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발아콩 및 목련박피 혼합추출물(SeleMix AN)에 의한 여드름 개선 효과

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Improving Effect for Acne with SeleMix AN Composed of Germinating Soy Bean and Magnolia Bark Extract

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요 약: *In vitro* 및 *in vivo*에서 모두 효과를 나타내는 새로운 여드름 개선 성분을 개발하였다. 특히, *in vitro*에서 효과가 검증된 많은 원료들이 실제 여드름환자에게서 실질적인 효능을 나타내지 못하는 경우가 많아, 본 연구에서는 임상에서 실질적인 효과를 보일 수 있는 원료의 개발에 초점을 맞추었다. 우선적으로 여드름에 효과가 높은 것으로 알려져 있거나 여드름개선 효과가 기대되는 천연물질의 추출물을 대상으로 소규모의 예비임상시험을 통해 효능을 확인하였다. 여러 가지 후보물질 중에서 발아콩과 목련박피 2종의 추출물이 여드름 및 여드름에 의한 홍조현상과 흉터에 뚜렷한 효과를 나타내어 이 두 천연물의 혼합추출물을 SeleMix AN이라 명명하였다. 200여 명의 예비임상시험을 통해서 SeleMix AN의 임상적 효과를 확인한 후 상기 물질의 여드름 개선 작용기작을 규명하기 위한 *in vitro* 효능테스트를 실시하여 *P. Acne* 성장억제 효과(시료농도 0.0125%), 16.9%의 히스타민 분비저해효과와 함께, 인간유래 섬유아세포의 활성을 대조군 대비 57% 높여 주는 실험결과를 확인하였다. 최종적으로 분당 서울대병원과의 공동연구를 통해 23명의 여성여드름환자를 대상으로 한 임상평가를 실시하여 새로운 여드름 개선성분의 효능을 검증하였다. 여름에 실시된 최종임상에서 SeleMix AN이 함유된 시료는 피지분비량이 증가하고 여드름 발병율이 높아지는 계절적인 영향에도 불구하고 4주 만에 특히, 염증성 병변을 대조군 대비 통계적으로 유의차 있게 감소시키는 뛰어난 여드름 개선 결과를 얻을 수 있었다.

Abstract: We investigated new ingredients with real efficacy in both *in vitro* and *in vivo* all together. Especially we focused on the real improving effect in the clinical experiments. Because most products containing effective materials evaluated *in vitro* failed to show a real improving effect in the human with acne. We evaluated the well-known ingredients in a small scale clinical experiment with half-finished goods containing each ingredient. Among these products, product formulating SeleMix AN composed with germinating soy bean and magnolias bark extract remarkably improved acne and acne scar. Moreover skin redness caused by severe acne was improved. There was statistical significance between placebo and sample. Two hundred volunteers participated in our pilot study with written informed consent. After then we performed *in vitro* efficacy test this ingredient. SeleMix AN inhibited the growth of *propionibacterium acnes* at a concentration of 0.0125% and suppressed histamine release by 16.9%. Moreover human fibroblast cell activity was increased by 57% compared to control. Lastly, we performed a clinical study. Consisting of groups of 23 volunteers. Although the period of the test was in summer accelerating sebum secretion and recurring a high rate of acne, inflammation lesions were especially improved after applying product containing SeleMix AN for 4 weeks.

Keywords: *acne, SeleMix AN, soy bean extract, magnolia bark extract, histamine*

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1. Introduction

To pursue the eternal beauty is the women's basic desire in the whole world. In this point of view, acne had been regarding as the eliminated skin disease. Acne is a common skin problem of the sebaceous glands due to genetic, hormonal and environmental factors. Therefore, acne was characterized by abnormal ductal keratinization, increased sebum secretion, abnormalities of the skin microflora, especially *propionibacterium acnes*, and lastly the induction of inflammation.

In general acne was divided two types, non-inflammatory (cormedones) and inflammatory lesions (papules, pustules and nodulocystic lesions)[1]. Inflammatory acne was very important in the view of beautiful face because acne scar was remained after a wrong treatment.

