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The effect of lupeol, a pentacyclic triterpene, on the treatment of acne

오환성 트리터핀계 화학물 루페올의 여드름 치료 효과

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의학과 피부과학전공

권 혁 훈

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The effect of lupeol, a pentacyclic triterpene, on the treatment of acne

by

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논문제목 : The effect of lupeol, a pentacyclic triterpene, on the treatment of acne

오환성 트리터핀계 화학물 루페올의 여드름 치료 효과

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Abstract

Acne vulgaris is a nearly universal cutaneous disease characterized by multifactorial pathogenic processes. Because current acne medications have various side effects, investigating new pharmacologically active molecules is important for treating acne. As natural products generally provide various classes of relatively safe compounds with medicinal potentials, we screened various medicinal plants to explore the novel acne medication. Among them, lupeol, a pentacyclic triterpene, from the hexane extract of *Solanum melongena L* was identifed. Lupeol targeted most of the major pathogenic factors of acne with desired physicochemical traits. It strongly suppressed lipogenesis by modulating IGF-1R/PI3K/Akt/SREBP-1 signaling pathway in SEB-1 sebocytes, and reduced inflammation by suppressing the NF-κB pathway in SEB-1 sebocytes and HaCaT keratinocytes. Lupeol exhibited only a minimal effect on cell viability and possibly modulated dyskeratosis of epidermal keratinocyte. It also decreased the viability of *P. acnes*. Finally, lupeol improved facial acne and was well tolerated in a 4-week double-blind, randomized, split-face, small-scale clinical trial. These results provide a therapeutic rationale for the use of lupeol in acne treatment.

Keyword: acne, lupeol, therapeutic development, activity guided purification, clinical trial

Student Number: 2013-30534

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List of abbreviations

TLR-2: Toll-Like Receptor-2

IL-1α: Interleukin-1 Alpha

IL-8: Interleukin-8

NF-κB : Nuclear Factor Kappa B

SREBP-1: Sterol Response Element Binding Protein-1

TNF- α: Tissue Necrosis Factor-Alpha

P. acnes: *Propionibacterium acnes*

GC-MS: Gas Chromatography-Mass Spectrometry

FAME: Fatty Acid Methyl Ester

FT-IR: Fourier Transform InfraRed

NMR: Nuclear Magnetic Resonance

HPLC: High Performance Liquid Chromatography

FFA: Free Fatty Acid

SM: Solanum Melongena L

ACC: Acetyl CoA Carboxylase

FAS: Fatty Acid Synthase

HMGCR: HMG-CoA Rreductase

HMGCS: HMG-CoA Synthase

Introduction

Acne vulgaris affects nearly 90% of adolescents worldwide and can leave permanent scarring if not properly treated (1,2). Although current medications are moderately effective in treating acne, they may be associated with various side effects (3,4). For example, oral isotretinoin, one of the most effective treatments, has potentially serious side effects including teratogenicity and dyslipidemia. Topical retinoids, as well as topical and systemic antibiotics may cause a burning sensation and antibiotic resistance. Therefore, the need to investigate new anti-acne ingredients has been growing, and natural products provide important clues for identifying novel drugs with relative safety that, so far, research has neglected (5). Natural compounds are a matchless source of novel drugs even compared with currently applied high-throughput combinatorial screening, while only a limited numbers have been tested for acne model. Because of the innate complexity of acne pathogenesis, a variety of candidate molecules should be screened for multiple pathogenic processes involving seborrhea, inflammation, *Propionibacterium acnes* (*P. acnes*), and follicular dyskeratosis (6). Therefore, stepwise fractionation and screening of natural products would be more efficient and systematic approach compared with hypothesis driven study for a single molecule.

To explore anti-acne ingredients from natural compounds, we screened several candidate medicinal plants known to be effective for acne from the literature or complementary medicine. Methanol extracts of each plant were separated based on polarity and acidity. Each fraction of these plant extracts was tested for biologic activities such as toxicity, anti-lipogenesis, anti-inflammation, and anti-microbial activities. Among them, the acidic hexane fraction of *Solanum Melongena L* (*SM)*

showed most desired results, and methanol extract of *Chamaecyparis Obtusa* also showed anti-acne effects. We demonstrated that Lactobacillus-Fermented *Chamaecyparis Obtusa* (LFCO) was rapid and effective for treating acne lesions compared with existing tea tree oil without notable side effects (7). Experimental findings including cellular and histopathological analysis correlated well with the clinical acne grade and treatment response. Instrumental analysis further identified constituents of LFCO. After identifying a new anti-acne agent, we further investigated the components of acidic hexane fraction of *SM* and purified lupeol as the most desirable component based on activity guided purification.

Lupeol (Lup-20(29)-en-3b-ol), a pentacyclic lupane-type tritperpene- is present in several species of the plant kingdom and is abundant in medicinal plants (8,9). The compound possesses wide-spectrum pharmacological activities, including lipid-lowering, anti-inflammatory, and anti-cancerous effects (10,11). Additionally, a few preclinical studies have suggested its potential as an anti-inflammatory or chemopreventive agent (12,13). These observations, along with the fact that acne is mainly related to lipogenesis and inflammation, suggest that it may improve acne.

In this research, we found lupeol, has potent previously unreported anti-acne traits, including sebosuppressive, anti-inflammatory effects on human SEB-1 sebocytes and HaCaT keratinocytes and beneficial effects on follicular dyskeratosis. Further biochemical and cellular studies indicated that modulation of IGF-1R/PI3K/Akt and NF-κB signaling pathways mediated its sebosuppressive and anti-inflammatory effects, respectively. We subsequently confirmed its efficacy and safety in a 4-week, double blind, randomized, split-face clinical trial. These data demonstrate that lupeol improves acne clinically with very few side effects by modulating major pathogenic factors.

Materials and Methods

■ Cell culture

The SEB-1-immortalized human sebocyte cell line was generated by transfection of secondary sebocytes with SV40 large T antigen, as previously described (14). SEB-1 cells were cultured and maintained in standard culture medium containing DMEM (Invitrogen, Carlsbad, CA, USA), 5.5 mM glucose/Ham's F-12 3:1 (Invitrogen), fetal bovine serum 2.5% (HyClone, Logan, UT, USA), adenine 1.8 x 10^{-4} M (Sigma, St Louis, MO, USA), hydrocortisone 0.4 mg/ml (Sigma), insulin 10 ng/ml (Sigma), epidermal growth factor 3 ng/ml (Austral Biologicals, San Ramon, CA, USA), and cholera toxin 1.2 x10^{-10} M (Sigma) at 37℃ in a 5% CO_2 incubator. The human keratinocyte cell line HaCaT was maintained in DMEM (Invitrogen) supplemented with 5% fetal bovine serum, 20 mM L-glutamine, 1mM sodium pyruvate, and antibiotic/antimycotic solution (10 U/m1 penicillin, 10 μg /ml streptomycin, and 0.25 μg /ml amphoterin; Invitrogen) at 37℃ in a 5% CO_2 incubator.

■ Western blot analysis

Protein was extracted using cell lysis buffer (Cell Signaling Technology, Beverly, MA, USA). Protein contents in lysates were determined using the BCA Protein Assay (Pierce, Rockford, IL, USA). Equal amounts of protein were run on 10% SDS-PAGE gels and then transferred to a polyvinylidene difluoride membrane. The blots were primarily probed with Akt rabbit antibody, Phospho-Akt (Thr308) rabbit antibody, Phospho-ACC (Ser79) rabbit antibody, Phospho-IRS-1 (Ser 307) rabbit antibody, IκBα rabbit antibody, Phospho-IκBα (Ser32/36) mouse antibody (Cell Signaling Technology), β-actin mouse antibody, NF-κB p65 mouse antibody, SREBP-1 rabbit antibody (Santa

Cruz Biotechnology, Santa Cruz, CA, USA), Phospho-IGF 1 receptor rabbit antibody, toll like receptor-2 rabbit antibody (Abcam, Cambridge, UK), and IL-1α mouse antibody (R&D Systems, Minneapolis, MN). Secondary anti-rabbit IgG and anti-mouse IgG antibody (Cell Signaling Technology) were used to detect primary antibodies. Blots were developed with WESTSAVE Up (LabFrontier, Seoul, South Korea) and exposed to the film. Films of blots were analyzed and quantified using a densitometric program (TINA; Raytest Isotopenmebgerate, Straubenhardt, Germany).

■ ELISA

SEB-1 sebocytes and HaCaT keratinocytes co-cultured with heat inactivated *P. acnes* for 24 hrs were treated with lupeol (5, 10 and 20 μM). IL-8 and IL-6 protein in the supernatants of co-cultured SEB-1 sebocytes and TNF-α and IL-6 in those of HaCaT keratinocytes were determined with the corresponding ELISA kit (Komabiotechnology, Seoul, South Korea) according to the manufacturers' instructions. Average value was calculated from the duplicate readings of individual standard and supernatants. Using a microtiter plate reader, the plate was read at 450 nm wavelength.

■ Thin-layer chromatography

SEB-1 cells were grown to 80% confluence in 60-mm dishes and treated with control or lupeol (5, 10 and 20 μM) for 24 hrs. In all experiments, cells were counted to normalize the data. The remaining cells were suspended in a DMEM solution containing 2 μCi ^{14}C-acetate and incubated for 2 hrs at 37℃ with agitation, and extracted twice with ethyl ether and nonradioactive carrier lipids. Samples were dissolved in a small volume of ethyl acetate and spotted on 20-cm silica gel thin-layer

chromatography plates (Merck, Darmstadt, Germany), which were run until the solvent front reached 19.5 cm in hexane, followed by 19.5 cm in benzene, and finally to 11 cm in hexane:ethyl ether:glacial acetic acid (69.5:30:1.5). Lipid spots were visualized, excised, and radioactivity in each spot was quantified in a liquid scintillation counter. Radio-TLC was also checked using TLC aluminum sheets 20 X 20 cm silica gel (Merck) for thin-layer chromatography as a stationary phase.

