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Original Research

Use of a highly standardized mixture of *Vitis vinifera*, *Ginkgo biloba* and *Melilotus officinalis* extracts in the treatment of cellulite: a biopharmaceutical approach

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Abstract

Cellulite currently remains a controversial topic to be defined and fully explained. The genesis of cellulite has a highly complex pathophysiology, which includes expansion of the subcutaneous adipose tissue, fibrotic dermal septa formation, progressive skin laxity, atrophy and structural modification of some dermal components. These alterations could also be caused by insufficiency of the precapillary sphincters affected by the development of cellulite. The study here encompassed a retrospective clinical trial that analyzed the results of 90 days of treatment in 21 adult women aged between 24 and 53 who underwent a motor programme for the treatment of cellulite along with using two different food supplements. The first was based on extracts of *Vitis vinifera*, *Ginkgo biloba* and *Melilotus officinalis*, with two subgroups at a dosage of two and three tablets per day, respectively; the second was based on carnosine and beta-alanine, constituting a control group. The weight, height, waist, hip, proximal thigh, mid-thigh, distal thigh and calf circumferences were measured for all subjects. There were no appreciable variations with respect to weight, BMI, waist circumference or the waist-to-hip ratio. The consumption of the mixture of *Vitis vinifera*, *Ginkgo biloba* and *Melilotus officinalis* extracts was associated with a greater reduction in hip circumference and thigh circumferences with a dosage of two tablets/day compared to three tablets/day, and compared to the control. The sample size yielded interesting but still not completely unequivocal results. Further studies with a greater number of subjects will be required to clarify the extent of the results obtained, while investigating the posology to determine the most effective treatment within the timeframe considered.

Keywords: Cellulite, Leucoselect, Lymphaselect, Ginkgoselect, Carvelin

Introduction

Despite numerous studies, cellulite remains a controversial topic to be defined and fully explained. Currently, the term 'cellulitis' is used in the scientific literature to indicate a spreading gangrenous infection of the subcutaneous cellular tissue [1]; in contrast, the term 'cellulite' was used for the first time according to several authors between 1850 and 1920 [2, 3] to describe an aesthetic alteration of the cutaneous surface. In an attempt to provide a more correct definition over time, many more descriptive terms have been used, and are considered by some authors to be synonyms of the term cellulite, including: panniculitis, lipodystrophy, oedematofibrosclerotic panniculitis, liposclerosis, lipoedema [1], adiposis oedematosa, dermopanniculosis deformans, status protrusus cutis, gynoid lipodystrophy (GLD) [2], nodular liposclerosis, oedematous fibrosclerotic panniculopathy and panniculosis [3]; a multitude of names for a pathology not yet fully understood.

Cellulite is estimated to affect between 85 and 98% of the female population [2]: such a high prevalence has led some authors to consider it almost as a secondary sexual characteristic [4].

The genesis of cellulite has a very complex pathophysiology, which includes expansion of the subcutaneous adipose tissue, fibrotic dermal septa formation, progressive skin laxity, atrophy and structural modification of some dermal components [4].

It is believed that fat cells take up an arrangement in chambers formed by bands of connective tissue called septa, which connect the muscular plane to the lower part of the dermis. Various factors such as inflammation and/or alteration of the microcirculation can favour expansion of the septa, stressing the connective components that will end up acting on the skin, which will be progressively tensed beyond its elastic capacity.

This process, which can continue with further weight gain and/or further gain of water, manifests as the appearance of dimples and skin with an 'orange peel' appearance, mainly in the gluteal, trochanteric, pelvic and abdominal areas [3, 4].

It is interesting that considerably older [3, 5] analyses of the interstitial fluid show an increased protein concentration (0.8–1.2 mg/ml; normal 0.2–5.1 mg/ml) and an increased interstitial pressure (150–200 mmH_20; normal physiological pressure 75–91 mmH_20).

