

## **Efficacy of the association of formulation with *Coffea canephora* extract and phonophoresis against gynoid lipodystrophy in women population: development, characterization and clinical study**

**Eficácia da associação de formulação contendo extrato de *Coffea canephora* e fonoforese na lipodistrofia ginóide na população feminina: desenvolvimento, caracterização e estudo clínico**

**Eficacia de la asociación de formulación de extracto de *Coffea canephora* y fonoforesis en lipodistrofia ginoide en población femenina: desarrollo, caracterización y estudio clínico**

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### **Abstract**

Gynoid lipodystrophy (GL), popularly known as cellulite, affects 85% of women after puberty. The aim of the study was to develop, characterize and evaluate the effectiveness of a topical formulation based on *Coffea canephora* green extract (GCE) applied by phonophoresis in the treatment of GL in young women. Formulations with 1.0% (w/w) and 3.0% (w/w) of GCE were developed and characterized by organoleptic analysis, pH, centrifugation, caffeine content, rheology, spreadability, preliminary stability and cytotoxic analysis. And after the selection of the formulation, in a longitudinal, randomized and blind study, with volunteers in separate groups, the Control Group (CG, n=15) used the formulation based on a copolymer of sodium acrylates and lecithin (Lec) and the Test Group (TG, n=15) the copolymer formulation of sodium acrylates and lecithin containing 3.0% (w/w) of GCE (Lec/GCE3). Volunteers were assessed before and after treatment for cellulite severity using the Cellulite Severity Scale (CSS) and quality of life using the Dermatology Life Quality Index (DLQI-BRA). The groups were treated with 5 sessions, twice a week, 20 min/session. As a result, the GT achieved a significant reduction (p=0.002) in cellulite severity according to the CSS and both groups showed significant changes in the DLQI-BRA after treatment, CG (p=0.001) and TG (p=0.001). We conclude that the LEC/GCE3 formulation applied by phonophoresis was effective for cellulite in young women.

**Keywords:** *Coffea canephora*; Cellulite; Phonophoresis; Clinical trial.

## Resumo

A lipodistrofia ginoide (LG), popularmente conhecida como celulite, acomete 85% das mulheres após a puberdade. O objetivo do estudo foi desenvolver, caracterizar e avaliar a eficácia de uma formulação tópica à base de extrato verde de *Coffea canephora* (GCE) aplicada por fonoforese no tratamento de LG em mulheres jovens. Formulações com 1,0% (p/p) e 3,0% (p/p) de GCE foram desenvolvidas e caracterizadas por análise organoléptica, pH, centrifugação, teor de cafeína, reologia, espalhabilidade, estabilidade preliminar e análise citotóxica. E após a seleção da formulação, em estudo longitudinal, randomizado e cego, com voluntários em grupos separados, o Grupo Controle (GC, n=15) utilizou a formulação à base de um copolímero de acrilatos de sódio e lecitina (Lec) e o Grupo Teste (GT, n=15) a formulação copolímero de acrilatos de sódio e lecitina contendo 3,0% (p/p) de GCE (Lec/GCE3). As voluntárias foram avaliadas antes e após o tratamento quanto à gravidade da celulite usando a Cellulite Severity Scale (CSS) e a qualidade de vida usando o Dermatology Life Quality Index (DLQI-BRA). Os grupos foram tratados com 5 sessões, duas vezes por semana, 20 min/sessão. Como resultado, o GT obteve redução significativa ( $p=0,002$ ) na gravidade da celulite de acordo com o CSS e ambos os grupos apresentaram alterações significativas no DLQI-BRA após o tratamento, GC ( $p=0,001$ ) e TG ( $p=0,001$ ). Concluímos que a formulação LEC/GCE3 aplicada por fonoforese foi eficaz para a celulite em mulheres jovens.