A lot of researches about acne had been doing by the dermatologists and cosmetic investigators. As a consequence of effort, raw materials for treating acne were developed and confirmed efficacy for acne *in vitro* test. But, most consumers with acne dissatisfied and disaffected finish products containing these materials because these will not lead to any practical result in the clinical demonstration.

In present study, laying stress on the real clinical improving effect for acne, we made half-finished products including ingredient known for *in vitro* fruitful effect for acne. We made a pilot clinical study to confirm and then we selected real ingredients for our finished product for acne. After then *in vitro* test for raw material was done because we know that *in vivo* improving effect was attributed to what kind of *in vitro* effect.

2. Materials and Methods

2.1. Subjects

All patients with acne were women in their age range from early twenties to late thirties. During the test period, all other treatments for acne were prohibited and the face divided into halves in the center of nose, right and left area. Self assessment was done by questionnaire.

2.2. Measurement of Sebum Excretion

Sebum secretion was measured by sebumeter SM810 (Courage + Khazaka electronic GmbH, Cologne, Germany) and sebutape (Cuderm Corporation, Dallas, TX, USA). Sebumeter was applied to the center of the forehead for 30 s to measure the casual sebum level. Sebutape was applied for 30 min after cleansing the forehead with an alcohol wipe. Sites of sebum secretion appear as clear dots in the white sebutape, which is then photographed under a light microscope. The area of the dots was measured using ImagePro image analysis software.

2.3. Determination of Antimicrobial Activity and Minimum Inhibitory Concentration

Propionibacterium acne was obtained from ATCC. The agar disc diffusion method was used for the determination of antimicrobial activity of the raw material against *P. acnes*. The agar dilution assay was used to determine the susceptibility of *P. acnes* to test material. Minimum inhibitory concentration (MIC) was defined as the lowest concentration of the test material where absence of growth was recorded. Each test was performed in triplicate and repeated twice.

2.4. Histamine Release Assay

RBL-2H3 cells were maintained in DMEM containing 10% FBS, 100 μg/mL penicillin and 100 U/mL streptomycin. For the histamine release assay, RBL-2H3 cells were seeded at 1×10^5 cells/well in 24-well culture dishes. The cells were incubated at 37℃ overnight and the medium was changed. After 72 h, the cells were washed twice in TGCM buffer [(TG buffer : 200 g NaCl, 5 g KCl, 1.25 g NaH_2PO_4, 1 g $NaHCO_3$, 1 g dextrose, 1 g gelatin (pH 7.4), after adding 5 mL of 0.2 M $CaCl_2$, 0.1 M $MgCl_2$ to TG buffer, TGCM buffer was completed.)] and sensitized for 1 h by 0.5 μg/mL anti-dinitrophenyl (DNP) mouse monoclonal IgE (Sigma, St. Louis, MO, USA). Next, the cells washed twice wit TGCM buffer and stimulated with DNP-BSA (Sigma, St. Louis, MO, USA). The histamine content was measured by Histamine EIA Kit (SPI bio, Massy Cedex, France). Histamine release was expressed as the percentage of total histamine content.

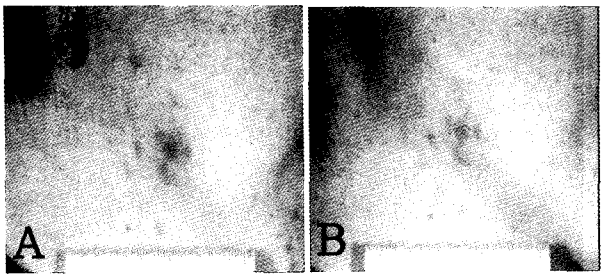

Figure 1. Acne and acne scar were remarkably improved after applying sample product formulating with germinating Soy bean and magnolia bark extract for 7 days. A: before treatment, B: after sample treatment for 7 days.