These alterations could be caused by insufficiency of the precapillary sphincters affected by the development of cellulite [5].

Laser Doppler fluxmetry carried out on patients with cellulite showed an average flow reduction of 35% in the affected regions compared to the unaffected regions [6].

Genetic and epigenetic factors are involved in the predisposition to development of cellulite:

Genetic factors [3, 7]:

- Sex (women are exclusively affected in the classical pattern);
- ethnicity (Caucasian women have a greater tendency than Asian, African or Afro-American women);
- biotype (Latina women develop cellulite on the hips, while Anglo-Saxon and Nordic women develop cellulite on the abdomen);
- fat tissue distribution;
- number, isoform and sensitivity of hormone receptors on the affected cells;
- predisposition to development of peripheral angiopathy (or susceptibility to circulatory insufficiency).

Exposomic factors [3]:

- An unbalanced diet (excessive intake of fats and carbohydrates);
- excessive salt intake (fluid retention);
- a fibre-poor diet (constipation and increased venous resistance);

- a sedentary lifestyle (causing a decrease in muscle mass, an increase in fatty mass, increased flaccidity of tendons and muscles, a decreased muscular pumping mechanism, thereby increasing stasis).

There are different methods of classifying cellulite. That proposed by Rossi and Vergnanini [3] yields four grades, with their respective characteristics as follows:

Grade I: The patient is asymptomatic, and there are no clinical alterations.

Grade II: There are no relief alterations at rest but after skin compression or after muscular contraction there is pallor, decreased temperature and decreased elasticity.

Grade III: Evident at rest there is padded skin and/or an orange peel appearance; palpable thin granulations in the deep levels of the dermis; pain to palpation; decreased elasticity and temperature; pallor.

Grade IV: As for grade III with more palpable, visible and painful nodules, adherence to the deep levels and an evident wavy appearance of the skin surface.

The purpose of the study here was to evaluate the effects of a formulation based on highly standardized and bioavailable extracts of *Vitis vinifera*, *Ginkgo biloba* and *Melilotus officinalis* on anthropometric parameters related to cellulite, and consequently, on the course of the pathology.

Materials and methods

Study overview

This was a retrospective clinical trial that analyzed the results of 90 days of treatment with 2 different food supplements in subjects who voluntarily underwent a structured lifestyle change programme on their own initiative. The experimental group was further divided into 2 different subgroups, based on the daily dosage (2 vs 3 tablets per day) of extracts of *Vitis vinifera*, *Ginkgo biloba* and *Melilotus officinalis*. The subjects receiving carnosine and beta-alanine constituted a control group.

The weight, height, waist, hip, proximal thigh, mid-thigh, distal thigh and calf circumferences were obtained for all subjects. The means of the data obtained within the respective groups were evaluated using Student's t test for hypothesis testing. Retrospective data studies and analysis were conducted in accordance with good clinical practice rules fixed by the Declaration of Helsinki and in accordance with the European Union Directive 2001/20/EC [8].

Each patient signed a consent form and privacy policy documents and approved data analysis and publishing.

Evaluated products and evaluation scheme

Of the 21 subjects whose data were examined, 7 took 2 tablets/day and 7 took 3 tablets/day of a mixture of *Vitis vinifera*, *Ginkgo biloba* and *Melilotus officinalis* extracts formulated in 3-layered tablets, produced by SIIT (Trezzano sul Naviglio, Milan, Italy), and notified to the Italian Ministry of Health as a food supplement by Pharmextracta SpA (Pontenure, PC, Italy) complying with law n°196-2004 (notification number: 22435), marketed under the name 'Carvelin'.

These contain: in the upper (30/45 minute-releasing) layer 100 mg of Leucoselect Phytosome® (from *Vitis vinifera*), in the middle (8 hour sustained-releasing) layer 50 mg of Lymphaselect® (from *Melilotus officinalis*) and in the lower (30/45 minute-releasing) layer 80 mg of Ginkgoselect Phytosome® (from *Ginkgo biloba*).