**Palavras-chave:** *Coffea canephora*; Celulite; Fonoforese; Ensaio clínico.

## Resumen

La lipodistrofia ginoide (GL), conocida popularmente como celulitis, afecta al 85% de las mujeres después de la pubertad. El objetivo del estudio fue desarrollar, caracterizar y evaluar la eficacia de una formulación tópica a base de extracto verde de *Coffea canephora* (GCE) aplicada por fonoforesis en el tratamiento del GL en mujeres jóvenes. Se desarrollaron formulaciones con 1,0% (p/p) y 3,0% (p/p) de GCE y se caracterizaron mediante análisis organolépticos, pH, centrifugación, contenido de cafeína, reología, esparcibilidad, estabilidad preliminar y análisis citotóxico. Y tras seleccionar la formulación, en un estudio longitudinal, aleatorizado y ciego, con voluntarios en grupos separados, el Grupo Control (GC, n=15) utilizó la formulación a base de un copolímero de acrilatos de sodio y lecitina (Lec) y el Grupo Test (GT, n=15) la formulación de copolímero de acrilatos de sodio y lecitina que contiene 3,0% (p/p) de GCE (Lec/GCE3). Los voluntarios fueron evaluados antes y después del tratamiento para determinar la gravedad de la celulitis utilizando la Escala de gravedad de la celulitis (CSS) y la calidad de vida utilizando el Índice de calidad de vida dermatológica (DLQI-BRA). Los grupos fueron tratados con 5 sesiones, dos veces por semana, 20 min/sesión. Como resultado, el GT logró una reducción significativa ( $p=0,002$ ) en la severidad de la celulitis según CSS y ambos grupos mostraron cambios significativos en DLQI-BRA después del tratamiento, GC ( $p=0,001$ ) y GT ( $p=0,001$ ). Concluimos que la formulación LEC/GCE3 aplicada por fonoforesis fue efectiva para la celulitis en mujeres jóvenes.

**Palabras clave:** *Coffea canephora*; Celulitis; Fonoforesis; Ensayo clínico.

## 1. Introduction

Gynoid lipodystrophy (GL) popularly known as cellulite, affects 85% of women after puberty according to the Clinically, it is characterized by topographical changes on the surface of the skin, giving it an “orange peel” appearance (Rawlings, 2006, Hexsel, Dal’Forno & Hexsel, 2009). GL manifests mainly in the gluteal regions and thighs (Rossi & Vergnanini, 2002, Almeida *et al.*, 2012) with multifactorial etiopathogenesis, hormonal influences, genetic, metabolic, gravitational and lifestyle factors (Afonso *et al.*, 2010, Teaima *et al.*, 2018). This condition impacts women psychologically, affecting their self-esteem and quality of life (Atamoros *et al.*, 2018) and it is one of the biggest bodily complaints among women, considerably moving the market of aesthetics (Emanuele, 2013).

A variety of topical products enriched with active principle, using plant extracts are used in order to improve the appearance of the GL, with caffeine being the most used in the aesthetic market, as it has a documented action (Teaima *et al.*, 2018, Cruz *et al.*, 2015). Green *Coffea canephora* extract (GCE) has significant antioxidant potential due to its high content of bioactives, such as caffeine and chlorogenic acids (Vignoli *et al.*, 2014).

Phonophoresis is applied through the therapeutic ultrasound device and is a strategy used to optimize the permeation of active compounds and the transport of active substances, increasing percutaneous absorption in the skin (Mardegan & Guirro, 2005, Petrilli & Lopez, 2018). There is, however, to date, a shortage in the scientific literature that demonstrates the real effects of therapeutic ultrasound, as well as phonophoresis, in the treatment of GL using GCE in formulation for topical applications (Cruz *et al.*, 2015, Pires-de-Campos *et al.*, 2018). The existing articles demonstrate a lack of standardization regarding the

intensities used in therapy.

Therefore, the need for studies in the area is evident. In this sense, the present work aimed to develop, characterize and evaluate a formulation containing green GCE obtained by *Coffea canephora* species applied by phonophoresis for the treatment of GL in young women.