■ Nile red staining

SEB-1 cells were treated with control and lupeol (5, 10 and 20 μM) for 24 hrs. SEB-1 cells were washed in PBS and fixed with 4% paraformaldehyde for 15 mins. Then, cells were stained with 1 μg/ml Nile red (Sigma, St Louis, MO, USA) working solution in the dark for 5 mins at 37℃. Observation was done using a fluorescence microscope with appropriate filters (excitation wavelength: 440-488 nm, emission wavelength: 550-590 nm) (X 400). Images used for quantification were captured so that pixel intensities could be linear with exposure time. The intensities were quantified using TINA (Raytest Isotopenmebgerate, Straubenhardt, Germany) program.

■ Fatty Acid Methyl Ester (FAME) analysis with GC

To analyze changes of specific fatty acid components after lupeol treatments, SEB-1 sebocytes treated with control or different concentrations of lupeol were grounded after freeze drying. Then, they were placed in tubes with Teflon caps. Pentadecaonic acid (15:0) is used as internal standard. Methylation and extraction steps were performed as previously described (15). Gas chromatography (7890A, Agilent, Santa Clara, CA, USA) with FID detector was used for detection.

■ Inhibitor treatment

For the experiments of inhibitor assays, SEB-1 sebocytes administered with 50 μM of LY 294002 (Santa Cruz biotechnology), cell permeable PI3-K inhibitor, for 30 mins prior to lupeol treatments were performed. The compound was suspended in DMSO.

■ Real time PCR

After 24 hrs of lupeol treatments on SEB-1 cell culture, total RNA was extracted, and the concentration was determined by measuring the A_{260} of the samples. RNA was reversely transcribed with RevertAid^{TM} First-Strand cDNA Synthesis kit (Fermentas, Pittsburg, PA, USA), and 1μg of cDNA was amplified by real time PCR. Primer sequences of SREBP-1a, SREBP-1c, SREBP-2, FAS, ACC, HMG-CoA reductase, and HMG-CoA synthase were recorded in Table 1 according to manufacturers' manual of SYBR Green/ROX q-PCR kit (Fermentas). Real time PCR was performed according to manuals from the manufacturer. For the experiment of HaCaT keratinocytes stimulated with heat inactivated *P. acnes*, primers for IL-1α, TLR-2, IL-8, TNF-α, and IL-6 (TaqMan® Applied Biosystems, Branchburg, NJ, USA) were also used. Real time PCR was performed according to manuals from the manufacturer. GAPDH was used as the normalizing gene in all experiments.

Table 1. Real time PCR primers

Gene		Primers (Sense & anti-sense sequences)
SREBP-1a	F	GCTGCTGACCGACATCGAA
	R	TCAAATAGGCCAGGGAAGTCA
SREBP-1c	F	GGAGCCATGGATTGCACTTTC
	R	ATCTTCAATGGAGTGGGTGCAG
SREBP-2	F	AACGGTCATTCACCCAGGTC
	R	GGCTGAAGAATAGGAGTTGCC
FAS	F	CCGAGGAACTCCCCTCAT
	R	GCCAGCGTCTTCCACACT
ACC	F	CCACTTGGCTGAGCGATT
	R	CCAGGTCCTCCAGCAGAA
HMG-CoA reductase	F	TACCATGTCAGGGGTACGTC
	R	CAAGCCTAGAGACATAATCATC
HMG-CoA synthase	F	CATTTGACCATCTCTCCAGC
	R	GTCCGGCTTCTACCAATCAA
GAPDH	F	CAAGGTCATCCATGACAACTTTG
	R	GTCCACCACCCTGTTGCTGTAG

■ MTT assay

SEB-1 sebocytes and HaCaT keratinocytes were seeded in each of the 96-well plate at a density of 5×10^3 cells per well. Both of cell lines were treated with lupeol (5, 10 and 20 μM), and medium alone was used as control. After 24 and 48 hr treatment, 50 mg/ml of MTT (Sigma, St Louis, MO, USA) was introduced into each well and incubated for 4 hrs at 37℃. The converted dye was solubilized with DMSO. The optical density of the wells was determined using a microplate reader (Multiscan v3.0, Lab systems, CA , USA) at 570 nm.

■ CCK-8 assay

Cell viability was again determined using the Cell Counting Kit-8 (CCK-8) (Dojindo Laboratories, Kumamoto, Japan) according to the manufacturer's instructions. SEB-1 sebocytes and HaCaT keratinocytes were seeded in each of the 96-well plate at a density of 5×10^3 cells per well. Both of cell lines were treated with lupeol (5, 10 and 20 μM), and medium alone was used as control. The CCK-8 solution (10 μl) was added to each well, and the cells were incubated for 24 and 48 hrs at 37℃, after which the optical density was measured at 450 nm using a microplate absorbance reader (Bio-Tek, Elx800, USA). Cells that stained positively with the CCK-8 solution were considered viable and are presented as a percentage compared with control cells.

■ Microbial experiments

P. acnes isolate ATCC 11828 was acquired from the Korea Research Institute of Bioscience and Biotechnology (Daejeon, Korea). *P. acnes* was cultured on thioglycollate medium and concentration was adjusted to the 0.5 McFarland turbidity. Lupeol was originally dissolved in the mixture of DMSO and ethanol (1:1) to the concentration of 2300 μM and diluted to 18 μM by two-fold serial dilution method. Then, samples were cultured at 37℃ in anaerobic chamber for 24 hrs, and results between lupeol containing solution and vehicle only solutions were compared, and each concentration of broth in both of two groups tested was repeatedly inoculated into new growth medium to confirm the bacterial viability. Experiments were repeated more than three times in the same way. For the experiments related with inflammation, *P. acnes* cultures were heat-killed by exposing to temperatures of 80℃ for 20 min.

■ Immunohistochemistry and immunocytofluorescence

From 8 patients participating in the clinical trial, punch biopsies (2 mm) were performed for histopathologic analysis. At baseline, biopsies were performed from typical acne lesions and non-involved skin lesions. At the finial 4-week visit, biopsies were performed from typical acne lesions of both 2% lupeol and vehicle treated sides. Demographic data of these patients are presented in the Table below. Immunohistochemical (IHC) analysis was performed employing the streptavidin-biotin amplification method. Tissue samples were processed for IHC staining for interleukin-8, interleukin-1α (R&D systems), sterol regulatory element binding protein-1, NF-κB p65, Keratin 16 (Santa Cruz Biotechnology), and TLR-2 (Abcam, Cambridge, UK). To compare the intensity of IHC staining before and after lupeol applications, pictures were taken from slide with microscope. The intensity of IHC staining was measured with Leica microsystem DFC290 (Leica Camera AG, Solms, Germany).

< Patient Information participating in skin biopsies>

Patient No.	Age	Gender	Region
1	21	M	Cheek
2	24	F	Cheek
3	19	M	Forehead
4	28	M	Cheek
5	21	M	Chin
6	20	F	Cheek
7	19	M	Cheek
8	26	F	Forehead

In immunocytofluorescence, cells were grown on 8-well chamber slides (Research Products International, Mt. Prospect, IL, USA) and fixed/permeabilized in ice-cold methanol:acetone. Following fixation, cells were blocked in 1% BSA and 5% normal horse serum PBS, stained with the primary antibody for one hr at 4℃, washed and then incubated for 30 mins with fluorescein isothiocyanate (FITC) conjugated secondary antibody. Coverslips were applied with ProLong® Gold Antifade Reagent with DAPI. Imaging was performed on a Nikon DiaPhot microscope with digital camera and using Spot Advanced software version 4.5 (Sterling Heights, Michigan, MI, USA).

■ Statistical analysis for in vitro assay

Data were analyzed using SPSS software (version 16.0, SPSS Inc., Chicago, IL, USA). The comparison of different groups in *in vitro* experiment was carried out using two-tailed unpaired Student's *t* test.

■ Clinical trial

a) Study design

To investigate the efficacy and tolerability of lupeol in the treatment of facial acne, a small-scale, randomized, double-blinded, split-face clinical trial over the course of 4 weeks was conducted. This study was prospectively registered in an officially approved clinical registry before patient enrollment (ClinicalTrials.gov ID #NCT02205892). We determined that 14 subjects would provide more than 90% power (with a 2-sided 5% significance level) to detect a difference of 35% in the number of inflammatory acne lesions between facial sides, assuming a standard deviation of 25%. To compensate for possible subject withdrawal, 16 patients were enrolled. This study was conducted at an outpatient dermatology clinic at Seoul National University Hospital between August and September, 2014.

