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ORIGINAL ARTICLE

Kaempferol enhances hair growth by regulating JAK3/STAT6 and TGF-β/Smad signaling in human dermal papilla cells and a C57BL/6 mouse model

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Abstract: Kaempferol, a naturally occurring flavonoid found in hair growth-promoting plants, was investigated for its direct effects on hair loss prevention. This study assessed its impact on human dermal papilla (HDP) cells, essential regulators of hair follicle activity. Kaempferol significantly enhanced HDP cell proliferation in a dose-dependent manner, exceeding the effect of minoxidil. It markedly inhibited TGF-β1-induced Smad-binding activity (65.8%) and IL-4-induced STAT6 activity (72.5%), compared to finasteride (35%) and tofacitinib (96%), respectively. Western blot analysis showed suppression of phosphorylated Smad2/3 and STAT6, along with upregulation of CDK2, CDK4, cyclin D3, ERK1/2, IGF-1R, and VEGFR-2. *In vivo*, topical application of *Kaempferol* in C57BL/6 mice significantly promoted hair growth, increased follicle numbers, and improved hair thickness. These findings suggest that *Kaempferol* enhances hair growth through modulation of TGF-β/Smad and JAK3/STAT6 pathways and may serve as a promising natural therapeutic agent for hair loss treatment.

Keywords: Kaempferol, hair growth, IL-4/JAK3/STAT6, TGF β1/Smad/SBE; HDP cells, C57BLmouse model

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1 Introduction

Hair loss (alopecia) is a prevalent condition that extends beyond cosmetic concern, often resulting in psychological distress such as low self-esteem, anxiety, and depression (Lindner et al., 1997). Although commonly associated with aging, alopecia also occurs in younger individuals due to genetic predisposition, medications, endocrine disorders, autoimmune diseases, chronic stress, and poor nutrition. Clinically, it is classified into non-scarring (reversible) and scarring (irreversible) types. Androgenetic alopecia and alopecia areata are common forms of non-scarring alopecia, whereas scarring alopecia involves permanent follicular destruction (Thom, 2016). Hair follicles (HFs) cycle through anagen (growth), catagen (regression), and telogen (resting) phases. These transitions are regulated by complex interactions among dermal papilla cells, epithelial matrix cells, and hair follicle stem cells, mediated through multiple signaling pathways. Dysregulation of these pathways contributes to HF miniaturization and progressive hair loss. A number of intracellular signaling pathways are involved in hair cycle regulation and the stimuli-specific activation or inactivation of some of these signaling pathways lead to hair loss. The Wnt/β-catenin-mediated cell signaling is one of the most extensively studied biochemical pathway that plays an essential role in the initiation and development of hair follicles (Banghong et al., 2024; Li et al., 2025). The activation of Wnt/β-catenin pathway promotes HF regeneration (Choi, 2020). Reddy et al. (2001) demonstrated that the deleting β-catenin attenuates HF progenitor cells proliferation and rapidly induces the catagen. Moreover, the amplification of the Wnt/β-catenin signaling showed extensive hair growth in mice (Andl et al., 2002). In response to the transforming growth factor β (TGF-β) interaction with its cognate receptor TGF-β receptor (TGFR1 and 2), and subsequent heterodimerization of TGFRs results in the phosphorylation of suppressor of mothers against decapentaplegic (Smad)-2/3, which then recruits Smad4 to form a transcription complex that translocates to the nucleus and binds the Smad binding element (SBE) to initiate transcription (Li et al., 2012). The transcribed genes then induce cell death. It has been reported that TGF-β/Smad signaling pathway inhibits cell growth and differentiation. The activation of TGF-β1 causes androgenic alopecia by inducing catagen in HF. Treatment with a TGF-β antagonist can promote hair growth via preventing catagen progression (Foitzik et al., 2000). The Janus-activated kinase (JAK) and the downstream signal transducer and activator of transcription (STAT) constitute

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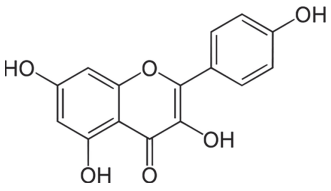

Figure 1. Chemical structure of kaempferol

another important intracellular signaling pathway that regulates the hair cycle. Xing et al. (2014) reported that the blockade of the JAK-STAT pathway induced regrowth of hair in both mouse and human alopecia areata. These authors also demonstrated that ruxolitinib, a clinically used JAK inhibitor, improved the hair growth in patients with alopecia. Another JAK inhibitor, tofacitinib, has also been reported to induce hair growth (Fisher et al., 2016). Thus, intracellular signaling cascades mediated via Wnt/β-catenin, TGFβ/Smad or JAK/STAT are valid molecular targets for developing therapy for hair loss prevention.

A number of hair growth therapies, such as minoxidil (Gupta et al., 2022) and finasteride (Keerti et al., 2023) are currently being used clinically, whereas some others (e.g., spironolactone) are under trial (Alkandahri et al., 2023). However, these therapies have limitations in terms of safety and tolerability, and there is an unmet need to find new therapies. Traditionally used herbal remedies are effective in promoting hair growth. There have been many reports of hair growth promoting effects of various plant extracts (Li et al., 2022; Rajan et al., 2023) that contain *Kaempferol* (Figure 1) as an active ingredient (Lin et al., 2012; Ko et al., 2020).

Kaempferol (3,4',5,7-tetrahydroxy flavone) is a natural flavonoid and is abundant in a variety of plants used for food or medical purposes (Choi, 2018). Numerous studies have demonstrated that *Kaempferol* has cardioprotective (Kamisah et al., 2023), hepatoprotective (Alkandahri et al., 2023), neuroprotective (Jin et al., 2023), anticancer (Amjad et al., 2022), antidiabetic (Yang et al., 2022), and antimicrobial (Periferakis et al., 2022) effects. However, the effect of *Kaempferol* on hair growth promotion have not been studied yet. In the present study, we report that *Kaempferol* inhibits TGF-β/smad- and JAK3/STAT6-mediated signaling activity in HDP cells to induce cell proliferation, suggesting that the compound may further be developed as a therapy for preventing hair loss.

