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쥐참외뿌리 추출물의 항산화 및 피부 세포에서의 세포 독성 연구

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Antioxidant and Cytotoxicity in Skin Cell of the Trichosanthis Cucumeroidis Radix Extract

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요 약 : 쥐참외뿌리 추출물이 항산화 활성 및 피부 세포에서의 독성을 확인하여, 피부에 효과적으로 사용할 수 있는 기능성 소재로써의 활용 가능성을 확인해 보고자 하였다. 쥐참외뿌리 추출물의 항산화 활성의 지표가 되는 총 폴리페놀과 총 플라보노이드 함량을 확인하였고, 피부에서의 Neutral red assay를 이용하여 세포 독성을 확인하였다. 연구 결과, 총 폴리페놀과 총 플라보노이드의 함량은 농도 의존적으로 증가하였다. 섬유아 세포인 HDF cell에서의 높은 생존율이 확인되었으며, B16F10 melanoma cel와 RAW 264.7 cell에서는 5 μg/mL부터 세포 생존율이 유의하게 낮아지는 것이 확인되었다. 본 연구 결과는 쥐참외뿌리 추출물의 항산화 활성 및 피부 세포에서의 기초적인 자료로 사용가능할 것으로 사료되어 진다.

주제어 : 항산화, 쥐참외뿌리추출물, HDF 세포, B16F10 멜라노마 세포, RAW 264.7 세포

Abstract : We tried to check the antioxidant activity and toxicity of trichosanthis cucumeroidis radix extracts in skin cells, and check the possibility of their use as a functional material that can be effectively used on the skin. Total polyphenol and total flavonoid content, which are indicators of antioxidant activity of trichosanthis cucumeroidis radix extracts, were confirmed, and cytotoxicity was confirmed using Neutral red assay in the skin. As a result of the study, the content of total polyphenols and total flavonoids increased concentration-dependent. High survival rates in fibroblast HDF cells were identified, and cell survival rates were significantly lowered from 5 μg/mL in melanocytes B16F10 melanoma cells and inflammation-related macrophages RAW 264.7 cells. He

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results of this study are believed to be available as basic data for antioxidant activity of trichosanthis cucumeroidis radix extracts and skin cells.

Keywords : Antioxidant Trichosanthis Cucumeroidis Radix Extract, HDF cell, B16F10 melanoma cell, RAW 264.7 cell

1. Introduction

As modern society changes rapidly, research is being conducted in various fields to improve the quality of life, and among them, interest in the cosmetics industry is increasing. Accordingly, many studies are being conducted to explore functionality from plant materials that can be used more effectively and safely than synthetic materials with side effects[1].

Among the changes in the skin, skin aging is a result of a combination of various changes in the epidermis, dermis, subcutaneous fat, and extracellular gasses, but typically, the main phenomenon of skin aging is associated with oxidative stress and increased inflammatory substances[2,3]. Reactive oxygen specifications (ROS) are typical of such oxidative stress or inflammatory substances. ROS is in close contact with various oxygen compounds derived from oxygen free radical, and all of which have high reactivity. They have unstable characteristics because they are not paired and lose electrons or try to get an electron from around them and go to a more stable state[4]. In particular, it is known to cause oxidation reactions in the body, causing DNA damage, cell membrane damage in the body, and various diseases[5,6].

Therefore, it is expected that a material having an antioxidant effect that suppresses the activity of active oxygen can be used as a material effective for the skin by suppressing the activity of pigmentation or wrinkle formation. Recently, many studies have been conducted to develop new materials using natural materials. In Korea, various types of herbal medicines and herbal medicines have been used since ancient times, and polyphenol compounds such as antioxidant, anticancer, amino acid, phenolic acid, flavonoid, and tannin are used as natural materials[7].

In this study, Trichosanthis Cucumeroidis Radix Extract of the medicinal herbs used in oriental medicine was used as this material. Trichosanthis Cucumeroidis Radix Extract refers to dried haneultari roots and has been known to have the effect of lowering heat since ancient times. Therefore, this study aims to provide basic data that can be effectively applied to the skin by identifying the antioxidant activity and toxicity of Trichosanthis Cucumeroidis Radix Extracts to skin cells.

2. Research Materials and Methods

2.1. Sample manufacturing

After adding 10 times of 70% ethanol to 100 g of dried Trichosanthis Cucumeroidis Radix, it was extracted in an incubator at 37℃ for 72 h. After 72 h, it was concentrated under reduced pressure with a rotary evaporator, and frozen and used in this experiment

2.2. Research method

2.2.1. Total polyphenol content

The total polyphenol content of Trichosanthis Cucumeroidis Radix Extract was quantified in color by modifying the Folin-Denis method[8]. The Trichosanthis Cucumeroidis Radix Extract used in this experiment was diluted for each

concentration, and then 400 μL of extract and 400 μL of Folin-Denis agent were mixed to react at room temperature for 3 min, and then 10% Na_2CO_3 of 400 μL was mixed to react 60 min in the dark room. After the 60 min reaction, 200 μL of the supernatant was divided into 96 well plates to measure absorbance at 760 nm. After obtaining a standard calibration curve using the standard substance caffeic acid, the total polyphenol content of trichosanthis cucumeroidis radix extract was obtained.

