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A Thesis

for the Degree of Master of Science in Medicine

Cytoprotective Effects of Americanin B against Oxidative Stress in Human Keratinocytes

Jian Zheng

Department of Medicine

Graduate School
Jeju National University

August, 2014

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인간 피부세포에서 산화적 스트레스에 대한 Americanin B의 보호효과

지도교수 현진원

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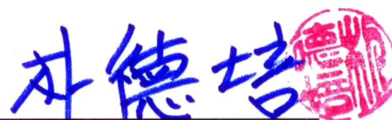
심사위원장 

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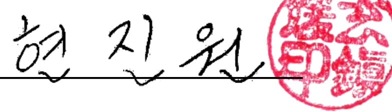
위 원 

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Cytoprotective Effects of Americanin B against Oxidative Stress in Human Keratinocytes

Jian Zheng

(Supervised by Professor Jin-Won Hyun)

A thesis submitted in partial fulfillment of the requirement for the degree of Master of Science in Medicine

2014. 08.

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Abstract

The aim of this study was to evaluate the cytoprotective effects of americanin B, a lignan compound, against hydrogen peroxide (H_2O_2)-induced cell damage. Americanin B decreased the level of DPPH radicals, superoxide anions, hydroxyl radicals and intracellular reactive oxygen species. Americanin B also attenuated DNA damage induced by H_2O_2 treatment, as shown by the inhibition of formation of comet tails indicative of DNA strand breakage, and prevented the oxidation of protein and peroxidation of lipid, as determined by protein carbonyls and 8-isoprostane. Furthermore, americanin B protected against H_2O_2-induced apoptotic cell death, as determined by a reduction in the numbers of apoptotic bodies stained with Hoechst 33342. These findings suggest that americanin B protects cells against oxidative damage by exerting antioxidant effects and inhibiting apoptosis.

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1. Introduction

Reactive oxygen species (ROS), are an unavoidable consequence of aerobic metabolism, which generates free radicals such as superoxide anions (O_2^-) and hydroxyl radicals (·OH), as well as non-radical molecules like hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) (Sharma et al. 2012). At high concentrations, ROS are extremely harmful to living organisms. Although possessing endogenous defense mechanisms responsible for reduction of ROS in organisms, lack of the capacity of cellular antioxidant systems or accumulation in ROS production can give rise oxidative damage to cellular components, potentially resulting in initiation of several pathologic processes (Bandyopadhyay et al. 1999). When cellular antioxidant capacity, which prevents oxidative injury, can't neutralize ROS generation, cells expose to condition termed "oxidative stress" (Tezel 2006). The accumulation of ROS in cytoplasm or organelle can disrupt internal balance of cells by causing peroxidation of lipids (Dmitriev and Titov 2010), oxidative modification of proteins (Lee and Yun, 2012), breaking the structure and function of nucleic acids (Mena et al. 2009), inhibition of antioxidant enzymes (Valko et al. 2007), activation of apoptotic pathways, and eventually cell dysfunction (Sinha et al. 2013).

In this study, the human keratinocyte (HaCaT) cell was chosen as cell model to examine the effect of oxidative stress induced by ROS. The skin is the largest organ of the body and has three main layers, the epidermis, the dermis and the hypodermis. The HaCaT cells are original from the most outer layer of skin, the epidermis, which serves as the physical and chemical barrier between the interior body and exterior environment (Proksch et al. 2008). In order to retard oxidative stress, cells have to precisely and dynamically keep the equilibrium between increase and reduction of ROS. H_2O_2, an inducer of oxidative stress, is capable of induction of cell injury both *in vitro* and *in vivo* (Hsu et al. 2013; Kim et al. 2012; Terashvili et al. 2012). Exogenous abruptly H_2O_2 can exacerbate oxidative stress beyond the counteraction capacity of endogenous antioxidant enzymes, thereby inducing apoptotic cell death by initiating

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mitochondrial dysfunction (Maroto and Perez-Polo 1997). On the other hand, UVB exposure also is a common inducer of oxidative stress, which can induce DNA damage by forming pyrimidine dimers directly (Bohr et al. 1985). In addition, the cytoprotective enzymes in cells protect critical macromolecules such as DNA, proteins, and lipids from ROS-mediated damage, and thus function as the line of defense against oxidative stress (Nguyen et al. 2013).

Plants are rich in flavonoids, stilbenes, and lignans, among which lignans are well studied because of their beneficial biological activity (Dixon 2004). Current work has gradually concentrated on lignans, which show antioxidants (Lee et al. 2009; Mei et al. 2009; Wang et al. 2005; Harper et al. 1999) and anti-inflammatory properties (Lee et al. 2012). Moreover, the pharmacological activity of lignans exerts beneficial health effects including inhibition of cyclooxygenase-2 (Schühly et al. 2009) as well as anti-neuroinflammatory (Kim et al. 2010), and antioxidant activities (Haraguchi et al. 1997; Lee et al. 2004). The skeletal structure of lignan is a group of dimeric phenylpropanoids, where two C6-C3 units are connected by its central C-8 carbon and the link of two C6-C3 units is taken place of carbon-carbon bond or ether oxygen atom in the absence of the C-8 bond, which is named after neolignan (Moss 2000). Lignan has many derivatives by adding different functional groups based on the primary framework and one of these derivatives, bearing electron-donating group or converting to electron-donating group of lignan, such as phenolic hydroxyl group, α, β-unsaturated carbonyl group or alkenyl by specific metabolites and biosynthesized ways (Cunha et al. 2012), can show antioxidant effects (Chen et al. 2002; Farombi et al. 2008). Americanin B ($C_{27}H_{24}O_9$) belonging to lignan possesses phenolic hydroxyl group and unsaturated carbonyl group (Yu et al. 2001) which are supposed to excert antioxidant activity. This study investigated the ability of americanin B to protect human keratinocytes against oxidative stress induced by H_2O_2.

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2. Materials and Methods

2-1. Reagents

N-acetyl cysteine (NAC), 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), 2',7'-dichlorodihydrofluorescein diacetate (DCF-DA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium) bromide (MTT), and Hoechst 33342 dye were purchased from Sigma Chemical Company (St. Louis, MO, USA). Diphenyl-1-pyrenylphosphine (DPPP) was purchased from Molecular Probes (Eugene, OR, USA). Americanin B was provided by Professor Sam Sik Kang (Seoul National University, Seoul, Korea). All other chemicals and reagents were of analytical grade.

