J. Cosmet. Sci., **62,** 305–316 (May/June 2011)

Evaluation of anti-cellulite efficacy: A topical cosmetic treatment for cellulite blemishes—A multifunctional formulation

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Synopsis

Cellulite is thought to affect 80-90% of postpubertal women, and is considered much of a cosmetic concern by the large majority of them. In this study, the objective was to assess the efficacy of a topical cosmetic product containing various active ingredients of botanical origin on cellulite blemishes on female volunteers affected by fat accumulations, as well as by slight-to-moderate cellulite in the lower limbs. The assessment was performed by means of various objective evaluations, including contact thermography, morphometric measures of thigh circumference, and microcirculation evaluation. The obtained results indicate that the use of synergistic botanical standardized extracts, through the exploitation of different mechanisms of action and acting on different biological targets, provides visible and measurable results in the improvement of cellulite signs and symptoms.

INTRODUCTION

Cellulite onset and recognition as a real pathology is still controversial, as well as its exact causes. On the one hand, it has been recently described as a physiological condition aimed to maximize adipose retention in order to ensure adequate caloric availability during pregnancy and lactation (1), but cellulite is also a complex problem, involving several different factors and mechanisms, such as metabolic imbalances, alterations in connective tissue structure, genetic factors, inflammatory conditions, reduced microcirculation, and hormonal factors. Although genetic predisposition to cellulite has been related to a particular polymorphism in the angiotensin-converting enzyme and hypoxia-inducible factor 1A genes (2), diet and lifestyle also play an important role in the improvement or worsening of the aspect of cellulitic areas.

From a metabolic standpoint, during the onset of cellulite, adipocytes become engorged with lipids and cluster together while an excess of subcutaneous fluids is retained. Also,

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inflammation of connective tissue and degradation of connective fibers occur. The subcutaneous adipose layer appears to be much thicker in women with cellulite compared to women devoid of cellulite (3). Also there are peculiarities in the architecture of fibrous septae compartmentalizing the adipose tissue, these being much more abundant in women affected by cellulite than in men, typically not affected by this disorder. Regardless of the number of women affected by cellulite, only a limited number of studies have been published in the scientific literature so far, botanical derivatives being one of the main groups of evaluated compounds for its treatment. A multi-target/multi-component strategy has been recently recognized as one of the best approaches to counteract the main cellulitic symptoms and signs (4). The botanical kingdom may offer several active ingredients, mainly in the form of standardized botanical extracts, able to act synergistically on different biological targets and to improve these sign and symptoms. Other crucial aspects not to be forgotten in cellulite treatment are represented by circulation in the lymphatic and venous systems and by capillary integrity. The aim of this study was to evaluate the cosmetic efficacy of a topical product on several cellulite signs and symptoms. The topical product (Formulation code: ACTIVE—anticellulite cream) contains a blend of botanical ingredients that, in different approaches and with different mechanisms of action, are intended to counteract cellulite at different levels.

The active ingredients selected are:

- Visnadine. Visnadine is a pure molecule extracted from the seeds and aerial part of *Ammi visnaga*, a plant widely used in Egyptian traditional medicine as an antispastic and vasodilator. It has shown anti-phosphodiesterase activity, maintaining elevated levels of cAMP and thus increasing lipase activity (5), which suggests a cosmetic application in the treatment of localized fatty deposits and cellulite. Pharmacological evidence consistent with traditional use has underlined a strong vascular activity of visnadine both on great and medium-size vessels (6). Visnadine also acts on peripheral vessels, inducing persistent vasokinetic activity with positive inotropic effects (7). In some previous experiences, the vasomotor activity of visnadine was assessed through the measurement of skin temperature by contact thermography and laser Doppler flowmetry (8). The topical application of this active ingredient is then reasonable in the treatment of peripheral vessel disorders. The well-documented phosphodiesterase-inhibiting activity of visnadine, combined with the relevant microcirculatory effects, suggests its potential application in the treatment of panniculopathies of the lower limbs.
- *Ginkgo biloba* Dimeric Flavonoids Phytosome®. Whereas *Ginkgo biloba* is being used worldwide as an herbal medicine for various circulatory disorders, the biflavonic fraction obtained from *Ginkgo biloba* leaf was shown to decrease cAMP phosphodiesterase and to increase microcirculation (9). Lipolysis stimulation was assessed *in vitro*, whereas blood flow improvement was evaluated in subjects affected by cellulite, and the evaluation comprised blood flow and capillary density objective measurements (10). The biflavonic fraction of *Ginkgo biloba* has been complexed with soy phospholipids to obtain the Phytosome® complex, a technology used to improve the topical bioavailability and efficacy of botanical extracts (11).
- Escin. Escin is a natural mixture of triterpene saponins obtained from the seeds of *Aesculus hippocastanum* (horse chestnut). Horse chestnut seed and leaf were traditionally used to treat varicose veins, hemorrhoids, and phlebitis, due to the activity of escin, which was shown to modulate vascular exchanges at a peripheral level, strengthening

