

Therapeutic effects of *Euonymus Sachalinensis* Extract on Atopic Dermatitis: Evidence from TNF-alpha/IFN-gamma-induced HaCaT Cells and a DNCB- induced Mouse Model

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Research Article

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Abstract

Background

Atopic dermatitis (AD) is a chronic inflammatory skin disorder characterized by immune dysregulation and skin barrier dysfunction. This study investigated the anti-inflammatory and barrier-restorative effects of *Euonymus sachalinensis* (ES) extract using *in vivo* and *in vitro* AD models.

Methods

A 1-chloro-2,4-dinitrobenzene (DNCB)-induced AD mouse model was used to assess the therapeutic effects of ES through histological analysis, Eczema Area and Severity Index (EASI) scores, and scratching behavior. Western blotting was performed to examine the key inflammatory pathways. *In vitro*, TNF- α /IFN- γ -stimulated HaCaT cells were treated with ES to evaluate inflammatory mediator suppression and skin barrier restoration.

Results

ES treatment significantly alleviated AD-like symptoms by reducing epidermal and dermal thickening, mast cell infiltration, and immune organ hypertrophy. The western blot analysis revealed MAPK suppression (ERK, JNK, p38) and NF- κ B (I κ B α , p65) signaling in dorsal skin tissues. *In vitro*, ES reduced COX-2 expression, inhibited MAPK phosphorylation, and prevented NF- κ B activation and PI3K/AKT pathway signaling. ES also downregulated inflammatory cytokines (IL-13 and IL-4) and chemokines (CCL22 and CCL17), while restoring filaggrin expression and improving skin barrier function.

Conclusion

The ES extract exerts anti-inflammatory and skin-protective effects by modulating key inflammatory pathways and enhancing epidermal integrity. Identification of bioactive compounds in ES highlights their therapeutic potential. Further studies are required to isolate the active constituents and validate their clinical applicability in AD treatment.

1 Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disorder affecting children and adults. While it is predominantly observed in childhood, studies have indicated that approximately 80% of affected children continue to experience symptoms into adulthood (1).

If inadequately managed, AD can significantly impair the quality of life of patients and their families. Additionally, AD is frequently associated with other atopic conditions such as asthma and allergic rhinoconjunctivitis (2). In rare cases, complications including vernal keratoconjunctivitis, giant papillary conjunctivitis, superficial punctate keratitis, atopic keratoconjunctivitis, and otitis externa or media may also develop (3). In severe cases, patients with AD may exhibit resistance to conventional topical

therapies, necessitating phototherapy or systemic immunosuppressant use (4). However, prolonged administration of these systemic treatments has been linked to various adverse effects, such as growth impairment, osteoporosis, cataracts, and lymphopenia (5).

AD pathogenesis is driven by complex immunological mechanisms involving T-lymphocyte activation and intricate interactions between keratinocytes, endothelial cells, eosinophils, and Langerhans cells (6). These interactions facilitate the release of various cytokines and inflammatory mediators, which play pivotal roles in disease progression (7). Immunoglobulin E (IgE)-mediated sensitization is a hallmark immunological feature of AD. Numerous studies have demonstrated that patients with AD exhibits increased serum levels of allergen-specific IgE, which is correlated with disease severity and chronicity (8).

Considering the limitations and side effects of conventional treatments, medicinal plants have gained increasing attention as alternative therapeutic options for treating AD. Numerous studies have explored the potential of plant medicines with low toxicity in the management of inflammatory skin disorders (9).

In this study, we investigated the therapeutic potential of *Euonymus sachalinensis* (ES) for AD treatment. Using a DNCB-induced AD mouse model and TNF- α /IFN- γ -stimulated human keratinocytes, we evaluated the effects of ES on inflammatory responses. Liquid chromatography-mass spectrometry (LC-MS) was used to identify the chemical constituents of the ES extract. To elucidate the underlying mechanisms, we conducted immunohistochemical analysis, western blotting, and metabolomic profiling, with an aim of examining key targets, signaling pathways, and metabolic changes. Our findings provide novel insights into the therapeutic efficacy of ES in the management of AD, potentially contributing to the development of safer and more effective treatment strategies.

2 Materials and Methods

2.1 Sample Preparation

The ES (*F. Schmidt*) Maxim. (Leaf) extract (KPM052-099) used in this study was obtained following a previously described extraction method and was provided by the Korea Plant Extract Bank at the Korea Research Institute of Bioscience and Biotechnology, Daejeon, Republic of Korea. Briefly, 100 g of powdered plant material was extracted in 1 L of 99.9% (HPLC grade) methyl alcohol for 15 min, followed by filtration to obtain the ES extract.

2.2 Reagents

1-chloro-2,4-dinitrobenzene (DNCB), dexamethasone, TNF- α , and IFN- γ were obtained from Sigma-Aldrich (St. Louis, MO, USA). COX-2 (cat no. 12282S), p-JNK (cat no. 9251S), JNK (cat no. 9252S), p-p38 (cat no. 4511S), p38 (cat no. 9212S), p-ERK (cat no. 9101S), ERK (cat no. 9102S), p-p65 (cat no. 3033S), p65 (cat no. 8242S), histone H3 (cat no. 4499S), p-IkBa (cat no. 2859S), IkBa (cat no. 4814S), GAPDH (cat no. 5174T), p-PI3K (cat no. 4228S), PI3K (cat no. 4292S), p-AKT (cat no. 4060S), and AKT (cat no.

9272S) antibodies were acquired from Cell Signaling Technology (Beverly, MA, USA). Antibodies against β -actin (cat no. sc-47778) and goat anti-mouse IgG-HRP (cat no. sc-516102) were purchased from Santa Cruz Biotechnology (Dallas, TX, USA). Goat anti-rabbit IgG (H + L) secondary antibody (cat no. 31460) was obtained from Thermo Fisher Scientific (Waltham, MA, USA).

2.3 Animal experiments

We used five-week-old male BALB/c mice (DBL Co., Ltd., Eumseong-gun, Chungcheonbuk-do, South Korea) in this study. All animal studies were performed according to protocols approved by the Institutional Animal Care and Use Committee (IACUC) of Kyung Hee University (confirmation number: KHSASP-24-402). Animals were housed under controlled temperatures (23–25°C), with a 08:00–20:00 light cycle. Food and water were provided ad libitum.

2.4 AD induction and treatment

After a 7-day acclimation period, the mice were randomly divided into five groups ($n = 5$): the Non-treated (NT) group served as the normal control treated with a vehicle; the DNCB group served as the positive control sensitized with DNCB; the DEX group served as a positive control sensitized with DNCB and treated with dexamethasone (DEX); and the ESL and ESH groups were sensitized with DNCB and treated with ES at a low dose (50 mg/kg) and high dose (100 mg/kg), respectively. To induce acute AD, 1% DNCB dissolved in acetone and oil (4:1) was topically applied to the dorsal skin and ears of the mice once daily for 4 days. A volume of 100 μ L was applied to the shaved dorsal skin, and 20 μ L was applied to each ear. After a rest period of 3 days, 0.5% DNCB was topically applied every alternate day for 15 days. During this period, the experimental groups were orally administered the test samples prepared at appropriate concentrations daily. A schematic of the model design is displayed in Fig. 1. Body weights were measured on days 0, 3, 7, 10, 13, 16, 19, and 22. After sacrifice, the weights of immune organs, including the spleen and lymph nodes, were measured and normalized to the body weight of each mouse prior to sacrifice. The dorsal and ear tissues were harvested for histopathological and biochemical analyses.

2.5 Evaluation of the severity of AD-like skin lesions and ear thickness

Dorsal skin severity and ear thickness were recorded on days 0, 7, 10, 13, 16, 19, and 22. The severity of AD symptoms was assessed using the Eczema Area and Severity Index (EASI) scoring system. The EASI scoring criteria accounts for erythema, edema/papulation, excoriations/scratching, and lichenification, evaluating symptoms on a scale of 0 (none), 1 (mild), 2 (moderate), or 3 (severe). Ear thickness was measured using a digital caliper. Scratching behavior was evaluated weekly in a blinded manner. After an appropriate acclimation period, each mouse was observed for 20 min and the frequency of scratching bouts were recorded.

2.6 Histological analysis

Skin tissue samples were collected from each mouse and fixed in a 10% formalin solution (Sigma-Aldrich, St. Louis, MO, USA) for 24 h. Fixed tissues were processed, embedded in paraffin, and sectioned at a thickness of 4 μm using a microtome.

For general histological examination, sections were stained with Hematoxylin and Eosin (H&E) to assess epidermal and dermal thickness. Toluidine Blue staining was performed to detect mast cell infiltration in the dermal layer. The stained sections were examined under a light microscope.

2.7 Biological analysis

Blood samples were collected in ethylenediaminetetraacetic acid-coated tubes. The samples were incubated at room temperature for 20 min, followed by centrifugation at 3,000 g for 20 min, and the supernatants were collected. Serum IgE levels were determined using an Enzyme-Linked Immunosorbent Assay (ELISA) kit (cat. no. 432401; BioLegend, San Diego, CA, USA). The plasma levels of IL-13 and IL-1 β were measured using the Luminex Performance Assay kit (cat. no. FCSTM20; R&D Systems, Minneapolis, USA). All procedures were performed according to the manufacturer's instructions.

2.8 Cell culture

The human skin keratinocyte cell line HaCaT was purchased from American Type Culture Collection (ATCC). HaCaT cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with low glucose (Cytiva, cat. no. SH30021.01), supplemented with 10% fetal bovine serum (Gibco, NY, USA) and 1% penicillin/streptomycin (Corning Inc., NY, USA). The cells were incubated at 37°C under 5% CO₂.