At the time of initial presentation, an evaluation of acne severity was performed using both individual counts of inflammatory and non-inflammatory acne lesions and an assessment of the Leeds revised acne grading system score. Then, the patients were randomly assigned to either left- or right-sided lupeol application, with the other side of the face assigned to vehicle-control application. A simple random allocation sequence was created using computer-based random number generators with a block size of 4. All dermatologists participating in outcome assessments, a physician assistant managing

trial enrollment and administration, medical statisticians analyzing the data, and patients were blinded to the assignments. Randomization codes were strictly secured in a safe in the administration office of the clinical research center until data entry was complete. The integrity of the blinding was ensured by packaging the study- and control products in identical tubes and by requiring a third party (other than the investigator/evaluator) to dispense the medication. In addition, both topical agents were identical in color and odor. All patients were instructed to apply each agent to all acne lesions on the respective facial side twice daily for 4 weeks. Efficacy and safety evaluations were performed at baseline and at weeks 2 and 4 (± 3 days). The study design was reviewed by medical statisticians before patient enrollment began.

b) Study procedure

At baseline and at the 2- and 4-week follow-up visits, individual lesion counts and Leeds revised acne grading system assessments were performed by 2 board-certified dermatologists. After discussion between the physicians, a single value was agreed upon for each measure; that value was used for statistical analysis. Standardized clinical photographs were taken at each visit, obtained using identical lighting conditions and digital-camera settings (Nikon D70; Nikon Corp., Tokyo, Japan). Patient compliance was monitored 3 times weekly using both telephone interviews and text messaging.

c) Efficacy and safety outcome measures

The primary outcome was the percent change in inflammatory lesions (papules, pustules, and nodules) at 4 weeks. The secondary outcomes were the percent change in non-inflammatory lesions (open and closed comedones) at 4 weeks, and the change in

Leeds revised acne grading system score at 4 weeks. Adverse effects (AE) were defined as all unintended and harmful signs, symptoms, and diseases, with the plan to record each AE in accordance with the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0. The safety- and side-effect profiles were assessed using both patient AE reporting and physicians' clinical skin examination at each visit.

d) Patients

Patients were eligible for study enrollment if they were over 15 years of age or older and experiencing mild to moderate acne vulgaris (Leeds revised acne grading system score 2–7) at baseline. Lesion counts were assessed on the face only, from the hairline to the jawline. Subjects were not allowed to use any systemic, topical, or light-based acne treatments during the course of the study. The exclusion criteria were as follows: known pregnancy; lactation; any medical illness that might influence the results of the study; a history of oral acne medication or surgical procedure, including laser treatment, within 6 months of study enrollment; use of topical acne medication within 4 weeks of study enrollment. Patients were free to withdraw from the study at any time and for any reason. All patients provided written informed consent before enrolling; a parent-agreement statement was also required for patients under 19 years of age. The study was approved by our institutional review board and complied with the Good Clinical Practice guidelines, the Declaration of Helsinki, and local laws.

A total of 23 patients were screened, and 16 patients were randomized and included in the ITT population; 15 patients (93.8%) completed the study. One patient dropped out at week 2 for personal reasons, not related to drug AEs. Data analysis was

performed using the ITT groups. The baseline patient characteristics are summarized in Table 2, and full dataset of patients are shown in Table 3. Facial ides were comparable for each patient on baseline dermatological evaluation.

Table 2. Baseline characteristic of patients enrolled in clinical trial

a) Demographic data

Demographic data	N=16
M / F	10/6
Age (mean ± SD, range)	22.9 ± 4.4 (15-34)
Race (%)	Korean (100%)
Skin types (III/ IV/V)	4/7/5

*Split-face study

b) Acne severity

Evaluation of acne severity	Lupeol side	Vehicle side
Inflammatory acne lesions	14.5 ± 2.1	14.3 ± 2.8
Non-inflammatory acne lesions	38.6 ± 12.2	37.9 ± 13.3
Leeds revised grade	4.5 ± 0.8	4.4 ± 0.9

* No significant difference found between two groups using a paired *t*-test ($P > 0.05$).

Table 3. Analysis of clinical data

a) ITT analysis

	Vehicle (mean ± SE)	Lupeol (mean ± SE)	Between group difference (Lupeol vs. vehicle, mean ± SE)	*P* value
Percent change of inflammatory acne lesions				
Week 2	-4.1 ± 4.4	-32.9 ± 3.9	-28.8 ± 4.8	<0.01
Week 4	-4.5 ± 4.0	-50.9 ± 3.1	-46.4 ± 5.4	<0.01
Percent change of non-inflammatory acne lesions				
Week 2	-4.1 ± 4.6	-23.0 ± 3.9	-17.9 ± 5.0	<0.01
Week 4	-5.7 ± 5.3	-36.1 ± 4.5	-29.4 ± 8.3	<0.01
Decrease of Leeds revised grade				
Week 2	-0.1 ± 0.2	-0.9 ± 0.1	-0.8 ± 0.2	<0.01
Week 4	-0.3 ± 0.1	-1.7 ± 0.1	-1.4 ± 0.2	<0.01

b) PP analysis

	Vehicle (mean ± SE)	Lupeol (mean ± SE)	Between group difference (Lupeol vs. vehicle, mean ± SE)	*P* value
Percent change of inflammatory acne lesions				
Week 2	-3.0 ± 4.3	-33.1 ± 4.1	-30.2 ± 4.9	<0.01
Week 4	-3.5 ± 4.1	-52.3 ± 3.0	-48.9 ± 5.2	<0.01
Percent change of non-inflammatory acne lesions				
Week 2	-2.3 ± 3.9	-22.2 ± 4.1	-19.9 ± 4.9	<0.01
Week 4	-4.0 ± 4.8	-36.1 ± 4.8	-32.1 ± 8.4	<0.01
Decrease of Leeds revised grade				

Week 2	-0.1 ± 0.2	-0.9 ± 0.1	-0.9 ± 0.2	<0.01
Week 4	-0.3 ± 0.2	-1.7 ± 0.1	-1.5 ± 0.1	<0.01

e) Statistical analysis

All analyses were carried out according to a pre-established analysis method. The ITT population included all randomized subjects who were dispensed study medication. The PP population included all ITT subjects who did not experience any major protocol deviations. LOCF analysis was used to evaluate the primary and secondary outcomes. The primary outcome was evaluated in the ITT population at the study endpoint (week 4). The baseline mean lesion counts and Leeds revised acne grading system scores for each side of the face were compared using a paired *t*-test. The percentage in lesion reduction and the change in Leeds score at subsequent visits were analyzed using linear mixed models. We were looking for a difference in facial-side acne status at each follow-up visit, as well as for an effect of time on the efficacy of either treatment, with adjustment for baseline values. The treatments and the clinic visits served as the repeated measures for these analyses. The linear mixed models used fixed factors including baseline values, treatments, clinic visits, interactions between treatments and visits, random intercepts, and general correlation structures. Multiple comparisons were adjusted using Bonferroni correction methods. The analysis for both primary and secondary endpoints was repeated for the PP population to confirm the results. All tests were 2-sided and used the 0.05 level to define statistical significance. All statistical analyses were performed using R software, version 2.15.3 (The R Foundation for Statistical Computing, Vienna, Austria).

Results

Lupeol decreased lipogenesis in human SEB-1 sebocytes

Although hyperseborrhea is critical in the pathogenesis of acne, few topical drugs currently used can reduce sebum secretion effectively. To examine whether lupeol modulates lipid synthesis in human SEB-1 sebocytes, intracellular neutral lipid content was analyzed by Nile Red staining after treating the cells with different concentrations of lupeol. Lupeol significantly decreased lipid by 58% in SEB-1 sebocytes treated with 20 μM lupeol (Figure 1a, b). Changes in specific free fatty acid (FFA) components, which play important roles in the innate immunity of acne inflammation, were further analyzed using quantitative FAME analysis with GC-MS (Figure 1c). Overall, fatty acid content was reduced as lupeol concentration increased, with significant decreases in major FFA components (palmitic acid [C16:0], stearic acid [C18:0], and oleic acid [C18:1]). Addition of lupeol robustly decreased ^{14}C acetate incorporation into fatty acids, cholesterol, and squalene, which are major components of human sebum, dose dependently after cell count normalization, further confirming the lupeol-induced reduction of intracelluar lipid synthesis (Figure 1d).

