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"A Study on the Effects of Hordeum vulgare extract on Hair Loss Improvement and Scalp Health"

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1. Introduction

As interest in appearance increases in modern society, hair loss is emerging as a major health problem that goes beyond just beauty problems to psychological and social effects. In addition, the population experiencing hair loss due to various causes such as stress, hormoal changes, genetic factors, and environmental factors is rapidly increasing. For these reasons, the demand for treatments and health functional foods for preventing and alleviating hair loss is increasing rapidly. Finasteride and Minoxidil have been approved for treatment of hair loss by the US Food and Drug Administration (FDA) [1]. However, due to their side effects as skin problems and sexual function problems, development of natural products for the treatment of hair loss is actively progressing [2, 3].

Therefore, in this study, we investigated the potential of Hordeum vulgare Extract (HVE) as a natural material for improving hair loss and scalp health. We evaluated its anti-oxidant, anti-inflammatory, moisturizing and its effects on cell proliferation and associated signaling factors in human hair cells.

2. Materials and Methods

Materials

The Hordeum vulgare extract used in the experiment was from Korea. The air-dried hordeum vulgare (100 g) were refluxed with 50% EtOH at 60℃ for 2 h, and then the extract was cooled and fitered through a Whatman No.2 filter. The extract was concentrated with a vaccum, freeze-dried, and stored at -20℃.

Quantitative analysis of marker compound

Quantitative analysis of the maker compound of HVE was performed using HPLC. First, HVE was dissolved at 20 mg/mL, dissolved for 30 minutes with an ultrasonic extractor, and filtered through a PTFE filter (0.45 uM), which was diluted 10 times and used as a test solution. As Standard, saponarin was purchased from Sigma and used. Saponarin was prepared at 1 mg/mL and then diluted to 5 to 200 ug/mL and used as a standard solution.

The analysis conditions are shown in the table below(Table 1).

Table 1. Conditions of HPLC for the confirmation of marker compound

Control Factor	Conditions
Detector	PDA detector (336 nm)
Column	Shim-pack GIS C_{18} (4.6 mm×250 mm, 5μm)
Mobile phase	Acetonitrile : 0.1% Trifluoroacetic acid(water) (Gradient)
Column flow rate	1.0 mL/min
Injection volume	10 μL
Colunm temperature	40 ℃

$ABTS^+$ radical scavenging activity

2'2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) cation ($ABTS^+$) radical scavenging activity experiment was performed by applying Re method (Re, 1999) [4]. The $ABTS^+$ radical solution was mixed with 7.4 mM ABTS and 2.6 mM potassium persulfate 1:1 and reacted for 16 h at room temperature and in the dark to form $ABTS^+$ radical. This solution was diluted with methanol and used in the experiment.

Cell culture

Raw 264.7 cells were obtained from ATCC (USA). The Raw 264.7 cells were culture in Dulbeccos's modified Eagle's medium (DMEM) high glucose medium with 10% FBS and 1% penicillin-streptomycin at 37℃ in a humidified atmosphere containing 5% CO_2. The cells were sub-cultured every 2~3 days to obtain enough quantity of cell for test.

Human Dermal Papilla (HDP) cells for cell proliferation assays were obtained from Cefobio (Korea) were cultured in CEFOgro™ Human Dermal Papilla Growth Medium and incubated same environment with Raw 264.7 cells. The HHDPC cells were sub-cultured every 3~4 days.

Human Follicle Dermal Papilla Cells (HFDPC) cells for VEGF quantification assays were obtained from Promocell (Germany). The HFDPC cells were cultured in Follicle dermal papilla cell growth medium and same environment with Raw 264.7 cells. The HFDPC cells were sub-cultured every 3~4 days.

HaCaT cells were obtained from Cell Lines Service (CLS, Germany). The HaCaT cells were culture in same media and environment with Raw 264.7 cells. The HaCaT cells were sub-cultured every 2~3 days.

Cell viability

Raw 264.7 cells, HDP cells, HFDPC cells and HaCaT cells were seeded according to the method specified in assay, and then cell viability was estimated using water-soluble tetrazolium salt (WST) assay.