## 2. Methodology

### 2.1 Preparation of the green *Coffea canephora* freeze-drying extract (GCE)

GCE was donated by Cia Iguacu de Café Solúvel (Cornélio Procópio, Paraná, Brazil), lot 17, sample 101012965. The extraction process was carried out in an industrial pilot plant following the conventional process of extraction of soluble coffee by percolation in columns and submitted to the freeze-drying process according to Dos Santos *et al.* (2017).

### 2.2 Preparation of formulations

Twelve semi-solid formulations for topical use were developed, as shown in Table 1, four bases, four with GCE 1.0% (w/w) and the other four with GCE 3.0% (w/w). For the bases, polymers were used: Carbomer (Carbopol® 940) (0.5%, w/w), Sodium Acrylate Copolymer (and) Lecithin (Lecigel®) (3.0%, w/w), hydroxyethylcellulose (Natrosol® 250HHR) (2.0%, w/w), and poloxamer 407 (Pluronic® F127) (20.0%, w/w).

The polymers dispersed in purified water under mechanical agitation at room temperature and after complete dispersion, the appropriate amount of potassium sorbate, glycerin, phenoxiethanol and parabens (methyl, propyl, butyl, and isobutylparaben), aminomethylpropanol, cyclomethicone and propylene glycol was added to the formulation, using different concentrations and in constant agitation (Draelos & Thaman, 2005). The GCE was added last, with homogeneous agitation.

**Table 1** - Composition of twelve formulations with four polymers (Carbopol® 940, Lecigel®, Natrosol® 250HHR, Pluronic® F127) without GCE (Base Carb, Base Lec, Base Nat, Base Plu), with 1.0% GCE (Carb/GCE1, Lec/GCE1, Nat/GCE1, Plu/GCE1), and with 3.0% GCE (Carb/GCE3, Lec/GCE3, Nat/GCE3, Plu/GCE3).

Formulations	Concentration (% w/w)							
	Carb	Lec	Nat	Plu	GCE	Gly	Sil	Prop
Base Carb	0.5	-	-	-	-	3.0	2.0	5.0
Carb/GCE1	0.5	-	-	-	1.0	3.0	2.0	5.0
Carb/GCE3	0.5	-	-	-	3.0	3.0	2.0	5.0
Base Lec	-	3.0	-	-	-	3.0	2.0	5.0
Lec/GCE1	-	3.0	-	-	1.0	3.0	2.0	5.0
Lec/GCE3	-	3.0	-	-	3.0	3.0	2.0	5.0
Base Nat	-	-	2.0	-	-	3.0	2.0	5.0
Nat/GCE1	-	-	2.0	-	1.0	3.0	2.0	5.0
Nat/GCE3	-	-	2.0	-	3.0	3.0	2.0	5.0
Base Plu	-	-	-	20.0	-	3.0	2.0	5.0
Plu/GCE1	-	-	-	20.0	1.0	3.0	2.0	5.0
Plu/GCE3	-	-	-	20.0	3.0	3.0	2.0	5.0

Carb = Carbopol® 940; Lec = Lecigel®; Nat = Natrosol® 250HHR; Plu = Pluronic® F127; GCE = *Coffea canephora* extract; Gly = Glycerin; Sil = Cyclomethicone; Prop = Propylene glycol. Source: Authors.

### 2.3 Characterization of formulations

The formulations were stored sealed and protected from light and moisture for 24 hours at a controlled room temperature ( $20 \pm 5$  °C), before being submitted to subsequent tests. They were submitted to the centrifugation test, pH value, organoleptic evaluation (appearance, color and odor), preliminary stability, spreadability test, rheological behavior, cytotoxic

analysis and determination of caffeine content (ANVISA, 2008; Borghetti & Knorst, 2006; ANVISA, 2004). All assays were evaluated in triplicate.

#### **2.4 Organoleptic evaluation**

Visual inspection of the formulations was performed in triplicate, placing 20 g of the respective samples in beakers with a capacity of 50 g and sealed. After being kept at rest for 24 h at controlled room temperature ( $20 \pm 5^\circ\text{C}$ ), the following changes were observed: appearance, color, odor and signs of instability such as creaminess and phase separation and classified as: normal, no change, slightly modified, modified or heavily modified, according to Brazilian Health Regulatory Agency (ANVISA, 2008).