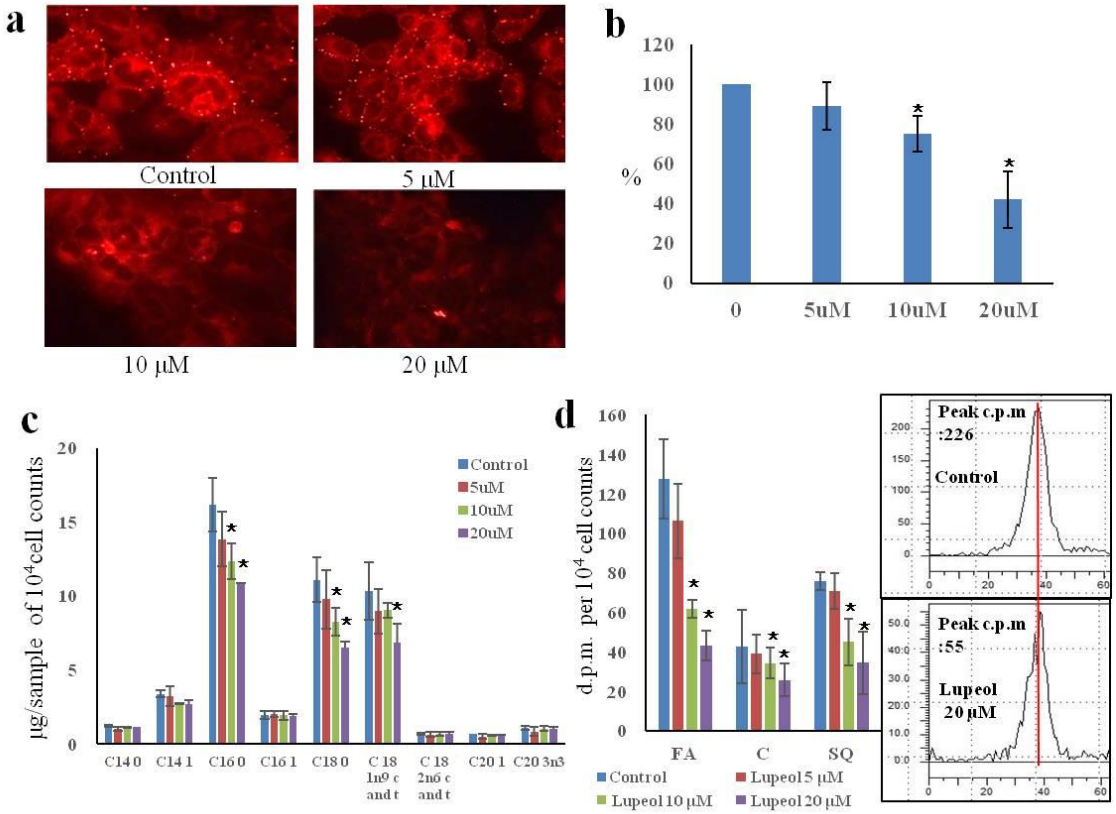


Figure 1. Lupeol decreased lipogenesis in SEB-1 sebocytes (**a**) Nile red assay was performed for SEB-1 sebocytes treated with lupeol for 24 hrs. (**b**) Relative percentile quantification after cell count normalization. (**c**) FAME-GC analysis was performed to detect quantitative changes in fatty acid profiles. (**d**) Changes of ^{14}C acetate incorporation levels in specific lipid components of SEB-1 cells after lupeol treatments were analyzed using thin-layer chromatography (FA: Fatty Acid, C: cholesterol, SQ: squalene) (left). Representative radio-TLC data for fatty acid (red line) of control and 20 μM lupeol samples (right). All experiments were repeated a minimum of three times. * $P < 0.05$ between each lupeol-treated group and control (Student's t-test).

Downregulation of IGF-1R/PI3K/Akt/SREBP pathway was responsible for the suppression of lipogenesis by lupeol in human SEB-1 sebocyte

We tested the hypothesis of whether sterol regulatory element-binding proteins (SREBPs), major transcriptional factors responsible for the regulation of cholesterol/fatty acid metabolism, were involved in the anti-lipogenic effects of lupeol. Lupeol significantly decreased both precursor and mature forms of SREBP-1 proteins after 24 hrs (Figure 2a). Quantitative real-time PCR showed that lupeol also decreased mRNA levels of SREBP-1a, SREBP-1c, and SREBP-2, as well as key downstream targets of all SREBPs, including fatty acid synthase, acetyl-CoA carboxylase, HMG-CoA reductase, and HMG-CoA synthase. These suggest that lupeol inhibited the expression of lipogenic molecules at the transcriptional level (Figure 2b).

To identify upstream regulators responsible for the lupeol-mediated suppression of the SREBP pathway, we focused on IGF-1R/PI3K/Akt pathway for the following reasons: (1) Previous studies show that lupeol mitigates PI3K/Akt pathway in a CD-1 mouse model and in AsPC-1 cell lines (16,17), and (2) IGF-1 treatment induces lipogenesis via activation of IGF-1R/PI3K/Akt pathway in SEB-1 sebocytes (18). Lupeol decreased IGF-1R/IRS-1/PI3K/Akt pathway in a dose-dependent manner after 3 hrs and subsequently downregulated SREBP-1 protein expressions (Figure 2a). Co-treatment with lupeol and the PI3K/Akt inhibitor LY 294002 potently blocked expression of phosphorylated Akt. Importantly, this inhibition did not synergistically decrease protein content of precursor and mature SREBP-1 or intracellular lipid content (Figure 2c, d), suggesting lupeol mainly suppressed lipogenesis through the PI3K/Akt pathway. Together, these data suggest that lupeol suppressed sebum mainly through inhibition of the IGF-1R/PI3K/Akt/SREBP-1 signaling pathway in human SEB-1 sebocytes.

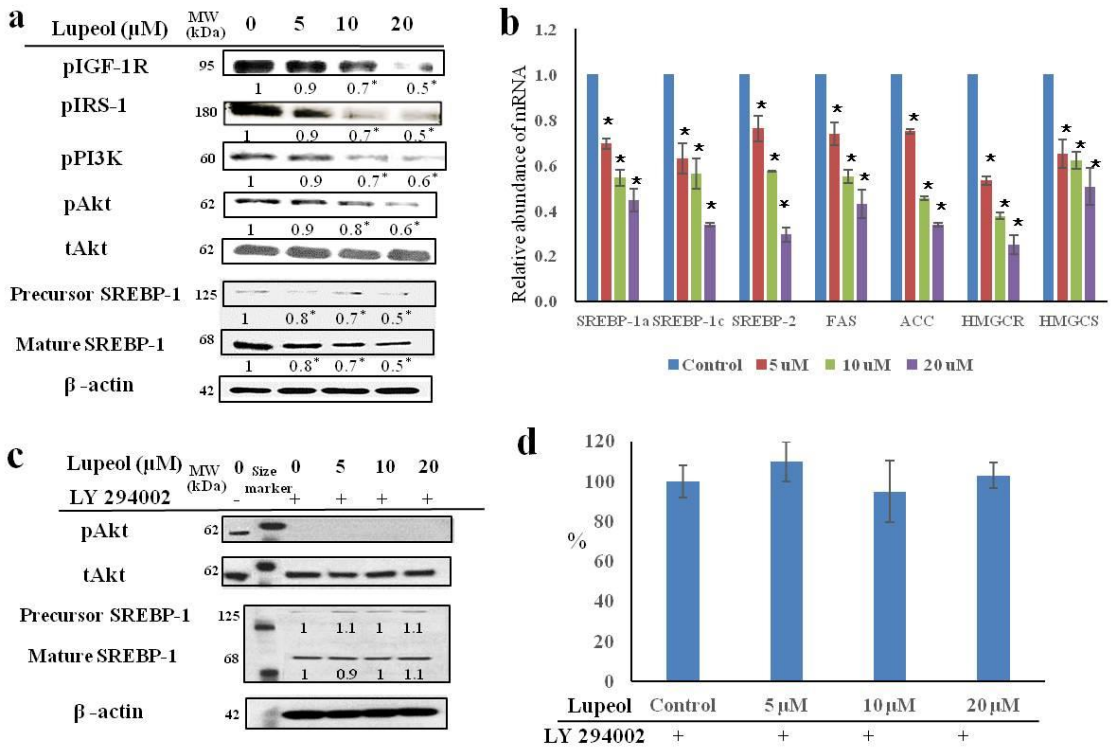


Figure 2. Downregulation of lipogenesis was mediated by suppressing IGF-1R/PI3K/Akt/SREBP signaling pathway (**a**) Western blotting of phospho-IGF-1R, phospho-IRS, phospho-PI3K, phospho-Akt, precursor and mature SREBP-1 was performed, (**b**) RNA was subjected to quantitative real-time PCR to determine the abundance of SREBP-1a, SREBP-1c, SREBP-2, FAS, ACC, HMGCR, and HMGCS. After pretreatment with LY294002 30 mins prior to lupeol treatment, (**c**) Western blotting of phospho-Akt, precursor and mature SREBP-1 was performed, and (**d**) quantification of Nile red assay was done. * $P < 0.05$ between each lupeol-treated group and control (Student's t-test).

Lupeol decreased inflammation induced by heat-inactivated P. acnes through the inhibition of NF-κB pathway in both SEB-1 sebocytes and HaCaT keratinocytes.

In acne, *P. acnes*-induced inflammatory response around pilosebaceous gland, mainly through the secretion of various pro-inflammatory cytokines, represents a key pathogenic factor leading to disease initiation and aggravation (19-21). We investigated anti-inflammatory effects of lupeol in SEB-1 sebocytes (Figure 3a-d) and HaCaT keratinocytes (Figure 3e-h), two major cutaneous parenchymal cells associated with acne. Heat-inactivated *P. acnes* induces several pro-inflammatory cytokines including IL-8 and IL-6 in SEB-1 sebocytes and tumor necrosis factor (TNF-α), IL-6, COX-2, and iNOS in HaCaT keratinocytes, respectively (Figure 3a, b, d, e, f).

To further examine underlying molecular mechanisms, we measured the activation of associated proteins related with NF-κB pathway. We found that protein expressions of NF-κB p65 and phospho-IκB were significantly increased after *P. acnes* treatment, strongly suggesting that the activation of NF-κB may induce pro-inflammatory cytokine expression (Figure 3c, g). Then, we tested the anti-inflammatory effects of lupeol in this *P. acnes* induced inflammatory model. Lupeol significantly decreased protein expression of cytokines, COX-2, and iNOS in a dose-dependent manner for both SEB-1 sebocytes and HaCaT keratinocytes, strongly supporting its anti-inflammatory effect. Messenger RNA expressions of pro-inflammatory cytokines in HaCaT keratinocytes showed similar patterns (Figure 3h). Furthermore, expression of phospho-IκB and NF-κB proteins were reduced with lupeol, confirming that lupeol inhibited heat-inactivated *P. acnes* signaling of NF-κB activation by mitigating innate immunity of two major cutaneous cells associated with inflammatory acne (Figure 3a-h).