2 Materials and Methods

2.1 Chemicals and Antibodies

Kaempferol was purchased from Sigma-Aldrich (St. Louis, MO, USA) (Figure 1). CellTiter-Glo® Luminescent Cell Viability Assay was purchased from Promega Corporation (Madison, WI, USA). Dulbeco's modified eagle's media (DMEM) and fetal bovine serum (FBS) were purchased from HyClone Laboratories Inc (Logan, UT, USA). β-actin antibody was purchased from Sigma-Aldrich (St. Louis, MO, USA). Cyclin D1 used as the primary antibody was purchased from Santa Cruz Biotechnology (Dallas, TX, USA), and β-catenin, phospho-β-catenin, STAT6, phospho-STAT6, smad2/3, phospho-smad2/3, Cyclin-dependent kinase (CDK) 4, Cyclin D3, CDK6, Extracellular signal-regulated kinases (ERK)1/2, phospho-ERK1/2, insulin-like growth factor 1 receptor (IGF-1R), Epidermal growth receptor (EGFR), and vascular endothelial growth factor receptor 2 (VEGFR2) were purchased from Cell Signaling (Danvers, MA, USA).

2.2 Cell Culture

HDP cells were purchased from Abm Inc. (Richmond, BC, Canada). The 3T3-Wnt reporter cell line (TCF/LEF luciferase) was obtained from Enzo Life Sciences (Lausen, Switzerland), and the HEK293-Blue-STAT6 (SEAP luciferase) and HEK293-TGF/Smad (SBE luciferase) reporter cell lines were purchased from BPS Bioscience (San Diego, CA, USA). HEK293 cells was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Stable reporter cell lines were maintained in selective media containing appropriate antibiotics for each selection marker: 10 μg/mL puromycin for the 3T3-Wnt cell line, 10 μg/mL blasticidin (Gibco, NY, USA) and 100 μg/mL zeocin (InvivoGen, San Diego, CA, USA) for the HEK293-Blue-STAT6 cell line, and 400 μg/mL geneticin (G418; HyClone™) for the HEK293-TGF/Smad cell line. All cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Corning, Corning, NY, USA) supplemented with 10% fetal bovine serum (FBS; HyClone™, Marlborough, MA, USA) and Antibiotic/Antimycotic Solution (100 U/mL penicillin, 100 μg/mL streptomycin, and amphotericin B; HyClone™). Cells were maintained in a humidified incubator at 37°C with 5% CO^2.

2.3 Cell Proliferation Assay

To evaluate the effect of *Kaempferol* on cell proliferation, human dermal papilla (HDP) cells were seeded into 96-well plates at a density of 3×10^3 cells/well and incubated for 24 hours. Subsequently, the cells were treated with various concentrations of *Kaempferol* (1–15 μg/mL) or 0.4 μM minoxidil (Sigma-Aldrich, St. Louis, MO, USA) as a positive control. Dimethyl sulfoxide (DMSO; Sigma-Aldrich) was used as the negative control. After 24 hours of treatment, cell proliferation was assessed using the CellTiter-Glo® Aqueous One Solution Cell Proliferation Assay (Promega, Madison, WI, USA) according to the manufacturer's protocol. This assay measures mitochondrial dehydrogenase activity, and the amount of formazan product—quantified by measuring absorbance at 490 nm—is directly proportional to the number of viable cells. Absorbance was recorded using a SpectraMax M2 microplate reader (Molecular Devices, Sunnyvale, CA, USA). The relative proliferation rate was calculated using the following formula: Cell proliferation rate (%) = (Absorbance of treated group/Absorbance of control group) × 100. To assess cytotoxicity, HEK 293 cells were seeded under identical conditions and treated with increasing concentrations of *Kaempferol* (1–50 μg/mL) for 24 hours.

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Cell viability was quantified using the CellTiter-Glo® Luminescent Cell Viability Assay (Promega), which measures ATP content as an indicator of metabolically active cells. Luminescence was measured using a LuBi luminometer (Berthold TEC GmbH & Co., Oak Ridge, TN, USA). All experiments were performed in triplicate, and data are expressed as mean ± standard deviation (SD).

2.4 Target Based Luciferase Reporter Gene Assay

3T3-Wnt cells transfected with the TCF/LEF-luciferase construct and HEK293 cells transfected with the SBE-luciferase construct were seeded into 96-well plates at a density of 3×10^3 cells/well and cultured for 24 hours in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Subsequently, 3T3-Wnt cells were treated with *Kaempferol* in the presence or absence of Wnt3a (50%, v/v), while SBE-luciferase-transfected HEK293 cells were treated with TGF-β1 (7 ng/mL) alone or in combination with *Kaempferol*. Cells were then incubated for an additional 48 hours. HEK-Blue cells transfected with a STAT6-luciferase reporter were seeded into 96-well plates at 5×10^3 cells/well and maintained for 16–20 hours in DMEM containing 10% FBS. These cells were subsequently treated with *Kaempferol*, either alone or in the presence of interleukin-4 (IL-4, 10 ng/mL; Sigma-Aldrich, St. Louis, MO, USA) and incubated for 48 hours at 37°C in a 5% CO^2 atmosphere. Luciferase activity was measured using the Luciferase® Reporter Assay System (Promega, Madison, WI, USA) according to the manufacturer's protocol. Following cell lysis, luminescence was detected using a LuBi luminometer (Berthold TEC GmbH & Co., Oak Ridge, TN, USA). All experiments were performed in triplicate, and data are expressed as mean ± standard deviation (SD).

2.5 Immunoblotting

Human dermal papilla (HDP) cells were seeded in 100-mM cell culture dishes at a density of 2.5×10^4 cells/mL and cultured in standard growth medium. After overnight attachment, cells were treated with Wnt3a, TGF-β1, or IL-4, either alone or in combination with *Kaempferol* (1–15 μg/mL), and incubated for 48 hours at 37°C under 5% CO^2. Following treatment, cells were lysed using RIPA buffer containing 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1% IGEPAL CA-630, 0.1% sodium dodecyl sulfate (SDS), and a protease inhibitor cocktail (1×; Sigma-Aldrich). Lysates were sonicated at 30% amplitude for 7 seconds and centrifuged at 13,000 rpm for 20 minutes at 4°C to obtain the clarified supernatant. Total protein concentrations were determined using the Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Equal amounts of protein (15–20 μg) were resolved by 8–10% SDS-PAGE or on Novex™ WedgeWell™ 4–20% Tris-Glycine mini protein gels (Invitrogen, Waltham, MA, USA), using Tris-Glycine SDS running buffer. Proteins were subsequently transferred onto polyvinylidene fluoride (PVDF) membranes using Pierce™ 10X Western Blot Transfer Buffer containing 20% methanol. Membranes were blocked with 5% skim milk in 1× TBS-T [10 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.05% Tween 20] for 1 hour at room temperature, followed by incubation with the appropriate primary antibodies overnight at 4°C. The following primary antibodies were used: Cyclin D1 (1:500), β-actin (1:5000), β-catenin, phospho-β-catenin, STAT6, phospho-STAT6, Smad2/3, phospho-Smad2/3, CDK4, Cyclin D3, CDK6, ERK1/2, phospho-ERK1/2, IGF-1R, EGFR, and VEGFR2 (all at 1:1000). After three washes with 1× TBS-T (15 minutes each), membranes were incubated with appropriate HRP-conjugated secondary antibodies at room temperature for 1 hour. Protein bands were visualized using the Immobilon™ Western Chemiluminescent HRP Substrate (Millipore, Burlington, MA, USA), and signals were detected with a ChemiDoc™ Imaging System (Bio-Rad, Hercules, CA, USA).