the capillary wall and preventing fluid leakage (12). The pharmacological activity is then a decrease in capillary permeability, and an anti-edema efficacy is observed. Escin also possesses significant anti-inflammatory properties, which can, however, be hardly separated from the anti-edema efficacy on pharmacological models. The combination of the mechanisms of action identified so far appoints escin as a relevant active ingredient in the treatment of cellulite.

MATERIALS AND METHODS

FORMULATION

The formulation of the topical product (ACTIVE) was developed with Sinerga Research Center Laboratories (Pero, Italy). The development took into account not only the efficacy of the active substances to counteract the causes of cellulite blemishes, but also the pleasantness of the final formulation in order to achieve the best possible compliance. The botanical active ingredients formulated in ACTIVE are visnadine (0.25% w/w), *Ginkgo biloba* Dimeric Flavonoids Phytosome® (0.5% w/w), and escin (1% w/w). The association of these active ingredients (described in patent WO2005/004858) has been selected according to the specific profile of each single substance.

TRIAL DESIGN

The aim of the study was to clinically assess the cosmetic efficacy of a topical treatment to be applied on the thighs over a period of four weeks. Morphometric analysis and instrumental evaluations were carried out. Twenty-five female volunteers (ages: 30–55 yrs), affected by fat accumulations and/or slight-to-moderate edematous-fibrosclerotic panniculopathy in the lower limbs, were selected for the study. The multifunctional product ACTIVE, containing the botanical ingredients visnadine (0.25%), *Ginkgo biloba* Dimeric Flavonoids Phytosome® (0.5%), and escin (1%) (obtained from Indena S.p.A., Italy), was compared to its relevant placebo formulation PLACEBO. All volunteers provided a written informed consent. The trial was conducted in a single-blind method with the comparison within subjects (each subject being its own control), and volunteers were required to apply the test products on the thigh twice a day, unilaterally, for a period of four consecutive weeks. They underwent two medical examinations, a baseline evaluation at T0 before the beginning of the test, and an evaluation at the end of the treatment period T4. Body weight, in the case of substantial differences between the beginning and the end of the trial, was considered as a dropout criterion. However, no dropouts due to relevant body-weight variations occurred (during the course of the study, the two dropouts quit the trial due to personal reasons independent from the trial itself). Additionally, at the beginning of the treatment, the overall tolerability of the treatment was also observed. Finally, the efficacy of the treatment was assessed both clinically and instrumentally.

CLINICAL EVALUATION

The clinical assessment was carried out at the upper, median and lower third of the thigh, according to the following evaluations:

(a) *Visual clinical score for assessment of the degree of cellulite (both visual appearance and appearance at pinching on the basis of a specific photographic reference scale)*: 0–no observable cellulite; 1–no panniculus, some mild depressions; 2–some panniculus separated from mild depressions; 3–several panniculus, separated from medium depressions; and 4–broad panniculus separated from deep depressions (0 and 4 were considered exclusion criteria).

(b) *Firmness of the inner thigh* (0–very low; 1–low; 2–moderate; 3–good; 4–very good).