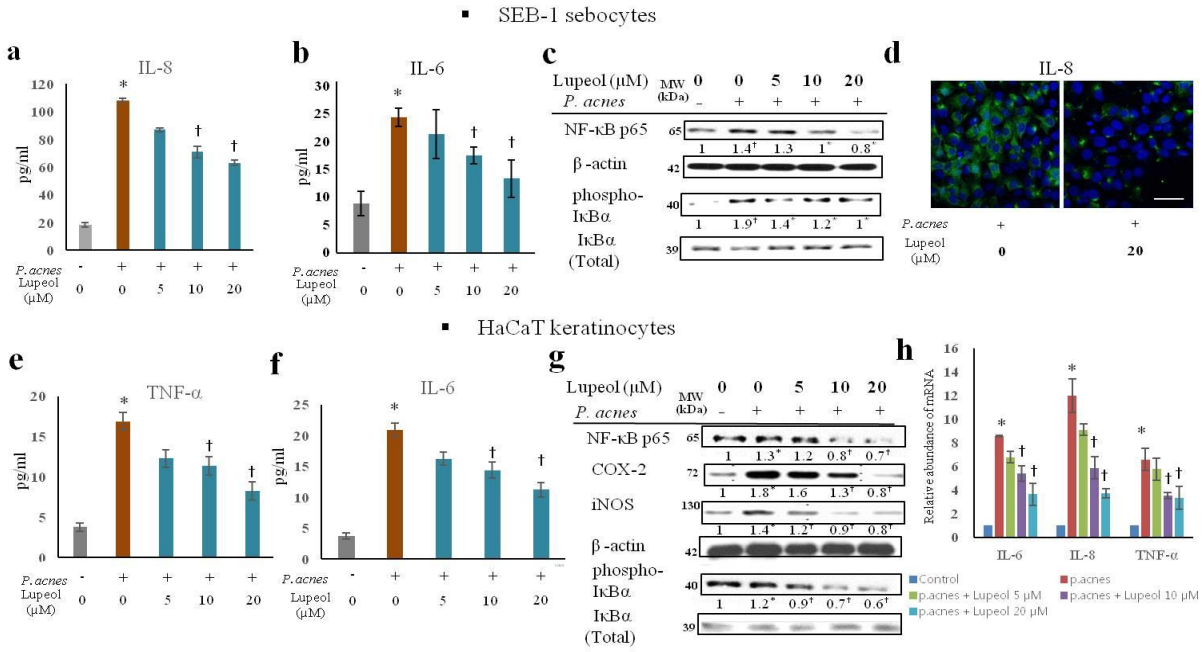


Figure 3. Lupeol decreased inflammation induced by heat-inactivated *P. acnes* through the inhibition of NF-κB pathway in both SEB-1 sebocytes and HaCaT keratinocytes ELISA was performed for (**a)** IL-8, and (**b**) IL-6 in the supernatant of SEB-1 sebocytes stimulated with heat-inactivated *P. acnes* before and after treatments with lupeol for 24 hrs. (**c**) Western blotting of NF-κB p65, and phospho-IκBα under the same condition. (**d**) Immunocytofluorescence staining was done for IL-8 (green) with cell nuclei counterstained with 4,6-diamidino-2-phenylindole (blue) (bar = 50 μm). Parallel experiments were performed with HaCaT keratinocytes. ELISA for (**e)** TNF-α, (**f**) IL-6, (**g**) Western blotting of NF-κB p65, COX-2, iNOS and phospho-IκBα and (**h**) Quantitative real-time PCR for IL-6, IL-8, TNF-α in HaCaT keratinocytes. All experiments were repeated a minimum of three times. * $P < 0.05$ between control and each *P. acnes*-stimulated group, † $P < 0.05$ between *P. acnes*-stimulated, lupeol non-treated group, and each lupeol-treated group (Student's t-test).

Lupeol exhibited only a minimal effect on cell viability

To test the possible toxicity of lupeol, we investigated the effect of lupeol treatment on the viability of SEB-1 sebocytes and HaCaT keratinocytes. Using both MTT and CCK-8 assays, we showed lupeol treatment was not cytotoxic in conditions identical to other cellular experiments (concentration range 0-20 μM for 24 hr incubation) in either cell type. Only a minimal effect on cell viability was observed at 20 μM after 48 hrs in SEB-1 seboycytes, and no toxicity was observed in HaCaT keratinocytes (Figure 4a and b).

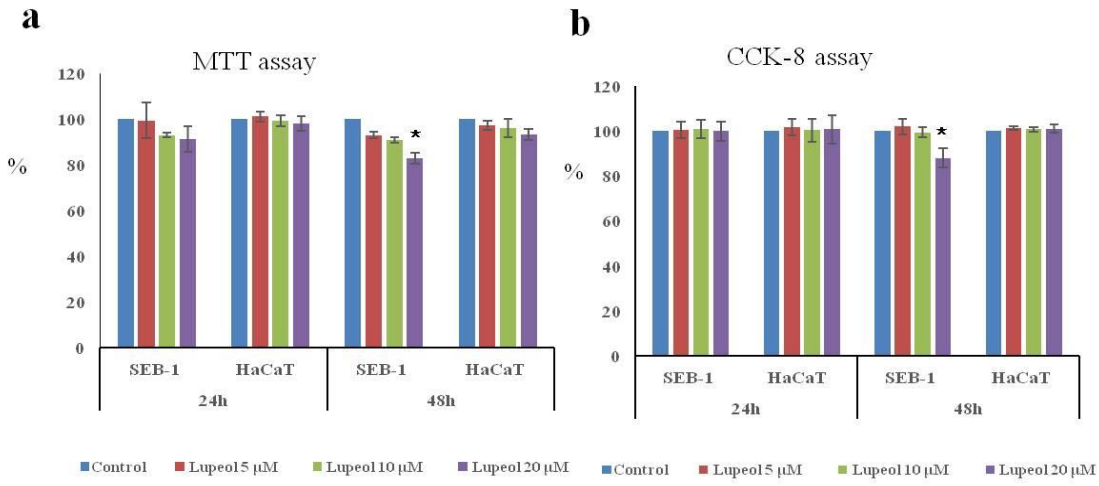


Figure 4. Lupeol exhibited only a minimal effect on cell viability Cell viability of both SEB-1 sebocytes and HaCaT keratinocyte was measured by both (**a**) MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and (**b**) CCK-8 (Cell Counting Kit-8) assays after treatment with lupeol for 24 and 48 hrs. All experiments were repeated a minimum of three times. * $P < 0.05$ between each lupeol-treated group and control (Student's t-test).

Lupeol possibly modulated dyskeratosis of epidermal keratinocyte

Because follicular epidermal dyskeratosis is another major triggering factor of acne pathogenesis, we investigated the possible beneficial effects of lupeol on the abnormal differentiation of epidermal keratinocytes. Lupeol significantly suppressed protein and mRNA expressions of IL-1α, a strong inducer of hypercornification of the infundibulm, and TLR-2, a key molecule in IL-1α release during comedogenesis, in HaCaT keratinocytes stimulated by heat inactivated *P. acnes* (Figure 5a, b). Consistent with these findings, immunohistochemical analysis confirmed decreases in IL-1α and TLR-2 in patient tissue after 4 weeks of lupeol application (Figure 5c, d; See below for clinical trial). Furthermore, the expression of K16, a marker of epidermal proliferation and abnormal differentiation, were significantly decreased in the epidermis, supporting the possible therapeutic role of lupeol in the prevention of comedogenesis (Figure 5e). The expression of these three proteins was significantly lower in non-acne skin than in skin containing acne lesions at both time points (IL-1α: 0.5, TLR-2: 0.5, K16: 0.3).