2.6. In vivo Hair Growth Assay: Preliminary Study

Seven-week-old female C57BL/6 mice were purchased from Oriental Bio Co. (Seoul, Republic of Korea). A total of 15 mice were obtained, and after a 7-day acclimation period under controlled environmental conditions (23 ± 3°C, 50 ± 10% relative humidity, and a 12-hour light/dark cycle), nine mice with uniform body weight and coat condition were selected for the experiment. The animals were randomly assigned to three groups (n = 3 per group) to evaluate the hair and hair follicle growth-promoting effects of kaempferol. The dorsal hair of each mouse was removed using an electric clipper. Kaempferol was dissolved in 50% ethanol and topically applied at a dose of 40 μg/mL in a volume of 200 μL per mouse, twice daily for 14 consecutive days. The total daily applied amount was approximately 8 μg per mouse. No local irritation, erythema, or other adverse skin reactions were observed throughout the study. All animal procedures were performed in accordance with the institutional procedural manual and internal regulations of Chungbuk, Republic of Korea. Because this was a preliminary feasibility study conducted prior to human application testing, formal IACUC approval was not required. For hair growth evaluation, on Day 14, the mice were sacrificed, and dorsal skin samples were harvested and fixed in 10% neutral-buffered formalin. The tissues were then embedded in paraffin, sectioned at 5 μM thickness, and stained with hematoxylin and eosin (H&E) for histological analysis. Hair growth was evaluated macroscopically by observing and photographing dorsal skin pigmentation and hair coverage. Histomorphometric analysis under light microscopy was performed to assess follicle number and size in the dermis. Quantitative analysis included the average number of hair follicles per high-power field (400× magnification) and follicle depth. These parameters were compared between the control and kaempferol-treated groups to determine the hair growth-promoting efficacy of the compound.

2.7 Statistical Analysis

Statistical analysis of the experimental results was performed by using SPSS Subscription (IBM SPSS Statistics, USA). Normality was evaluated through the Shapiro-Wilk test, and if normality was satisfied, a parametric test was performed, and

if normality was not satisfied, a non-parametric test was performed. Statistical analysis results confirmed significance at the 95% confidence interval. For comparison before and after, a paired t-test was performed as a parametric test, and the Wilcoxon Signed-rank test was used as a non-parametric test.

3 Results and Discussion

3.1 Kaempferol Enhances Proliferation of HDP Cells Without Cytotoxicity

Hair loss is a prevalent condition affecting both psychological and socio-economic well-being. Despite the limited therapeutic options, traditional medicine has long utilized plant-derived compounds to promote hair growth. *Kaempferol*, a bioactive flavonoid found in several plant extracts (Lin et al., 2012; Ko et al., 2020), has been identified as a potential agent in this context. We investigated the effect of *Kaempferol* on human dermal papilla (HDP) cells, which are crucial regulators of hair follicle cycling and growth. MTT and ATP assays showed that *Kaempferol* significantly promoted HDP cell proliferation in a concentration-dependent manner, reaching 121.9% and 127.3% activity respectively at 15 μg/mL, exceeding the effect of minoxidil (Figures 2A, 2B). Importantly, *Kaempferol* did not induce cytotoxicity in HEK293 cells even at the highest concentration tested (50 μg/mL) (Figure 2C). These results align with previous findings on the hair growth-promoting effects of *Kaempferol*-containing plant extracts (Li et al., 2022; Rajan et al., 2023) supporting its safety and efficacy.

3.2 The Effects of Kaempferol on the Transactivation of Various Luciferase-Reporter Genes

To elucidate the mechanisms underlying *Kaempferol*'s proliferative effects, we explored its influence on multiple signaling pathways related to hair cycle regulation. Interestingly, *Kaempferol* had no significant impact on the Wnt/β-catenin pathway, as indicated by its lack of effect on TCF/LEF luciferase activity and downregulation of β-catenin and phospho-β-catenin protein levels (Figures