2.9 Cell viability assay

Cytotoxicity was measured using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium (MTT) assay. HaCaT cells were seeded in a 96-well plate at the density of 1×10^4 cells/well and incubated for 24 h. Then, the cells were treated with different concentrations of ES (25–400 $\mu\text{g/mL}$) for 24 h. To assess HaCaT cell recovery against TNF- α (10 ng/mL) and IFN- γ (10 ng/mL), HaCaT cells were pretreated with ES (25–100 $\mu\text{g/mL}$) for 1 h before incubation with TNF- α and IFN- γ in DMEM for 24 h. Next, the cells were treated with MTT solution for 1 h. Formazan was dissolved in dimethyl sulfoxide (DMSO), and the absorbance of the solution was measured at 540 nm using a microplate reader (Bio-Rad, Hercules, CA, USA).

2.10 Western blot analysis

Western blotting was performed according to the protocol outlined in a previous study. Tissue samples were homogenized in a RIPA buffer (BioSesang Co., Ltd.). HaCaT cells (2×10^5 cells/well) were seeded and incubated in DMEM with different concentrations of ES (25–100 $\mu\text{g/mL}$) for 1 h. After pretreatment, the cells were treated with TNF- α (10 ng/mL) and IFN- γ (10 ng/mL) for 24 h. Cells were lysed in lysis buffer (Cell Signaling Technology, Danvers, MA, USA) (10). Cytoplasmic and nuclear fractions were obtained using NE-PER nuclear and cytoplasmic extraction reagents (Thermo Fisher Scientific, Waltham,

MA, USA) (11). After performing sample separation by 10–15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), the protein bands were electrotransferred onto a nitrocellulose membrane. The membrane was blocked using 3% bovine serum albumin (BSA) and subsequently incubated with primary antibodies overnight at 4°C. The membrane was then exposed to secondary antibodies, and the color was developed in an enhanced chemiluminescence detection solution.

2.11 Cytokine analysis

Cytokine analysis was performed as described in a previous study (12). Total RNA was isolated from HaCaT cells using the RiboEX and GeneAll Hybrid-RNA purification kits. RNA was quantified using NanoDrop (Thermo Fisher Scientific, Waltham, MA, USA), and cDNA was synthesized using Maxime RT PreMix (inNtRON Biotech, Seongnam-si, Korea). RT-PCR was conducted using the Applied Biosystems StepOne system (Applied Biosystems, USA). The gene expression level was quantified as the relative expression of the target gene contrasted to the *GAPDH* gene ($2^{-\Delta\Delta C_t}$). The primer sequences used for RT-PCR are listed in Table 1.

Table 1
List of primer sequences used for RT-PCR

Gene	Forward (5'–3')	Reverse (5'–3')
Human <i>FLG</i>	TGAAGCCTATGACACCACTGA	TCCCCTACGCTTTCTTGTCTT
Human <i>IL-4</i>	GCCAAGACCCCTTCGAGAAAT	CCGATCCTGTTATCTGCCTCC
Human <i>IL-13</i>	GGGCATTGAAGCGAAGATACA	GCCCAGGAACCTTTGAACTTCTG
Human <i>CCL17</i>	GACCTTGGTAAACTGCAAGCA	CCTTTTTGGAATCCCCATTCTCC
Human <i>CCL22</i>	ATCGCCTACAGACTGCACTC	GACGGTAACGGACGTAATCAC
Human <i>GAPDH</i>	TGTGGGCATCAATGGATTTGG	ACACCATGTATTCCGGGTCAAT

2.12 Liquid chromatography-mass spectrometry (LC-MS) analysis

Based on a previous study (13), ES component analysis was conducted using LC-MS. The analysis was conducted using an Agilent 1290 Infinity LC System with a Waters C18 column (2.1 mm × 100 mm, 1.7 μm) at 30°C. The system was connected to Agilent 6550 Q-TOF with a dual AJS ESI source operating in both the positive and negative ion modes. Mass spectra were recorded at 100–1700 m/z. Data analysis was performed using the MassHunter Qualitative Analysis software.

2.13 Statistical analysis

All experiments were repeated at least thrice. The data were presented as the mean ± SEM. Statistical analyses were performed with ANOVA and unpaired *t*-test using the GraphPad Prism 8 software (GraphPad Software, San Diego, CA, USA). Statistical significance was set at *p* < 0.05.

3 Results

3.1 Effects of ES on the skin lesions and immune organ in DNCB-induced BALB/c mice

To investigate the anti-inflammatory effects of ES on AD, a BALB/c mouse model was used. Acute AD-like lesions were induced with the topical application of 1% DNCB for four days, followed by chronic exposure to 0.5% DNCB. Prior to DNCB treatment, no significant differences in body weight were observed between the NT and DNCB-induced groups. However, following DNCB induction, the body weight of DNCB-treated mice significantly decreased, likely due to the reduced quality of life and increased pruritus. Notably, the ESL, ESH and DEX groups exhibited less weight loss than that observed in the DNCB-induced group (Fig. 2A). In DNCB-induced mice, the weight of immune organs, such as the spleen and lymph nodes, increased, indicating systemic immune activation. However, in mice treated with ES, the weights of these immune organs were significantly reduced (Fig. 2B, C). Ear thickness, a key marker of AD-like lesions, was significantly increased by day 7 following 1% DNCB treatment. However, continuous ES administration accelerated recovery, leading to a marked reduction in ear thickness (Fig. 2D, E, F).

AD is characterized by dry skin, erythema, pruritus, and a frequent scratching behavior. Disease severity was evaluated using the EASI scoring system and a behavioral test to measure scratching behavior (14). The EASI score increased in the DNCB-induced group but was significantly reduced in the ESL, ESH, and DEX groups (Fig. 2G). The scratching frequency increased in mice in the DNCB-induced group, and decreased in mice from the ESL, ESH, and DEX groups, suggesting an alleviation of pruritus (Fig. 2H).

3.2 Effects of ES on thickness and mast cell infiltration in DNCB-induced BALB/c mice

Compared with the NT group, the DNCB-induced group exhibited more pronounced inflammatory responses, edema, erythema, and skin lesions. Similarly, increased swelling and redness were observed in the ear, along with a significant increase in thickness (Fig. 3A). H&E staining revealed that both epidermal and dermal thicknesses were markedly increased in the DNCB-induced group in both the ear and back, whereas these changes were alleviated in the ESL, ESH, and DEX groups (Fig. 3B). Mast cell expression is increased in various inflammatory skin disorders, including AD. Toluidine blue staining revealed sparse, fewer mast cells in the NT group, whereas the DNCB-induced group showed dense aggregation and a significant increase. However, following treatment, the number of mast cells in both the back and ear significantly decreased in both the ES- and DEX-treated groups compared to the DNCB-induced group (Fig. 3C). These results indicate that ES notably enhances skin conditions in AD mice and facilitates recovery.

3.3 Effects of ES on IgE and mRNA levels in DNCB-induced BALB/c mice

Elevated IgE levels are a key immunological feature of AD (15). IgE induces mast cell degranulation and histamine secretion, exacerbating allergic reactions (16). Thus, inhibiting IgE secretion reduces inflammatory responses. Mouse serum IgE levels detected by ELISA increased in DNCB-induced mice. However, the IgE levels showed a decreasing trend in the ESH group (Fig. 4A). Further, biological analysis was conducted to evaluate the effects of ES on inflammatory cytokines. ES treatment significantly downregulated the expression of inflammatory cytokines, including IL-13 and IL-1 β , in the serum of DNCB-induced AD mice (Fig. 4B, C). These findings suggest that ES attenuates inflammatory responses, reinforcing its therapeutic potential in AD.

3.4 Effects of ES on the MAPK/NF- κ B signaling pathway in DNCB-induced BALB/c mice

Mitogen-activated protein kinase (MAPK) phosphorylation is associated with inflammatory responses, including those observed in allergic reactions (17). The activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) is dependent on the degradation of NF-kappa-B inhibitor alpha (I κ B α) (18). Once activated through I κ B degradation, NF- κ B translocates to the nucleus, where it promotes inflammation by upregulating key inflammatory genes (19). To investigate the potential inhibitory effects of ES on MAPK and NF- κ B activation, western blot analysis was performed on dorsal tissue. Compared to the DNCB-induced group, the protein levels of p-JNK/JNK, p-p38/p38, and p-ERK/ERK were significantly reduced in the ES-treated groups. Additionally, the levels of p-p65/p65 and p-I κ B α /I κ B α were also decreased in the ES-treated groups compared to the DNCB-induced group. These findings indicate that ES administration modulates the MAPK and NF- κ B signaling pathways, thereby suppressing the inflammatory response.

3.5 Effect of ES on the viability of TNF- α /IFN- γ -treated HaCaT cells

To investigate its specific mechanism of action, we evaluated the efficacy of ES in HaCaT cells. The MTT assay was performed to analyze the cytotoxicity of ES in HaCaT cells. The cells were treated with ES up to a concentration of 400 μ g/mL, and no cytotoxicity was observed until 100 μ g/mL (Fig. 6A). Based on the result of the MTT assay, we selected ES concentrations in the range of 25–100 μ g/mL for the subsequent experiments. Afterward, we sought to confirm the protective effect of ES on TNF- α /IFN- γ -treated HaCaT cells. Cell viability decreased to 77.0% following TNF- α /IFN- γ treatment; however, a 1-hour pre-treatment with ES prompted a dose-dependent recovery of viability.

3.6 Effect of ES on the COX-2 Expression in TNF- α /IFN- γ -treated HaCaT cells

Oxidative stress promotes the expression of inflammatory genes, including *COX-2*. *COX-2* expression is upregulated by proinflammatory signals leading to increased prostaglandin synthesis, which facilitates the inflammatory cascade (20). To evaluate the anti-inflammatory effects of ES, the expression levels of COX-2 were analyzed using western blotting (Fig. 7A, B). TNF- α /IFN- γ -treated HaCaT cells exhibited a significant increase in COX-2 expression compared to untreated cells. ES treatment effectively reduced the relative expression of COX-2 in a dose-dependent manner (Fig. 7B).