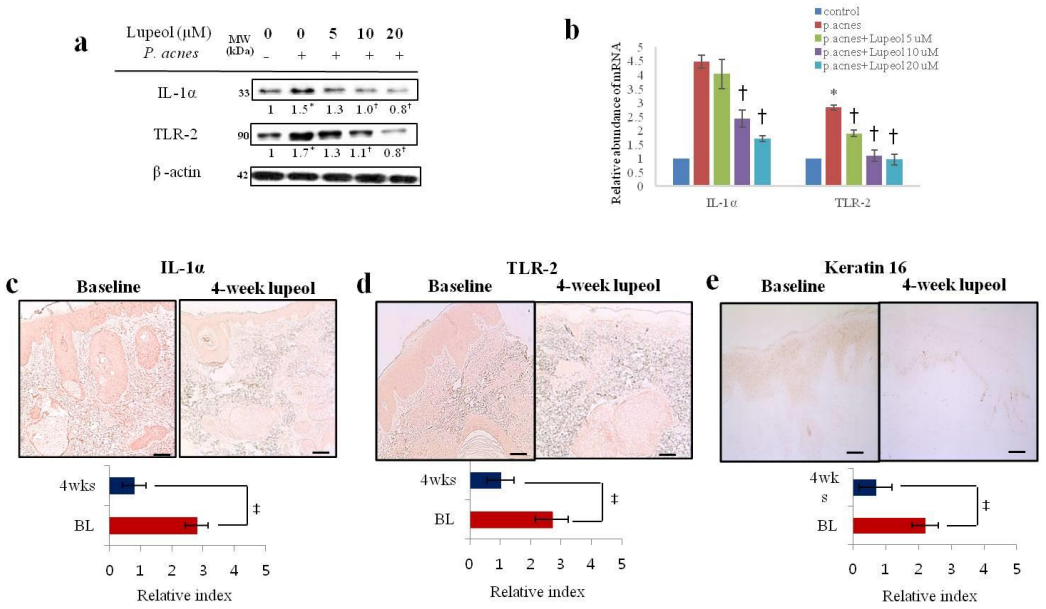


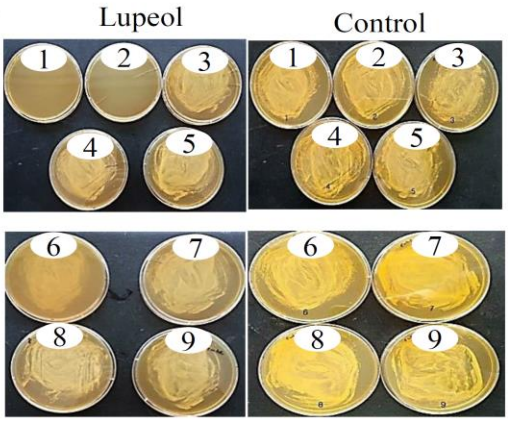
Figure 5. Lupeol possibly modulated dyskeratosis of epidermal keratinocyte For HaCaT keratinocytes stimulated with heat-inactivated *P. acnes* before and after treatments with lupeol for 24 hrs, (**a**) Western blotting of IL-1α and TLR-2 and (**b**) Quantitative real-time PCR were performed. Immunohistochemical analysis of (**c**) IL-1α, (**d**) TLR-2, and (**e**) Keratin 16 from patient skin tissues acquired at baseline and final visits (bar = 100 μM). All experiments were repeated three times (mean ± SEM). * $P < 0.05$ between control and each *P. acnes*-stimulated group, † $P < 0.05$ between *P. acnes*-stimulated, lupeol non-treated group, and each lupeol-treated group. ‡ $P < 0.05$ compared with baseline (Student's t-test).

Lupeol inhibited the growth of *P. acnes*

Previous findings report that lupeol exerts antimicrobial activity against a wide range of community encountered microbes (22,23). This, along with the critical roles of *P. acnes* in innate immunity of acne, led to our hypothesis that lupeol demonstrates antibacterial effects against *P. acnes.* To test this hypothesis, the minimal concentration

of lupeol needed to inhibit *P. acnes* growth in culture medium was compared to vehicle control used for dissolution. Only Lupeol containing solution effectively inhibited growth of *P. acnes* at concentrations more than 1150 μM, suggesting it may also target abnormal colonization of *P. acnes* in acne development (Figure 6a, b). In addition to directly suppressing inflammation by targeting the NF-κB pathway in skin parenchymal cells, lupeol may relieve acne inflammation synergistically through its antimicrobial activities against *P. acnes*.

a



b

No	1	2	3	4	5
Conc. (μM)	2300	1150	575	287.5	143.8
Lupeol	-	-	+	+	+
Control	+	+	+	+	+
No	**6**	**7**	**8**	**9**	
Conc. (μM)	71.9	35.9	18.0	0 (control)	
Lupeol	+	+	+	+	
Control	+	+	+	+	

Figure 6. Lupeol inhibited *P. acnes* growth (**a**) *P. acnes* was incubated with increasing concentrations of lupeol and vehicle control (**b**) Antibacterial effects of them were repeatedly measured and minimal inhibition concentration of lupeol was determined. Only Lupeol containing solution effectively inhibited growth of *P. acnes* at concentrations more than 1150 μM, suggesting it may also target abnormal colonization of *P. acnes* in acne development. All experiments were repeated a minimum of three times.

Lupeol improves acne without notable side effects in a double blinded, randomized, small scale clinical trial

To investigate the clinical efficacy and tolerability of lupeol for the treatment of facial acne, we conducted a small-scale, randomized, double-blinded, split-face clinical trial over the course of 4 weeks. Fifteen of 16 enrolled patients completed the study, in which affected areas on half each patient's face were treated with 2% lupeol twice daily while the other side of the face was treated with a vehicle control. Clinical visits were scheduled at baseline and at 2 and 4 weeks. The primary outcome of the study was the percent change in inflammatory lesions (papules, pustules, and nodules) at 4 weeks. The secondary outcomes were the percent change in non-inflammatory lesions (open and closed comedones) and the change in the Leeds revised acne grading system score at 4 weeks. AEs were defined as all unintended and harmful signs or symptoms; these were assessed by both patients' self-reporting and physicians' skin examination at each visit. Results were analyzed using ITT groups. The PP population consisted of the 15 patients who did not have any major protocol deviations. LOCF analysis was used to evaluate the primary and secondary outcomes. CONSORT diagram is described in Figure 7. Detailed information on this clinical trial is described in the materials and methods.

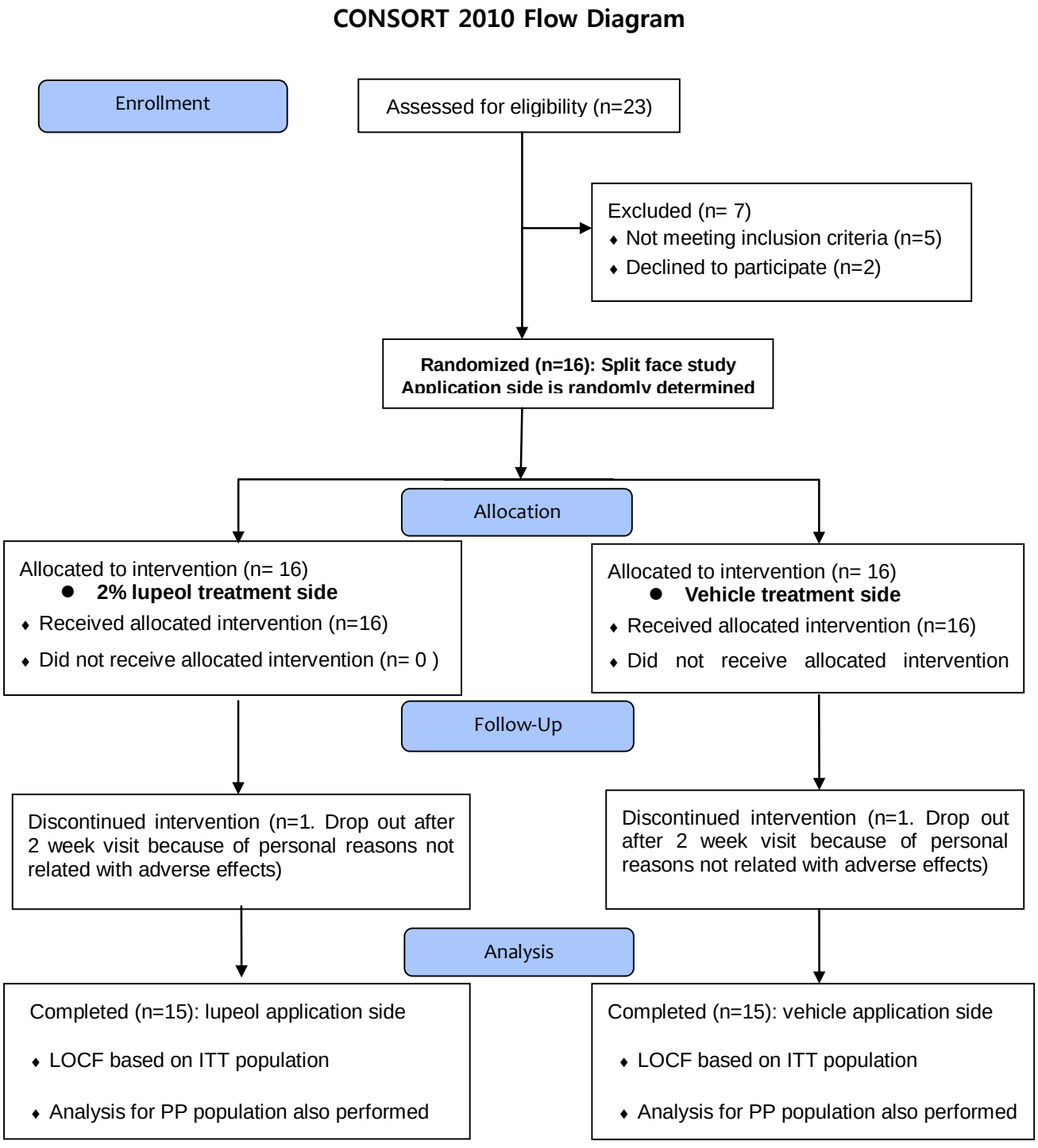


Figure 7. Flow diagram of clinical trial

The treatment groups were comparable with respect to demographic characteristics and baseline dermatological scores (Table 1). As a result of clinical trial, patients improved acne only in acne side (Figure 8a). At the 4-week visit, patients experienced a mean reduction in inflammatory lesions of 50.9% (95% confidence interval, 41.7–60.1%) in lupeol application side of the face, which was a significant decrease compared with baseline ($P < 0.01$). No significant change was observed on the vehicle-control side of the face, leading to a significant difference between facial-side groups at both 2 and 4 weeks of application ($P < 0.01$) (Figure 8b). Non-inflammatory lesions also significantly decreased by 36.1% (95% confidence interval, 22.5–49.6%) after 4 weeks ($P < 0.01$) in lupeol side, and a significant difference in the percent reduction between groups was found after both 2 and 4 weeks of application ($P < 0.01$) (Figure 8c). Consistent with these lesion-count results, the mean Leeds revised acne grading system score, used as a global severity assessment, decreased significantly by 1.67 (95% confidence interval, 1.30–2.04) after 4 weeks ($P < 0.01$) in lupeol side, and a significant difference in the reduction between groups was also found after both 2 and 4 weeks of application ($P < 0.01$) (Figure 8d). Endpoint analysis for all 3 efficacy measures was repeated for the PP population to confirm the results. Lupeol was generally well tolerated; no AEs were reported.

