indicate that dietary habits can influence the incidence of oxidative stress diseases (De *et al.*, 1997; Orgogozo *et al.*, 1997; Golbe *et al.*, 1998; Lemeshow *et al.*, 1998; Youdim *et al.*, 2002). Dietary flavonoids are natural antioxidants (Kandaswami and Middleton, 1994) and found in various plant products such as fruits, leaves, seeds, oils and plant-derived beverages such as tea and wine (Larson, 1988). Humans have consumed flavonoids and other dietary phenolics compounds and over 4000 different natural flavonoids have been described (Middleton and Kandaswami, 1994).

Correspondence to: Jin Won Hyun, Department of Biochemistry, College of Medicine, Cheju National University, Jeju-si 690-756, Korea
E-mail: jinwonh@cheju.ac.kr

Antioxidants, such as fat-soluble vitamin E and more hydrophilic flavonoids, possess free radical scavenging properties. Experimental studies support that dietary antioxidants (eg, vitamin E, vitamin C, β-carotene, and other phytochemicals) as well as endogenous antioxidants (eg, glutathione) that neutralize or trap ROS act as cancer preventive agents (Borek, 1997; Borek *et al.*, 1986). Recent evidences suggested that vitamin E and its analogues may not only protect cells from free radical damage but also induce apoptotic cell death in malignant cell lines and inhibit tumorigenesis *in vivo*

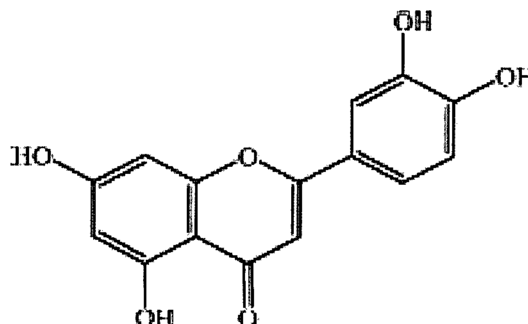

Fig. 1. Chemical structure of luteolin.

(Birringer *et al.*, 2003; Stapelberg *et al.*, 2004).

In present study, we have tested the antioxidant effect from luteolin (Fig. 1), a polyphenolic flavonoid compound, against radicals. In addition, it was investigated whether it may show protective effect against oxidative damage on V79-4 cell and cytotoxic effect against various cancer cells.

MATERIALS AND METHODS

Compound

Luteolin was obtained from Dr. Sam Sik Kang (Seoul National University, Seoul, Korea).

Reagents

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, 2',7'-dichlorodihydrofluorescein diacetate (DCF-DA), and [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium] bromide (MTT) were purchased from Sigma Chemical Company (St. Louis, MO, USA).

Cell culture

To study the antioxidative effects of luteolin against oxidative stress, it was used V79-4 (Chinese hamster lung fibroblasts cells). To study the cytotoxic effect of luteolin against cancer cells, NCI-H460 (human non-small cell lung), HeLa (human cervix cancer cell), U937 (human monocytic leukemia cell) and MCF-7 (human breast cancer cell) from the American Type Culture Collection were used. These cancer cells were maintained at 37°C in an incubator with a humidified atmosphere of 5% CO_2 and were cultured in Dulbecco's modified Eagle's medium and RPMI 1640 medium containing 10% heat-inactivated fetal calf serum, streptomycin (100 μg/ml) and penicillin (100 units/ml).

DPPH radical scavenging activity

Luteolin were added to a 1×10^{-4} M solution of DPPH radical in methanol, and the reaction mixture was shaken vigorously. After 1 h, the amount of residual DPPH was determined at 520 nm using a spectrophotometer (Lo *et al.*, 2004).

Intracellular ROS measurement

The DCF-DA method was used to detect the intracellular ROS level (Rosenkranz *et al.*, 1992). 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. Sixteen hours after plating, the cells were treated with luteolin 1 h 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(PerkinElmer Life Science Inc., Foster City, CA USA).

Cell viability

The cell viability of luteolin against various cancer cells and oxidative damage are 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 (Carmichael *et al.*, 1987). Luteolin were treated and incubated for 24 h. The 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.