3.7 Effects of ES on the MAPK Signaling Pathway in TNF- α /IFN- γ -treated HaCaT cells

Next, western blot analysis was performed to investigate the effects of ES on MAPK signaling pathways, which are closely associated with inflammation. The phosphorylation levels of JNK (A), p38 (B), and ERK (C) were significantly increased in TNF- α /IFN- γ -treated HaCaT cells; however, treatment with 25–100 μ g/mL of ES effectively suppressed their phosphorylation in a dose-dependent manner.

3.8 Effects of ES on the NF- κ B signaling pathway in TNF- α /IFN- γ -treated HaCaT cells

In the NF- κ B signaling pathway, p65 is phosphorylated following the degradation of I κ B α in the cytoplasm. Phosphorylated p65 then translocates to the nucleus where it causes the overexpression of inflammatory factors. Our results indicated that ES treatment attenuated the expression of p-p65 in the nuclear fraction of TNF- α /IFN- γ -treated HaCaT cells (Fig. 9A). Furthermore, ES effectively inhibited the degradation of I κ B α in the cytosolic fraction of these cells (Fig. 9B). Consequently, our findings suggest that ES exerts anti-inflammatory effects in TNF- α /IFN- γ -treated HaCaT cells by inhibiting the activation of the NF- κ B signaling pathway.

3.9 Effects of ES on the PI3K/AKT signaling pathway in TNF- α /IFN- γ -treated HaCaT cells

The phosphatidylinositol 3-kinase (PI3K)/AKT signaling pathway plays a critical role in the pathogenesis of chronic immune-mediated skin diseases (21). PI3K/AKT signaling is activated by inflammatory responses, and its inhibition is associated with the suppression of NF- κ B signaling through the regulation of downstream effectors (22). Therefore, western blot analysis was performed to examine the effects of ES on the PI3K/AKT signaling pathway in HaCaT cells (Fig. 10A, B). The results showed that TNF- α /IFN- γ treatment induced the activation of PI3K and AKT, whereas ES treatment effectively suppressed the activation of the PI3K/AKT pathway. These findings suggest that ES inhibits I κ B α degradation and NF- κ B activation via the PI3K/AKT pathway.

3.10 Effects of ES on mRNA levels in TNF- α /IFN- γ -treated HaCaT cells

RT-PCR was conducted to evaluate the effects of ES on the mRNA expression of inflammatory cytokines, chemokines, and skin barrier-related factors in HaCaT cells treated with TNF- α /IFN- γ . A significant increase in *IL-13* (Fig. 11A) and *IL-4* (Fig. 11B) mRNA levels upon TNF- α /IFN- γ stimulation confirmed the induction of inflammation. Similarly, the expression of the chemokines *CCL22* (Fig. 11C) and *CCL17* (Fig. 11D) was upregulated following TNF- α /IFN- γ stimulation. However, pretreatment with ES markedly suppressed the TNF- α /IFN- γ -induced increases in the levels of these inflammatory mediators. Additionally, the expression of *FLG* (Fig. 11E) was significantly reduced by TNF- α /IFN- γ stimulation, but was restored upon ES treatment. FLG is a key factor in skin barrier function and alleviates AD-like skin inflammation (23). These results suggest that ES exerts anti-inflammatory effects by downregulating cytokine and chemokine expression, while enhancing skin barrier-related gene expression.

3.11 LC-MS Analysis of ES

LC-MS analysis was conducted to investigate the separation and identification of the bioactive compounds in ES. The chromatogram displayed multiple peaks, each corresponding to a distinct compound in the extract. Most components were effectively detected in both positive (ESI⁺) and negative (ESI⁻) electrospray ionization modes. The x-axis represents the retention time (min), and the y-axis indicates the intensity of the ion signals. The compounds associated with these peaks are summarized in Table 2, which highlights key bioactive constituents that may contribute to the pharmacological effects of ES. Evonine and Isoevonine are alkaloids, whereas Strophanthidin belongs to the cardenolides and is a steroid aldehyde. Nicotinamide, a form of vitamin B3, can improve skin conditions such as atopic dermatitis by modulating inflammatory responses (24). These findings elucidated the molecular mechanisms underlying the anti-inflammatory effects of ES and highlighted its potential as a natural anti-inflammatory agent.

Table 2
ES compound list

No.	R.T (min)	Compound	Molecular Formula	Mass	Experimental Mass (m/z)	Selected Ion Species
1	6.483	strophanthidine	C23H32O6	404.22	427.195 449.204	(M + Na)+ (M + HCOO)-
2	7.393	nicotinamide	C6H6N2O	122.05	123.045 121.030	(M + H)+ (M-H)-
3	10.338	Evonine	C34H41NO16	719.24	720.251 754.2119 718.2352	(M + H)+ (M + Cl)- (M-H)-
4	11.827	Isoevonine	C36H43NO17	761.25	762.2685	(M + H)+

4 Discussion

AD is a chronic inflammatory disease that affects both children and adults and significantly impairs the quality of life, imposing a substantial disease burden (25). Additionally, it may lead to the development of subsequent conditions, such as asthma and allergic rhinitis (26, 27). In AD, Th2 cytokines, such as IL-4, are markedly elevated. These cytokines suppress the expression of FLG and other barrier-related proteins, contributing to epidermal barrier dysfunction (28). Moreover, they promote the IgE production, which facilitates mast cell degranulation and initiates inflammatory signaling pathways (29). Numerous natural compounds demonstrate anti-inflammatory effects with minimal toxicity, making them promising candidates for atopic dermatitis treatment (30).

The present study demonstrated that the ES extract exerts significant anti-inflammatory and protective effects in both *in vitro* and *in vivo* AD models. These findings highlight the ability of ES to suppress proinflammatory cytokine production, modulate signaling pathways associated with AD pathogenesis, and restore skin barrier integrity.

In the DNCB-induced AD mouse model, ES effectively reduced epidermal and dermal thickening, decreased mast cell infiltration, and alleviated immune organ hypertrophy. The improvement in AD-like lesions was further confirmed by a reduction in the EASI scores and scratching behavior. These results suggest that ES mitigates inflammatory responses and pruritus, which are hallmarks of AD. Additionally, inflammatory responses were regulated through the MAPK and NF- κ B signaling pathways in dorsal skin tissues— western blot analysis of dorsal skin extracts revealed that ES treatment significantly reduced the phosphorylation levels of ERK, JNK, and p38, indicating the suppression of MAPK signaling.

Additionally, ES decreased the phosphorylation of I κ B α and p65, suggesting inhibition of NF- κ B signaling. These molecular changes corroborated the histopathological findings, further supporting the anti-inflammatory effects of ES *in vivo*. *In vitro* experiments were conducted to further elucidate the underlying mechanisms.

ES significantly reduced COX-2 expression and inhibited phosphorylation of MAPK pathway components, including ERK, JNK, and p38, in TNF- α /IFN- γ -stimulated HaCaT cells. MAPK signaling plays a crucial role in regulating inflammatory responses by controlling the production of pro-inflammatory cytokines in keratinocytes (31). These results indicated that ES effectively interferes with pro-inflammatory signaling cascades, thereby suppressing the production of inflammatory mediators. The MAPK signaling pathway is involved in the pathogenesis of AD and contributes to the downregulation of FLG expression (32, 33). Furthermore, ES treatment downregulated NF- κ B activation by preventing the degradation of I κ B α , which is essential for nuclear translocation of NF- κ B subunits. NF- κ B activation, observed in patients with AD, plays a crucial role in the pathogenesis of the disease (34). These molecular events contribute to the attenuation of inflammatory responses in keratinocytes.

Additionally, ES modulated the PI3K/AKT pathway, a crucial regulator of cellular survival and immune responses. The PI3K/Akt pathway has been implicated in the pathogenesis of various human diseases and is associated with dermatological manifestations. Given its critical role in inflammatory skin disorders, this signaling pathway has emerged as a potential therapeutic target, prompting extensive research into pharmacological agents aimed at its modulation (35). Phosphorylation of PI3K in mast cells facilitates cytokine production by activating AKT and promoting the signaling of nuclear factor kappa B (NF- κ B), particularly its p65 and p50 subunits (36). The inhibition of PI3K/AKT signaling suggests that ES may exert broader immunomodulatory effects beyond NF- κ B suppression (37).

Moreover, ES reduced the expression of inflammatory cytokines (IL-13 and IL-4) and chemokines (CCL22 and CCL17). In addition, ES restored the expression of FLG, a key protein in maintaining epidermal barrier integrity, which was compromised in TNF- α /IFN- γ -treated keratinocytes. IL-13 and IL-4 are key cytokines associated with type 2 immune responses and play pivotal roles in AD pathogenesis (38). Additionally, CCL22 and CCL17 are released by keratinocytes, mast cells, and eosinophils, and are thought to contribute to the chronic progression of AD (39, 40). CCL22 and CCL17 are chemokines that recruit T cells to inflamed sites, thereby exacerbating skin inflammation (41, 42). The reduced expression of FLG is closely associated with AD. Inflammatory cytokines, including TNF- α and IFN- γ , suppress FLG expression, contributing to skin barrier dysfunction in AD (43). Our findings indicate that ES not only reduces inflammation, but also reinforces skin barrier function, which is a crucial aspect of AD management.

LC-MS analysis identified multiple bioactive compounds in the ES extract that were likely to contribute to its pharmacological activity. Future studies should focus on isolating the specific active constituents responsible for the observed anti-inflammatory and barrier-restorative properties. Additionally, clinical validation is necessary to confirm the translational potential of ES in the treatment of human AD.

5 Conclusions

In summary, our findings demonstrate that *Euonymus sachalinensis* extract exerts significant anti-inflammatory and skin barrier-restorative effects in both in vitro keratinocyte models and in vivo AD-like mouse models. ES modulates multiple signaling pathways, including MAPK, NF- κ B, and PI3K/AKT, thereby reducing inflammatory cytokine production and restoring FLG expression. These results suggest that ES may offer a safer and effective natural alternative for the treatment of atopic dermatitis.

Further studies are warranted to isolate and characterize the active constituents within the ES extract and to evaluate their efficacy and safety in clinical trials. These findings suggest that ES is a promising natural therapeutic candidate for the management of AD, offering a safer alternative to conventional treatments.