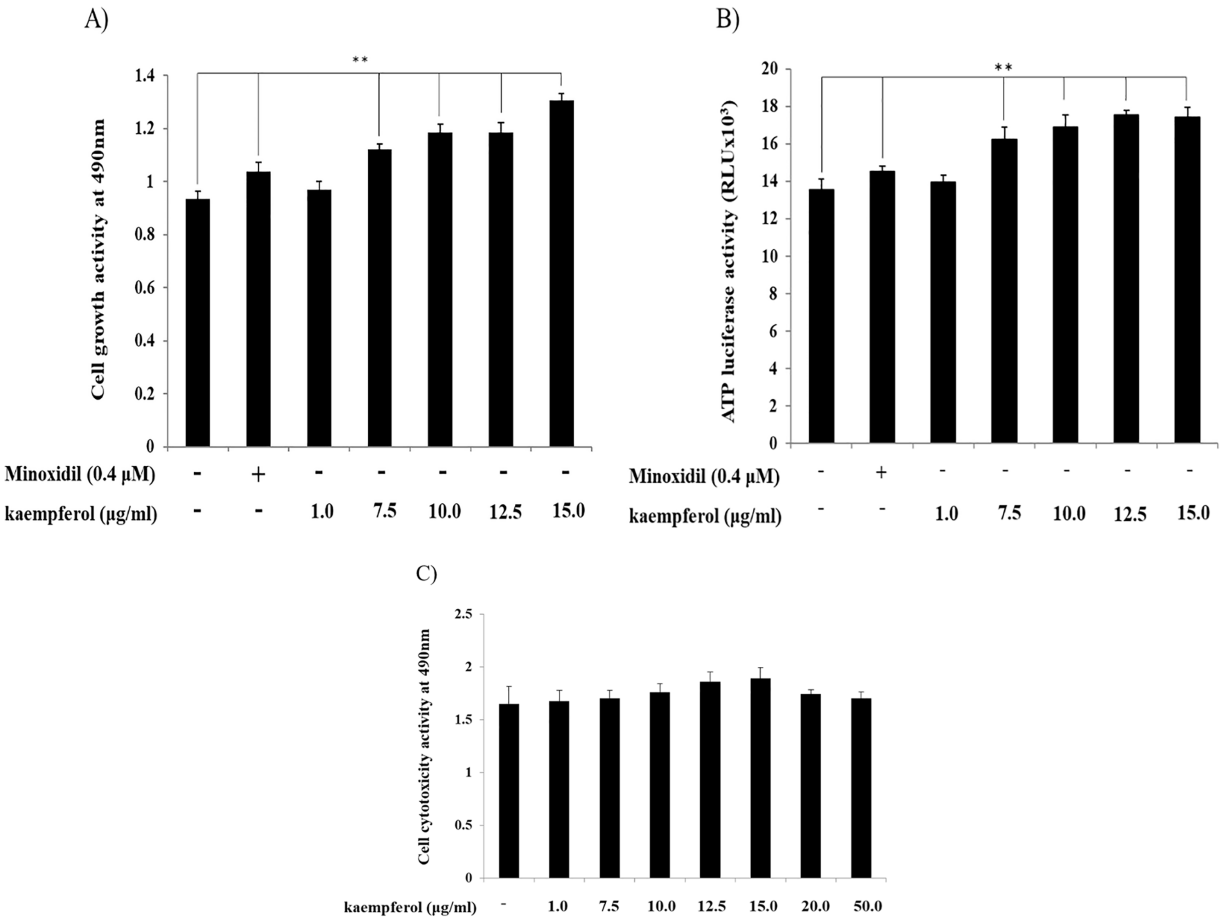

Figure 2. Effects of *Kaempferol* on cell growth and cytotoxicity assay. The proliferative effects of *Kaempferol* on human dermal papilla (HDP) cells were evaluated using the MTT assay and the ATP-based luminescent cell viability assay. (A) For the MTT assay, HDP cells (1×10^3 cells/well) were seeded in 96-well plates and treated with *Kaempferol* (1, 7.5, 10, 12.5, or 15 μg/mL) for 24 hours. (B) For the ATP assay, cells (3×10^3 cells/well) were seeded and treated under the same conditions. Minoxidil (0.4 μM) was used as a positive control. (C) To evaluate the cytotoxicity of *Kaempferol*, HEK 293 cells were seeded into 96-well plate at a concentration of 3×10^3 cells/well, treated with *Kaempferol* (1, 7.5, 10, 12.5 or 15 μg/mL), and cultured for 24 h. All experiments were conducted in triplicate. Data are presented as mean ± SD. Asterisks indicate statistically significant differences compared to the control group (**$p < 0.05$).

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3A, 4A). This suggests that proliferation is promoted through alternative pathways. The compound significantly inhibited TGF-β1-induced activation of the Smad-binding element (SBE)-luciferase reporter and reduced Smad2/3 phosphorylation in a dose-dependent manner (Figures 3B, 4B), indicating suppression of the TGF-β/Smad pathway, a key mediator of apoptosis in HDP cells. These anti-apoptotic effects are consistent with previous reports of *Kaempferol* attenuating TGF-β1-induced Smad2/3 phosphorylation in hepatic stellate cells and fibroblasts (Xu et al., 2019). In support, kaempferol downregulated Bax and preserved Bcl-2 expression (Figure 4D), consistent with the known role of Bcl-2 in hair follicle survival (Phillips et al., 2017). In addition, *Kaempferol* effectively inhibited IL-4-induced STAT6 activation, both at the reporter gene level and via reduced STAT6 phosphorylation (Figures 3C, 4C). The pattern mirrored that of tofacitinib, a JAK1/3 inhibitor known for its hair regrowth potential in alopecia (Harel et al., 2015). Collectively, these findings suggest kaempferol modulates the JAK/STAT6 axis, contributing to its protective and regenerative effects. In our assays, kaempferol exerted stronger inhibitory effects on TGF-β/Smad and JAK3/STAT6 signaling than finasteride and showed efficacy broadly comparable to minoxidil. This aligns with prior reports demonstrating that kaempferol directly suppresses TGF-β/Smad activation via ALK5 inhibition and attenuates IL-4–induced STAT6 signaling through JAK3 modulation (Li et al., 2016; Cortes et al., 2007). In contrast, finasteride primarily reduces androgen-induced TGF-β signaling indirectly, while minoxidil acts via KATP channel activation and ERK/AKT pathways with