Anti-inflammatory effect

Raw 264.7 cells were seeded to 24-well plates by 1 X 10^5 cells per well, and incubated for 24 h. Then the cells were treated with various concentrations of HVE containing lipopolysaccharide (LPS) 1 μg/mL and incubated for 24 h. And then, to measure the nitric Oxide (NO) production amount, 100 μL of cell supernatants and 100 μL Griess reagent (1% sulfanilamide, 0.1% naphtylethylene-diamine in 2.5% phosphoric acid) were mixed in 96-well plates and reacted at 37℃ for 10 min.

Cell proliferation effect

HDP cells were seeded to 96-well plates by 3 x 10^4 cells per well, and incubated for 24 h. Then the cells were treated with various concentrations of HVE in serum-free culture media, after that incubated for 72 h. The percentage viability was estimated by WST-1 assay.

Effect on VEGF Secretion

HDP cells were seeded to 24-well plates by 2 x 10^4 cells per well and incubated for 24 h. Then the cells were treated with various concentrations of HVE in serum-free culture media, after that incubated for 24 h. The cell supernatants were analyzed for VEGF using a Human VEGF Quantikine ELISA Kit (USA, R&D systems).

Moisturizing effect

HaCaT cells were seeded to 24-well plates by 1 X 10^5 cells per well and incubated for 24 h. Then the cells were treated with various concentrations of HVE in serum-free culture media, after that incubated for 24 h. The cell supernatants were analyzed for HA using a Hyaluronan Quantikine ELISA Kit (USA, R&D systems).

3. Results

Quantitative analysis of marker compound

Saponarin is known as a flavonoid with anti-oxidant, anti-inflammatory, and anti-allergic effects [5, 6]. These properties of saponarin are thought to help suppress oxidative stress, inflammatory reactions, and damage to hair follicle cells, which are causes of hair loss. Analysis of the saponin content in HVE showed that it contained 19.71 mg/g.

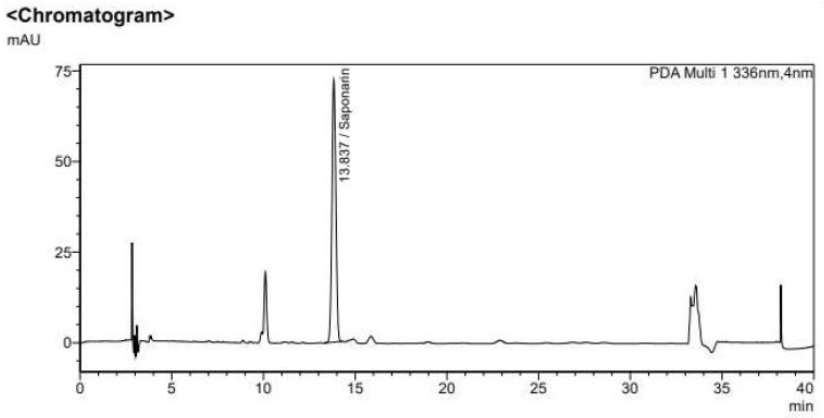


Figure 1. Analytical HPLC chromatogram of saponarin.

Effect on Anti-oxidant of HVE

Radical scavenging activity is important to prevent the harmful role of free radicals in various diseases, including aging. To investigate the effect of HVE on anti-oxidative acitivity, $ABTS^+$ radical scavenging assay was performed. This results showed that SE showed $ABTS^+$ radical scavenging activity concentration-dependently, and the SC_{50} value of SE was 167.59 μg/mL(Figure 2). Ascorbic acid was used as positive control (5 μg/mL).