Abbreviations

Abbreviation	Full Term
AD	Atopic dermatitis
ES	<i>Euonymus sachalinensis</i>
DNCB	1-chloro-2,4-dinitrobenzene
EASI	Eczema Area and Severity Index
H&E	Hematoxylin and Eosin
COX-2	Cyclooxygenase-2
MAPK	Mitogen-activated protein kinase
ERK	Extracellular signal-regulated kinase
JNK	c-Jun N-terminal kinase
NF- κ B	Nuclear factor kappa-light-chain-enhancer of activated B cells
I κ B α	NF-kappa-B inhibitor alpha
PI3K	Phosphatidylinositol 3-kinase
IL	Interleukin (e.g., IL-4, IL-13)
CCL	Chemokine (C-C motif) ligand (e.g., CCL17, CCL22)
FLG	Filaggrin
TNF- α	Tumor necrosis factor-alpha
IFN- γ	Interferon-gamma
IgE	Immunoglobulin E
LC-MS	Liquid chromatography-mass spectrometry
DMEM	Dulbecco's Modified Eagle Medium
FBS	Fetal bovine serum
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
DMSO	Dimethyl sulfoxide
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
ELISA	Enzyme-linked immunosorbent assay
RT-PCR	Real-time polymerase chain reaction
BSA	Bovine serum albumin

Declarations

Consent for publication

Not applicable.

Availability of data and materials

The data presented in this study are available on request from the corresponding author.

Competing interests

The authors declare no conflict of interest. Graphical Abstract was created with BioRender software (<https://biorender.com>).

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Authors' contributions

Conceptualization, N.K. and H.-J.J.; software, N.K.; validation, N.K.; formal analysis, N.K.; data curation, N.K., W.J., S.W.K., and M.K.; writing—original draft preparation, N.K.; writing—review and editing, N.K., S.K. and H.-J.J.; visualization, N.K.; project administration, H.-J.J.; funding acquisition, H.-J.J. All authors have read and agreed to the published version of the manuscript.

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Figures

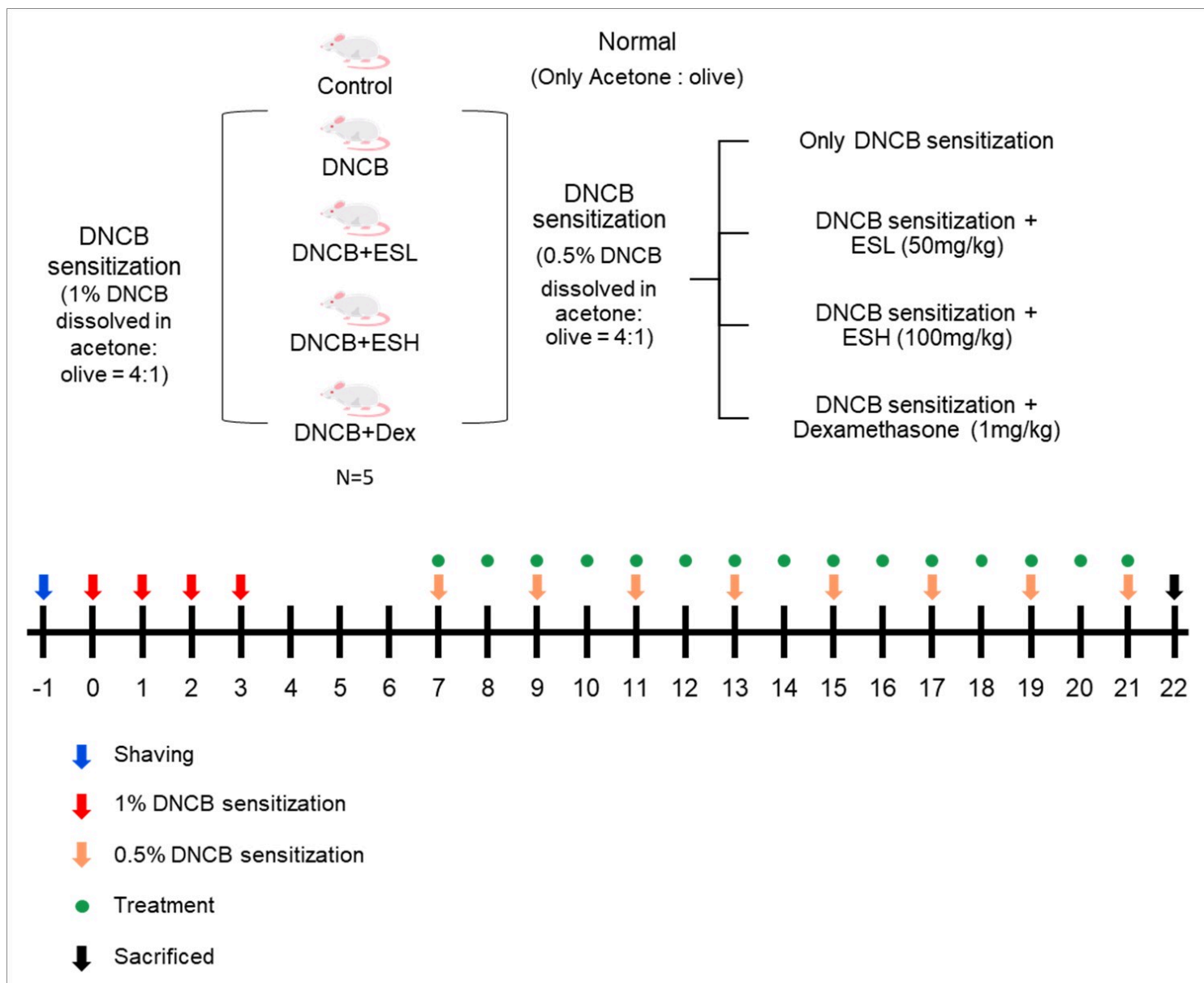


Figure 1

Scheme of the experimental design of atopic dermatitis (AD) skin lesion mouse model. DNCB (1%) was applied for sensitization for four days, with repeated application of 0.5% DNCB every other day for two weeks, except in the control group (NT). *E. sachalinensis* extract (ES) was administered orally for two weeks to the ESL (50 mg/kg/day) and ESH (100 mg/kg/day) groups. The DEX group was orally administered for two weeks with dexamethasone (1 mg/kg/day).

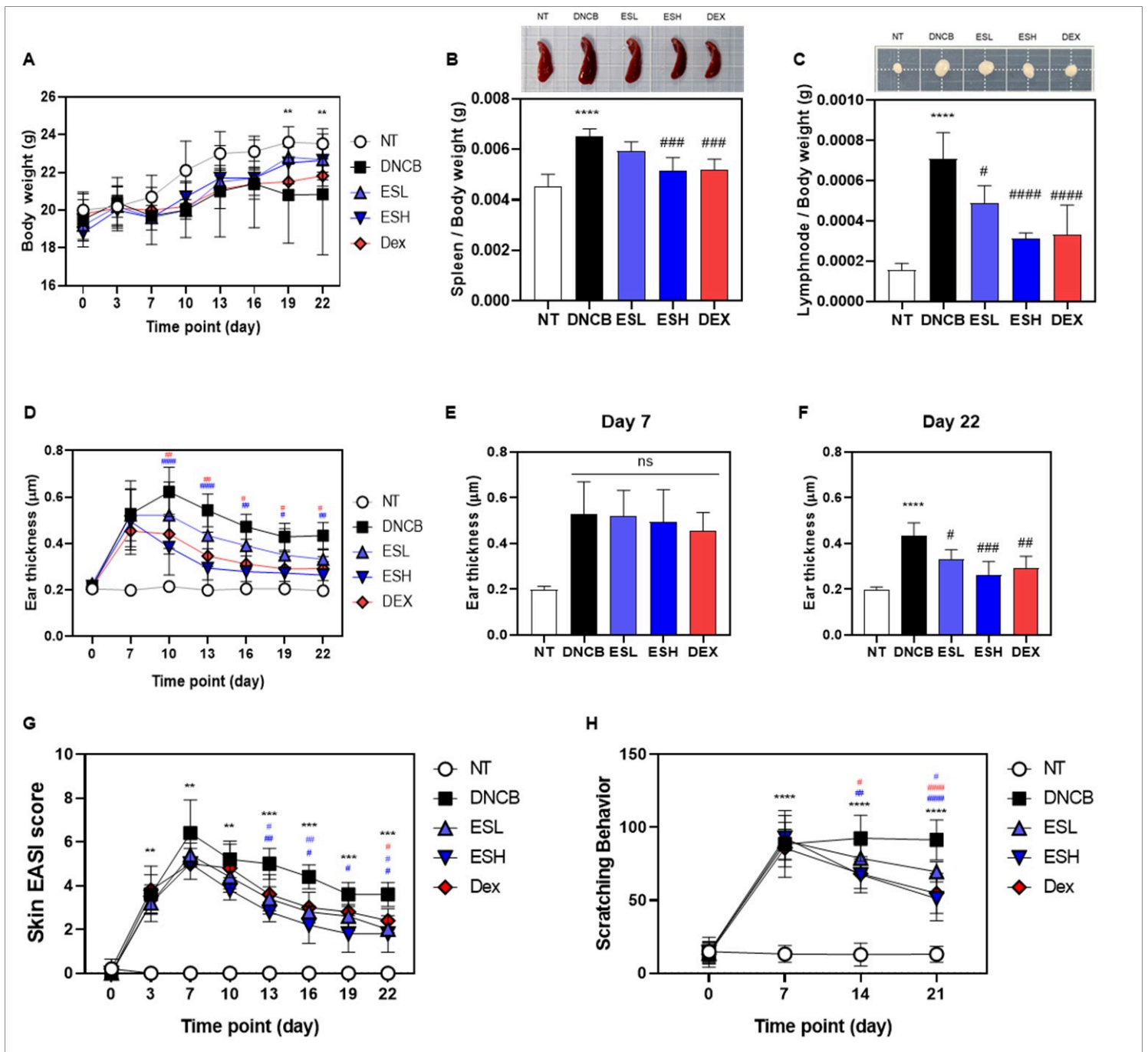


Figure 2

The effect of ES on body weight (A), relative spleen weight (B), and relative lymph node weight (C). Effect of ES on ear thickness (D). The ear thickness was measured immediately after DNCB sensitization (E) and before sacrifice following drug treatment (F). Scratching behavior was assessed weekly as part of a behavioral test (H).