The characteristics of the samples were visually observed, checking if there were macroscopic changes in relation to the reference samples (formulation with and without GCE) or if there were changes like separation, precipitation or turbidity. The color analysis of the formulations was colorimetry performed by visual comparison, under a white light source. The sample color results were photographed and compared to the standard color. On the other hand, the odor of the samples was evaluated directly through the sense of smell (ANVISA, 2004).

#### **2.5 Centrifugation Test and pH value determination**

After 24 hours of production, the samples were subjected to the centrifugation test, in triplicate, where 5,0 g of each formulation was placed in a graduated conical test tube and centrifuged (Centrifuge I Fanem 206-BL, São Paulo, Brazil) at 3200 rpm for 30 min at controlled room temperature ( $20 \pm 5^\circ\text{C}$ ). This assay help to detect visible changes or instabilities such as phase separation, compact sediment formation and coalescence, visually assessed (ANVISA, 2008).

The pH value was verified in triplicate, where pH was determined using a digital pH meter (pH 21 pH/mV meter-HANNA, Brazil) at room temperature ( $20 \pm 5^\circ\text{C}$ ). For formulation determinations, the electrode inserted directly into the diluted sample (1:10) according to ANVISA (2008).

#### **2.6 Determination of Caffeine content in the Formulation**

About 0.20 g of each formulation was slowly added to a 100 mL volumetric flask with Milli-Q water until complete homogenization. Determined amounts of aliquots were withdrawn and filtered (Millex 0.45  $\mu\text{m}$ ) and proceed with sample injection (20  $\mu\text{l}$  loop) by High Performance Liquid Chromatography analysis. Chromatographic conditions: mobile phase (water: methanol; 80:20),  $40^\circ\text{C}$ , mobile phase flow (1 mL/min), injection volume (20  $\mu\text{L}$ ), chromatographic column Agilent Scalar C18 4.6 x 100 mm, 5 mm (Part Number 449905-902), and UV/VIS detector at 272 nm (Boger *et al*, 2022).

#### **2.7 Rheological characterization**

The rheological performances of the samples were determined in triplicate, at controlled room temperature ( $20 \pm 5^\circ\text{C}$ ), using a Brookfield Viscometer spindle T-F (23). The samples were placed in a container measuring 1.9 cm in diameter and 6.3 cm in height (from the equipment itself) and using speeds of 0.1; 0.2; 0.3; 0.4; 0.5; 0.6 and 0.7 rpm. Each speed was maintained for 3 minutes, and at the end of every 45 seconds, torque (%), viscosity (cP) shear rate (1/sec) and shear stress ( $\text{D}/\text{cm}^2$ ) data were collected, totaling between 3 and 4 readings for each speed.

#### **2.8 Spreadability test**

Spreadability was analyzed according to Borghetti and Knorst (2006), using a glass plate on a millimeter scale to determine the surface that the sample covers by measuring the perpendicular diameters, with subsequent calculation of the

mean diameter, at controlled room temperature ( $20 \pm 5^\circ\text{C}$ ).

The calculations were performed from the Equation 1:

$$Ei = \frac{d^2 \cdot \pi}{4} \quad (1)$$

Where  $Ei$  ( $\text{mm}^2$ ) is the spreadability of the sample for weight  $i$ , and  $d$  is the average diameter (mm) reached by the sample after the overlap of each plate.

The spreadability values as a function of the added weights were determined in triplicate, calculating the average, using the plate with a weight of 23.82 g and weights of 2.0 g, 4.0 g and 10.0 g in a time of 1 minute.

## 2.9 Preliminary stability study

Preliminary stability study was carried out in triplicate.<sup>21</sup> The samples were placed in a neutral, transparent glass bottle with a tight-fitting lid, without completing the total volume of the package. The formulations were subjected to stress conditions, at a temperature of  $40 \pm 2^\circ\text{C}$  and  $5 \pm 2^\circ\text{C}$ , in 24-hour cycles, for 15 days. Then they were evaluated in physical-chemical and organoleptic (appearance, color and odor) tests (ANVISA, 2008).