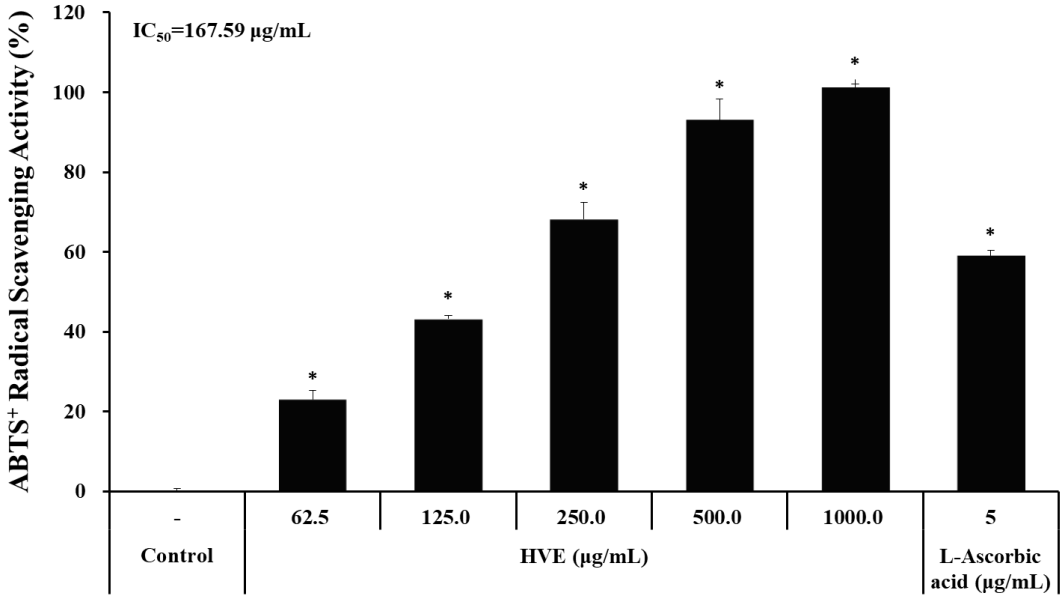


Figure 2. $ABTS^+$ radical scavenging activity of HVE. The data are expressed as a percentage of control and represent the mean ± SD of triplicate experiments. *p < 0.05 compared with control group.

Effect on anti-inflammatory of HVE

Nitric Oxide (NO) is kinds of reactive oxygen whose over-expression of NO is related to inflammation [7]. To investigate the effect of SE on NO inhibition of Raw 264.7 cells, the cells were treated with HVE at non-cytotoxic concentrations. Our results showed that HVE showed concentration-dependent NO inhibition. The HVE treatment group at concentrations of 125, 250, 500, 1000 and 2000 μg/mL was inhibited NO production by 2.33(±2.99) %, 19.10(±4.47) %, 24.57(±3.62) %, 56.70(±3.04) % and 89.43(±1.67) % as compared with the control (Figure 3). 2-AM (2-Amino-4-methylpyridine) (2 μM) was used as positive control of NO inhibition.