(c) *Skin smoothness* (0–very low; 1–low; 2–moderate; 3–good; 4–very good).

(d) *Degree of pain at pinching* (0–absent; 1–mild; 2–moderate; 3–severe; 4–very severe).

INSTRUMENTAL EVALUATION

The instrumental evaluations were carried out through:

(a) *Contact thermography for the assessment of the thermographic stage of cellulite (13).* At each control, contact thermography was conducted by means of liquid crystal thermographic plates that can detect a temperature range form 28° to 34°C through color visualization (from the coldest to the warmest: black–brown–yellow–green–light blue–pink–dark green–blue). The clinical meaning of contact thermography reflects the amount of heat that is transmitted to the plate by the contact with the skin. The skin temperature is a clear indication of cutaneous microcirculation, and its variations allow assessing the efficacy of vasokinetic treatments: skin temperature increases in the case of vasodilation, increased number of open capillaries, and increased local metabolism, while it decreases following vasoconstriction, a decreased number of blood vessels, and a fat tissue increase. One thermographic aspect typical of panniculopathy is dysthermia, with wide hypothermic areas. The panniculopathy evaluation was based upon the following classification: 0–homogeneous "warm" aspect of the thermographic image; 1–dysthermiae; 2–"venous" lakes; 3–wide cold areas; and 4–"cold" aspect of the thermographic image.

(b) *Morphometric measurements of thigh circumferences (upper, median, and lower third).* All circumferences were measured in standardized conditions at the upper, median, and lower third levels of the thigh thanks to a specific electro-optical system able to define the volunteer's position. Measurements were taken three times for each site; both single values and the median value were recorded. The electro-optical system (composed of a support, a horizontal bar with two laser beams, and a graduated panel in front of the support) allowed us to establish precisely the volunteer's position with respect to the graduated panel behind him (1 mm approximation). In order to determine the coordinates, the subject stood in front of the graduated panel and the operator drew the feet position in order to put him back in the same position during the following visits. Then the laser beam was set to tangentially touch the volunteer's leg and was pointed on the graduated panel. The same point was then marked on the volunteer's skin (by means of a dermographic pencil) and the measurement of the thigh circumference was taken.

(c) *Skin plastoelasticity measured on the inner thigh for the evaluation of elasticizing/firming efficacy (14).* The evaluation of skin plastoelasticity was carried out at the inner thigh level by the means of a dermal torque meter (Diastron LTD). The instrument applies the technique of *in vivo* torsion by a probe composed of two concentric circles 3 mm apart. The inner circle, spinning slowly, determines a constant torsion on the skin. When the skin resistance reaches 9mNm, the torsion stops. The torsion time is 1 second. The equipment

measures the resulting torsion angle at the maximum of mechanical stimulation and at the end of the stimulus (return phase).

For each of the curves, the cutaneous rotation can be measured allowing the definition of the following parameters:

- U_e: immediate extensibility (measured at 0.02 sec)
- U_f: maximal extensibility (measured at 0.9 sec)
- U_v: viscoelasticity
- U_r: immediate elastic return (at 0.02 sec on the return phase)

A typical torsion curve example is provided in Figure 1. Among all the available methods to assess cutaneous elasticity, the method involving torsion appears to be one of the most interesting, as it is very sensitive to the variations of the mechanical properties of the stratum corneum (14)

(d) *Ultrasonography performed on the outer thigh to measure the thickness of the panniculus adiposus (in mm) (15,16).* Ultrasonography allowed the measurement of the *panniculus adiposus* of the upper third of the outer thigh by means of the equipment Body Metrix BX2000 (Genex). In this system, a probe generates high-frequency sound waves, transmitting them within the human body. The waves cross tissues and are reflected at the tissue interfaces. By recording the echoes of the reflected waves, the equipment defines the thickness of a certain tissue, and this is made possible by measuring the time it takes for the signal to reach an interface and by multiplying it by the speed of the waves in that specific tissue (in the adipose tissue, the speed is around 1400m/s). A watery gel is applied on the probe in order to minimize wave dispersion.