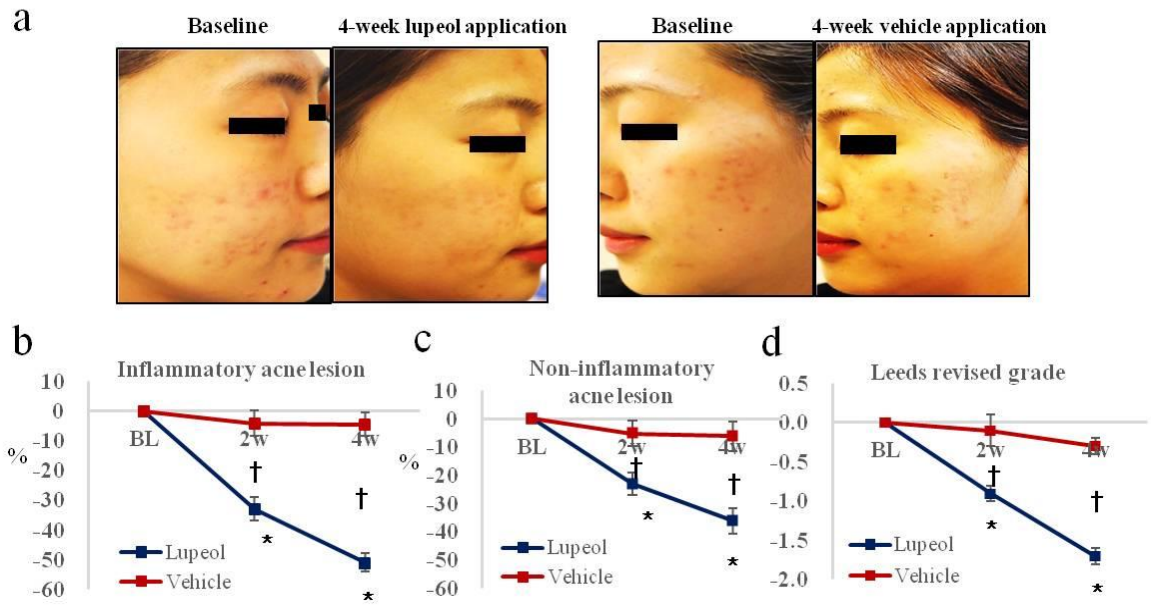


Figure 8. Topical application of 2% lupeol significantly improved acne in a 4-week human clinical trial (**a**) In a small-scale, randomized, double-blinded, split-face clinical trial over the course of 4 weeks, patients with mild to moderate acne applied 2% lupeol to one side of the face, and vehicle to the other side. Percent changes of (**b**) inflammatory (papules, pustules, and nodules), and (**c**) non-inflammatory (whiteheads and blackheads) lesions were counted. (**d**) Changes of Leeds revised grade for general acne severity were recorded. * $P < 0.05$ between lupeol-treated group and baseline, † $P < 0.05$ between each lupeol-treated group and vehicle treated group (linear mixed models).

Histopathologic changes after applying lupeol for 4 weeks are consistent with experimental data in cellular levels

Furthermore, we examined histological changes of acne lesions for *in situ* expression of SREBP-1, NF–κB p65, and IL-8 to determine consistency with our *in vitro* findings and provide clinical relevance. Skin specimens were acquired from

typical acne lesions at baseline and after 4 weeks of lupeol application. Consistent with results from our cellular assays and clinical trial, lupeol significantly decreased expression of SREBP-1, NF-κB p65, and IL-8 as measured by immunohistochemistry (Figure 9a, b, c). The expression of these three proteins in non-acne involved skin was significantly lower than in the skin with acne lesions at both time points (SREBP-1: 0.5, NF-κB p65: 0.8, IL-8: 0.8). These immunohistochemical results confirmed that lupeol decreased lipogenesis and inflammation, two critical steps in acne pathogenesis, in acne lesions *in vivo*.

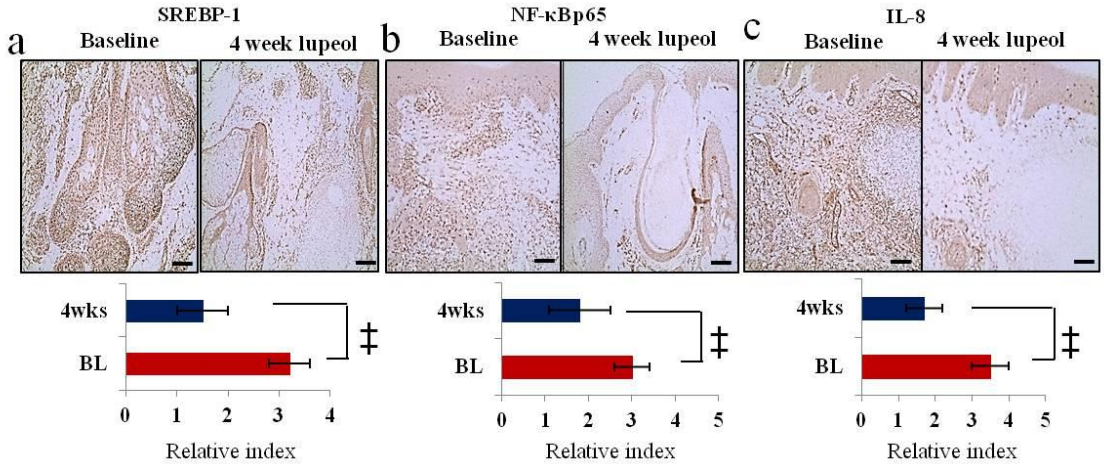


Figure 9. Histopathological analysis before and after patients' application of lupeol confirmed *in vitro* results Histological analysis of patient skin tissues for (**a**) SREBP-1, (**b**) NF-κB p65, and (**c**) IL-8 were performed at baseline and final visits (bar = 100 μM). ‡ $P < 0.05$ compared with baseline (Wilcoxon signed rank test).

Based on these results, we illustrated underlying therapeutic mechanisms of lupeol as a schematic diagram in Figure 10.

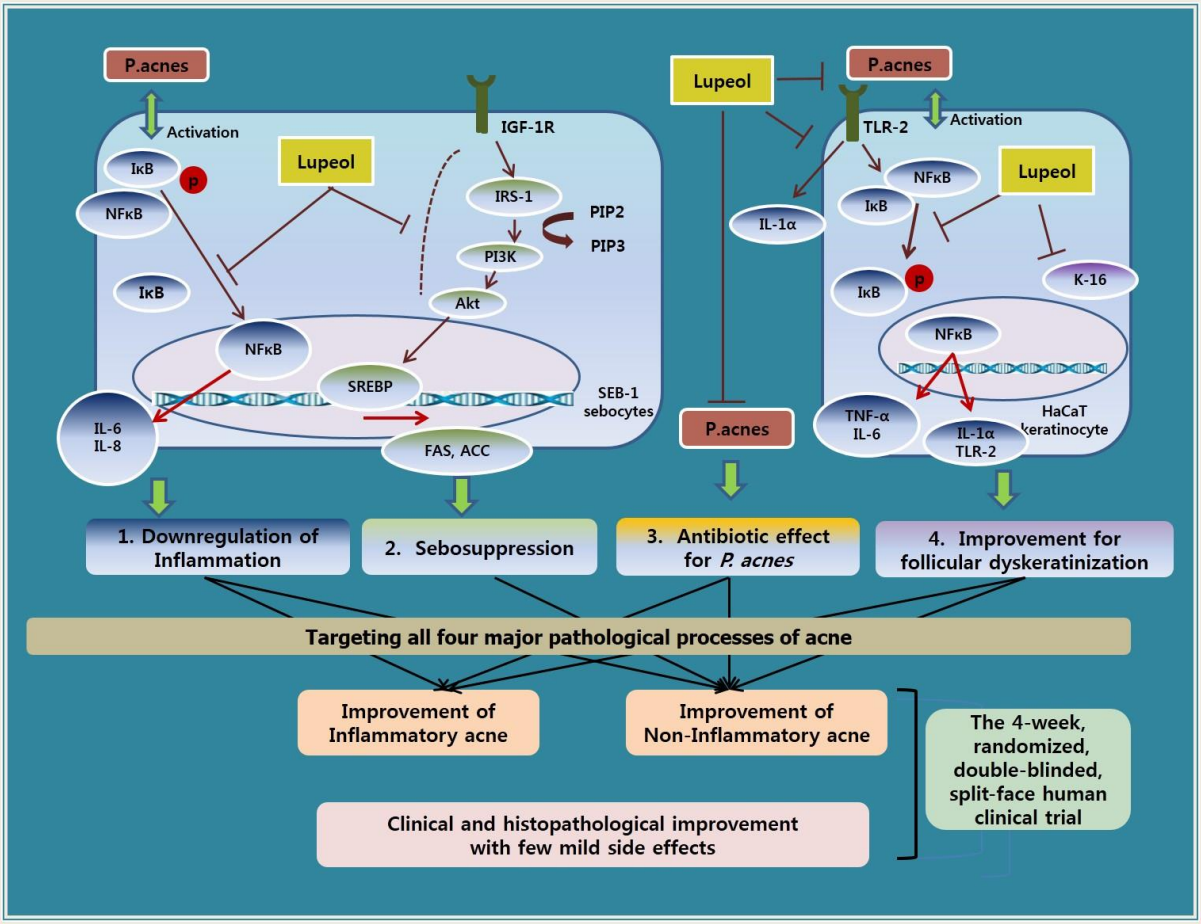


Figure 10. Possible therapeutic mechanisms based on experimental results These mechanisms may elucidate the efficacy of lupeol in our 4-week randomized, double-blinded, split-face human clinical trial.

