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Clinical Study of an Anti-Cellulite Cosmetics Containing Solubilized System of Poorly Soluble Caffeine and a Skin Penetrating Peptide

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Abstract
The aim of the study was to determine the clinical efficacy and safety on anti-cellulite cosmetics containing solubilized system (12.0% caffeine; 8.0% mixed hydrotropes; niacinamide and vanillin) of poorly soluble caffeine and a transdermal penetrating peptide (100ppm arginine oligomer peptide, R6). Dermal and subcutaneous lipid interface length measurement, skin roughness measurement, researcher visual assessment and thigh normal photo shoot were used. This study was conducted on 23 adult females with cellulite skin aged 20~50 years. A total of 23 subjects who met the criteria for selection and who did not meet the exclusion criteria and who were consenting to participate in the human body test were used for 6 weeks at the designated site. Clinical results was summarized as followed: 1) the dermis and subcutaneous lipid interface length was statistically significantly decreased after 3 and 6 weeks using the product ($p<0.05$); 2) the skin roughness value of the thigh was statistically significantly decreased 3 and 6 weeks ($p<0.05$); 3) the cellulite grades of researcher's visual evaluation in the thigh area were statistically decreased ($p<0.025$) after 6 weeks; 4) subjective questionnaire evaluation scores increased significantly ($p<0.025$) after 3 and 6 weeks; 5) There was no skin adverse event reported after using the product during the study period. Therefore, the test containing a caffeine complex and a skin penetrating peptide is have beneficial effects in reducing the temporary cellulite in the thigh for 6 weeks.

Keywords*: Anti-cellulite, Efficacy, Hydrotrope, Caffeine Complex, Transdermal Penetrating Peptide.*

1. INTRODUCTION

Cellulite, also known as gynoid lipodystrophy has been a cosmetically unacceptable disorder that most women experience at some point in their lifetime. It occurs mainly on the lower limbs, pelvic region and abdomen and is characterized by an 'orange peel' or 'cottage cheese' appearance. Approximately 85% of women over the age of 20 have some degree of cellulite [1, 2]. It has been described as a normal physiological state in post-adolescent women which maximizes adipose retention to ensure adequate caloric availability for pregnancy and lactation [3]. This disorder should not be confused with obesity where only adipocytes hypertrophy and hyperplasia occurs.

Natural Coffee has been for decades the most commercialized food product and most widely consumed beverage in the world. The nonvolatile fraction of green coffee is composed primarily of

water, carbohydrates and fiber, proteins and free amino acids, lipids, minerals, organic acids, chlorogenic acids, trigonelline, and caffeine. Caffeine stimulates the central nervous system as an adenosine-receptor antagonist. Caffeine is used as a good compound in anti-cellulite products because it prevents excessive accumulation of fat and is widely used as cellulite-degrading cosmetics. Caffeine is involved in lipolysis [4-7] by increasing cAMP levels in adipocytes and activating hormone-sensitive lipase (HSL), which leads to the degradation of triglycerides in the lipolysis process and takes part in the reduction of cellulite. Caffeine also stimulates the draining lymph systems in fatty tissue by removing accumulated fat, toxin and unnecessary substances arising during the lipolysis process, which all together may impede the microcirculation in blood vessels and foster the emergence of cellulite [8, 9]. However, caffeine has a low solubility in water of 2.1% at room temperature but has a very low solubility which is significantly reduced to 0.1% at 2°C and is known to be insoluble in non-polar solvents [10]. It is limited in cosmetic formulations containing high concentrations of caffeine. Hydrotrope is a unique and unprecedented solubilization technique in which certain chemical components termed as hydrotropes can be used to affect a several fold increase in the solubility of sparingly soluble solutes under normal conditions. This increase in solubility in water is probably due to the formation of organized assemblies of hydrotrope molecules at critical concentration. Hydrotropes in general are water soluble and surface-active compounds, which can significantly enhance the solubility of organic solutes such as acids, esters, alcohols, aldehydes, ketones, hydrocarbons and fats.