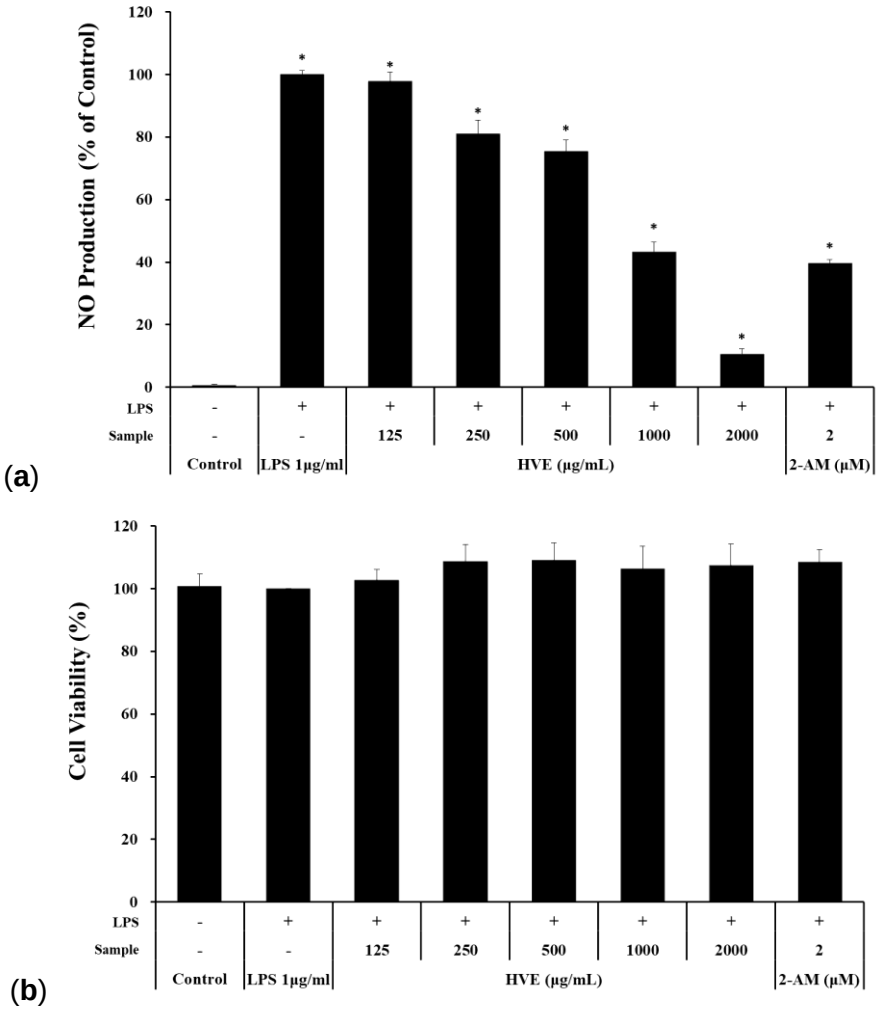


Figure 3. Effect on NO inhibition (a) and cell viability (b) of HVE in LPS-induced Raw 264.7 cells. The cells were treated with the indicated concentrations of HVE with LPS (1 μg/mL) for 24 h. The data represent the mean ± SD of triplicate experiments. *$p < 0.05$ compared with control group.

Effect on cell proliferation of HVE

HDP cell proliferation has been widely tested to determine the hair growth-promoting or hair-loss inhibiting effects from natural extracts [8]. In this study, cell proliferation effect of HVE was investigated on the dermal papilla cell culture systems. Our results showed that HVE showed cell proliferation effect, concentration-dependently. The HVE treatment group at concentrations of 200, 400 and 800 μg/mL was increased cell viability by 6.39(±2.13) %, 15.09(±0.53) % and 87.10(±3.53) %, as compared with the control (Figure 4).

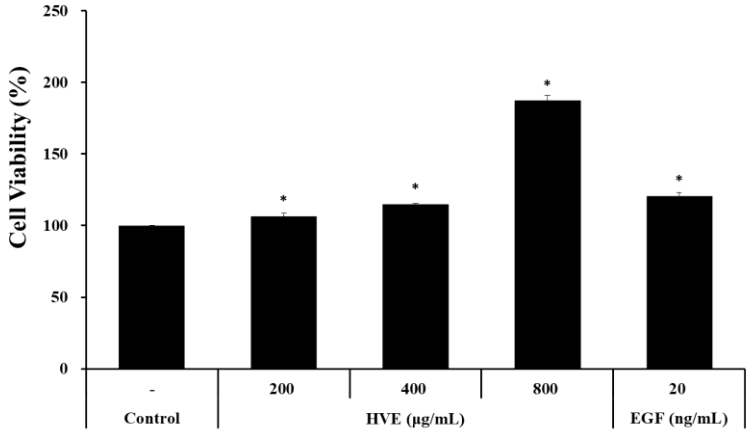
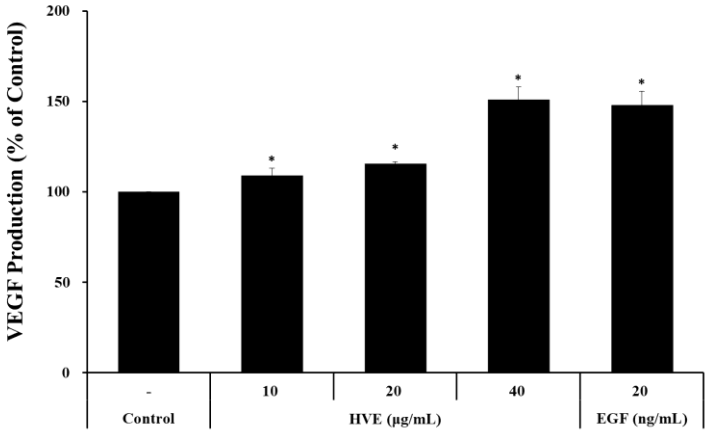