(e) *Spectrophotometric analysis for the assessment of the activity on surface microcirculation (17).* The area of the inner knee represents the most sensitive zone subject to microcirculation variations, where the presence of cellulite is best shown in terms of circulatory stasis. For this reason, the efficacy of the test product was evaluated with a spectrophotometric measurement of the skin color on the inner knee. Spectrophotometric evaluations employed a spectrophotometer for the spectra of visible, infrared, and ultraviolet (λ 300-900 nm) using a tungsten halogen lamp and a deuterium lamp compliant to CIE (Commission Internationale de l'Eclairage). The light source was turned on 30 minutes prior to the use of the equipment in order to stabilize the lamp emissions. The inclination of the probe

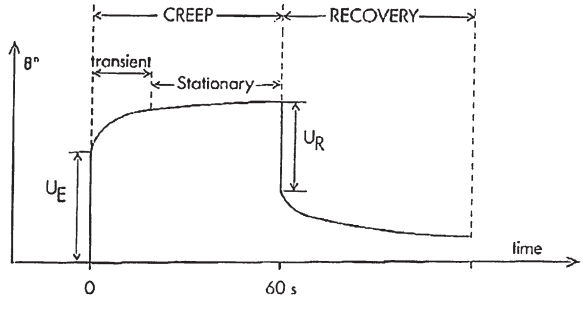

Figure 1. Skin angular deformation versus time upon application of constant torque. U_e: immediate deformation; U_r: immediate recovery upon torque switching off.

was 90° on the surface to be examined, on an area of *ca.* 2 mm^2. The wavelength range was 380–780 nm, corresponding to the visible spectrum.

SAFETY

The tolerability of the active ingredients visnadine (0.25%), *Ginkgo biloba* Dimeric Flavonoids Phytosome® (0.5%), and escin (1%) (all obtained from Indena S.p.A., Italy), formulated in the finished dosage form under the name of ACTIVE (manufactured by Sinerga S.p.A., Italy), was assessed by occlusive patch test prior to the beginning of the efficacy evaluation.

Twenty-three female volunteers, aged 30 to 55 years, used the test product over a period of four weeks, twice daily. In the occlusive patch test, the product was applied in a standardized amount of 20 µl on a Fixomull stretch patch (BSN Medical), and the medium irritation index resulted in 0 at all the considered times (15 min, and 24, 48, and 72 hours from patch removal). Although the trial population was limited to sensitization evaluations, no irritation was observed during the occlusive patch application.

RESULTS

Twenty-three volunteers completed the study, as two subjects left the trial for personal reasons independent from their participation in the trial at week 2. The clinical evaluation (visual appearance and appearance at pinching, firmness of inner thigh, skin smoothness, and degree of pain at pinching) confirmed that the active product induced a statistically significant reduction in visual appearance and appearance at pinching in comparison with placebo-induced variations, thus indicating that the "orange peel-like" appearance was found to be less evident at the end of the trial (Tables I, II). Non-significant results were obtained for the visual score in the placebo-treated area (compared to baseline), apart from the smoothness evaluation, which was found to be statistically significant ($p<0.001$) both in the active and the placebo application versus baseline.

Table I
Statistical Analysis—Comparison of ACTIVE vs Placebo

Clinical evaluations of thigh—upper third	Wilcoxon test		
	Time	*p*-value	Significance
Visual appearance	T0	0.3388	NS
	T4	0.0268*	$p<0.05$
Appearance at pinching	T0	0.3388	NS
	T4	0.0017**	$p<0.01$
Firmness	T0	NA	—
	T4	0.0481*	$p<0.05$
Smoothness	T0	NA	—
	T4	0.3388	NS
Pain at pinching	T0	NA	—
	T4	0.1667	NS

Table II
Statistical Analysis—Comparison of ACTIVE vs Placebo

Clinical evaluations of thigh—median/lower third	Wilcoxon test		
	Time	*p*-value	Significance
Visual appearance	T0	NA	-
	T4	0.0268*	$p<0.05$
Appearance at pinching	T0	0.1667	NS
	T4	0.0151*	$p<0.05$
Firmness	T0	NA	—
	T4	0.08	NS
Smoothness	T0	NA	—
	T4	0.3388	NS
Pain at pinching	T0	NA	—
	T4	0.088	NS

These data suggest an efficaceous activity on the smoothness parameter provided by the formulation vehicle (Tables III–VI).