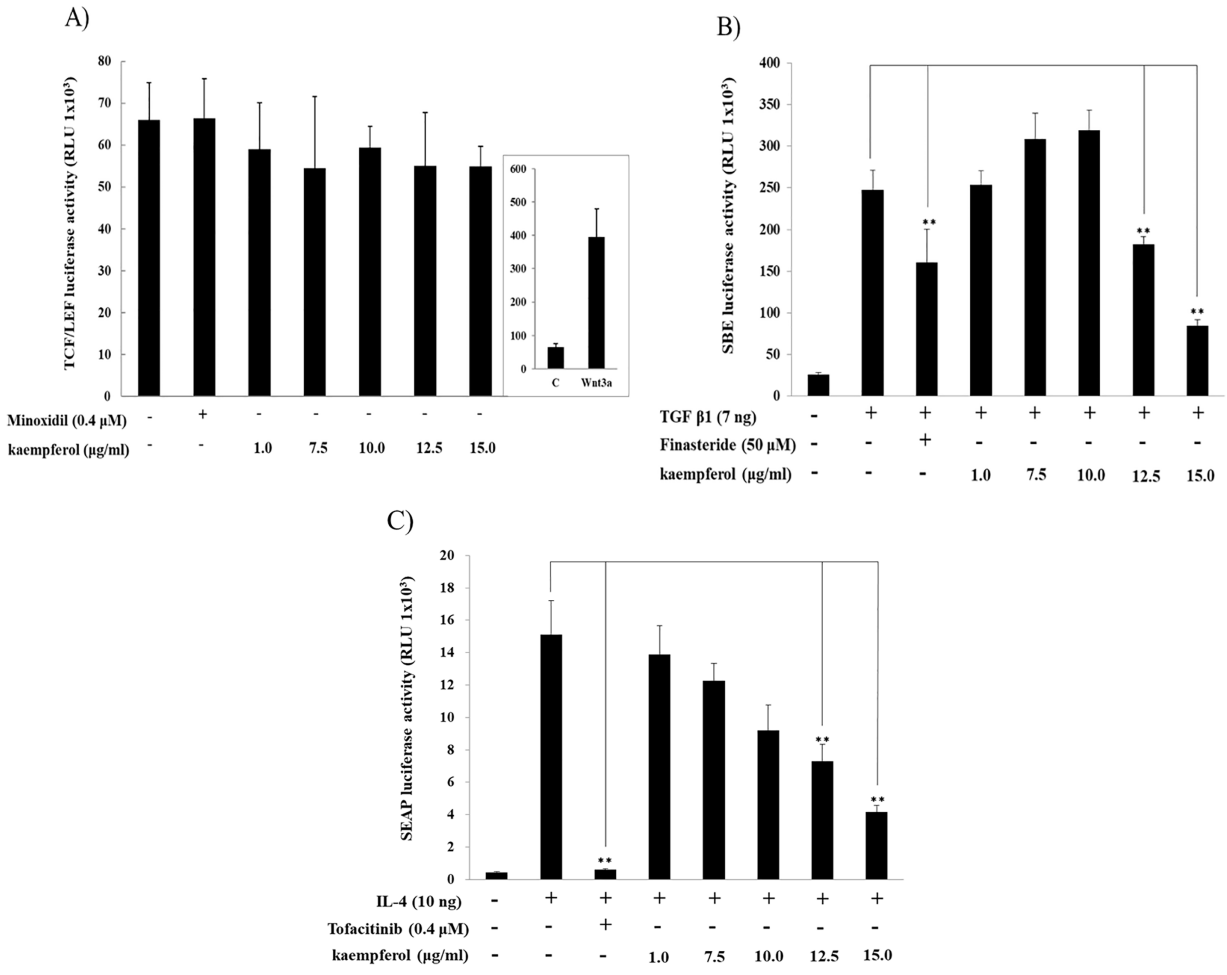

Figure 3. Evaluation of *Kaempferol*'s effects on the Reporter gene activity. (A) The effect of *Kaempferol* on Wnt/β-catenin signaling was assessed using 3T3-Wnt cells transfected with a TCF/LEF-luciferase reporter construct. Cells were seeded into white 96-well plates at a density of 3×10^3 cells/well, pre-incubated for 24 hours, and then treated with *Kaempferol* (1, 7.5, 10, 12.5, or 15 μg/mL), with or without Wnt3a (50%, v/v). After 48 hours, luminescence was measured following substrate addition. (B) To assess the modulation of TGF-β/Smad signaling, HEK293 cells transfected with an SBE-luciferase construct were seeded into white 96-well plates (3×10^3 cells/well) and treated with TGF-β1 (7 ng/mL) alone or in combination with *Kaempferol* (1–15 μg/mL). After 48 hours of incubation, luciferase activity was quantified. The calculated IC_{50} value of *Kaempferol* in this assay was 14 μg/mL. (C) To evaluate the inhibitory effect of *Kaempferol* on IL-4-induced STAT6 activation, HEK293 cells transfected with a STAT6-luciferase construct were seeded at 5×10^3 cells/well and treated with IL-4 (10 ng/mL) alone or in combination with *Kaempferol* (1–15 μg/mL). After 48 hours, luminescence was measured. The IC_{50} value for *Kaempferol* in this assay was 13 μg/mL. All experiments were performed in triplicate, and results are expressed as mean ± SD. Asterisks indicate statistically significant differences compared to the control group (**$p < 0.05$).