In addition, transdermal delivery should be required that caffeine could penetrate to the epidermis, dermis, and subcutaneous fat to exhibit anti-cellulite effects. Recently, several studies have represented that cell-penetrating peptides (CPPs) can enhance the transdermal delivery of biomaterials [11-13]. Short arginine oligomer peptides enabled transport across the epidermis, when applied topically to either mouse or human skin [14]. It was reported by the present author [15-16] that caffeine aqueous solutions could be successfully prepared using mixed hydrotropes containing niacinamide and vanillin and transdermal penetration was improved when high concentration of caffeine complex is contained with a short arginine oligomer (R6).

In this study, we examined the clinical efficacy and safety on anti-cellulite cosmetics containing solubilized system (12.0% caffeine; 8.0% mixed hydrotropes; niacinamide and vanillin) of poorly soluble caffeine and a transdermal penetrating peptide (100ppm arginine oligomer peptide, R6).

2. MATERIAL AND METHODS

2.1 Test formulation

The main ingredients of test formulation 12.0% caffeine; 4.5% niacinamide and 1.5% vanilin as mixed hydrotropes; 0.01% R6 as a skin penetrating peptide (arginine oligomer peptide). The other ingredients contained emulsifier, oil, humectants, fragrance and deionized water in test products. Caffeine and niacinamide were purchased from Daejung Chemicals and Metals Co., Ltd., in Korea and have a purity of at least 98.5% and 98.0%, respectively. Vanilin (purity, 99.0%) was purchased from Samchun Chemicals Co., Ltd., in Korea.

2.2 Study Protocol

KC Skin Research Center conducted the body efficacy evaluation according to the tenets of the Declaration of Helsinki and complied with the Guideline of Bioethics and Safety Act by the Ministry of Health and Welfare. The study was approved by the Institutional Review Board of

KC Skin Research Center Co., Ltd., in Korea (KC-IRB-028).

This study selected voluntarily participating healthy women aged 20 to 50 who were BMI (Body Mass Index) 18 or higher and cellulite grade 1 or higher (based on Standard photograph) and the test site was set on both thighs. They were fully informed of all the information related to this human body test, and the subjects were willing to make a written consent and participated. We excluded women who were pregnant or breastfeeding, who did not agree with the protocols of prevention of conception, who were allergic to cosmetics, medicines, and daily light exposure, who used the steroid contained ointment for more than 1 month to treat skin disease, who with no more than 6 months after participating in the same test, and who with identical or similar cosmetics or products on the study sites 3 months prior to this test. The subjects who satisfied the criteria of selecting the subjects and those who did not have the criteria for selection exclusion were selected by homogeneity test for those who have relatively similar life environment, skin condition, skin care and cosmetics use. The human body test was conducted for a total of 6 weeks

2.3 Evaluation Method

The length of the interface of the dermis and subcutaneous fat layer on thighs was measured before using the test product, 3 weeks after using the product, and 6 weeks after using the product by Skin Scanner DUB (taberna pro medicum GmbH, Germany). The length of the interface of the dermis and subcutaneous fat layer on images were analyzed and used as temporary cellulite reduction effect assessment data. The unit of the length of the interface of the dermis and subcutaneous fat layer is in mm and the analytical value and the extent of cellulite reduction are proportional, so the smaller analytical value, the more temporary cellulite reduction effect.

The skin roughness was measured using PRIMO lite (LMI Technologies GmbH, Canada) on thighs before using the product, 3 weeks after using the product, and 6 weeks after using the product. The photographed images were analyzed by using PRIMOS-specific software to determine the volume of cavities on site. The unit of the skin roughness analysis value is mm^3, and the analytical value is inversely proportional to the skin roughness improvement degree, which means that the skin roughness is improved as the analysis value is decreased.