Discussion

Despite the near-universal prevalence of acne and corresponding demands for treatment, the development of effective anti-acne medications has been hampered by the complexity of acne pathogenesis. In our previous study, we discovered that epigallocatechin-3-gallate improves acne by modulating multiple pathogenic factors, including hyperseborrhea, inflammation, and *P. acnes* overgrowth (24). In spite of great efficacy in properly controlled condition, relative molecular instability of this anti-oxidant could be affected by various external factors including solvent, pH, temperature, ionic strength and oxidative stress (25). Possible molecular degradation during drug formulation or delivery processes may lead to significant decreases of therapeutic potentials. To resolve this issue by exploring new class of small molecules, we screened various extracts of medicinal plants, and finally isolated lupeol showing desirable therapeutic effects against all major targets of acne. This molecule is quite lipophilic, has no ionizable moiety in the physiologic pH, and chemically stable (26). It is also useful in maintaining skin texture and integrity in animal studies (27). These physicochemical and biological properties would enhance absorption rate through the solid skin barrier and accumulation around the pilosebaceous unit during a long period of time without notable side effects (28).

Excessive sebum secretion is crucial in acne pathogenesis, and only oral isotretinoin and hormonal therapy have been shown to reduce seborrhea (29). In our current study, lupeol had sebosuppressive effects mainly through the downregulation of IGF-1R/PI3K/Akt/SREBP-1 signaling pathway in SEB-1 sebocytes with a minimal effect on cell viability. Significant decreases in typical FFAs, including palmitic acid and oleic acid, were remarkable because these FFAs enhance innate immune system by inducing antimicrobial peptides (30,31). However, concentration of linoleic acid, a

polyunsaturated FFA that attenuates related inflammation by decreasing NF-κB-mediated host immune response (32), did not change with lupeol treatment. Therefore, changing patterns of these fatty acid components may subsequently alleviate inflammation. We confirmed this anti-lipogenic effect from other lipid-laden cell lines by showing that lupeol also decreased intracellular lipid accumulation in adipocytes without notable cytotoxicity. Although lupeol normalizes the serum lipid profiles in animals fed a high-fat and cholesterol diet, associated cellular mechanisms have never been investigated extensively (33,34). These *in vitro* results may at least partially explain the reduction of the non-inflammatory lesion counts in our patients during a 4-week clinical trial. Taken together, lupeol may provide another therapeutic strategy to target lipogenesis in acne or seborrhea itself.

Many cutaneous diseases are associated with chronic inflammation, which also aggravates acne and frequently leaves remnant scars (35,36). We found that lupeol alleviated the inflammatory response in well-established *in vitro* models of inflammatory acne by inhibiting the NF-κB pathway (37). Although the exact mechanism by which lupeol mitigates NF-κB remains elusive, the decrease in phospho-IκB that we observed strongly suggests that lupeol acts upstream of IκB degradation (38). The uniqueness of acne inflammation is not in the nature of the signaling cascade but in the localization of the process (specialized sebaceous follicles) (20). Downregulation of NF-κB pathway, a representative indicator of inflammation, may provide insight on the molecular mechanism behind lupeol's effects on inflammatory acne lesions in our clinical trial. Consistent with our results, downregulation of NF-κB is the major mechanism behind the anti-cancer properties of lupeol against various cancer cell lines (10,16). Since NF-κB and Akt pathways are functionally inter-connected (39), lupeol may target a protein commonly shared by these two pathways.

We found high levels of the pro-inflammatory cytokine IL-1α in acne lesions *in vivo*. Previous works have shown that exposing isolated infundibula to IL-1α *in vitro* induces comedone formation (40-42), and a recent report suggests that activation of TLR-2 in basal and infundibular keratinocytes provokes release of IL-1α, thereby initiating comedogenesis (43). Remarkably, lupeol decreased both IL-1α and TLR-2 in inflamed HaCaT keratinocytes in our study. In accordance with these findings, 4 weeks of lupeol application decreased immunohistochemical markers of IL-1α, TLR-2, and K16 around epidermal keratinocytes. K16 is the type I keratin partner of K6 in the intermediate filament heterodimer formation and is upregulated in all abnormally differentiating and hyperproliferative suprabasal keratinocytes (44,45). Therefore, these results suggest lupeol reduces acne by modulating a comedogneic process in the early phase of acne development, although further studies should be conducted to support these findings. We also discovered that *P. acnes*, a major Gram-positive anaerobic bacterium that strongly triggers host innate immune system activity, was vulnerable to lupeol. Minimal inhibitory concentration was higher compared with the concentration range shown in other experiments, indicating that further sophisticated studies investigating antimicrobial activities should test the therapeutic applicability in both *in vivo* and *in vitro* conditions.

Following the encouraging results of our *in vitro* studies, a small-scale clinical trial was conducted to examine the therapeutic potential of lupeol on acne. Application of 2% lupeol significantly improved both inflammatory and non-inflammatory acne lesions. The efficacies of lupeol in this relatively small study were satisfactory, and the overall occurrence of side effects observed was much lower compared with currently used topical medications (46,47). Based on current global guidelines, the degree of local tolerability is a major factor determining patient adherence for the early initiation of

topical treatments (48,49). The superior tolerance of lupeol makes it an attractive therapeutic option as a topical anti-acne agent. Previous studies have provided convincing evidences that lupeol is a non-toxic material in animal models while further safety profiles in human should be thoroughly investigated (8). And additional studies such as measuring effective dosage of lupeol compared with currently used topical medications and devising optimal formulations should be followed for its clinical uses. This clinical trial generally provides the clinical rationale for the use of lupeol in acne, while following large scale studies with long-term follow up should be also followed.

With all these biological and clinical effects of lupeol against acne, we strongly believe that further chemical studies should be conducted for this intriguing molecule. In recent years, libraries based on natural products have received wide attention since they give an easy access to analogues having superior therapeutic potential than the parent scaffold (50). One group in India already demonstrated an appreciable increase in the antimalarial activity of lupeol using combinatorial approach based on solid-phase synthesis (51). Addition of diverse chemical moiety to the hydroxyl group of lupeol might successfully change the biological and physicochemical traits depending on the potential uses of lupeol. Since permeability barrier impairment of stratum corneum could occur inherently in facial skin of acne patients (52), increased hydrophilicity of lupeol derivative might enhance effective drug delivery in the specific microenvironment.

In summary, lupeol modulated the key pathological factors contributing to acne, including hyperseborrhea, inflammation, follicular dyskeratosis, and *P. acnes* overgrowth. Thus, this molecule may be a viable option for the effective and rapid treatment of both inflammatory and non-inflammatory acne.

CLINICAL TRIAL REGISTRATION

This clinical trial was performed in accordance with the Declaration of Helsinki (2000) and approved by the Institutional Review Board of Seoul National University Hospital. Written informed consent was obtained from each participant before study enrollment. The study was registered with ClinicalTrials.gov under registration ID # NCT02205892

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국문 초록

여드름은 복합적인 병인 요소들에 의해서 발생하는 질환으로서 청소년-청년기의 유병율이 매우 높은 대표적인 피부 질환이다. 현재 여러 약제들이 사용되고 있으나 각각은 다양한 부작용을 동반하고 있는 것이 사실이기 때문에, 새로운 개념의 항여드름 약리 활성을 가지는 물질을 찾는 것은 피부과학에서 절실하게 요구되는 분야이다. 천연물은 다양한 생물학적 활성을 가지면서도 비교적 안전한 약리 분자들을 꾸준히 제공하였다. 이 연구에서는 다양한 약용 식물의 추출물로부터 항여드름 성분을 가지는 물질을 발견하기 위해 스크리닝을 진행하였다. 그 결과, 가지의 헥산 추출물로부터 분리한 오환성 트라이터핀 계통의 루페올 분자가 가장 활성이 뛰어난 것을 발견하였다. 구체적으로, 세포 실험에서 루페올은 SEB-1 피지 세포주에서 IGF-1R/PI3K/Akt/SREBP-1 신호 경로를 조절하여 지질 합성을 강하게 억제시킴을 입증하였다. 또한 SEB-1 피지 세포주와 HaCaT 각질세포주에서 NF-κB 신호 경로를 조절하여 여드름 염증을 조절하였고, 여드름 발생의 초기 단계에 관계하는 피부의 이상 각화 역시 억제하는 효과를 보였다. 루페올은 피부의 주요 세포들에 대해서 독성을 보이지 않았으며 여드름 원인균인 프로피오니박테리움 아크네스의 성장도 억제하였다. 불포화/포화 지방산의 비율, IGF-1 경로의 변화 등 최근 주목을 받고 있는 여드름 유발 요소에 대해서도 이로운 방향으로 작용하였다. 이상의 실험 결과를 바탕으로, 루페올이 실제 여드름 환자에서 항여드름 효과가 있는지를 판단하기 위하여, 4 주간의 이중 맹검 무작위 배정 임상 연구를 시행하였고, 그 결과 2% 루페올을 도포한 면에서 대조면에 비해서 특별한 불편감 없이 뚜렷하게 염증성-비염증성 여드름을 호전시킴을 확인할 수 있었다. 이러한 결과를 통해 루페올이 새로운 여드름 치료제로서 역할을 할 수 있을 것으로 판단된다.

주요어: 여드름, 루페올, 치료제 개발, 활성 유도 분리법, 임상시험

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