2-2. Cell culture

The human keratinocyte cell line HaCaT was supplied by the Amore Pacific Company (Gyeonggi-do, Korea) and maintained at 37°C in an incubator supplemented with an atmosphere containing humidified 5% CO_2 plus 95% air. These cells were grown in RPMI 1640 medium which contains 10% fetal calf serum, streptomycin (100 μg/ml), and penicillin (100 units/ml).

2-3. Cell viability assay

The effect of americanin B on the viability of HaCaT cells was applied by the MTT assay. Cells were seeded on a 96-well plate at a density of 1×10^5 cells/ml, cultured for 16 h, and then treated with 1, 5, 10, or 20 μM of americanin B. After incubation for a further 16 h, MTT stock solution (50 μl, 2 mg/ml) was added to each well to yield a total reaction volume of 250 μl. Four hours later, the supernatants were sucked, and application of the dimethylsulfoxide (DMSO) dissolved formazan crystals in each well, and the absorbance of solution was read at 540 nm on a scanning multi-well spectrophotometer (Carmichael et al. 1987).

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2-4. DPPH radical scavenging activity

Americanin B (1, 5, 10, or 20 μM) or NAC (1 mM) was added to a solution of DPPH (0.1 mM) dissolved in methanol. These mixtures were shaken vigorously and reacted at room temperature for 3 h. After 3 h, the amount of residual DPPH was measured at 520 nm using a spectrophotometer (Brand-Williams et al. 1995). The DPPH inhibition (%) was calculated as follows: % inhibition of DPPH = (absorbance of control - absorbance of americanin B or NAC)/absorbance of control × 100.

2-5. Detection of the superoxide anions

The xanthine/xanthine oxidase system produces superoxide anions, which reacted with a nitrone spin trap, DMPO and formed the reaction product the DMPO/·OOH adducts. The DMPO/·OOH adducts were detected by using a JES-FA electron spin resonance (ESR) spectrometer (JEOL, Tokyo, Japan) (Ueno et al. 1984; Kohno et al. 1994). ESR signaling was read 2.5 min after 20 μl of xanthine oxidase (0.25 U/ml) was mixed with 20 μl each of xanthine (10 mM), DMPO (3 M), and americanin B (10 μM). The ESR spectrometer parameters were set as follows: magnetic field = 336.8 mT, power = 1.00 mW, frequency = 9.4380 GHz, modulation amplitude = 0.2 mT, gain = 500, scan time = 0.5 min, scan width = 10 mT, time constant = 0.03 sec, and temperature = 25°C.

2-6. Detection of hydroxyl radicals

The Fenton reaction ($H_2O_2 + FeSO_4$) system produces hydroxyl radicals, which reacted with a nitrone spin trap, DMPO and formed the reaction product the DMPO/·OH adducts (Li et al. 2003, 2004). The ESR spectrum was recorded 2.5 min after phosphate buffer solution (pH 7.4) was reacted with 20 μl each of 0.3 M DMPO, 10 mM $FeSO_4$, 10 mM H_2O_2, and 10 μM americanin B. The ESR spectrometer parameters were set as follows: magnetic field = 336.8 mT, power = 1.00 mW, frequency = 9.4380 GHz, modulation amplitude = 0.2 mT, gain = 200,

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scan time = 0.5 min, scan width = 10 mT, time constant = 0.03 sec, and temperature = 25°C.

2-7. Detection of intracellular ROS

After seeding in 96-well plate at a density of 1 × 10^5 cells/ml, cells were incubated in incubator for 16 h. Later, they were treated with americanin B and the compound NAC (2 mM), as an antioxidant, was performed for a positive control. After incubation of samples for 1 h at 37℃, H_2O_2 (1 mM) was added to these wells, and the plates were again incubated for 30 min at 37℃. Thirty minutes later, DCF-DA solution (25 μM) was added and waited for ten minutes in dark, and then the fluorescence of 2',7'-dichlorofluorescein (DCF) was detected and recorded with PerkinElmer LS-5B spectrofluorometer (PerkinElmer, Waltham, MA) (Rosenkranz et al. 1992). For imaging analysis of the generation of intracellular ROS, cells were seeded on a 4-well chamber slide at a density of 2 × 10^5 cells/ml. After plating for 16 h, these cells were exposed to 10 μM americanin B. After 1 h, 1 mM H_2O_2 was added to the plate. After 2 h, 100 μM of DCF-DA was added to each well, and the cells were incubated for an additional 30 min at 37°C. After washing with PBS, the stained cells were mounted on the chamber slide with mounting medium (DAKO). The images were obtained on a confocal microscope using the Laser Scanning Microscope 5 PASCAL software (Carl Zeiss).

2-8. Single-cell gel electrophoresis (comet assay)

The breakdown of DNA strand is assessed by the comet assay (Rajagopalan et al. 2003; Singh et al. 2000). The cell pellet was suspended with 0.5% low-melting agarose (LMA, 75 μl) at 39°C, and then it was transfered to a microscopic slide pre-coated with 200 μl of 1 % normal melting agarose. After solidification of the agarose, the slide was covered with another 75 μl of 0.5% LMA and then immersed in a lysis solution (2.5 M NaCl, 100 mM Na-EDTA, 10 mM Tris, 1% Triton X-100, 10% DMSO, pH 10) for 1 h at 4°C. The slides were subsequently placed in a gel electrophoresis apparatus containing 300 mM NaOH and 10 mM

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Na-EDTA (pH 13) for 40 min to allow for DNA unwinding and the expression of alkali-labile damage. An electrical field was then applied (300 mA, 25 V) for 20 min at 4°C to draw the negatively charged DNA towards the anode. The slides were washed three times for 5 min at 4°C in a neutralizing buffer (0.4 M Tris, pH 7.5), stained with ethidium bromide, and observed under a fluorescence microscope and image analyzer (Kinetic Imaging, Komet 5.5, UK). The percentage of the total fluorescence in the comet tails and the tail lengths of 50 cells per slide were recorded.

2-9. Protein carbonyl formation

Cells were treated with 10 μM of americanin B, followed 1 mM of H_2O_2 was added to the plate 1 h later, and the mixture was incubated for 12 h. The amount of carbonyl formation in protein was determined using an Oxiselect^{TM} protein carbonyl enzyme-linked immunosorbent assay kit purchased from Cell Biolabs (San Diego, CA).