2.2.2. Total flavonoid content

The total flavonoid content of trichosanthis cucumeroidis radix extracts was measured using the Moreno method[9]. After diluting the trichosanthis cucumeroidis radix extract by concentration, 100 μL of the extract, 20 μL of 10% aluminum nitrate, 20 μL of 1M potassium acetate, and 860 μL of ethanol were sequentially mixed and reacted at room temperature for 40 min. After 40 min, the suspension was settled with a centrifuge, and then the absorbance was measured at 415 nm by dispensing 200 μL on a 96 well plate. After obtaining a standard calibration curve using quercetin, the total flavonoid content of trichosanthis cucumeroidis radix extract was obtained.

2.2.3. Cell culture

HDF Cell, B16F10 Melanoma Cell, and RAW 264.7 Cell were purchased from Korea Cell Bank, and 10% fetal bovine serum, 1% phenicillin, and 1% streptomycin were added to a high glucose Dulbecco's modified Eagle's medium medium medium to maintain incubation volume at 37℃, 362℃.

2.2.4. Cytotoxicity Measurement Using Neutral Red Assay

To confirm the cytotoxicity of the Trichosanthis Cucumeroidis Radix Extract, it was measured using a neutral red (NR) assay. RAW 264.7 cells were divided into 96 well plates at a concentration of 3×10^4 cells/well per well and cultured in a incubator supplied with 37℃ and 5% CO2 for 24 hours. After 24 hours, the trichosanthis cucumeroidis radix extract was diluted by concentration and treated, and then cultured for 48 hours. The cultured cell culture solution was replaced with a serum-free medium containing 1% of NR solution, and the presence or absence of crystallization of NR under a microscope was determined after 3 hours of incubation. A 10% formaldehyde solution was added to phosphate buffered saline and treated to be fixed for 20 minutes by dispensing 100 μL in each well, and then NR desorb solution was dispensed with 100 μL in each well to extract NR in the cell. Absorption was measured at 540 nm using a microplate reader.

2.3. Statistical processing

The results of this experiment were all measured three or more times independently under the same conditions and used, and were expressed as mean ± standard deviation (Mean ± SD). As a result of the experiment, when the p value was less than 0.05, it was determined that there was a statistically significant difference and marked.

3. Result

3.1. Total polyphenol content measurement

According to many studies, antioxidant activity such as electron donor capacity increases in proportion to the content of phenolic substances such as polyphenols and flavonoids in natural products[10]. In this experiment, the total polyphenol content of trichosanthis cucumeroidis radix extract was confirmed to confirm the antioxidant activity of trichosanthis cucumeroidis radix extract (Fig. 1). As a result of the experiment, the total concentration-dependent polyphenol content of 24.4, 67.2, 117.3, 200.1, and 308.2 mg/g was confirmed for each concentration of

trichosanthis cucumeroidis radix extract. Polyphenol compounds contained in many oriental medicine materials are reported to have strong antioxidant activity[11], and 0trichosanthis cucumeroidis radix extracts are also considered to have high antioxidant activity.

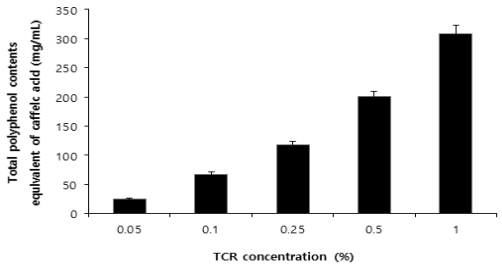

Fig. 1. Total polyphenol content of trichosanthis cucumeroidis radix extractextract.

3.2. Total flavonoid content measurement

In this experiment, in order to confirm the antioxidant activity of trichosanthis cucumeroidis radix extract, the total flavonoid content of trichosanthis cucumeroidis radix extract was confirmed (Fig. 2). As a result of the experiment, the total concentration-dependent flavonoid content of 17.8, 38.1, 53.1, 70.4, and 120.7 mg/g was confirmed for each concentration of trichosanthis cucumeroidis radix extract. Through the above-described results, the trichosanthis cucumeroidis radix extract is considered to have excellent

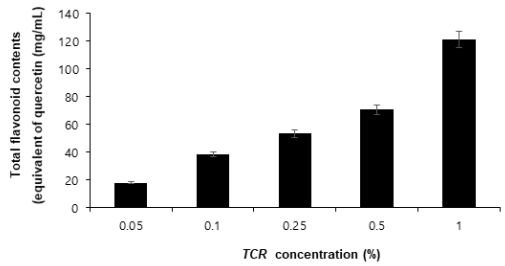

Fig. 2. Total flavonoid content of trichosanthis cucumeroidis radix extract.

antioxidant activity, considering that the total polyphenol and flavonoid contents, which are phenolic compounds, are high.

