Antioxidant effects of geraniin via scavenging reactive oxygen species

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Abstract

The present study was to investigate the antioxidant effect of geraniin against free radicals. We found that geraniin scavenged the hydroxyl radicals, which were measured by electron spin resonance (ESR) spectrometry. Geraniin scavenged the DPPH radicals in cell free system. Furthermore geraniin reduced intracellular reactive oxygen species (ROS) detected by 2',7'-dichlorodihydrofluorescein (DCF-DA) dye. Our data suggested that geraniin showed antioxidant effects via scavenging reactive oxygen species. (J Med Life Sci 2009;6:373-375)

Key Words : geraniin, antioxidant effect, reactive oxygen species

Introduction

Reactive oxygen species (ROS) are free radicals such as superoxide anion (O_2^-), hydroxyl radical (OH.) and hydrogen peroxide (H_2O_2). It is highly reactive due to the presence of unpaired valence shell electrons. ROS form as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling[1, 2]. However, Overproduction of ROS is known to cause oxidative modification to DNA, proteins, lipids, and small intracellular molecules. Furthermore, the oxidative stress induced by

overproduction of ROS causes many diseases such as lung cancer, asthma, lung toxicity[3-5]. Gerannin is a flavonoid compound, and has been reported to have antioxidant effect and anticancer effect[6-7]. Many researchers have studied the effect of geraniin in a variety of cells. However, antioxidant effect of geraniin in lung cells has not been reported until now. The present study investigates the antioxidant effect of geraniin via the reduction of ROS.

Materials and methods

Reagents

Geraniin (Fig. 1) was obtained from Professor Nam Ho Lee of Jeju National University. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical and 2',7'-dichlorodihydrofluorescein diacetate (DCF-DA) were purchased from Sigma Chemical Company.

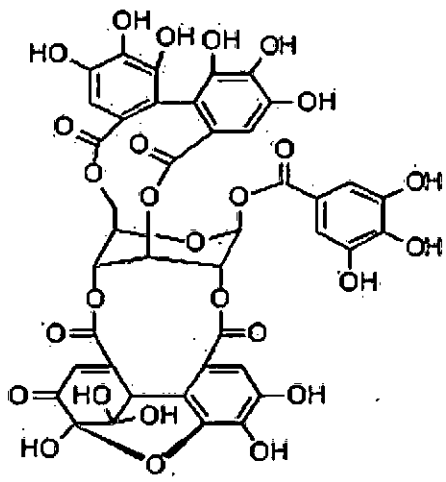
Cell culture

The cells were maintained at 37℃ 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㎍/ml) and penicillin (100 U/ml).

Detection of hydroxyl radical

Hydroxyl radicals were generated by the Fenton reaction (H_2O_2+$FeSO_4$), which were then quickly reacted with a nitrone spin trap, 5,5-dimethyl-1-pyrroline-N-oxide (DMPO). The resultant DMPO-OH adducts were detected

Figure 1. Chemical structure of geraniin.



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using an ESR spectrometer. The ESR spectrum was recorded using JES-FA ESR spectrometer (JEOL, Tokyo, Japan), at 2.5 min after being mixed in a phosphate buffer solution (pH 7.4) with 0.2 ml of 0.3 M DMPO, 0.2 ml of 10 mM $FeSO_4$, 0.2 ml of 10 mM H_2O_2, and 2.5μg/ml luteolin. The parameters of the ESR spectrometer were set at the following conditions: magnetic field of 336.5 mT, power of 1.00 mW, frequency of 9.4380 GHz, modulation amplitude of 0.2 mT, gain of 200, scan time of 0.5 min, scan width of 10 mT, time constant of 0.03 s, and a temperature of 25℃ [8, 9]

DPPH radical scavenging activity

For detection of DPPH radical, geraniin 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.

Intracellular reactive oxygen species measurement

The V79-4 cells were treated with geraniin and 30 min later, 1 mM H_2O_2 was addedto the plate. After 30 min, DCF-DA solution was added and the fluorescence was detected at 485 nm excitation and at 535 nm.