Figure 4. Cell proliferation effect of HVE in HDP cells. The cells were treated with the indicated concentrations of HVE for 72 h. At the end of incubation, cell viability was estimated using WST assay. The data represent the mean ± SD of triplicate experiments. *$p < 0.05$ compared with control group.

Effect on VEGF secretion of HVE

VEGF is an important mediator of angiogenesis in hair follicles by improving perifollicular vascularization and increasing hair follicle size [9-11]. To investigate the effect of HVE on VEGF production of HFDPC cells, the cells were treated with HVE at non-cytotoxic concentrations. Our results showed that HVE showed concentration-dependent VEGF production. The HVE treatment group at concentrations of 10, 20 and 40 μg/mL was increased VEGF production by 8.97(±3.99) %, 15.57(±1.09) % and 50.90(±7.02) %, as compared with the control(Figure 5). In particular, HVE was found to significantly increase VEGF expression at low concentrations.



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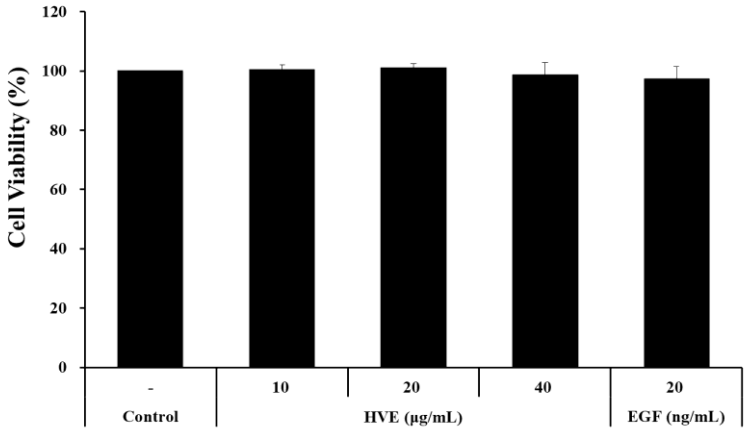

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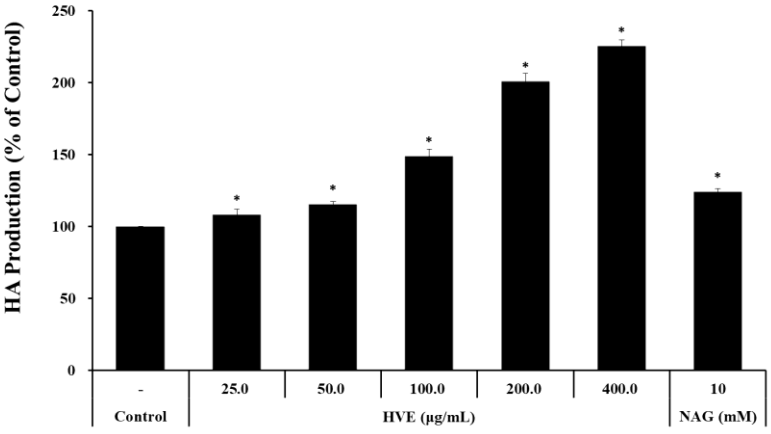
Figure 5. Effects of HVE on VEGF secretion (a) and cell viability (b) in HFDPC cells. The cells were treated with the indicated concentrations of HVE for 24 h. At the end of incubation, cell supernatants were analyzed for VEGF using an enzyme-linked immunosorbent assay (ELISA) kit. The data represent the mean ± SD of triplicate experiments. *p < 0.05 compared with control group.