2-10. Lipid peroxidation assay

8-Isoprostane, an indicator of lipid peroxidation, is formed in medium secreted from cells and measured by a commercial enzyme immune assay (Cayman Chemical, Ann Arbor, MI, USA) (Beauchamp et al. 2002). Lipid peroxidation was also detected by the image analysis after staining of DPPP (Okimoto et al. 2000). Cells were treated with 10 μM americanin B for 1 h, followed by exposure to 1 mM H_2O_2. Five hours later, 5 mM DPPP was added and incubated for 30 min in the dark. Images of DPPP fluorescence were saved with a Zeiss Axiovert 200 inverted microscope at an excitation wavelength of 351 nm and an emission wavelength of 380 nm.

2-11. Nuclear staining with Hoechst 33342

Hoechst 33342, the DNA-specific fluorescent dye, can interact with DNA and mark the damaged DNA within cells undergoing apoptosis. Cells were treated with americanin B (10

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μM) or NAC (2 mM), and H_2O_2 (1 mM) was added to the plate 1 h later. After additional 12 h incubation at 37°C, cells were incubated with Hoechst 33342 (1.5 μl of a 10 mg/ml stock) for 10 min at 37°C. The bright color of being dyed cells (apoptotic cells) were visualized under a fluorescence microscope equipped with a CoolSNAP-Pro color digital camera.

2-12. Statistical analysis

All measurements were performed in triplicate, and all values are expressed as means ± standard error. The results were subjected to an analysis of variance using Tukey's test to analyze differences between means. In each case, a p-value < 0.05 was considered statistically significant.

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3. Results

3-1. Americanin B attenuates ROS generation.

MTT assay revealed that americanin B itself was not cytotoxic towards HaCaT cells at any concentration used, up to 20 μM (Fig. 1A). Americanin B scavenged the DPPH radical in a concentration-dependent manner– 4% of radicals at 1 μM, 16% at 5 μM, 36% at 10 μM, and 58% at 20 μM. By comparison, the well-known ROS scavenger NAC scavenged 90 % of radicals at a concentration of 2 mM (Fig. 1B). Next, ESR spectrometry was used to investigate the ability of americanin B at 10 μM to eliminate the superoxide anions and hydroxyl radicals. The ESR outcomes indicated that the xanthine/xanthine oxidase system distinctly enhanced the value of superoxide anion signal from 957 in the control to 3637; however, americanin B treatment reduced the superoxide anion signal to 2834 (Fig. 1C). In the same way, americanin B treatment attenuated the production of the hydroxyl radicals from 3011 in the Fenton reaction (H_2O_2 + $FeSO_4$) to 2231 (Fig. 1D).

Next, the ROS-removing activity of americanin B in cells treated with H_2O_2 was explored by probe the intensity of fluorescent dye DCF-DA. The fluorescence spectrometric data revealed that the reduction of intracellular ROS of americanin B was 7% at 1 μM, 31% at 5 μM, 36% at 10 μM, and 45% at 20 μM, compared with 70% for 2 mM NAC (Fig. 1E). Furthermore, confocal microscopy revealed that americanin B at 10 μM ameliorated the increase of intensity caused by H_2O_2 treatment (Fig. 1F), indicating that americanin B treatment reduces ROS generation and this compound possesses ROS-scavenging properties.

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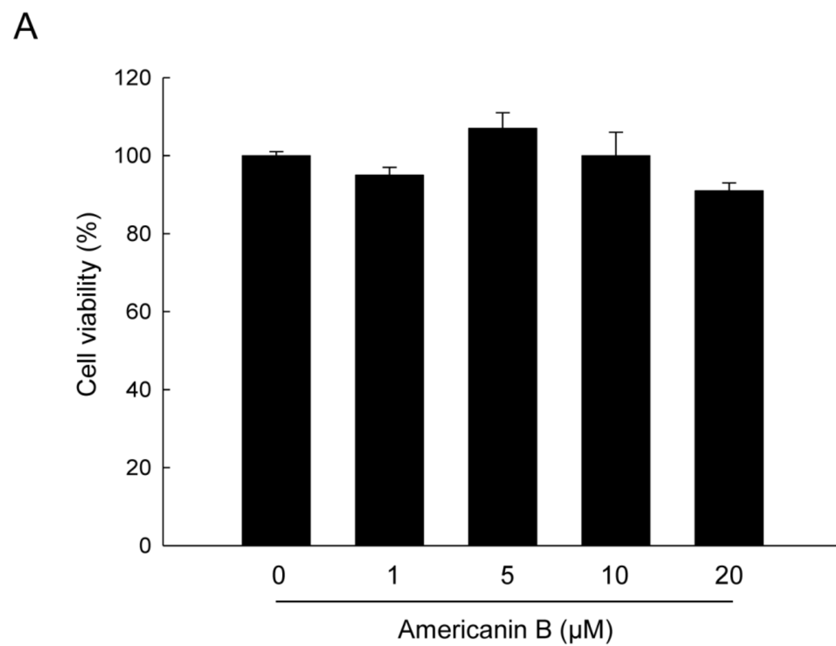
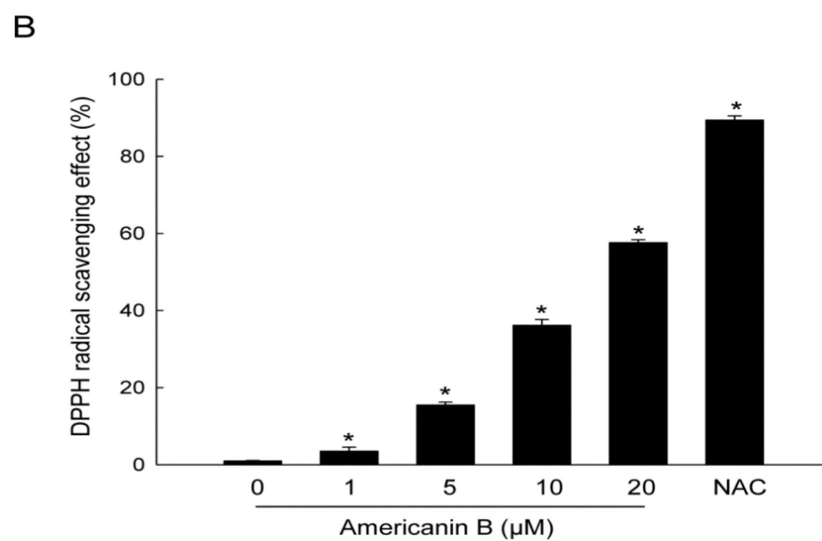
Based on the results of these experiments, the study chose a dose of 10 μM americanin B for subsequent investigations.