Nuclear staining with Hoechst 33342

Cells were placed in a 24 well plate at 1×10^5 cells/ml. At 16 h after plating, the cells were treated with luteolin at 10 μg/ml and after further incubation for 1 h, 1 mM H_2O_2 was added to the culture. Cells were placed in a 24 well plate at 1×10^5 cells/ml. The cells were treated with luteolin at 10 μg/ml. 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.

Statistical analysis

All the measurements were made in triplicate and all values were represented as means ± standard error. The results were subjected to an analysis of the variance (ANOVA) using the Tukey test to analyze the difference. $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

To find the antioxidant effects from luteolin, we tested the scavenging effect of DPPH radical and intracellular ROS. As shown in Table 1, luteolin showed DPPH radical scavenging effect, 2.2%, 11.3% and 65.7% at 0.1, 1 and 10 μg/ml, respectively. In intracellular ROS scavenging effect, luteolin showed 24.4%, 38.0% and 60.2% at 0.1, 1 and 10 μg/ml, respectively (Table 2). And then it was detected using MTT assay whether luteolin has

Table 1. Effect of luteolin on scavenging DPPH radical

Luteolin (μg/ml)	Scavenging %
0	0 ± 0.7%
0.1	2.2 ± 1.2%
1	11.3 ± 0.5%*
10	65.7 ± 5.2%*

The amount of DPPH radicals was determined spectrophotometrically. The measurements were made in triplicate and values are expressed as mean ± standard error. *Significantly different from control ($p < 0.05$).

Table 2. Effect of luteolin on scavenging intracellular ROS induced by H_2O_2

Luteolin (μg/ml)	Scavenging %
0	0 ± 2.4%
0.1	24.4 ± 4.9%*
1	38.0 ± 2.2%*
10	60.2 ± 1.3%*

The intracellular ROS was detected by DCF-DA method. The measurements were made in triplicate and values are expressed as mean ± standard error. *Significantly different from control ($p < 0.05$).

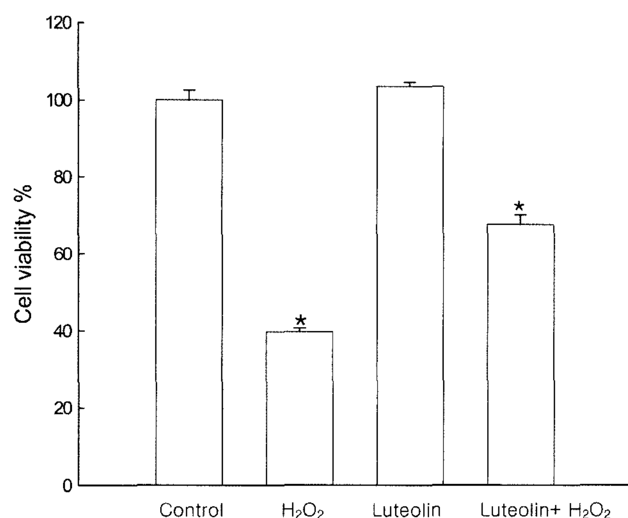

Fig. 2. Cytoprotective effect of luteolin on H_2O_2 induced V79-4 cells damage. The viability was determined by MTT assay. The measurements were made in triplicate and values are expressed as means ± standard error. *Significantly different from control ($p < 0.05$).

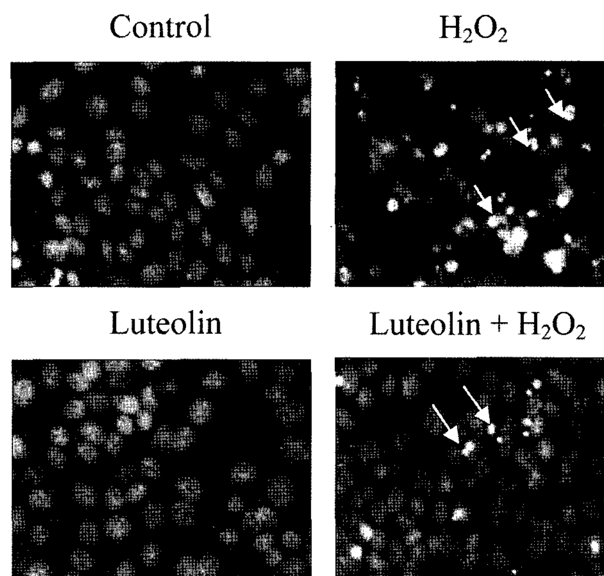

Fig. 3. Cytoprotective effect of luteolin on H_2O_2 induced V79-4 cells damage. Apoptotic body formation was observed under a fluorescent microscope after Hoechst 33342 staining and apoptotic bodies are indicated by arrows.