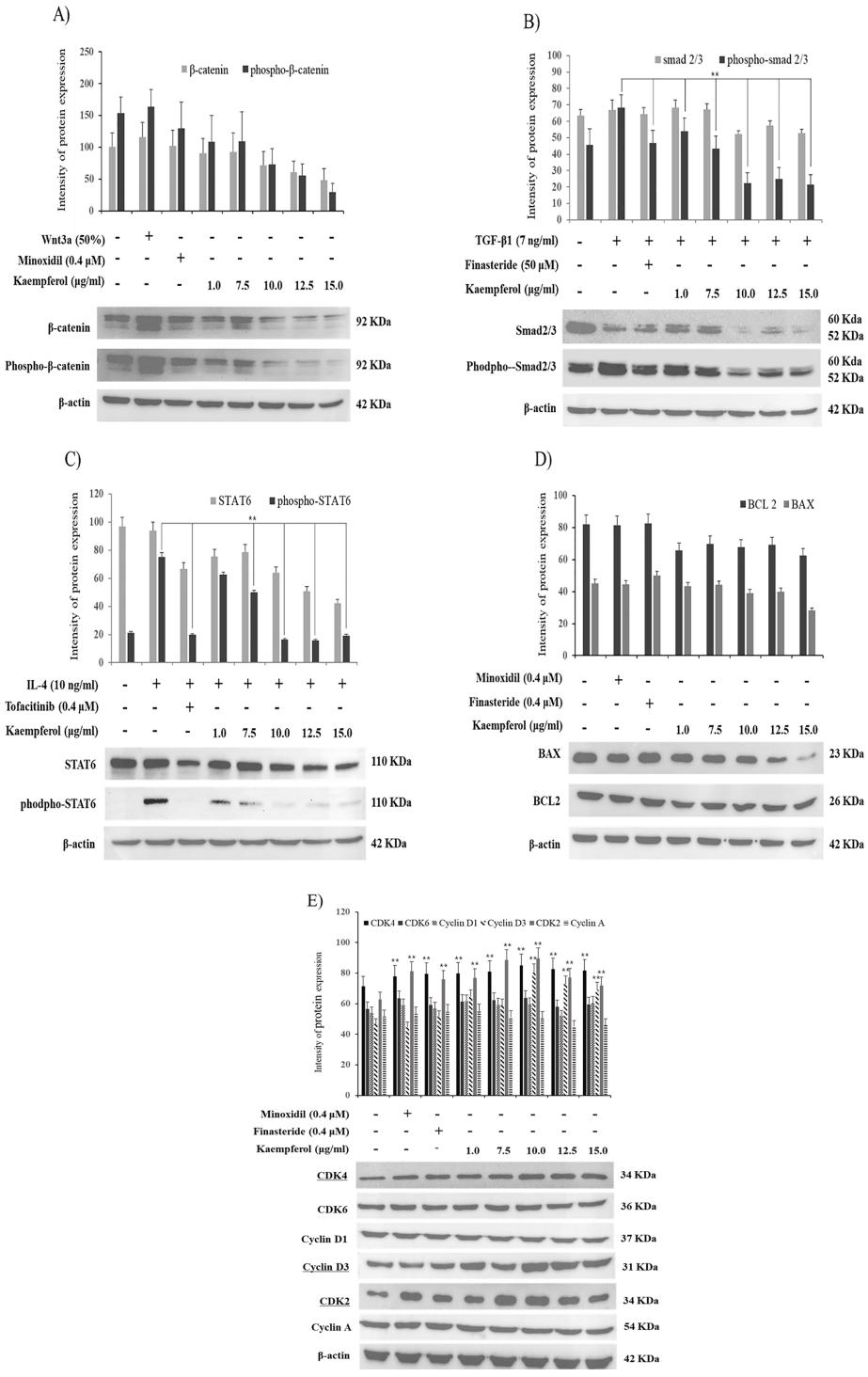

Figure 4. Effects of *Kaempferol* on the expression of intracellular signaling proteins. To investigate the molecular mechanisms underlying *Kaempferol*'s effects on hair growth, HDP cells (2.5×10^4 cells/mL) were treated with Wnt3a, TGF-β1, or IL-4, either alone or in combination with *Kaempferol* (1, 7.5, 10, 12.5, or 15 μg/mL) for 48 hours. Positive controls included minoxidil (0.4 μM), finasteride (50 μM), and tofacitinib (0.4 μM). (A) Expression levels of total β-catenin and phospho-β-catenin were assessed following co-treatment with Wnt3a (50%). High concentrations of *Kaempferol* resulted in a dose-dependent reduction in both total and phosphorylated β-catenin. (B) Smad2/3 and phospho-Smad2/3 were evaluated after co-treatment with TGF-β1 (7 ng/mL). *Kaempferol* markedly suppressed phospho-Smad2/3 levels, showing a stronger inhibitory effect than finasteride. (C) The effect on STAT6 and phospho-STAT6 was examined following IL-4 (10 ng/mL) stimulation. *Kaempferol* inhibited phospho-STAT6 in a concentration-dependent manner. (D) Bax and Bcl-2, apoptosis-related proteins, were analyzed. Bcl-2 expression remained unchanged, whereas Bax expression was notably reduced at the highest *Kaempferol* concentration. (E) Cell cycle regulators were examined. CDK6, cyclin D1, and cyclin A levels were unaffected by *Kaempferol*, while CDK4, CDK2, and cyclin D3 expression levels increased in a dose-dependent manner. All experiments were conducted in triplicate, and results are expressed as mean ± SD. Asterisks denote statistically significant differences compared to control (**$p < 0.05$).

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limited direct impact on STAT6 (Kim et al., 2019; Han et al., 2004).

3.3 Kaempferol Regulates Cell Cycle-Related Proteins to Promote Hair Follicle Activation

Cell cycle progression is essential for hair follicle growth, and cyclins/CDKs play critical roles in this process. *Kaempferol* treatment increased expression of CDK2, CDK4, and cyclin D3 (Figure 4E), key regulators of the G1 to S phase transition, thereby supporting active proliferation of HDP cells (Luo et al., 2012; Yu et al., 2013). While *Kaempferol* inhibited pro-apoptotic signaling pathways, it concurrently activated cell cycle-related proteins, collectively promoting follicular regeneration. Although *Kaempferol* has previously been shown to suppress other STAT pathways such as STAT1/3 in keratinocytes and airway epithelial cells (Li et al., 2024), its potential action on these STATs in HDP cells remains to be further explored, and future studies should clarify their contribution to kaempferol's efficacy.

3.4 Kaempferol Selectively Enhances IGF-1R Expression and Growth Factor Signaling

Growth factor signaling is central to hair follicle maintenance and angiogenesis. While *Kaempferol* did not significantly