In the upper third of the thigh the reduction provided by the product ACTIVE was 14% with regard to visual appearance and 21% with regard to appearance at pinching (statistically significant vs placebo), with at least one degree of improvement in the visual scores (Figure 2), while in the median/lower third of the thigh the reduction was found to be 23% for visual appearance and 13% for appearance at pinching (statistically significant vs placebo), with at least one degree of improvement in the clinical scores.

As far as the other cellulite-related symptoms are concerned, the following clinical results were observed:

- A statistically significant improvement ($p<0.05$ vs baseline) in the firmness of the inner thigh in the upper third (at least one degree of improvement).
- A statistically significant reduction ($p<0.05$ vs baseline) in the pain at pinching, both in the upper third (-82%) and, to even a greater extent, in the median/lower third (-100%) of the thigh (Figure 3). On the other hand, no significant variation was observed in the placebo-treated areas.

Table III
Statistical Analysis—Comparison of Placebo T0 to T4

Clinical evaluations of thigh—upper third	Wilcoxon test			
	T0	T4	*p*-value	Significance
Visual appearance	1.87 ± 0.757	1.87 ± 0.757	1.0000	NS
Appearance at pinching	2.43 ± 0.507	2.39 ± 0.499	0.7769	NS
Firmness	1.87 ± 0.757	2.00 ± 0.798	0.0880	NS
Smoothness	2.30 ± 0.926	3.48 ± 0.790	0.0001	$p<0.001$ (**)
Pain at pinching	0.22 ± 0.518	0.13 ± 0.344	0.1667	NS

Table IV
Statistical Analysis—Comparison of Placebo T0 to T4

Clinical evaluations of thigh—median/lower third	Wilcoxon test			
	T0	T4	*p*-value	Significance
Visual appearance	1.30 ± 1.020	1.22 ± 1.085	0.1667	NS
Appearance at pinching	2.04 ± 0.767	1.96 ± 0.825	0.1667	NS
Firmness	2.30 ± 0.703	2.30 ± 0.703	1.0000	NS
Smoothness	2.30 ± 0.926	3.48 ± 0.790	0.0001	$p<0.001$ (**)
Pain at pinching	0.17 ± 0.388	0.13 ± 0.344	0.3388	NS

Table V
Statistical Analysis—Comparison of ACTIVE T0 to T4

Clinical evaluations of thigh—upper third	Wilcoxon test		
	Time	*p*-value	Significance
Visual appearance	T0-T4	0.0151	$p<0.05$
Appearance at pinching	T0-T4	0.0109	$p<0.05$
Firmness	T0-T4	0.0086	$p<0.05$
Smoothness	T0-T4	0.0001	$p<0.001$ (**)
Pain at pinching	T0-T4	0.0481	$p<0.05$

Table VI
Statistical Analysis—Comparison of ACTIVE T0 to T4

Clinical evaluations of thigh—median/lower third	Wilcoxon test		
	Time	*p*-value	Significance
Visual appearance	T0-T4	0.0086	$p<0.05$
Appearance at pinching	T0-T4	0.0151	$p<0.05$
Firmness	T0-T4	0.0880	$p<0.05$
Smoothness	T0-T4	0.0001	$p<0.001$ (**)
Pain at pinching	T0-T4	0.0481	$p<0.05$

As far as skin smoothness is concerned, the highly significant and clinically important improvement observed at the end of the trial on the whole thigh was similar for both tested products, active and placebo, suggesting a smoothing activity promoted by the vehicle.