Leucoselect Phytosome® corresponds to a highly standardized mixture of oligomeric procyanidins (OPC – proanthocyanidins: 25.0–30.0%), extracted from the outer part of Vitis vinifera seeds, complexed with distearoylphosphatidylcholine from soy (*Glycine max*).

Lymphaselect® corresponds to a highly standardized extract from aerial parts of *Melilotus officinalis* titrated to yield ≤20% coumarins.

Ginkgoselect Phytosome® corresponds to a highly standardized extract from *Ginkgo biloba* leaves (ginkgoflavonglycosides ≥7.0%, ginkgoterpenes ≥2.0%, bilobalide ≥0.8%, ginkgolides ≥0.8%, total ginkgolic acids ≤5 ppm), complexed with distearoylphosphatidylcholine from soy (*Glycine max*). Carvelin was used under the rationale of supporting circulatory function through the synergistic action of its components.

The remaining 7 participants took a galenic preparation containing 250 mg of carnosine and 250 mg of beta-alanine, taken under the rationale of supporting the buffer function of skeletal muscle by promoting the execution of the physical activity programme; this group was used as a control. Anthropometric data such as height, weight and circumferences were obtained according to the standard methods.

Patients

Data from 21 adult women aged between 24 and 53 who underwent a motor programme for the treatment of cellulite were examined. Normal weight and overweight subjects were considered (BMI between 19 and 29.7 kg/m^2), with gynoid fat accumulation according to the method proposed by Björntorp [9], as the literature seems to indicate that these parameters are not decisive for the development and progression of cellulite [1, 10, 11]. All 21 subjects evaluated according to the method proposed by Rossi and Vergnanini [3] had a cellulite framework, with 17 classifiable as grade II and 4 classifiable as grade III. No subjects classified with grade I or IV were included in the study, as they were characterized by excessively different conditions compared to the other subjects evaluated. Patient characteristics are reported in **Table 1.**

	Carvelin (3 tablets; n=7)		Carvelin (2 tablets; n=7)		Control (n=7)	
	Mean	SD	Mean	SD	Mean	SD
Age (years)	38	± 9	35	± 7	38	± 4
BMI (kg/m^2)	22.58	± 1.27	25.18	± 3.07	22.29	± 2.42
WHR	0.69	± 0.03	0.71	± 0.04	0.74	± 0.04

Table 1 Patient demographics and anthropometric characteristics; data are represented as the mean ± standard deviation (SD). BMI = body mass index; WHR = waist-to-hip ratio

Inclusion and exclusion criteria

All women had to be affected by grade II or III cellulite, according to the scale proposed by Rossi and Vergnanini [3], and still in the presence of menstrual activity. The motor programme path had to be started no more than 3 days since the beginning of data recording.

Ongoing pathologies, pregnancy, treatment with oestrogen-progestogen drugs, anticoagulants, anti-inflammatories, phlebotropics, the execution of other specific treatments for cellulite, changes with respect to usual food consumption and behaviour (smoking, alcohol consumption) and the carrying out of further physical activity in addition to that scheduled (2 weekly aerobic exercise sessions, lasting approximately 45 min) constituted exclusion criteria.

Results

At the end of the evaluation period, considering the extent of the variations and the standard deviations, there were no appreciable variations in weight, BMI, waist circumference or the waist-to-hip ratio (WHR).

The circumference of the hips was reduced by 2 cm ± 3.1 (p>0.05), 4.1 cm ± 2.1 (p>0.05) and 0.8 cm ± 1.6 (p>0.05), respectively, in the Carvelin 3, Carvelin 2 and control groups.