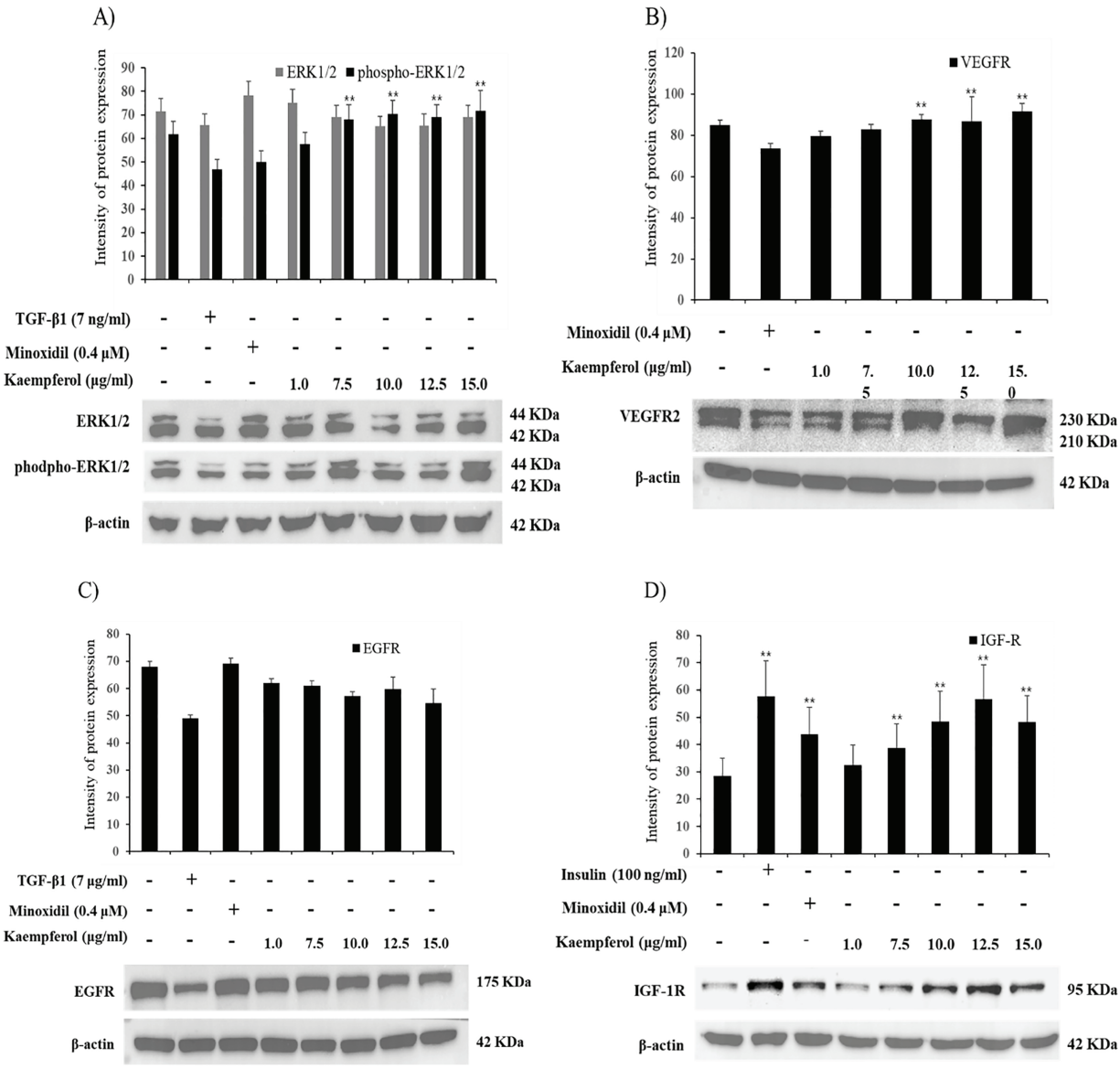

Figure 5. Effects of *Kaempferol* on the expression ERK, phospho-ERK and growth factor receptors in HDP cells. Cells were seeded in 100 mM petri dish and treated with *Kaempferol* as described in legend of Figure 3. (A) ERK1/2 and p-ERK1/2 was measured by incubation with *Kaempferol* and TGF-β1 (7 ng/mL), respectively, and showed a weak increase in expression at the highest concentration. (B) VEGFR2 expression was increased at the highest concentration of *Kaempferol*. (C) *Kaempferol* had no effect on the expression of EGFR. (D) IGF-1R was measured in cultures treated with *Kaempferol* and insulin (100 ng/mL) alone. *Kaempferol* increased expression in a concentration-dependent manner. Each experiment was quantified with β-actin. All experiments were repeated at least three times. The asterisk indicates a significant statistical significance (**$p < 0.05$)

affect VEGFR2 or EGFR expression (Figures 5B, 5C), it markedly enhanced IGF-1R expression (Figure 5D), comparable to that induced by insulin or minoxidil. IGF-1R activation is known to facilitate the G1–S transition and prevent catagen entry (Nan et al., 2020; Premanand & Reena Rajkumari, 2018), providing a plausible pathway through which *Kaempferol* supports follicular growth. *Kaempferol* also increased ERK1/2 and phospho-ERK1/2 expression (Figure 5A), suggesting a link between IGF-1R and MAPK pathway activation. These pathways contribute to the angiogenic and proliferative environment necessary for active hair growth. Although VEGFR2 expression was only modestly increased, it may still contribute to angiogenesis during the anagen phase (Ren et al., 2019; Calderon-Montano et al., 2011).

3.5 Effect of Kaempferol on Hair Growth in an In Vivo Model

As a preliminary study prior to human application testing, an *in vivo* experiment was conducted using a C57BL/6 mouse model to evaluate the hair growth-promoting effect of *Kaempferol*. After shaving the dorsal skin of the mice, animals were divided into three groups: a negative control group (50% ethanol), a positive control group (5% minoxidil), and a *Kaempferol*-treated group (40 μg/mL). Each treatment was topically applied for 14 consecutive days. *Kaempferol*-treated mice exhibited significantly enhanced hair growth compared to controls, with increased follicle number and coverage, and effects comparable to minoxidil. Histopathology revealed increased follicle diameter and depth, indicating enhanced development and transition to anagen (Figures 6A–6C). The topical dose of kaempferol (40 μg/mL, 200 μL per mouse, twice daily) was determined based on the effective in vitro concentration range (1–15 μg/mL) observed in HDP cells. Although the applied concentration was higher than the cellular dose, this adjustment was necessary to compensate for limited percutaneous absorption through the mouse epidermis. Preliminary solubility testing showed that concentrations above 40 μg/mL were unstable or poorly dissolved in 50% ethanol; therefore, 40 μg/mL was selected as the maximal feasible and physiologically relevant dose. The total daily amount applied per mouse was approximately 8 μg. While these results provide important evidence, we acknowledge the inherent limitations of this model, including the small sample size, the short treatment period, and the fact that the mouse hair cycle does not fully replicate the complexity of human alopecia. Therefore, these findings should be considered preliminary. Further long-term studies, particularly human clinical trials, are needed to confirm the efficacy and safety of kaempferol in restoring hair growth.