Instrumental evaluations confirmed the efficacy of the active formulation in counteracting the signs and symptoms of cellulite:

- *Contact thermography*. The tested product induced a significant ($p<0.01$ vs baseline) reduction of 17% in the mean score of the thermographic stage of "cellulite." The result proved to be close to statistical significance in comparison to the placebo ($p=0.0564$).

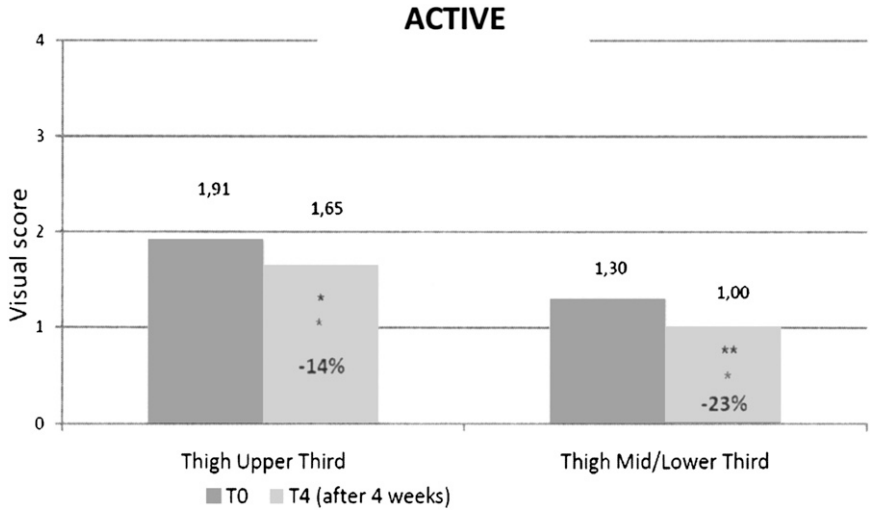

Figure 2. Visual appearance with test cream [Wilcoxon test, $p<0.05$ (*) vs T0; $p<0.01$ (**) vs T0; $p<0.05$ (*) T4 ACTIVE vs T4 Placebo].

- *Morphometric measurements of the thigh circumferences (upper, median, and lower third)*. A statistically significant reduction, both versus baseline and placebo, was observed, in particular: 0.9-cm mean reduction in the upper third, 1.2-cm mean reduction of the median third, and 0.6-cm mean reduction in the lower third with the treatment (Figure 4).
- *Skin plastoelasticity measured on the inner thigh for evaluation of elasticizing/firming efficacy*. The torsiometric measurements (U_e, immediate extensibility; U_f, maximum extensibility; U_v, viscoelasticity; U_r, immediate elastic recovery) were performed on the upper third of the inner thigh at T0 and T4. The active product resulted capable of inducing an

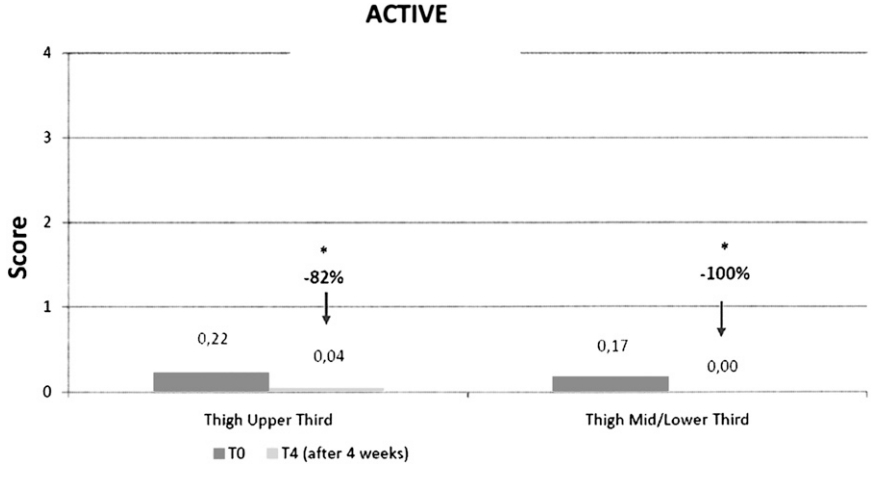

Figure 3. Pain at pinching with test cream [Wilcoxon test, $p<0.05$ (*) vs, T0].