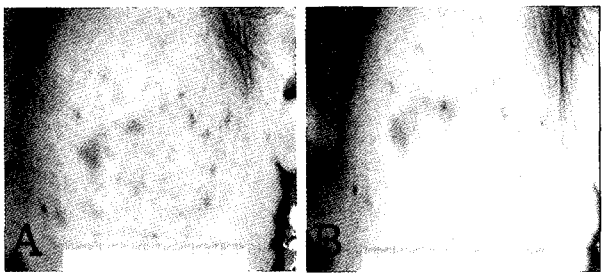

Figure 2. Skin redness caused by acne was remarkably improved after applying sample product formulating with germinating Soy bean and magnolia bark extract for 7 days. A: before treatment, B: A: before treatment, B: after sample treatment for 7 days.

2.5. Cell Proliferation Assay(2)

Human fibroblast (HF) was obtained from foreskin by the primary cell culture. HF was maintained in DMEM containing 10% FBS, 100 μg/mL penicillin and 100 U/mL streptomycin. The cells was transferred to 96-well plates at 1 × 10^4 cells/well and incubated at 37℃, 5% CO_2 for 48 h. Cell proliferation was measured using the MTT test. Absorbance was measured at 570 nm in a plate reader (ELISA reader Amersham Biosciences Biotrack ELISA system, Amersham Biosciences UK Ltd., Little chalfont, Buckinghamshire, UK).

3. Results

Among these products, product formulating SeleMix AN composed with germinating soy bean and magnolias bark extract remarkably improved acne and acne scar (Figure 1). Moreover skin redness caused by severe acne was improved (Figure 2). There was statistical significance between placebo and sample. After then

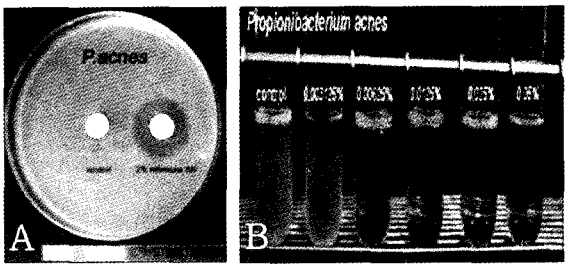

Figure 3. Disc diffusion susceptibility test. SeleMix AN prevented the growth of *P. acne*. The MIC showed 0.0125%. A: disc diffusion method, B: broth dilution method.

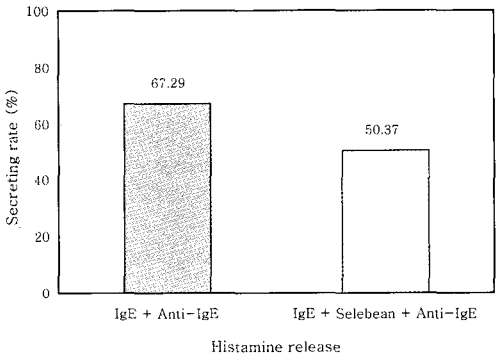

Figure 4. Selebean, one of the composition of SeleMix AN, suppressed the histamine release by 16.9%.

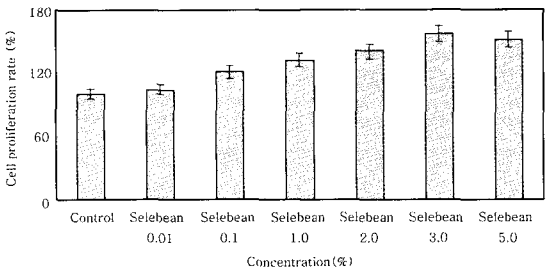

Figure 5. Selebean, one of the composition of SeleMix AN, proliferated human fibroblast by 57% compared to control.

we made *in vitro* efficacy test to examine how this ingredient act on acne. SeleMix AN inhibited the growth of *propionibacterium acnes* on the 0.0125% concentration (Figure 3) and the release of histamine suppressed by 16.9% (Figure 4). Moreover human fibroblast cell proliferation was increased by 57% compared to control (Figure 5). But, the inhibition effect of sebum secretion was not expressed. Lastly, we made a validated again with finished product through the clinical test on the department of derma-