Table 3. Cell viability of luteolin of various cancer cells

Cell line	Luteolin
NCI-H460	63.2 ± 0.8%*
HeLa	34.7 ± 5.4%*
U937	18.4 ± 0.2%*
MCF-7	71.4 ± 4.4%*

The cell viability of control was estimated as 100% using MTT assay. The measurements were made in triplicate and values are expressed as mean ± standard error. *Significantly different from control ($p < 0.05$).

cytoprotective effect against H_2O_2-induced V79-4 cells damage and cytotoxic effect against various cancer cells. Luteolin at 10 μg/ml showed the cell viability of 67.6%, compared to 39.7% in only H_2O_2 treatment (Fig. 2). In order to study the cytoprotective effect of luteolin on cytotoxicity induced by H_2O_2, nuclei of V79-4 cells were stained with Hoechst 33342 for microscopy. The microscopic pictures in Fig. 3 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 luteolin for 1 h prior to H_2O_2 treatment, a dramatic decrease in nuclear fragmentation was observed. Therefore luteolin has cytoprotective effect on H_2O_2-induced oxidative damage. And luteolin at 10 μg/ml showed the cell viability of 63.2%, 34.7%, 18.4% and 71.4% against NCI-H460, HeLa, U937 and MCF-7, respectively (Table 3). In order to study the cytotoxic effect of luteolin on cancer cell, nuclei of U937 cells were stained with Hoechst 33342 for microscopy. The microscopic pictures in Fig. 4 showed that cells were treated with luteolin for 24 h treatment, a dramatic increase in nuclear fragmentation was observed. Therefore, luteolin shows sensitive to U937 cells among tested cell lines. Flavonoids are plant polyphenolic components and are contained high concentrations in tea, apples, grapes, and vine

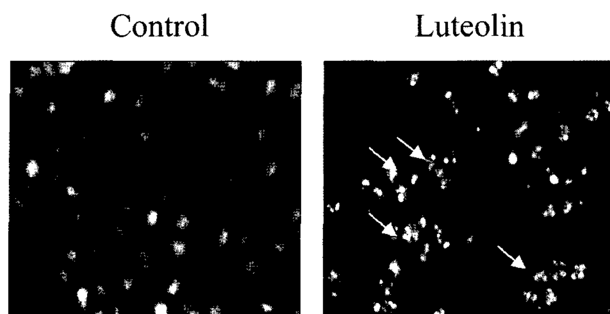

Fig. 4. Cytotoxicity effect of luteolin on U937 leukemia cells. Apoptotic body formation was observed under a fluorescent microscope after Hoechst 33342 staining and apoptotic bodies are indicated by arrows.

(Tichopad *et al.*, 2005). Polyphenols have an ideal and intrinsic structure of capturing of free radicals and electron delocalization, causing higher antioxidant activity (Sangeetha *et al.*, 1990). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet-oxygen quenchers (Lenton and Greenstock, 1999). It is composed of a phytyl chain and a chromanon ring may be incorporated into the biological membrane, thus contributing to their physical stability, which can be a function of interaction with ROS, preventing lipid peroxidation and subsequent membrane disorganization (Young and Lowe, 2001; Rodemann *et al.*, 1999). Luteolin belongs to the flavone subclass of flavonoids, usually occurring as glycosylated forms in celery, green pepper, perilla leaf and camomile tea (Shimoi *et al.*, 1998, 2000). It has been reported to display antimutagenic, antiplatelet aggregation and anticancer effects (Lu *et al.*, 2001; Casagrande and Darbon, 2001). Recently, many reports indicated that ROS participated in cancer cell proliferation and apoptosis (Arai *et al.*, 2003; Wu *et al.*, 2004). Therefore, luteolin-induced cancer cell cytotoxicity is closely associated with antioxidant properties of the luteolin.

In summary, our experiments provide evidence that luteolin has antioxidant and cytotoxicity effect on various cancer cell lines.

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