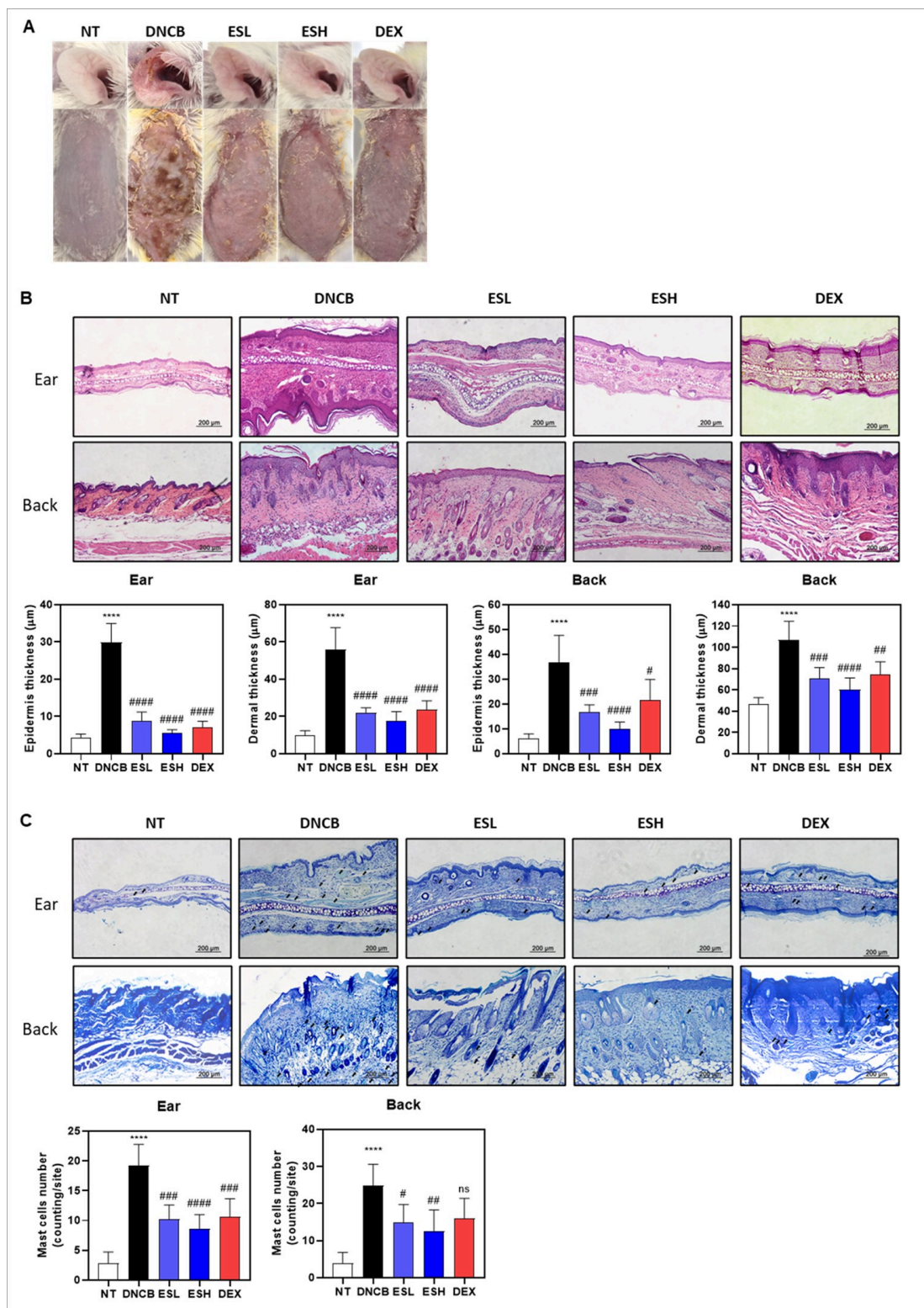


Figure 3

Anti-inflammatory effect of ES at the back region and ear of AD mice (A). The result of H&E staining of the back and ear skin (B). Toluidine blue staining showing mast cell infiltration (C). All values are expressed as the mean $\pm$ SEM. **** $p < 0.0001$ compared with the control group; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, and #### $p < 0.0001$ compared with only the DNCB-induced group.

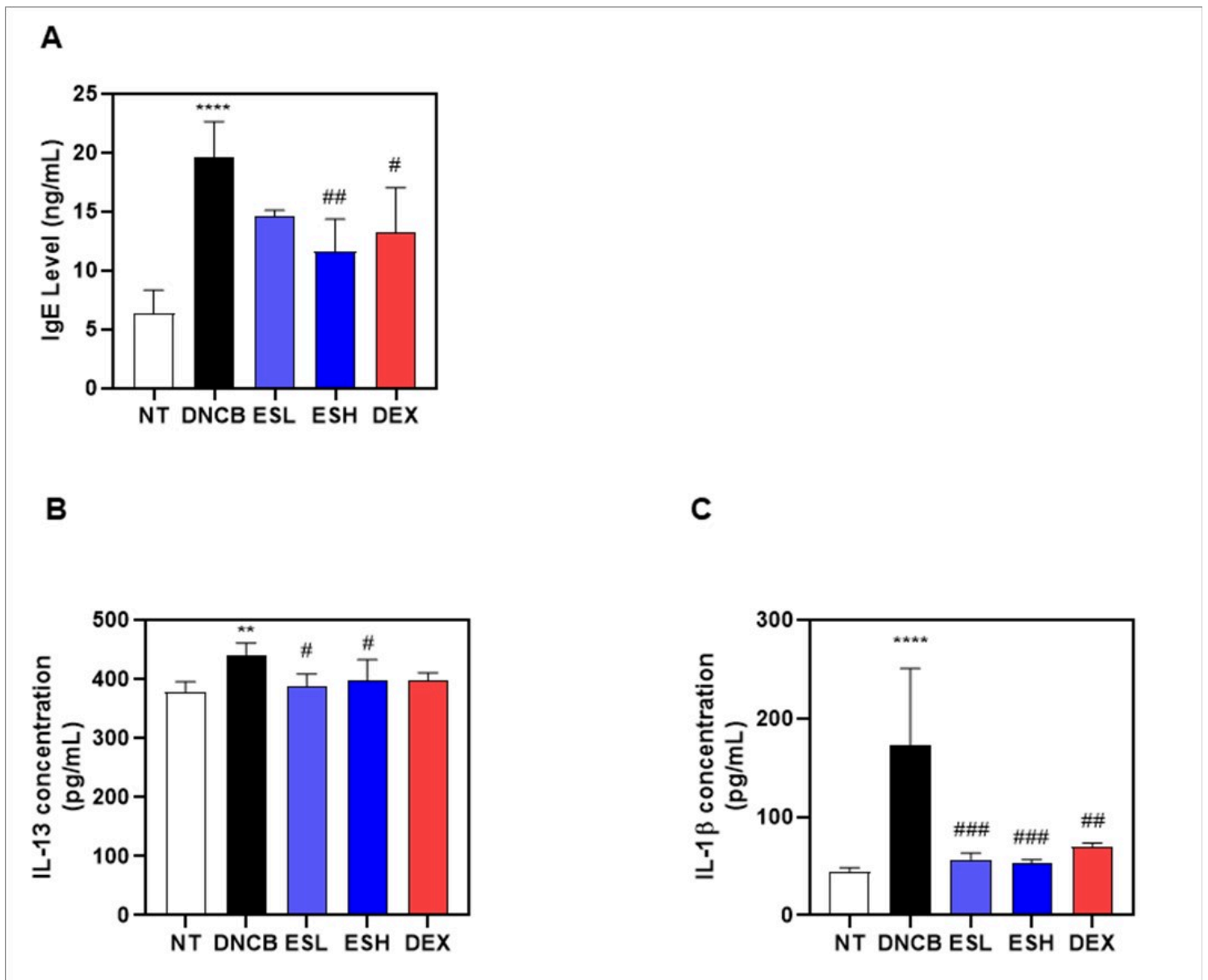


Figure 4

Effects of ES on the IgE and cytokine levels. The levels of IgE and inflammatory cytokines were measured to evaluate the anti-inflammatory effects of ES. Serum IgE levels (A) and the expression of cytokines (B–C) were analyzed. All values are expressed as the mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ compared with the control group; # $p < 0.05$, ## $p < 0.01$, and ### $p < 0.001$ compared with the DNCB-induced group.