Assessment of the circumferences of the thigh showed a respective reduction for the

Carvelin 3, Carvelin 2 and control groups as follows: proximal thigh 1.1 cm ± 1.1 (p>0.05), 2.3 cm ± 1.3 (p>0.05), 0.1 cm ± 1.5 (p>0.05); mid-thigh 1.3 cm ± 2.1 (p>0.05), 1.4 cm ± 1.3 (p>0.05), 0.6 cm ± 1.6 (p>0.05); distal thigh 1.1 cm ± 0.9 (p>0.05), 1.3 cm ± 0.5 (p>0.05), 0.3 cm ± 0.5 (p>0.05). At the calf level, no significant alterations were detectable.

The scale of Rossi and Vergnanini [3] is based on clinical and symptomatic evaluations aimed at assigning the patient to 1 of 4 classes.

As the possibility of intermediate scores is not envisaged, means and standard deviations are not applicable. At T0 in the Carvelin 3 group there were 5 subjects classified as grade II and 2 subjects as grade III; by T90 1 subject originally classified as grade II was reclassified as grade I and 1 subject was reclassified from grade III to grade II. Similarly, in the Carvelin 2 group, at T0 there were 5 subjects classified as grade II and 2 subjects as grade III; by T90 2 subjects originally classified as grade II were reclassified as grade I and 1 subject was reclassified from grade III to grade II. In the control group, at T0 there were 7 subjects classified as grade II; by T90 1 subject originally classified as grade II was reclassified as grade I. Therefore, even in the evaluation according to the Rossi and Vergnanini scale, the Carvelin 2 group demonstrated a superior outcome. All data are summarized in **Table 2**.

Discussion

At the end of the 90 days, the two subgroups that took Carvelin showed a greater reduction in the circumferences of the hips and thigh compared to the group that took the control product, although this was not statistically significant. Compared with each other, the group that took two tablets/day of Carvelin showed superior results to the group that took three tablets. In the initial speculative hypothesis, it is possible to consider the role of the components of Carvelin as inducers of the activity of different cytochrome enzymes;

	Carvelin (3 tablets; n=7)							Carvelin (2 tablets; n=7)							Control (n=7)						
	T0	±	T90	±	Δ	±	*p*	T0	±	T90	±	Δ	±	*p*	T0	±	T90	±	Δ	±	*p*
Weight (kg)	60.4	2.8	60.7	3.4	0.3	1.1	>0.05	68.5	3.0	66.7	2.5	-1.8	2.1	>0.05	62.8	4.4	62.1	4.8	-0.7	1.6	>0.05
BMI (kg/m^2)	22.6	1.3	22.7	1.4	0.1	0.4	>0.05	25.2	3.1	24.5	2.9	-0.7	0.8	>0.05	22.3	2.4	22.1	2.7	-0.2	0.6	>0.05
Waist (cm)	68.3	2.1	69.3	2.6	1.0	1.5	>0.05	75.6	5.4	74.2	4.7	-1.4	1.4	>0.05	74.2	5.8	73.9	6.2	-0.3	1.6	>0.05
Hips (cm)	99.3	2.7	97.3	5.2	-2.0	3.1	>0.05	106.9	4.5	102.8	3.9	-4.1	2.1	>0.05	99.9	3.8	99.1	4.2	-0.8	1.6	>0.05
WHR	0.7	0.0	0.7	0.0	0.0	0.0	>0.05	0.7	0.0	0.7	0.0	0.0	0.0	>0.05	0.7	0.0	0.7	0.0	0.0	0.0	>0.05
Thigh P (cm)	57.3	3.1	56.3	3.9	-1.1	1.1	>0.05	61.4	3.3	59.1	3.2	-2.3	1.3	>0.05	56.2	3.5	56.2	3.4	-0.1	1.5	>0.05
Thigh M (cm)	52.2	2.4	50.8	3.2	-1.3	2.1	>0.05	53.6	4.0	52.1	4.2	-1.4	1.3	>0.05	50.6	3.3	50.0	3.8	-0.6	1.6	>0.05
Thigh D (cm)	39.1	1.7	38.0	2.4	-1.1	0.9	>0.05	41.6	2.1	40.4	2.4	-1.3	0.5	>0.05	38.1	2.4	37.8	2.4	-0.3	0.5	>0.05
Calf (cm)	35.2	1.6	35.1	1.7	-0.1	0.2	>0.05	36.8	1.7	36.6	2.0	-0.2	0.6	>0.05	34.9	2.6	35.1	2.8	0.3	0.4	>0.05
Rossi and Vergnanini grade [3]	No. Subjects T0		No. Subjects T90		Δ Subjects			No. Subjects T0		No. Subjects T90		Δ Subjects			No. Subjects T0		No. Subjects T90		Δ Subjects		
I	0		1		+1			0		2		+2			0		1		+1		
II	5		5		-1/+1			5		4		-2 /+1			7		6		-1		
III	2		1		-1			2		1		-1			0		0		0		
IV	0		0		0			0		0		0			0		0		0		