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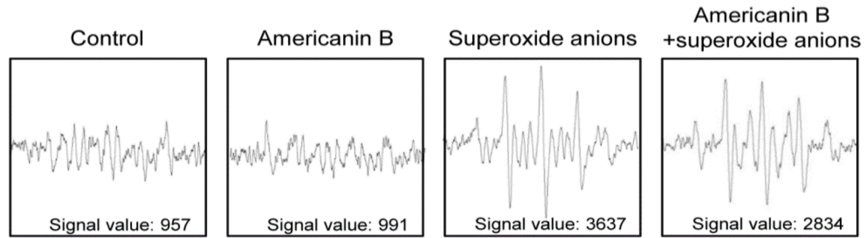

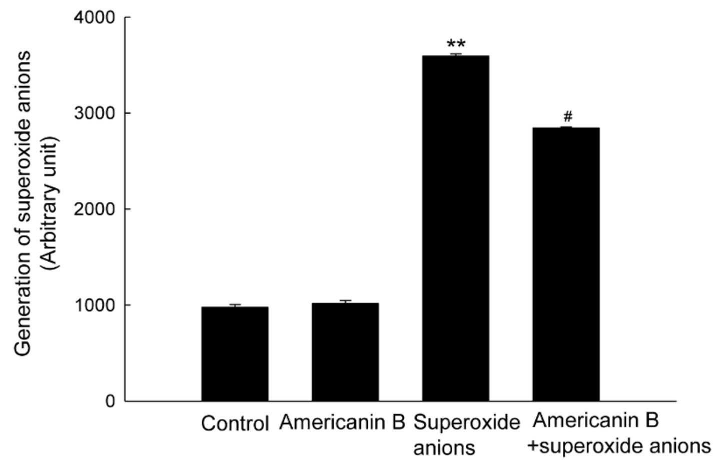

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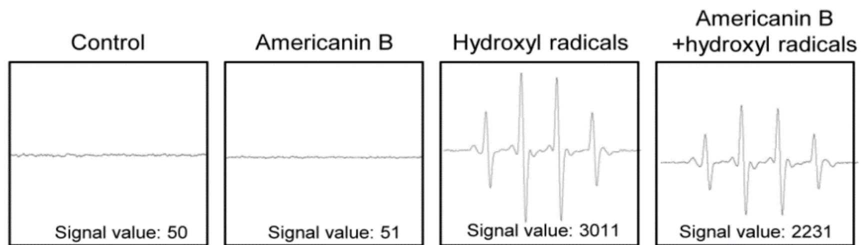

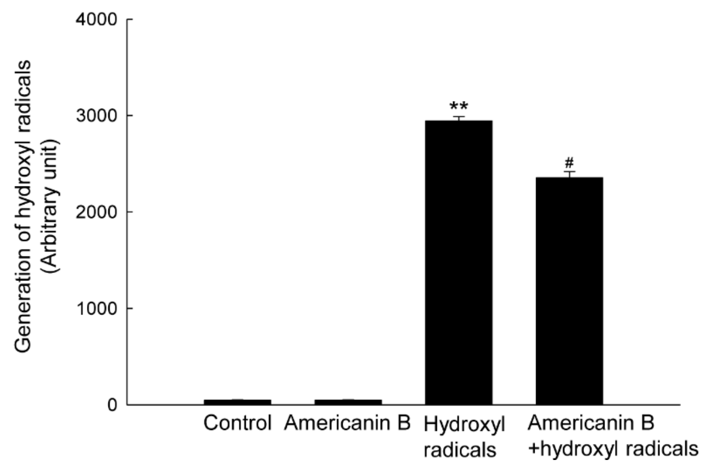

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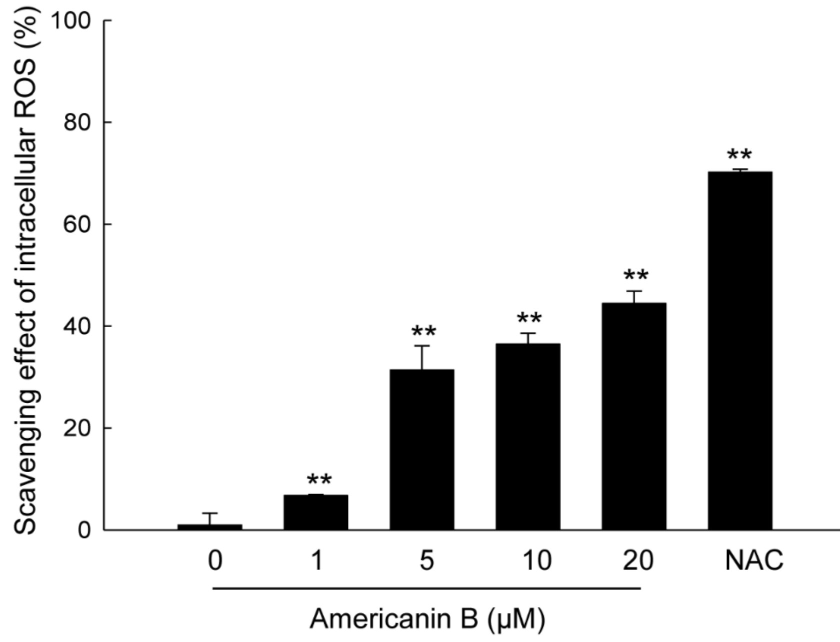

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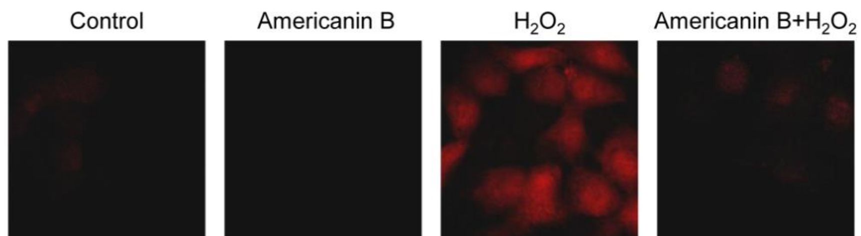