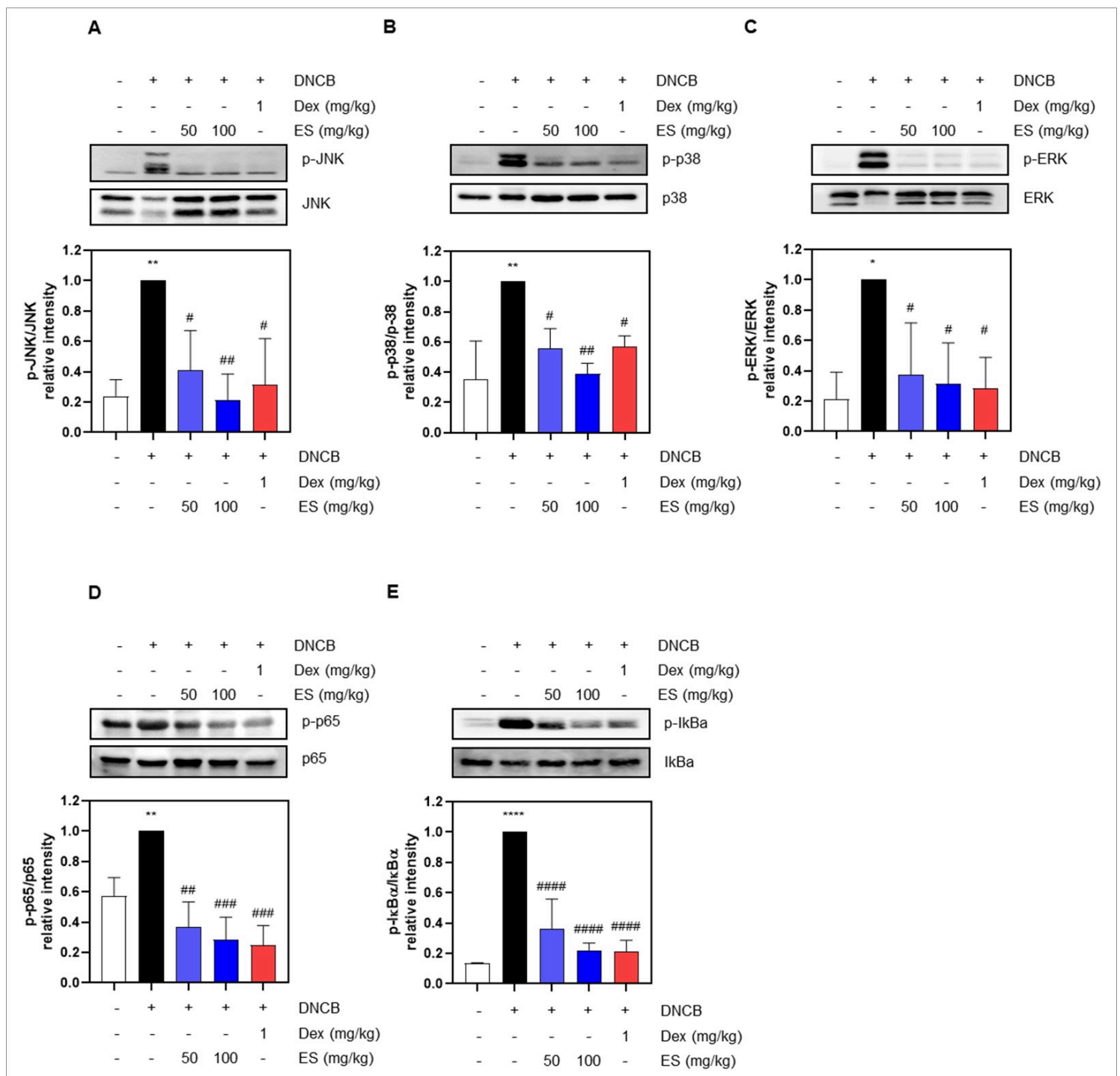


Figure 5

Effects of ES on the expression of proteins involved in the MAPK and NF- κ B pathways in the skins of DNCB-induced atopic dermatitis mice. The collected skin tissues were homogenized, and protein expression levels were determined using western blot analysis. The levels of MAPK factors including p-JNK/JNK (A), p-p38/p38 (B), and p-ERK/ERK (C) were quantified using the ImageJ software. The levels of NF- κ B factors including p-p65/p65 (D) and p-I κ B α /I κ B α (E) were also measured using ImageJ. All values are expressed as the mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, and **** $p < 0.0001$ compared with the

control group; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, and #### $p < 0.0001$ compared with the DNCB-induced group.

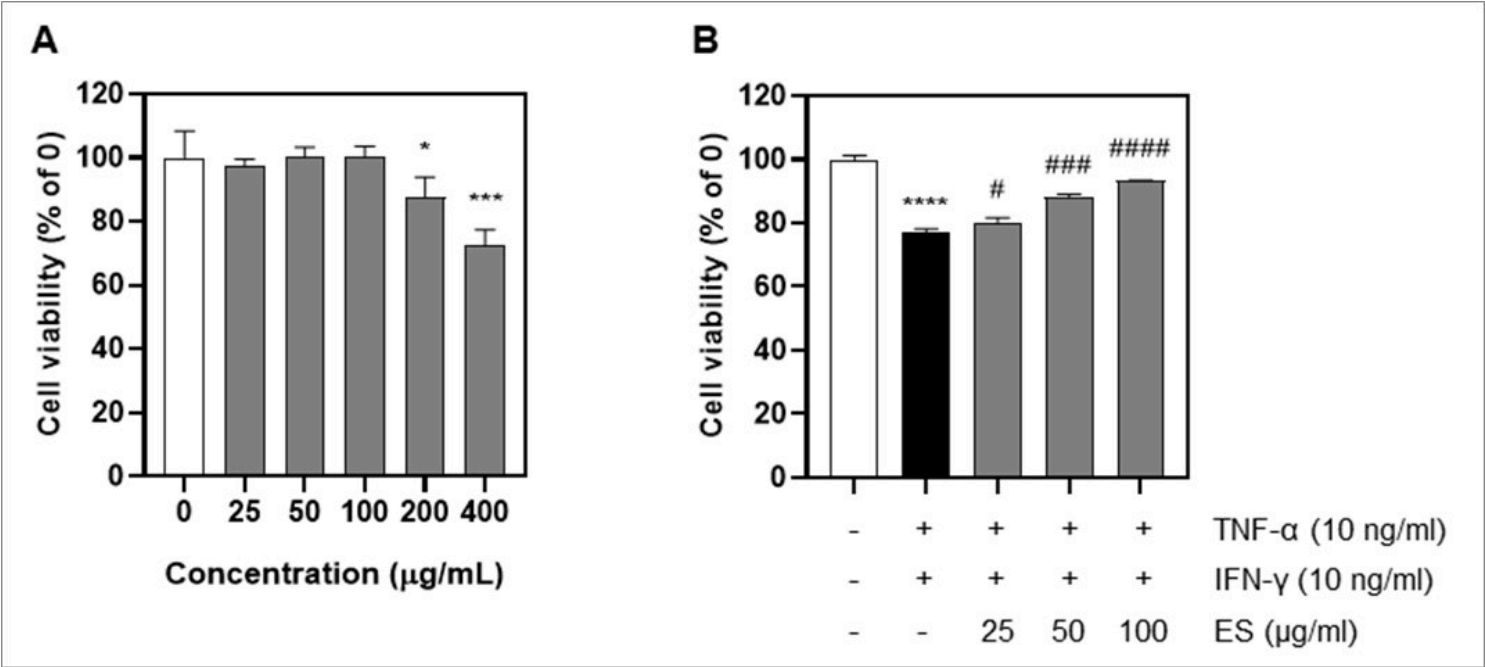


Figure 6

Effects of ES on HaCaT cells. Cell viability of HaCaT cells treated with ES for 24 h (A). Cell viability of HaCaT cells pretreated with ES for 1 h and treated with TNF-α/IFN-γ for 24 h. All values are expressed as the mean ± SEM. * $p < 0.05$, *** $p < 0.001$, and **** $p < 0.0001$ compared with the control group; # $p < 0.05$, ## $p < 0.001$, and #### $p < 0.0001$ compared with the TNF-α/IFN-γ-treated group.

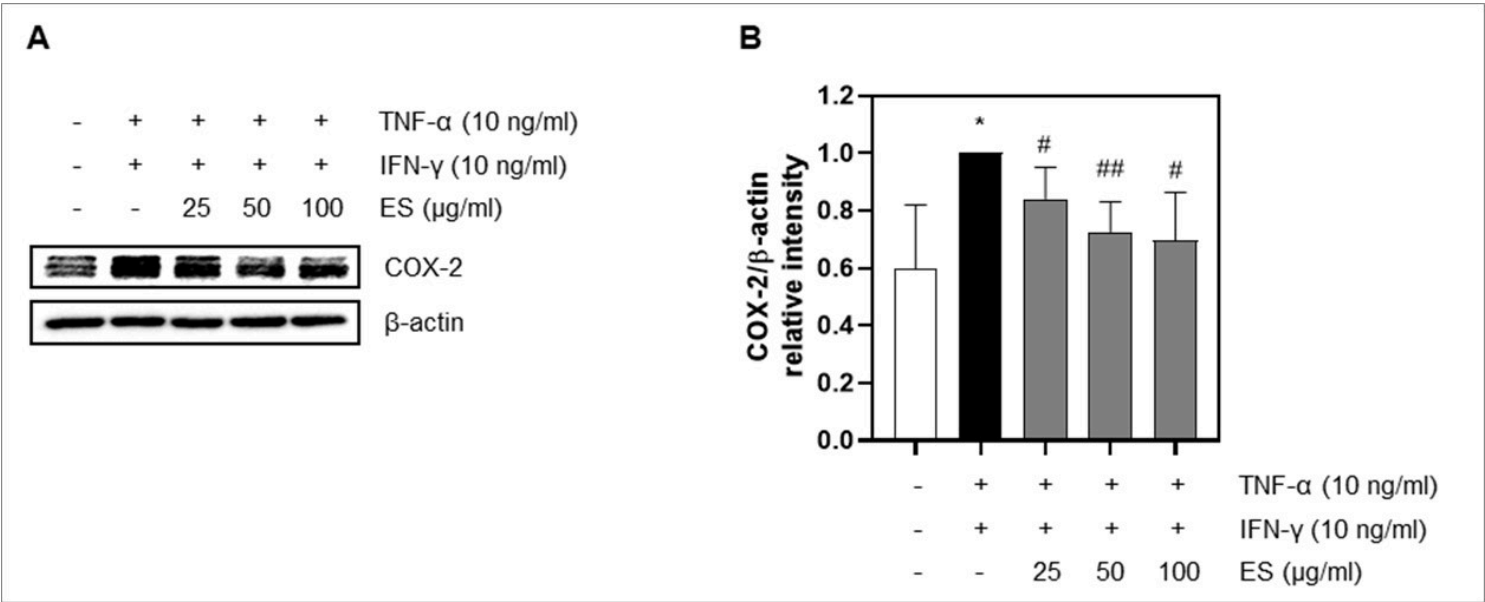


Figure 7

Effects of ES on COX-2 protein expression. All values are expressed as the mean $\pm$ SEM. * $p < 0.05$ compared with the control group; # $p < 0.05$ and ## $p < 0.01$ compared with the TNF- α /IFN- γ -treated group.