3.3. Cell viability in HDF cells

To confirm the availability of trichosanthis cucumeroidis radix extracts for the skin, cytotoxicity of trichosanthis cucumeroidis radix extracts using Neutral redassay was confirmed. HDF cells, which are typically used to identify aging and wrinkles, were used, and cytotoxicity was confirmed by treating trichosanthis cucumeroidis radix extracts in cells at concentrations of 1.25, 2.5, 5, and 10 μg/mL (Fig. 3). As a result of the experiment, a cell survival rate of 85% or more was confirmed at all concentrations.

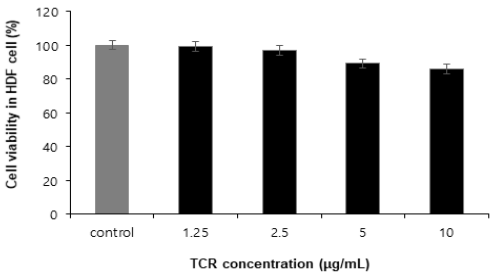

Fig. 3. Survival rate of trichosanthis cucumeroidis radix extract in HDF cells.

3.4. Cell viability in B16F10 melanoma cells

To confirm the availability of trichosanthis cucumeroidis radix extracts for the skin, cytotoxicity of trichosanthis cucumeroidis radix extracts using Neutral redassay was confirmed. B16F10 melanoma cell, which is typically used to confirm whitening activity, was used, and the cell was treated with trichosanthis cucumeroidis radix extracts at concentrations of 1.25, 2.5, 5, and 10 μg/mL to confirm cytotoxicity (Fig. 4). As a result of the experiment, cytotoxicity was confirmed as the concentration increased, 15.7% at 5 μg/mL and 29% at 10 μg/mL.

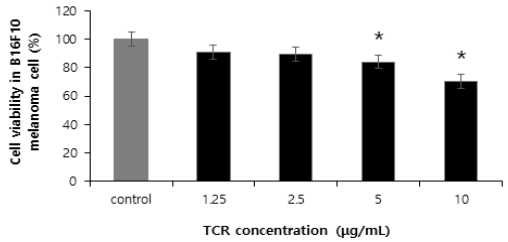

Fig. 4. Survival rate of trichosanthis cucumeroidis radix extract in B16F10 melanoma cells.

3.5. Cell viability in RAW 264.7 Cells

To confirm the availability of trichosanthis cucumeroidis radix extracts for the skin, cytotoxicity of trichosanthis cucumeroidis radix extracts using Neutral redassay was confirmed. RAW 264.7 cells, which are typically used to confirm anti-inflammatory activity, were used, and cytotoxicity was confirmed by treating trichosanthis cucumeroidis radix extracts in cells at concentrations of 1.25, 2.5, 5, and 10 μg/mL (Fig. 5). As a result of the experiment, cytotoxicity was confirmed as the concentration increased, 23.6% at 5 μg/mL and 25.1% at 10 μg/mL.

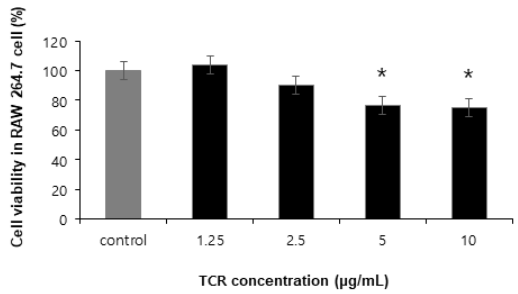

Fig. 5. Survival rate of trichosanthis cucumeroidis radix extract in RAW 264.7 cells.

4. Conclusion

In this study, trichosanthis cucumeroidis radix extract was extracted with 70% ethanol and used after freeze-drying. Total polyphenols and total flavonoids, which are indicators of antioxidant activity, were confirmed, and cytotoxicity to skin cells was to be confirmed. As a result of the antioxidant experiment, the total concentration-dependent total polyphenol and total flavonoid content of the trichosanthis cucumeroidis radix extract were confirmed. As a result of confirming cytotoxicity, a cell survival rate of 85% or more at all concentrations was confirmed in HDF cells. Cytotoxicity was significantly identified in B16F10 melanoma cells and RAW 264.7 cells from 5 μg/mL concentration. Through these results, trichosanthis cucumeroidis radix extract is considered to be effective in suppressing active oxygen due to excellent antioxidant activity, and based on this study, it is considered that it is possible to be a natural herbal material with low side effects and excellent effect by using it at an appropriate concentration.

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