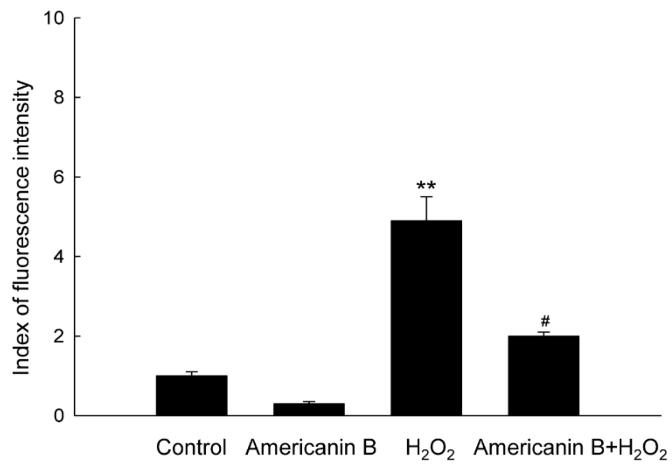

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Figure 1. Americanin B attenuates ROS generation. (A) HaCaT cells were treated with 0, 1, 5, 10, or 20 μM americanin B, or with 2 mM NAC. After 16 h, cell viability was determined by the MTT assay. (B) Levels of the DPPH radicals scavenged by various concentrations of americanin B were measured spectrophotometrically at 520 nm. *$p<0.05$, significantly different from control. (C) Superoxide anions generated by the xanthine/xanthine oxidase system were reacted with DMPO, and the resulting DMPO/ ·OOH adducts are detected by ESR spectrometry. The results are expressed as representative peak data and histogram. Control: PBS + DMPO; americanin B: PBS + americanin B + DMPO; superoxide anions: PBS + xanthine + xanthine oxidase + DMPO; americanin B + superoxide anions: americanin B + xanthine + xanthine oxidase + DMPO. **$p<0.001$, significantly different from control; #$p<0.001$, significantly different from superoxide anions. (D) The hydroxyl radicals generated by the Fenton reaction (H_2O_2 + $FeSO_4$) were reacted with DMPO, and the resulting DMPO/ ·OH adducts were detected by ESR spectrometry. Results are expressed as representative peak data and histogram. Control: PBS + DMPO; americanin B: PBS + americanin B + DMPO; hydroxyl radicals: PBS + $FeSO_4$ + H_2O_2 + DMPO; americanin B + hydroxyl radicals: americanin B + $FeSO_4$ + H_2O_2 + DMPO. **$p<0.001$, significantly different from control; #$p<0.001$, significantly different from hydroxyl radicals. (E) HaCaT cells were treated with 0, 1, 5, 10, or 20 μM americanin B, or with 2 mM NAC. One hour later, 1 mM H_2O_2 was added to the plate. After an additional 30 min, cells were stained with DCF-DA, and intracellular ROS were detected using a spectrofluorometer. **$p<0.001$, significantly different from control. (F) Representative confocal images illustrated that H_2O_2 treatment increased the red fluorescence intensity of DCF (produced by ROS) compared to the control, and americanin B treatment of H_2O_2-treated cells reduced the fluorescence intensity. **$p<0.001$, significantly different from control; #$p<0.001$, significantly different from H_2O_2-treated cells.

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3-2. Americanin B protects cell components against H_2O_2-induced oxidative damage to lipids, proteins, and DNA.

The studies investigated whether americanin B was capable of inhibition damage to macromolecules in H_2O_2-treated cells; it firstly monitored H_2O_2-induced DNA damage by using the alkaline comet assay. The length of comet tails in microscopic images and the percentage of cellular fluorescence were shown in Fig. 2A. After treatment of cells with H_2O_2, comet tail length was distinctly elongated, as well as the ratio of injury DNA strand outside of nucleus. Nevertheless, treatment of H_2O_2-treated cells with americanin B clearly recovered the portion of injury DNA from 47% to 23%. Second, protein carbonylation was measured, which is reaction taken place during the process of oxidation in protein to form carbonyl groups (Pirinccioglu et al. 2010). Management of H_2O_2 obviously increased the content of carbonyl moieties, whereas pre-treatment with americanin B in H_2O_2-treated cells notably suppressed formation of protein carbonyls (Fig. 2B). Finally, the degree of 8-isoprostane was examined, a hallmarker of lipid peroxidation, which is released from the oxidative cells into the culture medium. As shown in Fig. 2C, cells treated with H_2O_2 secreted higher levels of 8-isoprostane than untreated cells, but pre-treatment with americanin B in H_2O_2-treated cells significantly reduced the 8-isoprostane level. In addition, lipid peroxidation was also verified by fluorescent product DPPP oxide produced from DPPP (Okimoto et al. 2000). The intensity of DPPP oxide was dramatically increased in H_2O_2-treated cells relative to control. Pre-treatment with americanin B in H_2O_2-treated cells led to a reduction in fluorescence intensity (Fig. 2D). Taken together, these results presented in Fig. 2 confirm that americanin B significantly protects lipids, proteins, and DNA against oxidative damage induced by H_2O_2.

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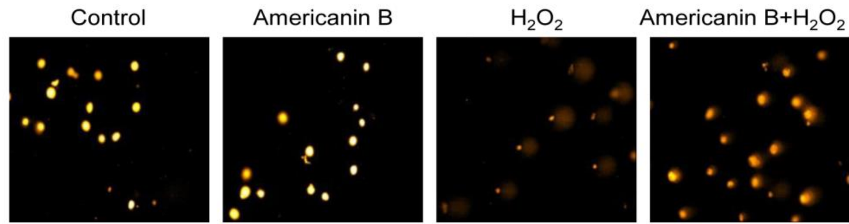
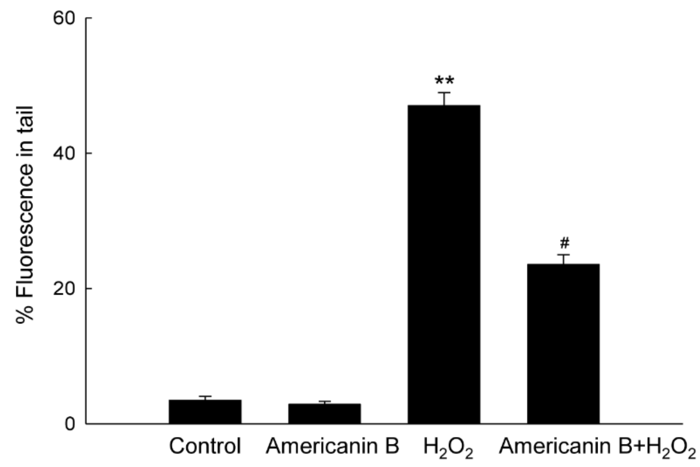
A