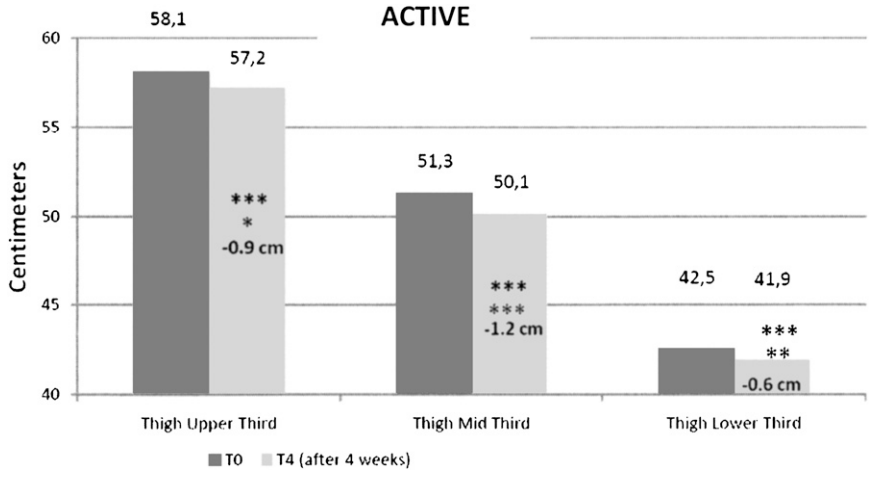

Figure 4. Morphometric measurements of the thigh circumferences (upper, median, and lower third) [$p<0.001$ (***) vs T0; $p<0.05$ (*) T4B vs T4B Placebo ; $p<0.01$ (**) T4B vs T4B Placebo; $p<0.001$ (***) T4B vs T4B Placebo].

important and statistically significant reduction of 20% ($p<0.001$ versus T0 and $p<0.05$ versus the placebo) of parameter U_r, which is an indicator of skin tone. This was also confirmed by the results from the visual assessment of the firmness of the inner thigh.

- *Ultrasonography performed on the outer thigh to measure the thickness of the panniculus adiposus (in mm).* The product ACTIVE induced a statistically significant reduction of 9.4% in the *panniculus adiposus* with regard to the baseline measurements ($p<0.01$), corresponding to an average reduction of 1 mm. No significant variation occurred in the placebo-treated areas.
- *Spectrophotometric analysis for the assessment of the activity on surface microcirculation.* Spectrophotometry results highlighted a highly significant improvement ($p<0.001$ versus baseline) in the general vascular condition, mainly related to the massage activity of the cream application. Both the active and the placebo product improved the vascular condition, but it is important to underline that with the treatment there was a statistically significant 3% difference versus the placebo. This result suggests that, besides the massaging stimulation of the cream application, the active product seems to possess an intrinsic activity in improving skin microcirculation, reducing interstitial excess fluids and improving cell oxygenation and filtration/re-absorption processes in the capillaries.

CONCLUSIONS

Both treatments (active and placebo) were generally well tolerated, apart from one single case of slight folliculitis that regressed spontaneously after 3–4 days' discontinuation and was classified as "aspecific skin reactivity," not imputable to the active product. Cosmetic acceptance in terms of consistency, spreadability, and absence of greasiness, was generally good for both the active and the placebo creams. In terms of efficacy, the following results

were demonstrated for the product ACTIVE in comparison with the placebo and/or in comparison to baseline:

- a significant reduction of cellulite both in visual appearance and at pinching, as well as a considerable increase in skin firmness
- a significant reduction in the thermographic degree of cellulite, indicating an anti-cellulite activity
- a significant reduction in thigh circumferences, indicating a slimming efficacy
- a significant and clinically considerable increase in inner-thigh tonicity
- an important reduction in the *panniculus adiposus* thickness in the upper third of the outer thigh, suggesting an important fat-reducing efficacy
- a significant increase in blood circulation in the skin of the inner-knee area, which is more sensitive to variations in skin microcirculation and where venous stasis could be better assessed, suggesting a vasokinetic activity.