The thigh photographs were taken on the left sides of the subject's thighs at the same conditions (before use, after 3 weeks of use, and after 6 weeks of use) at each visit using a high-resolution digital camera (DSLR: Canon Inc, Taiwan). The visual evaluation of the researchers was carried out by two researchers according to the criteria of cellulite grade. Before the test product was used, a visual evaluation by the researchers was performed to determine whether the selection criterion was appropriate (cellulite grade 1 or higher) and checked the change in the grade after 3 weeks using the product and 6 weeks using the product. If the researchers had different assessments, the low-grade was chosen.

The circumference of the left and right thighs was measured by InBody Co., Ltd., Korea) before using the product, 3 weeks after using the product, and 6 weeks after using the product. The unit of the circumference of the measured thighs is cm. The measured value and the circumference of the thighs are proportional, so the change in the thigh circumference according to the degree of cellulite reduction can be seen.

If the test site shows any adverse reactions or other symptoms such as erythema, edema, stinging, burning, tightness, prickling, or irritability, it is stated on individual case report form (CRF) with degree of the symptom: mild, moderate, or severe.

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3. RESULTS AND DISCUSSION

3.1 Dermis and Subcutaneous Fat Layer Interface Measurement

The changes in the dermis and subcutaneous fat layer were measured 3 times, including before application (0 weeks), after 3 weeks, and after 6 weeks. Measurement of its layer after use of the test product showed a decrease to 22.035 ± 3.676 mm after 3 weeks and 21.865 ± 3.875 mm after 6 weeks, from 21.786 ± 3.736 mm before product use (Table 1).

To analyze the rates of improvements, when the degrees of change on the 3nd and 6th week were 100% based on the degree of the interface length before the use of the product, the degree of its length decreased by 3.239% after 3 weeks, 4.079% after 6 weeks (Table 1). Therefore, it was concluded that as results of measuring the interface length of the dermis and the subcutaneous fat layer of the thighs, it was statistically significantly decreased after 3 weeks and 6 weeks using the product ($p<0.05$).

Table 1: Results of the length of the interface of dermis and subcutaneous fat layer measurements

Time	Average ± STD (mm)	Improvement rate[a] (%)	Probability[b] (*p* value)
Before use	23.322 ± 4.822	-	-
After 3 weeks	22.765 ± 4.689	-2.313	0.143
After 6 weeks	22.415 ± 4.602	-3.669	0.019 **

• Improvement rate[a] (%) = [(After product use –Before product use) / Before product use] x 100

• Probability[b] (*p value*) **: $p<0.05$ by Repeated measured ANOVA, post hoc Bonferroni correction

3.2 Skin Roughness Measurement

The changes in the skin roughness were measured 3 times, before using the product (week 0), after 3 weeks, and after 6 weeks. The results showed that the skin roughness decreased from 164.779 ± 23.535 to 159.557 ± 23.262 after 3 weeks and to 157.941 ± 22.221 after 6 weeks (Table 2, $p<0.05$).

The degrees of the improvement in the 3rd and 6th weeks were calculated as percentage to analyze improvement rate, by setting the skin roughness after using test product as 100%. Skin roughness decreased by 3.130% after 3 weeks, while skin roughness decreased by 4.000% after 6 weeks (Table 2). From these results, it was found that as results of measuring the skin roughness value of the thigh, it was statistically significantly decreased after 3 and 6 weeks ($p<0.05$).

Table 2: Results of the skin roughness measurement

Time	Average ± STD (mm^3)	Improvement rate[a] (%)	Probability[b] (*p* value)
Before use	164.779 ± 23.535	-	-
After 3 weeks	159.557 ± 23.262	-3.130	0.006 **
After 6 weeks	157.941 ± 22.221	-4.000	0.001 **

• Improvement rate[a] (%) = [(After product use –Before product use) / Before product use] x 100

• Probability[b] (*p value*) **: $p<0.05$ by Repeated measured ANOVA, post hoc Bonferroni correction

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3.3 Results of Cellulite Grade

Changes in cellulite grade were assessed 3 times, including before using the test product, after 3 weeks, and after 6 weeks of using the test product. Using the test product resulted in a decrease of its index from 2.773 ± 1.066 before use, to 2.545 ± 0.912 after 3 weeks and to 2.364 ± 0.902 after 6 weeks [17-19].