Effect on HA production of HVE

Hyaluronic acid is a natural substance that is present in animals' bodies, especially in the extracellular matrix of the skin. This has moisture binding power enough to contain more than 1,000 times the unit weight, which plays an excellent role in moisturizing and transporting moisture in tissues [12]. To investigate the effect of HVE on HA production of HaCaT cells, the HaCaT cells were treated with HVE at non-cytotoxic concentrations. Our results showed that HVE showed concentration-dependent HA production. The HVE treatment group at concentrations of 25, 50, 100, 200 and 400 μg/mL was increased HA production by 8.28(±3.75) %, 15.23(±2.33) %, 48.72(±4.96) %, 100.60 (±5.79) %, and 125.18 (±4.57) %, as compared with the control(Figure 6). NAG (N-acetyl-D-glucosamine) (10 mM) was used as positive control of HA production.



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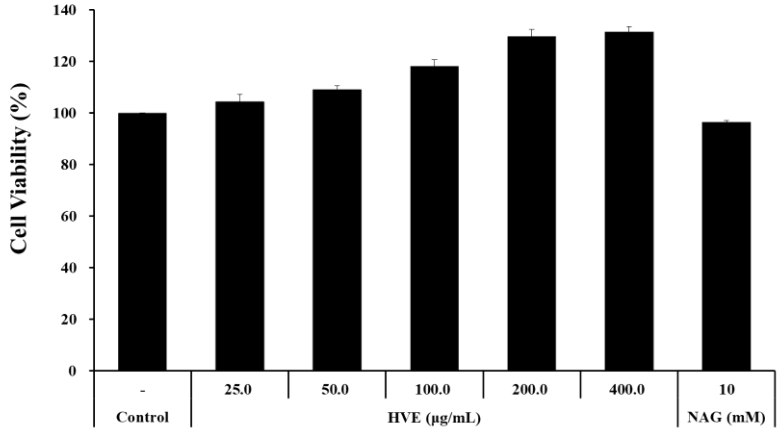

(**b**)

Figure 6. Effects of HVE on HA production (a) and cell viability (b) in HaCaT cells. The cells were treated with the indicated concentrations of HVE for 24 h. At the end of incubation, cell supernatants were analyzed for HA using an enzyme-linked immunosorbent assay (ELISA) kit. The data represent the mean ± SD of triplicate experiments. *$p < 0.05$ compared with control group.

4. Discussion

This study was conducted to investigate the possibility of HVE, a natural product, as a material for improving hair loss and scalp environment. Hair loss is a disease caused by the involvement of various factors, and this study confirmed the effects of cell proliferation and hair growth factor production, along with antioxidant, anti-inflammatory, and moisturizing effects, which are therapeutic factors for hair loss.

HVE showed anti-oxidant efficacy concentration-dependently in $ABTS^+$ radical scavenging assay. Also, HVE inhibited NO production, a major inflammatory mediator in macrophages, in a concentration-dependent manner. In addition, HVE was found to increase hyaluronic acid, the most representative moisturizing factor, in a concentration-dependent manner. These results suggest that HVE has the effects of improving the scalp environment such as dry scalp, excessive oxidation, and inflammatory reactions, which are considered to be the cause of hair loss.

HVE showed cell proliferation effect in Human follicle dermal papilla cells and increase the production of VEGF, which is known to promote hair growth. This suggests that HVE has the effect of improving hair loss.

In addition, as a result of quantitative analysis using HPLC, it was found that HVE contained 1.97 mg/g of saponarin. Saponarin is well known as a natural antioxidant of the flavonoid family, and its anti-oxidant and anti-inflammatory effects are also known. Taken together, HVE is considered to have the potential as an effective natural material for improving the scalp environment based on active ingredients including saponarin.

5. Conclusion

This study shows that HVE has hair growth promoting effects, anti-oxidant, moisturizing, and anti-inflammatory effects. Based on these results, it was suggested that HVE could be a potential candidate for improving the hair loss and scalp environment in cosmeceutical.

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