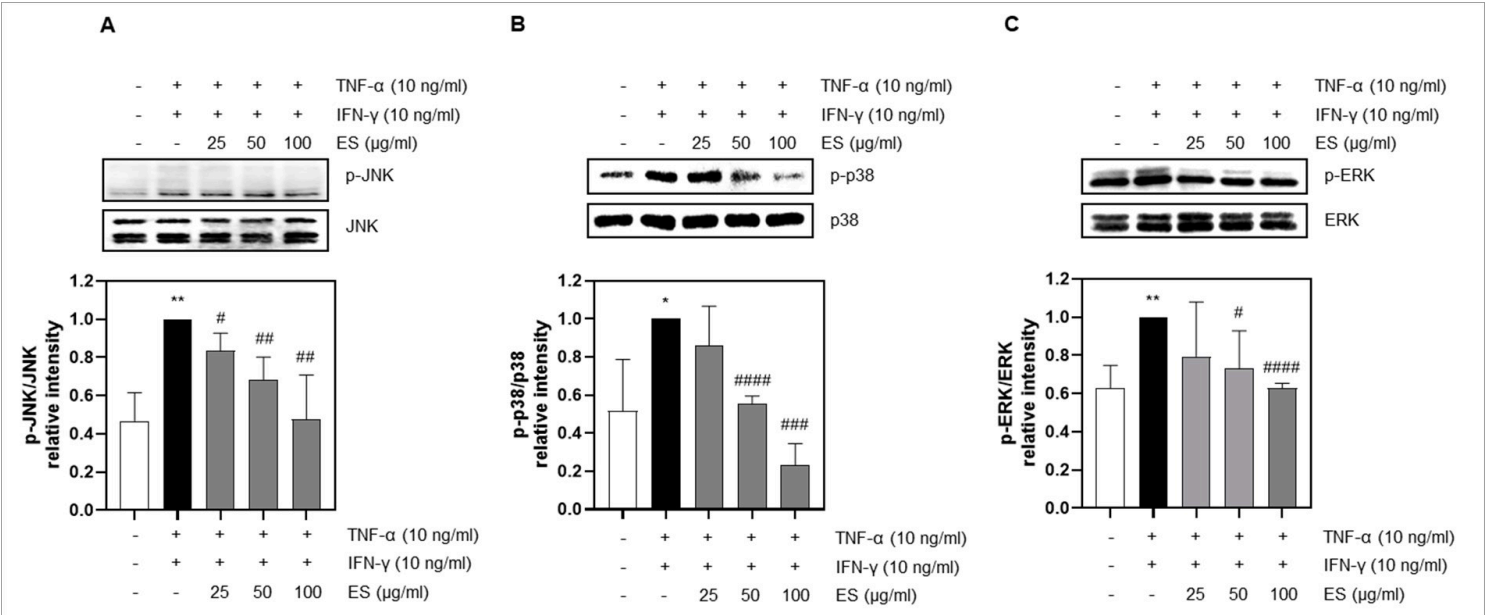


Figure 8

Effects of ES on MAPK pathways. The levels of p-JNK/JNK (A), p-p38/p38 (B), and p-ERK/ERK (C) were measured using the ImageJ software. All values are expressed as the mean $\pm$ SEM. * $p < 0.05$ and ** $p < 0.01$ compared with the control group; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, and #### $p < 0.0001$ compared with the TNF- α /IFN- γ -treated group.

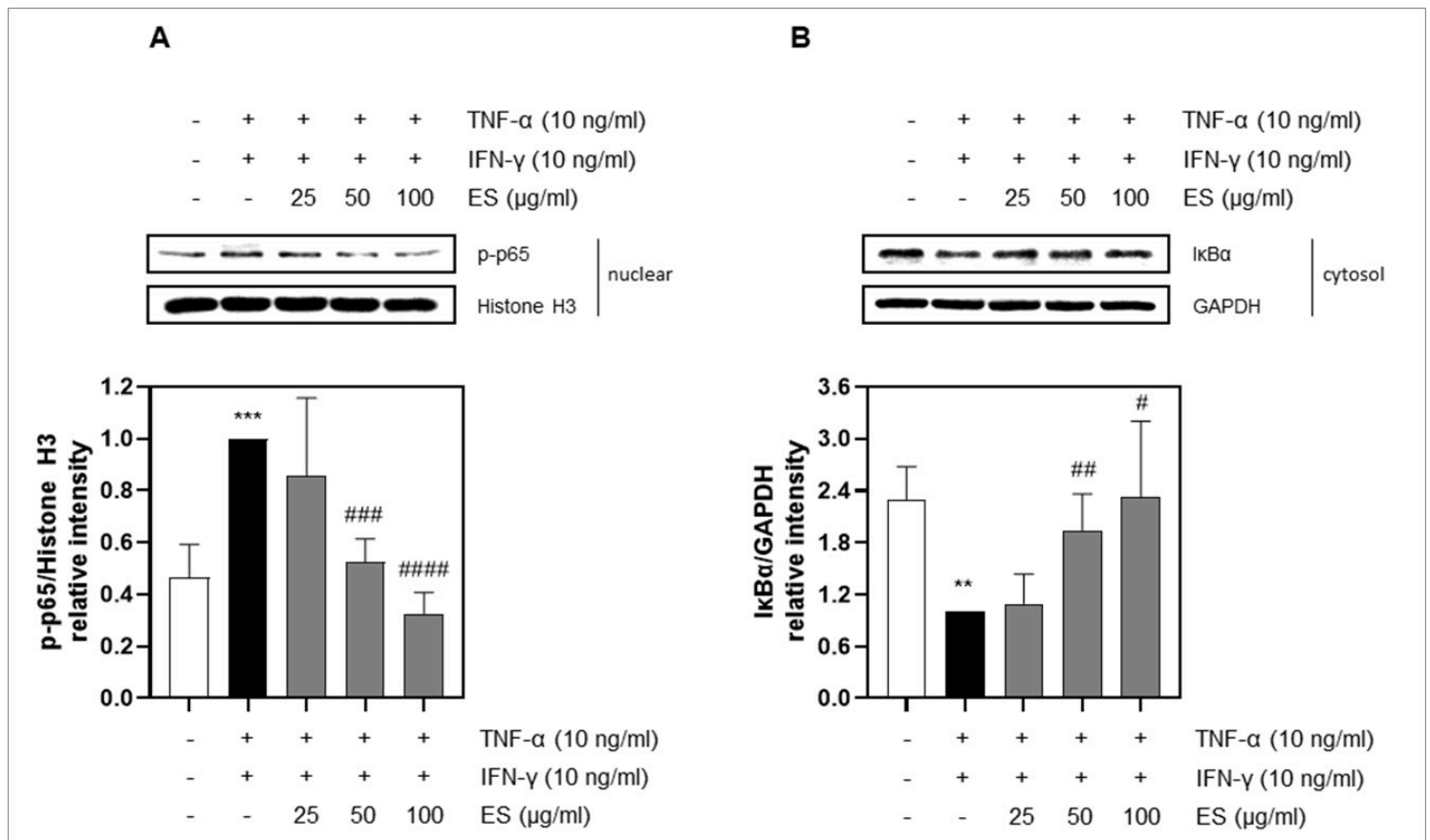


Figure 9

Effects of ES on NF- κ B pathways. The levels of p-p65/Histone H3 (A) and I κ B α /GAPDH (B) were measured using the ImageJ software. Harvested cells were separated into cytoplasmic and nuclear fractions and examined using immunoblotting analysis. All values are expressed as the mean $\pm$ SEM. ** $p < 0.01$ and *** $p < 0.001$ compared with the control group; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, and ##### $p < 0.0001$ compared with the TNF- α /IFN- γ -treated group.