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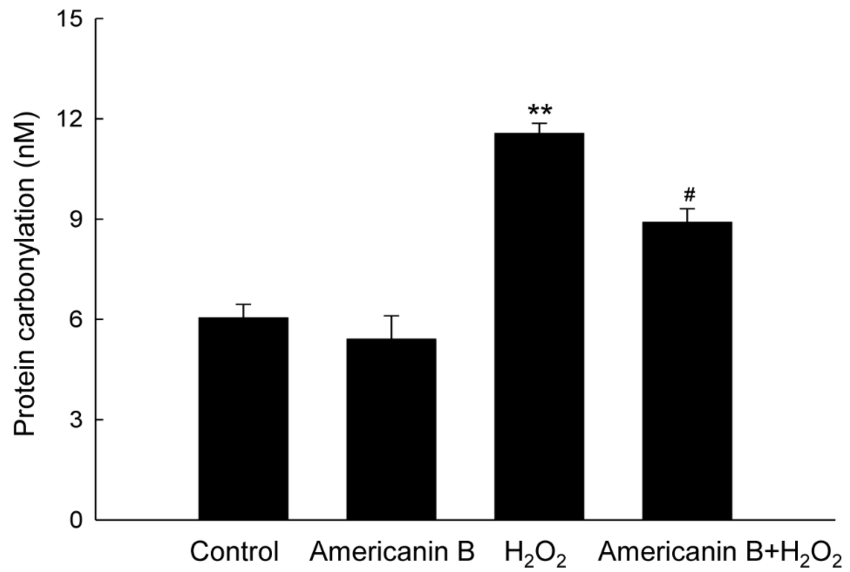

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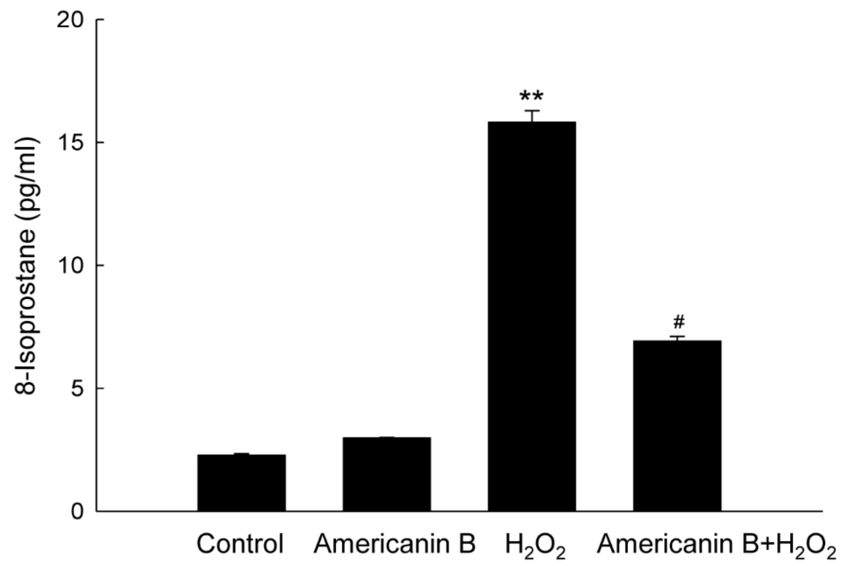

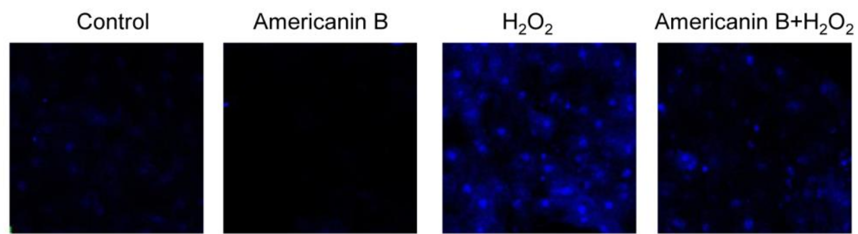

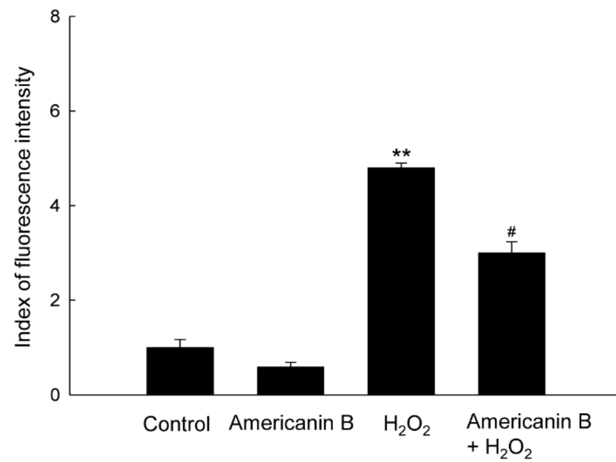

Figure 2. Americanin B protects against H_2O_2-induced oxidative lipid, protein, and DNA damage. HaCaT cells were treated with 10 μM americanin B for 1 h, and then exposed to

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H_2O_2. (A) The comet assay was performed to assess DNA damage. Representative images and percentage of cellular fluorescence within comet tails are shown. (B) Protein oxidation was assayed by measuring the amount of carbonyl formation. Lipid peroxidation was assayed by (C) measuring 8-isoprostane levels in the conditioned medium and (D) detecting lipid hydroperoxide by fluorescence microscopy after the DPPP reaction. $^{**}p<0.001$, significantly different from control; $^{\#}p<0.001$, significantly different from H_2O_2-exposed cells.