Result

The hydroxyl radicals generated by the Fenton reaction ($FeSO_4$+H_2O_2) in a cell-free system, which was detected by ESR spectrometery. The ESR data revealed that a signal was not observed for the control and geraniin at 10 μg/ml, however, the signal of the hydroxyl radical increased up to 3891 in the $FeSO_4$+H_2O_2 system. Pretreatment of geraniin decreased hydroxyl radical signal to 2861 (Fig.2). The scavenging effect of garaniin on DPPH free radical was measured. As shown in Fig 3, the DPPH radical scavenging activity of garaniin showed the dose-dependent manner. Moreover, pretreatment of geraniin reduced the intracellular ROS levels at dose-dependent manner. (Fig. 4). These data demonstrate that geraniin showed antioxidant effects via scavenging reactive oxygen species

Discussion

Flavonoids are a family of diphenylpropanes most commonly found in a variety of fruits, vegetables. In addition, flavonoids have been reported to exert ROS scavenging effects, antitumor effects and antioxidant effects [10-12]. Although many studies have reported the antioxidant effects of flavonoids, there is no report on the antioxidant effects ofgeraniin in lung fibroblast cells. In this study, we investigate the effect of geraniin in lung fibroblast cells. H_2O_2 is one of the reactive oxygen species and causes the oxidative damage. Hydroxyl radicals generated by the Fenton

Figure 2. Effect of geraniin on the scavenging of hydroxyl radicals. Hydroxyl radicals generated by the Fenton reaction (H_2O_2+$FeSO_4$) were reacted with DMPO, and the resultant DMPO-OH adducts were detected by ESR spectrometry.

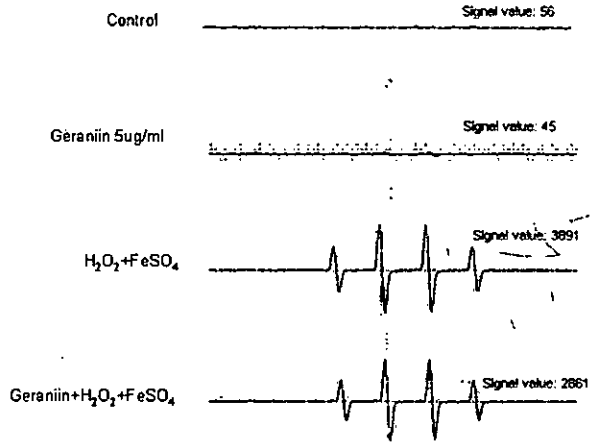


Figure 3. Effect of geraniin on the scavenging of DPPH radicals. The amount of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals was determined spectrophotometrically.

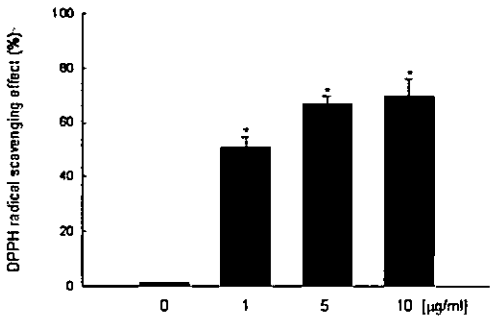
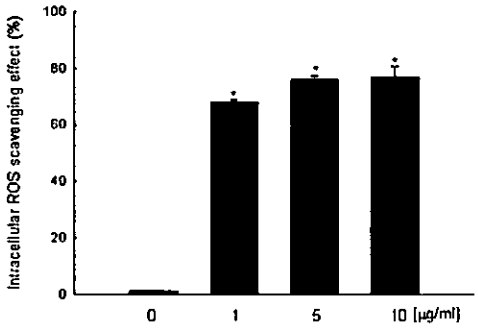


Figure 4. Effect of geraniin on the scavenging of intracellular ROS. Intracellular ROS was detected by the DCF-DA method.



reaction ($FeSO_4+H_2O_2$) in a cell-free system, which was detected by ESR spectrometry. In this study, geraniin scavenged the hydroxyl radicals in a cell-free system. Moreover, geraniin increased the DPPH radical scavenging activity in a dose dependent manner. In addition, geraniin reduced intracellular ROS formation. Taken together we have shown that geraniin can inhibit the ROS formation in Chinese hamster lung fibroblast cells. (V79-4).

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