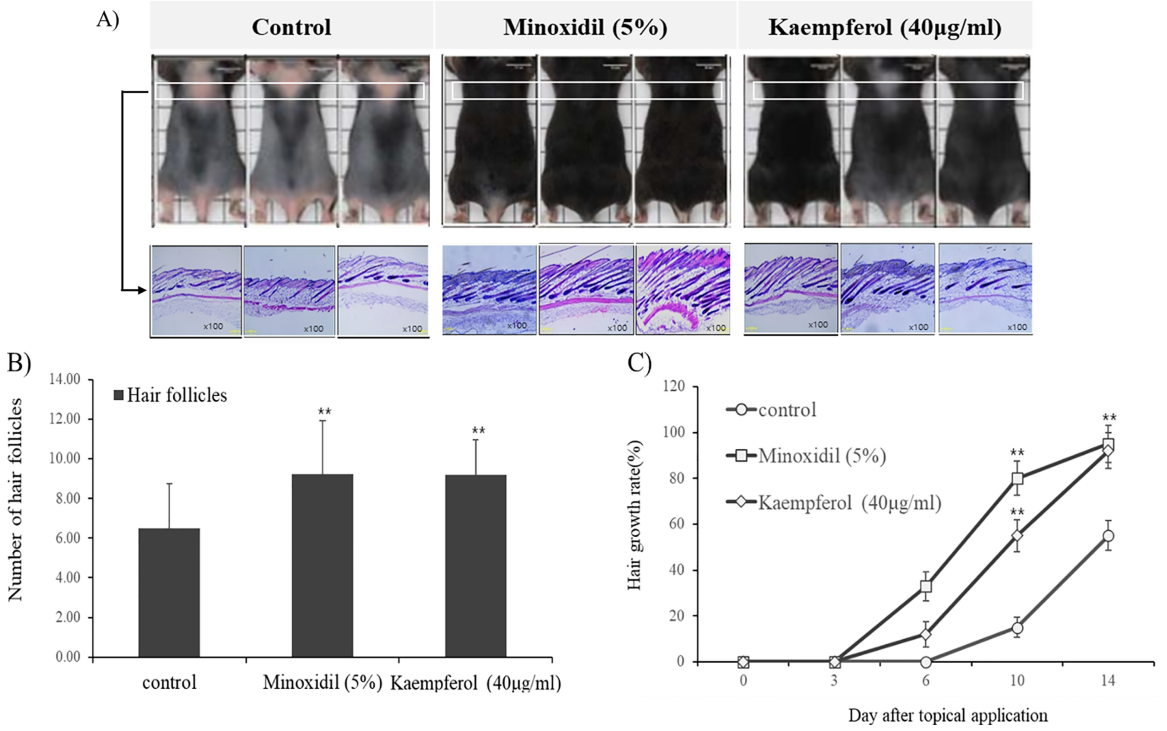

Figure 6. Effect of *Kaempferol* on hair growth in C57BL/6 mice. (A) Representative macroscopic images and histological sections of dorsal skin after 14 days of topical treatment. Mice were treated with 50% ethanol (control), 5% minoxidil, or *Kaempferol* (40 μg/mL) twice daily. Hair regrowth was visibly enhanced in both the minoxidil and *Kaempferol* groups compared to the control. Corresponding H&E-stained skin sections show increased number and depth of hair follicles in the treated groups (original magnification: ×100, scale bar = 100 μM). (B) Quantification of hair follicle number per high-power field. Both minoxidil and *Kaempferol* treatments significantly increased the number of hair follicles compared to control. Data are presented as mean ± SD (n = 3); $p < 0.01$ vs. control. (C) Time-course analysis of hair growth rate (%) over 14 days. *Kaempferol* and minoxidil groups showed significantly accelerated hair regrowth compared to the control group. Data are expressed as mean ± SD (n = 3); $p < 0.01$ vs. control.

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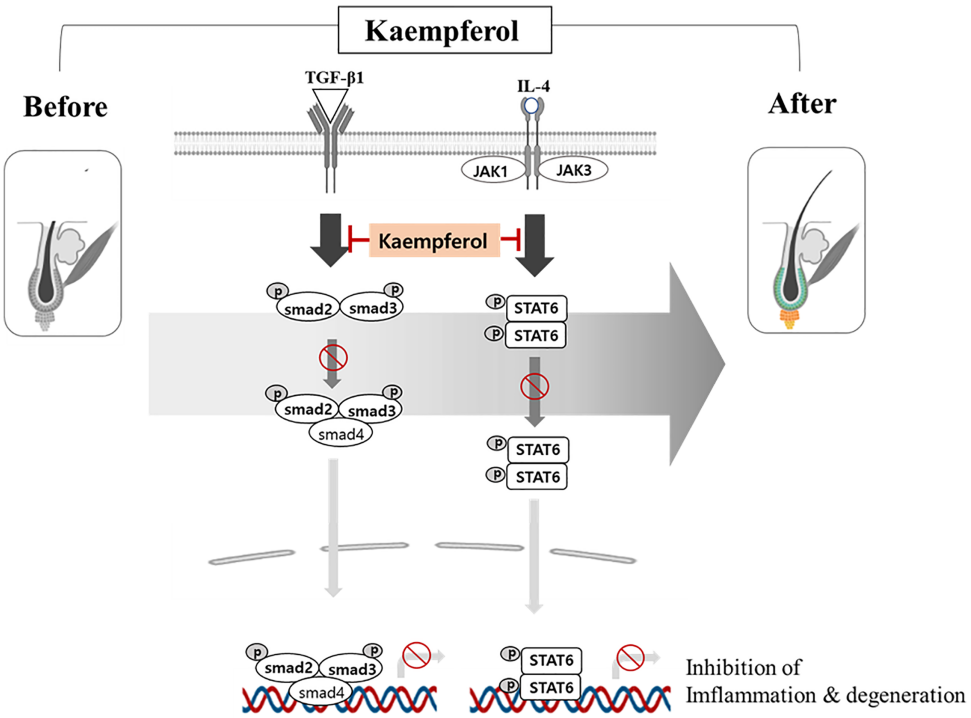

Figure 7. Summary of efficacy of *Kaempferol* on hair follicular cell proliferation and degeneration. JAK/STAT and TGF-β1 signaling are important molecular targets in hair loss, which progresses through inflammation and degeneration of hair follicles

These results suggest that *Kaempferol* may exert potent hair growth-promoting activity *in vivo*, comparable to or greater than that of minoxidil. These *in vivo* findings are consistent with our *in vitro* results, which demonstrated that *Kaempferol* promotes proliferation of human dermal papilla (HDP) cells and modulates key signaling pathways involved in hair follicle regulation. The observed increase in hair follicle number, depth, and diameter in *Kaempferol*-treated mice suggests that the compound effectively induces anagen phase entry and supports follicular maturation. This study demonstrates that *Kaempferol* promotes proliferation and survival of human dermal papilla (HDP) cells by suppressing TGF-β/Smad and JAK/STAT6 signaling pathways, as shown in both *in vitro* and *in vivo* models (Figure 7).

These effects are mediated independently of the canonical Wnt/β-catenin signaling pathway. The ability of kaempferol to simultaneously modulate multiple regulatory axes highlights its therapeutic potential as a novel agent for hair loss treatment. However, further well-designed clinical studies are warranted to establish its efficacy and safety in the context of hair growth restoration.

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Author Contributions

This study was solely conceived, designed, conducted, and written by the author.

Availability of Data and Materials

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Ethics Approval

All animal experimental procedures were performed in accordance with the institutional guidelines for the care and use of laboratory animals at Chungbuk National University, Republic of Korea. This work was conducted as a preliminary feasibility study involving a limited number of animals.

Conflicts of Interest

The authors declare that they do not have any conflict of interest.

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products.

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