All the above-mentioned results were obtained in the absence of remarkable body weight variations (data not shown), which might have otherwise been considered as dropout criteria.

The above-mentioned results indicate that the use of synergistic botanical standardized extracts, through the exploitation of different mechanisms of action and acting on different biological targets, provides visible and measurable results in the improvement of cellulite signs and symptoms. Although some of the results showed statistical significance compared to the baseline and not to the placebo, in most cases the placebo data versus baseline were not at all significant, indicating an overall difference between the two treatments.

REFERENCES

(1) M. P. Goldman, Cellulite: A review of current treatments, *Cosmet. Dermatol.*, **15,** 17–20 (2002).
(2) E. Emanuele, M. Bertona, and D. Geroldi, A multilocus candidate approach identifies ACE and HIF1A as susceptibility genes for cellulite, *J. Eur. Acad. Dermatol. Venereol.* (2010).
(3) B. Querlux, C. Cornillon, O. Jolivet, and J. Bittoun, Anatomy and physiology of subcutaneous adipose tissue by *in vivo* magnetic resonance imaging and spectroscophy: Relationship with sex and presence of cellulite, *Skin Res. Technol.,* **8,** 118–124 (2002).
(4) A. V. Rawlings, Cellulite and its treatment, *Int. J. Cosmet. Sci.,* **28,** 175–190 (2006).
(5) O. Thrastrup, B. Fjalland, and J. Lemmich, *Acta Pharmacol. Toxicol.* **52,** 246 (1983).
(6) H. Erbring, H. Uebel, and G. Vogel, *Arzneim Forsch.,* **1,** 283 (1967).
(7) H. Eyraud, and M. Aurousseau, *Arneim Forsch. (Drug Res.),* **23,** 201 (1973).
(8) E. Bombardelli, M. Spelta, and S. B. Curri, Aging skin: Activity on the smallest skin blood vessels of an *Ammi visnaga* purified extract, *16th IFSCC Congress, October 8–11, 1990, New York.*
(9) M. Dell'Agli and E. Bosisio, Biflavones of *Ginkgo biloba* stimulate lipolysis in 3T3-L1 adipocytes, *Planta Med,* **68,** 76–79 (2002).
(10) M. Dell'Agli, G. V. Galli, and E. Bosisio, Inhibition of c-GMP-phosphodiesterase-5 by biflavones of *Ginkgo biloba*, *Planta Medica,* **72,** 468–470 (2006).
(11) E. Bombardelli, A. Cristoni, and P. Morazzoni, Phytosome in functional cosmetics, *Fitoterapia,* **65**(5), 387–401 (1994).
(12) C. R. Sirtori, Aescin: Pharmacology, pharmacokinetics and therapeutic profile, *Pharmacol. Res.,* 44(3), 183–193 (2001).
(13) D. A. Sandler and J. F. Martin, Liquid crystal thermography as a screening test for deep vein thrombosis, *Lancet* (March 23, 1985).

(14) "Twistometry: Measurement of Skin Elasticity" in *Non-Invasive Methods and the Skin* (CRC Press, Boca Raton, FL, 1995), pp. 319–328.
(15) M. F. Ramirez, Measurements of subcutaneous adipose tissue using ultrasound images, *Am. J. Phys. Antrop.,* **89**(3), 347–357 (1992).
(16) A. Bellissari, Sonographic measurement of adipose tissue, *J. Diagn. Med. Sonogr*., **9**(1), 11–18 (1993).
(17) G. Zomios, J. Bykowski, and N. Kollias, Skin melanin, hemoglobin and light scattering properties can be quantitatively assessed in vivo using reflectance spettroscopy, *J. Invest. Dermatol.,* **117**, 1452–1457 (2001).