Table 1. The Comparison of Number of Inflammation Lesions on the Placebo-Application and Sample-Application Site Through the Visual Scoring by the Specialist (n = 23)

	Before	After 1 week	After 2 weeks	After 4 weeks
A	5.57 ± 3.31	4.78 ± 2.78	3.70 ± 2.18	4.00 ± 2.13
B	5.57 ± 3.72	4.65 ± 2.52	3.48 ± 2.17	3.00 ± 1.83
A-B	0.00 ± 3.57	0.13 ± 2.53	0.22 ± 1.83	1.00 ± 1.57
p value	1.000	0.807	0.575	0.006

Probability p (paired t - test, statistical significance: $p < 0.05$)

Table 2. The Comparison of Number of Non-Inflammation Lesions on the Placebo-Application and Sample-Application Site Through the Visual Scoring by the Specialist (n = 23)

	Before	After 1 week	After 2 weeks	After 4 weeks
A	15.57 ± 9.28	14.39 ± 8.76	14.26 ± 9.43	15.39 ± 9.89
B	15.78 ± 9.12	15.43 ± 9.19	15.35 ± 9.90	15.83 ± 9.62
A-B	-0.22 ± 3.03	1.04 ± 3.91	1.09 ± 4.75	-0.43 ± 2.73
p value	0.744	0.214	0.284	0.453

Probability p (paired t - test, statistical significance: $p < 0.05$)

tology in the Seoul National University Bundang Hospital. 23 volunteers with written consent took part in the test. Although the period of the test was in summer accelerating sebum secretion and recurring a high rate of acne, inflammation lesions were especially improved after applying finished product for 4 weeks. There was statistical significance (Table 1). But this product was not efficient in the non-inflammatory lesions (Table 2).

4. Discussion and Conclusion

In general, it was known acne was caused by four reasons, sebum over production, ductal keratinocyte hyperproliferation and abnormal differentiation, microbial invasion such as *Propionibacterium acnes* and hormone. All researches for acne were focused on these four view points. Many *in vitro* efficient ingredients were known for us and then many finished products were included one or complex of these materials. But most of consumers complained product for acne because they did not practically improved acne. We thought that these mistakes were contributed to emphasize *in vitro* effect. Therefore, we investigated with the clinical effect as the central figure. As the result of this test process, we searched the satisfactory ingredients, SeleMix AN, and made a finished - product having good effect for acne. People used this product satisfied not only improving effect for acne but also keeping the moisturizing skin for a long time use.

Selebean, one of the composition of SeleMix AN, was simply extracted from germinating soybean. Although, Selebean had the effect of cell proliferation and anti- histamine, product including this ingredient also had the same improving effect for acne in the clinical test. In the recent research, Vahideh Yavari reported antihistamines have been found useful to be used in treatment of special kinds of acne, especially inflammatory acne[5]. Until now, exact mechanism was not shown. But, he simply guessed that antihistamine was took part in the inflammatory procedure in the pathogenesis of acne. Further study was demanded for making brighter exact mechanism.

References

1. D. B. Holland and A. H. T. Jeremy, The role of inflammation in the pathogenesis of acne and acne scarring, *Semin Cutan Med Surg.*, **24**, 79 (2005).
2. K. Y. Ho and C. C. Lin, Antimicrobial activity of honokiol and magnolol isolated from *Magnolia officinalis*, *Phytother. Res.*, **15**, 139 (2001).
3. F. Minervini, A. Giannoccaro, A. Cavallini, and A. Visconti, Investigations on cellular proliferation induced by zearalenone and its deratives in relation to the setrogenic parameters, *Toxicology Letters.*, **159**, 272 (2005).
4. K. Goishi, K. Mizuno, H. Nakanishi, and T. Sasaki, Involvement of Rab27 in antigen-induced histamine release from rat basophilic leukemia 2H3 cells, *Biochem Biophy Res Commu.*, **324**, 294 (2004).
5. V. Yavari, Antihistamines may be used as an "adjuvant" in treatment of all kinds of acne, Medical Hypotheses., **66**, 447 (2006).