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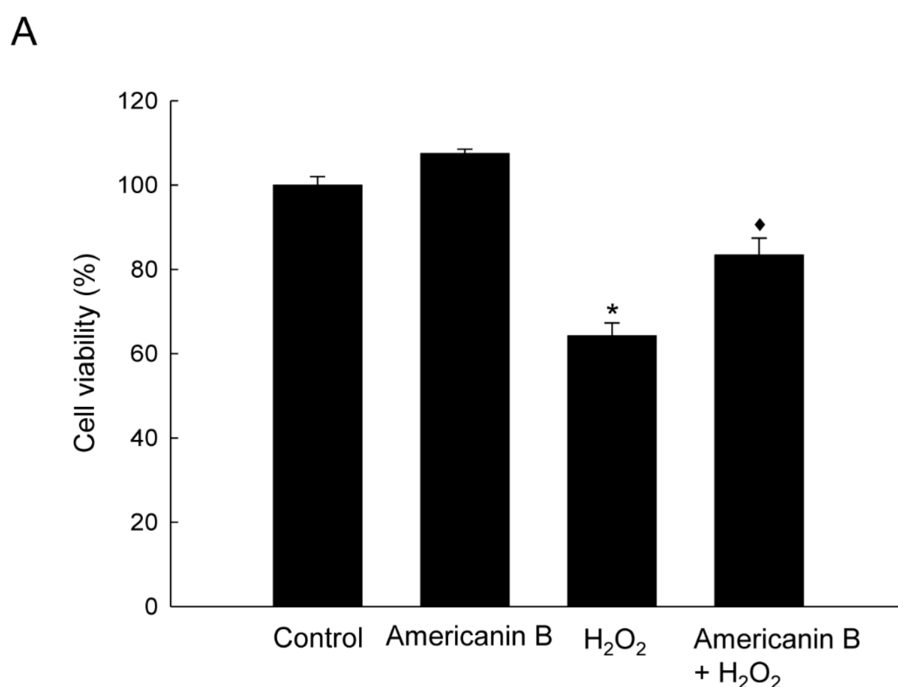
3-3. Americanin B reduces apoptosis induced by H_2O_2.

To elucidate the cytoprotective effect of americanin B against H_2O_2-induced apoptosis, the research examined the viability of HaCaT cells exposed to 1 mM H_2O_2, either pre-treated or not with 10 μM americanin B. In H_2O_2-treated cells, cell viability was reduced to 64% relative to the control group, but pre-treatment with americanin B recovered the viability to 83% (Fig. 3A). In parallel, cells were stained with nuclei with Hoechst 33342, and then assessed the cells by microscopy. In control groups or americanin B-treated cells, normal nuclei could be visualized, whereas significant nuclear condensation was found in H_2O_2-treated cells (apoptotic index = 6). However, when these cells were pre-treated with americanin B or NAC, the number of nuclear fragmentation was declined (americanin B: apoptotic index = 2.7; NAC: apoptotic index = 2) (Fig. 3B). These results indicate that americanin B protects cells against H_2O_2-induced apoptosis.



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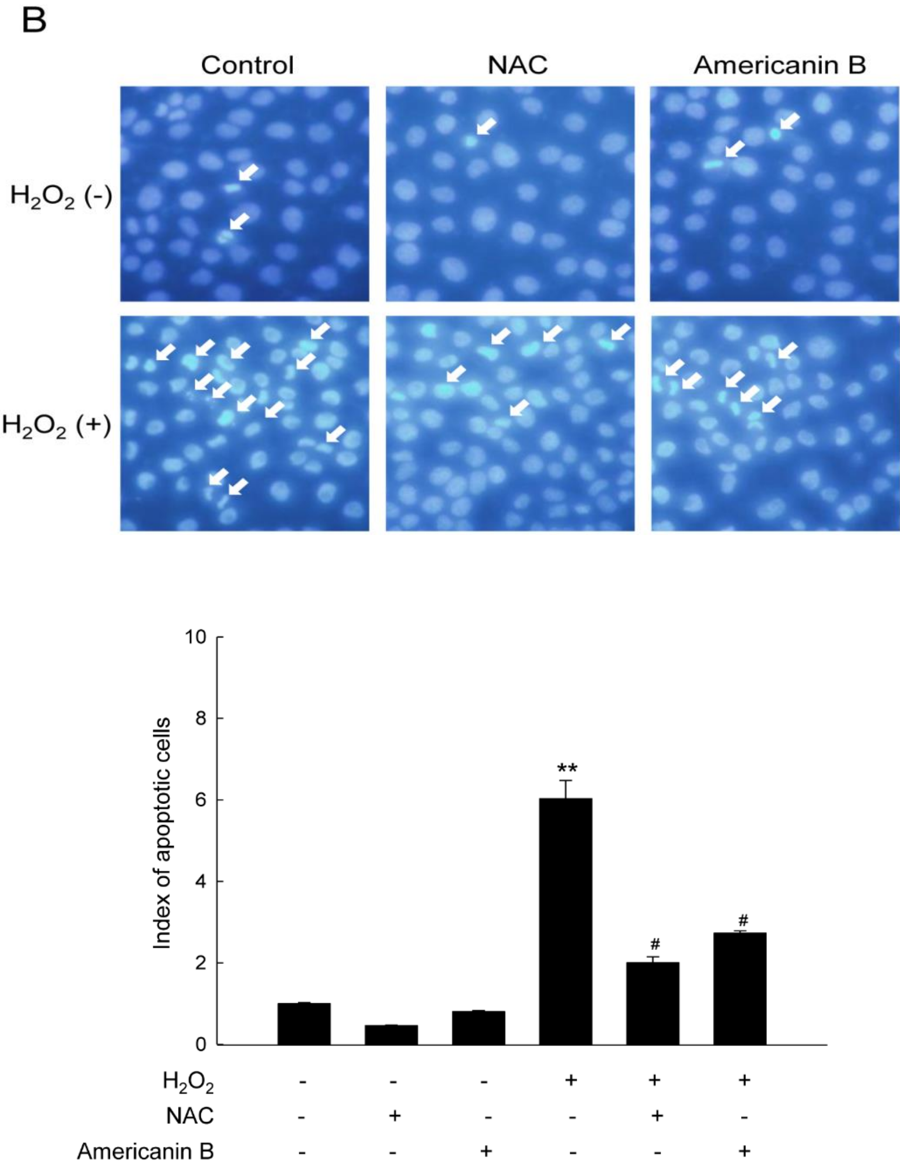

Figure 3. Americanin B reduces apoptosis induced by H_2O_2. (A) Cells were treated with 10 μM americanin B and exposed to H_2O_2 1 h later. After incubation for a further 24 h, cell viability was determined by the MTT assay and is expressed as a percentage of the control. **p*<0.05, significantly different from control; ♦*p*<0.05, significantly different from H_2O_2-treated cells. (B) Cells were stained with Hoechst 33342 dye and observed by fluorescence microscopy; apoptotic bodies were quantitated. ***p*<0.001, significantly different from control; #*p*<0.001, significantly different from H_2O_2-treated cells.