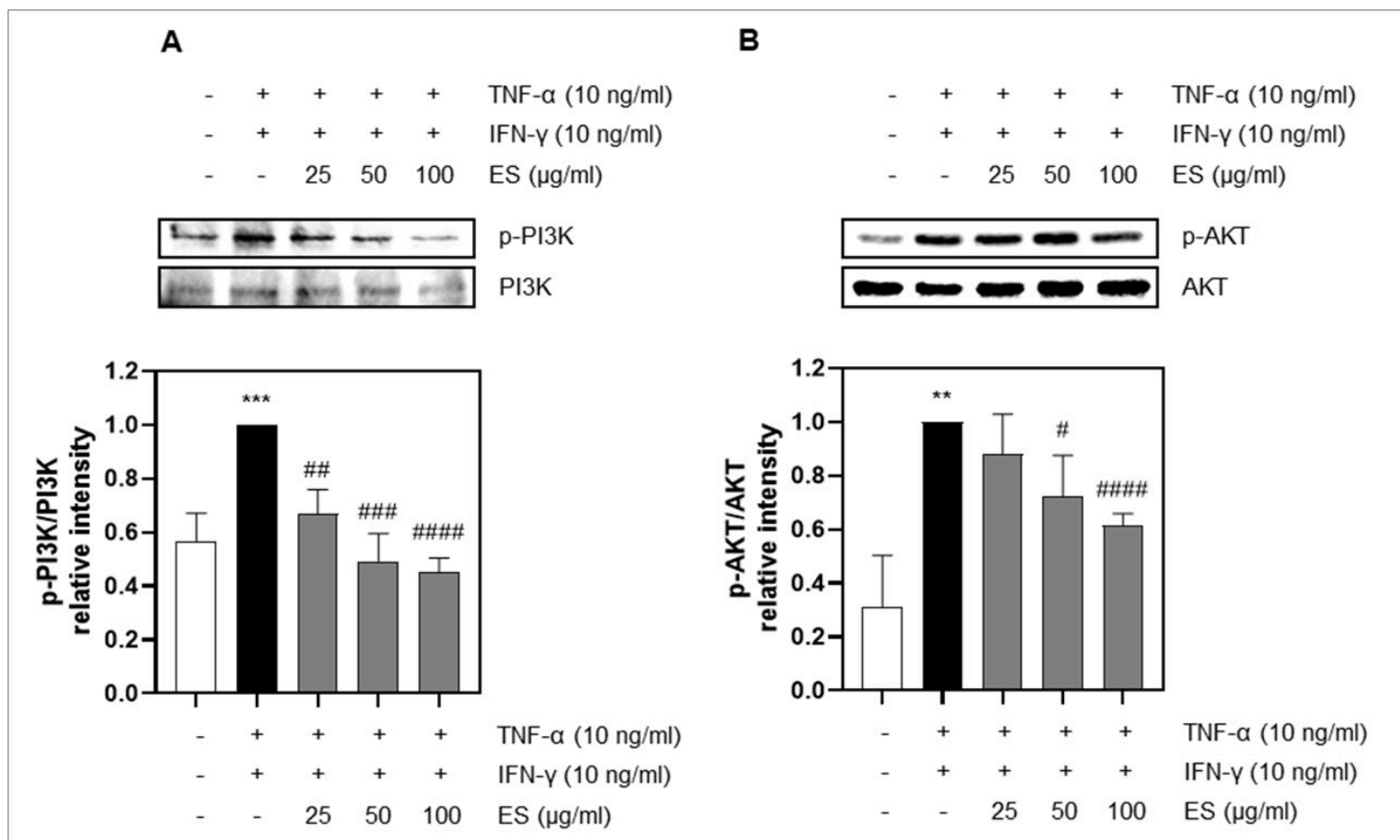


Figure 10

Effects of ES on PI3K/AKT pathways. The levels of p-PI3K/PI3K (A) and p-AKT/AKT (B) were measured using the ImageJ software. All values are expressed as the mean $\pm$ SEM. ** $p < 0.01$ and *** $p < 0.001$ compared with the control group; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, and #### $p < 0.0001$ compared with the TNF- α /IFN- γ -treated group.

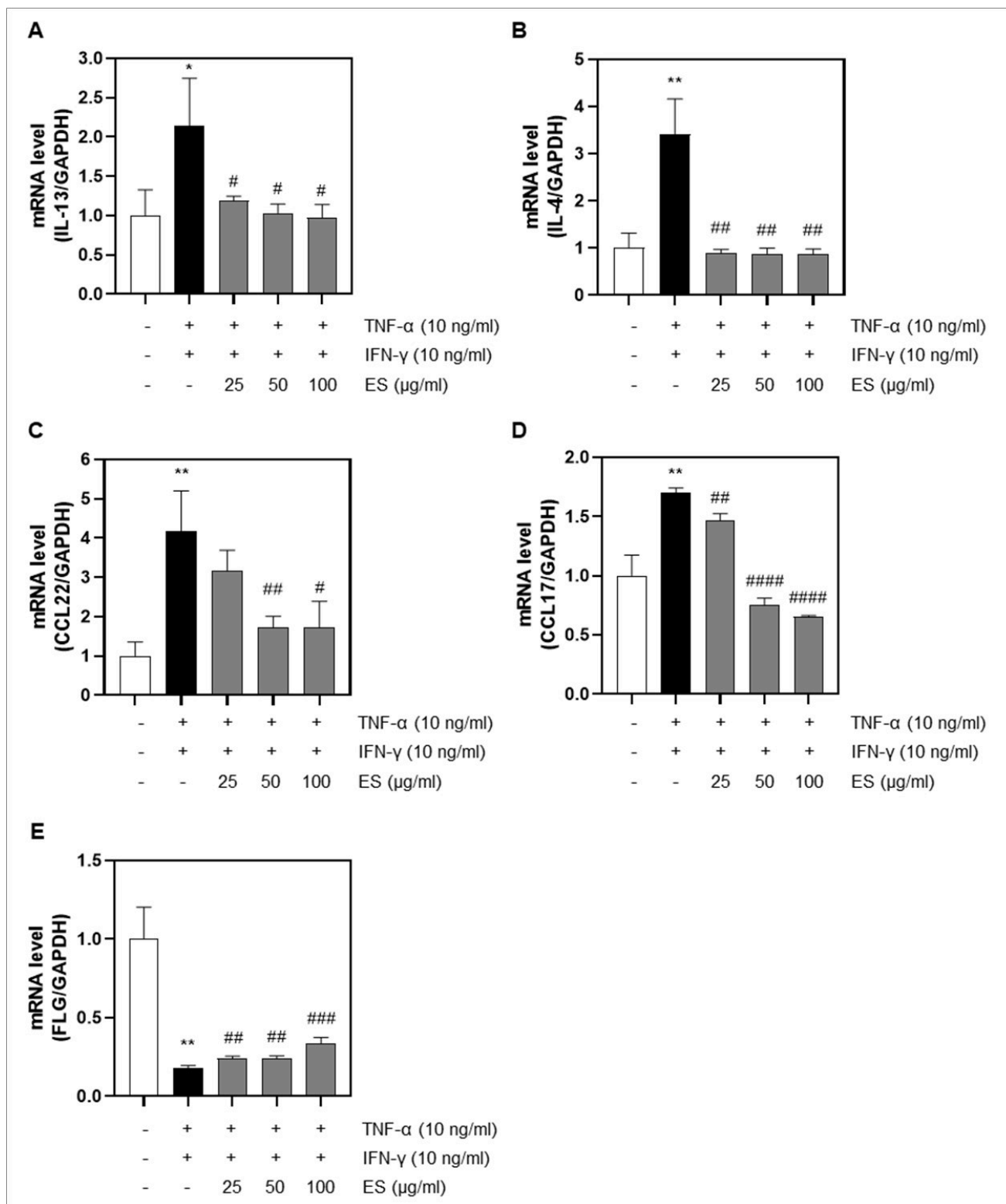


Figure 11

Effects of ES on mRNA expression. The levels of the inflammatory cytokines IL-13 (A) and IL-4 (B) were measured using RT-PCR. Additionally, the expression of the chemokines CCL22 (C) and CCL17 (D), as well as the skin barrier-related factor FLG (E), was analyzed using RT-PCR. All values are expressed as the mean $\pm$ SEM. * $p < 0.05$ and ** $p < 0.01$ compared with the control group; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, and #### $p < 0.0001$ compared with the TNF- α /IFN- γ -treated group.

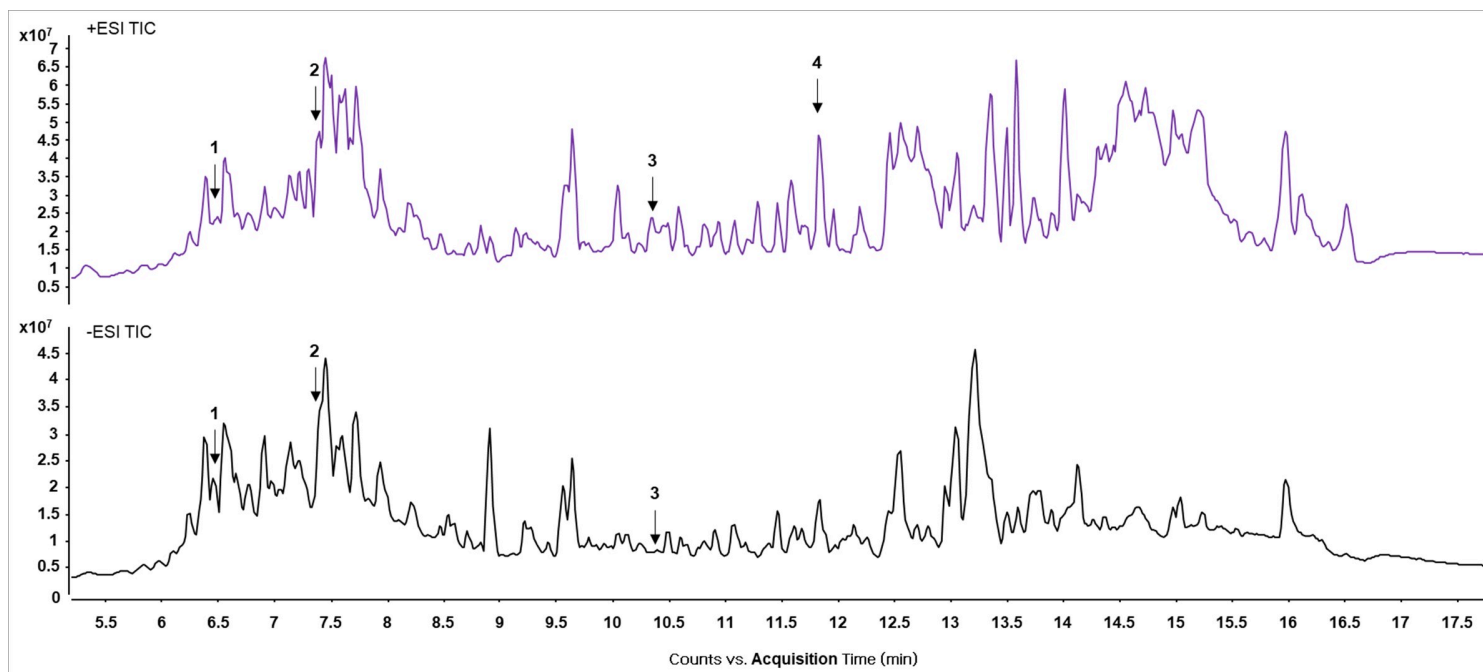


Figure 12

LC-MS chromatogram of *Euonymus Sachalinensis*. The chromatogram displays multiple detected peaks, each representing a compound that may contribute to its bioactivity. The identified compounds corresponding to each peak are listed in Table 2.

Supplementary Files

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