Analysis of the rate of improvement in cellulite grade revealed that the percentage of change between the 3nd and 6th weeks was 100% and the extent of cellulite grade decreased by 6.742% after 3 weeks and 13.182% after 6 weeks. Therefore, it was concluded that as results of cellulite grade on the thighs after using test product, the visual evaluation by researchers was statistically reduced 6 weeks (Table 3, p<0.05), after using the product compared to before use.

Table 3: Results of cellulite grade

Time	Average ± STD (mm)	Improvement rate[a] (%)	Probability[b] (*p* value)
			Effects in individual subjects
Before use	2.773 ± 1.066	-	0.000 -
After 3 weeks	2.545 ± 0.912	-6.742	0.025
After 6 weeks	2.364 ± 0.902	-13.182	0.003†

• Improvement rate[a] (%) = [(After product use –Before product use) / Before product use] x 100

• Probability[b] (*p value*) †: *p*<0.025(5%/2) by Friedman test, post hoc Wilcoxon signed rank test

3.4 Measurement of the Circumference of the Thighs

As results of the measurement of the circumference of the thighs after using the product (Table 4)., this measurement results after 3 weeks and 6 weeks of product use showed no statistically significant difference (*p*<0.05).

Table 4: The change of the circumference of the thighs

Time	Average ± STD (cm)	Improvement rate[a] (%)	Probability[b] (*p* value)
Before use	53.391 ± 2.503	-	-
After 3 weeks	53.764 ± 2.565	0.707	0.220
After 6 weeks	53.232 ± 2.339	-0.276	0.973

• Improvement rate[a] (%) = [(After product use –Before product use) / Before product use] x 100

• Probability[b] (*p value*) **: *p*<0.05 by Repeated measured ANOVA, post hoc Bonferroni correction

3.4 Evaluation of Skin Adverse Reactions

In the test subjects, the presence of adverse skin reactions such as erythema, edema, scaling, itching, stinging, burning, tightness, ting (rickets, swelling, scurvy, itching, aching, burning, stiffness, tingling) among others was investigated every time subject presented themselves for analysis. No specific skin adverse events were observed in all subjects participating that participated in the present study (Table 5).

Table 5: Assessing skin adverse events

Time	Erythema	Edema	Scaling	Itching
After 3 weeks	-	-	-	-
After 6 weeks	-	-	-	-

Time	Stinging	Burning	Tightness	Prickling

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After 3 weeks	-	-	-	-
After 6 weeks	-	-	-	-

Step=1: Weak, 2: Medium, 3: Severe

4. CONCLUSION

In this study, we examined the clinical efficacy and safety on anti-cellulite cosmetics containing solubilized system (12.0% caffeine; 8.0% mixed hydrotropes; niacinamide and vanillin) and a transdermal penetrating peptide. In this study, dermal and subcutaneous lipid interface length measurement, skin roughness measurement, researcher visual assessment (cellulite grading) and thigh normal photo shoot were used. This study was conducted on 23 adult females with cellulite skin aged 20~50 years. As a result of total 22 subjects, excluding 1, used the test product containing a caffeine complex and a skin penetrating peptide 6 weeks in cellulite skin area and clinical results was summarized as follows: 1) the dermis and subcutaneous lipid interface length was statistically significantly decreased after 3 weeks and 6 weeks using the product ($p<0.05$); 2) the skin roughness value of the thigh was statistically significantly decreased 3 weeks and 6 weeks using the product ($p <0.05$); 3) the cellulite grades of researcher's visual evaluation in the thigh area were statistically decreased ($p <0.025$) after 6 weeks of using the product; 4) subjective questionnaire evaluation scores increased significantly ($p <0.025$) after 3 weeks and 6 weeks using the product use; 5) There was no skin adverse event reported after using the product during the study period. Therefore, the test product containing a caffeine complex and a transdermal penetrating peptide is have beneficial effects in reducing the temporary cellulite in the thigh for 6 weeks.

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