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4. Discussion

Previous researches show that plenty of natural compounds exert pharmaceutical activity such as anticancer, antioxidant, or anti-inflammatory activities because of containing active pharmaceutical ingredient phenolic groups (Chung et al. 1998; Cassidy et al. 2000; Tapiero et al. 2002). Moreover, the antioxidant activities of many natural compounds are related to their phenolic structures (Rice-Evans et al. 1996; Heim et al. 2002). Several lines of evidences suggest that the protective mechanism is based on formation of phenoxyl radical by upon donating a hydrogen atom from phenolic hydroxyl groups. The resultant radical can effectively act as antioxidant to quench reactive oxygen and nitrogen species by giving radicals to them and forming the stable state (Valentão et al. 2002, 2003; Heim et al. 2002; Payá et al. 1992; Choi et al. 2002), and interdict the process of lipid peroxidation (Cheng et al. 2003; Foti et al. 2001) by inhibition of the cycle of radicals. The number and position of aromatic hydroxyl groups strongly affect the property of phenolic antioxidants (Cai et al. 2006; Lien et al. 1998; Tyrakowska et al. 1999). The main requirement for effective radical quenching in aspect of structure is the bearing an ortho-dihydroxy group (catechol structure), which confers intrinsic antioxidant properties by providing an easily donated electron (Kang et al. 2012).

Recent studies have shown that the presence of a catechol group is associated with a high capacity to protect DNA against oxidative damage (Melidou et al. 2005; Noroozi et al. 1998). The antioxidant capacity of phenolic compounds has also been attributed to their ability to chelate metal ions involved in the production of free radicals (Yang et al. 2001). Furthermore, a great many studies have certified that an unsaturated group within its structure (e.g., carbon-carbon double bond, carbon-oxygen double bond, or conjugated system) can enhance the antioxidant capacity of a compound. Compounds bearing an electrophilic α, β-unsaturated carbonyl group can interact with nucleophiles, including ROS such as the superoxide anions, hydroxyl radicals, H_2O_2, and singlet oxygen (Farombi et al. 2008).

Americanin B, a lignan compound, has both catechol moieties and α, β-unsaturated carbonyl

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group in its structure (Yu et al. 2001), and it is likely that these groups provide it with its intrinsic antioxidant properties. Based on its structural properties, americanin B appears to exert its antioxidant effects by directly quenching ROS. When recognizing ROS, the catechol moieties of americnanin B become rich-electronic groups by releasing one hydrogen atom and form large conjugated groups with the help of benzene ring, providing some free radicals to unstable molecules because of lacking of electron in electron shell. In addition, α, β-unsaturated carbonyl group itself is a conjugated group and forms covalent bonds with ROS molecules via sharing one or more electron pairs to stable structures in outer electrons. ROS disable the ability to attack cells or organism when they become steady state through been given or sharing electrons from americanin B. Consistent with this assumption, the investigation showed that americanin B decreased generation of free radicals such as DPPH radicals, superoxide anions and hydroxyl radicals as well as intracellular ROS.

In H_2O_2-treated cells, americanin B markedly decreased the tail length and the proportion of damaged DNA. H_2O_2 promoted the modification of protein-bound carbonyl groups and disrupted cell function; americanin B blocked the formation of protein carbonyl following H_2O_2 treatment. Similarly, americanin B also protected membrane lipids from H_2O_2-induced peroxidation, as shown by the DPPP data and levels of 8-isoprostane. These protective effects of americanin B against DNA, lipid, and protein damage ultimately help to protect cells against oxidative stress-induced cell death. Cells exposed to H_2O_2 exhibited the distinct nuclear fragmentation of apoptosis, but americanin B suppressed these manifestations of apoptosis and increased cell viability.

In this study, these data showed that americanin B is an antioxidant that protects cells against the oxidative damage caused by H_2O_2 via scavenging ROS and inhibiting apoptosis. In future experiments, the subsequent investigation will explore the mechanisms by which americanin B blocks ROS generation and prevents apoptosis.

The thesis for *Degree of Master of Science in Medicine* referring to experimental data

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and contents is from *"Americanin B protects cultured human keratinocytes against oxidative stress by exerting antioxidant effects"*, which has been published in the journal of *"In Vitro Cellular & Developmental Biology - Animal"* with *"DOI 10.1007/s11626-014-9759-9"* in 2014.

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6. Abstract in Korean

이 연구는 리그난계 화합물인 americanin B 의 hydrogen peroxide (H_2O_2)로 유도된 세포 손상에 대한 세포 보호 효과를 평가하였다. Americanin B 는 DPPH radicals, superoxide anions, hydroxyl radicals 의 레벨을 감소시킬 뿐만 아니라 세포 내 ROS 의 레벨도 감소시킨다. Americanin B 는 또한 DNA 가닥 파괴를 나타내는 comet tails 형성의 억제에 의해 나타낸 바와 같이, H_2O_2 처리에 의해 유도된 DNA 손상을 감소시키며, protein carbonyls 및 8–isoprostane 의 측정을 통해 단백질의 oxidation 과 지질의 peroxidation 을 보호한다는 것을 나타내었다. 또한, americanin B 는 H_2O_2 에 유도된 apoptotic cell death 에 대한 보호를 하며, 이것은 Hoechst 33342 로 염색된 apoptotic bodies 의 숫자의 감소에 의해 측정되었다. 이러한 연구 결과는 americanin B 가 antioxidant effects 와 apoptosis 억제를 발휘함으로써 oxidative damage 에 대한 세포를 보호하는 것을 제안한다.

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