Table 2 Comparison of the parameters evaluated at time 0 (T0) and 90 days after the start of the programme (T90); data are represented as the mean ± standard deviation (SD) or the number of subjects (cellulite grading). BMI = body mass index; WHR = waist-to-hip ratio; Thigh P = proximal thigh; Thigh M = mid-thigh; Thigh D = distal thigh. The Rossi and Vergnanini scale [3] is based on clinical and symptomatic evaluations aimed at assigning the patient to 1 of 4 classes; as the possibility of intermediate scores is not envisaged, the mean and SD are not applicable

Ginkgo biloba has a known *in vitro* role as an inducer of CYP2B1/2, CYP3A1, CYP3A2 and CYP3A4 [12–15]. *Vitis vinifera* is known to bring about the *in vitro* induction of CYP3A4 [16], while *Melilotus officinalis* is known to be metabolized *in vitro* by CYP2C9 [17]. These actions could only potentially play a role in the metabolism of substances with reduced bioavailability, such as those associated with plant extracts, compared to the metabolism of drugs for which there are no clinically confirmed interactions. For example, in the study of the *Ginkgo biloba* special extract EGb 761® no relevant effect on the in vivo activity of the major CYP enzymes in humans has been reported, and therefore, this has no associated potential to cause metabolic drug– drug interactions [15].

This possibly explains how a lower dosage in this case could potentially correspond to a greater effect. Although size reductions were found, a p value <0.05 was not reached in any of the statistical evaluations. Among other factors, this most likely could be related to the small sample size of the study. Another significant limitation to take into consideration is the fact that the study was retrospective, based on data collected during the execution of a structured and homogeneous lifestyle programme.

The future execution of a double-blind, randomized, placebo-controlled study where, in addition to anthropometric evaluation, a bio-impedance and haematochemical evaluation is also performed, could contribute significantly to the solidification of our results while also clarifying, if confirmed, the reason for greater efficacy with a lower dose.

Conclusions

The consumption of a mixture of *Vitis vinifera*, *Ginkgo biloba* and *Melilotus officinalis* extracts with high bioavailability, in a biopharmaceutical formulation, is related to a greater reduction in hip circumference and thigh circumferences when taking a dosage of two tablets/day compared to three tablets/day, and compared to a control consisting of a galenic preparation containing 250 mg of carnosine and 250 mg of beta-alanine. Taking into account the sample size here, the study has yielded promising results. This approach constitutes a potentially interesting solution within the perspective of a global programme for the treatment of cellulite, which can provide a further element to support medical treatments, pharmacological and nutritional therapies and structured physical activity. Further studies with a greater number of subjects will be needed in the future to clarify the results obtained, while investigating which dose represents a more effective treatment in the timeframe considered.

Author contributions

All authors contributed equally to writing of the manuscript; all authors read and approved the final version of the manuscript.

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Conflict of Interest

Alexander Bertuccioli works as a scientific consultant for the company responsible for developing Carvelin.

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