## 2.10 Cytotoxicity assay

The MTT colorimetric test [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was performed to quantify viable cells in Hep-2 cell culture. Previously  $2.5 \times 10^5$  cells/ml were cultured in 96-well plates for 48 h. When cells are confluent, non-adherent cells were washed away with 0.01 M PBS. A volume of 100  $\mu\text{l}$  of RPMI medium containing different concentrations of compounds was added to the wells, and the plate was incubated for 24h. Each concentration was tested in triplicate, as was done with the control (cells grown in medium without antimicrobial compound). After the incubation time, the culture medium was removed and the wells were washed with 0.01 M PBS. MTT stock solution was added and incubated at  $37^\circ\text{C}$  for 4 hours in a 5%  $\text{CO}_2$  atmosphere. The concentration of compound required to inhibit cell viability by 50% was determined (IC50) (Izumi *et al.*, 2012).

## 2.11 Human volunteers: inclusion, exclusion and loss criteria

The general skin performance evaluation of a topical formulation with and without GCE applied by phonophoresis for the treatment of GL was evaluated in young women volunteers. The protocols for experiments on humans followed the ethical principles of the Declaration of and were approved by the Standing Committee on Ethics in Research Involving Human Subjects of the University Center of Philadelphia, Londrina, Paraná, Brazil, according to Resolution No. 466/2012 CAAE: 3697130.1.000.5217, with Opinion No. 4,718.403 (Mlosek *et al.*, 2021).

The following inclusion criteria adopted female volunteers aged between 20 and 35 years, with complaints of GL in the gluteal region, who maintained their eating habits and/or routine of physical activities as usual, in the period from onset to the end of the study. The study excluded volunteers who were allergic to coffee and/or caffeine, who had contraindications to the application of phonophoresis, who had pyrexia on the day of assessment, pregnant or lactating women, history of surgical intervention in the gluteal region, and those who used cosmetic or gluteal medications during the study.

The study had an exclusion criterion. Thus excluded from the research were the dropouts, pregnancy, those who started any medical treatment that could influence weight gain or loss, or who underwent any aesthetic procedure and/or others

in the gluteal region, those who started diets to gain or losing weight, changing physical activity routine and missing two consecutive sessions. After being informed about the procedures and objectives of the work, the volunteer's participation was affected by signing the free and informed consent form for the present study.

### **2.12 Clinical Protocol**

The study was carried out in the form of a longitudinal, randomized and blind study, including thirty women recruited from the local community of the city of Londrina/Paraná-Brazil, from January to March 2021. The volunteers were randomly, through separate draws, in two groups (n=30). The GC group (Control Group) with application of base formulation applied by phonophoresis (n=15), coded randomly by numbers from 16 to 30, and the other group called TG (Test Group) with application of base formulation plus GCE applied by phonophoresis (n=15), randomized coded by numbers from 1 to 15. Each volunteer was analyzed using the Cellulite Severity Scale (CSS), Dermatology Quality of Life Index (DLQI-BRA) and then photographic documentation was performed. The evaluation aimed to verify the effectiveness of the proposed therapy (Sreeraj, Bharati & Ipseeta, 2015).

Phonophoresis was applied by the direct method, using the HTM UST brand, Sonic Compact model (ANVISA registration nº 80212480001), at a frequency of 3 MHz, in continuous mode, with an intensity of 0.6 W/cm<sup>2</sup>, for 10 minutes. The application was made in the lower outer quadrant of each buttock (right and left), in an area of 70 cm<sup>2</sup>, using an effective radiation area (ERA) of the head of 7 cm<sup>2</sup>. Both groups were treated with five sessions (2X/week), for 5 weeks, where each session lasted 20 minutes. 10 g of the formulation were used for the session, 5 g for each buttock. After the consultations, the volunteers were reassessed and photographed again.

### **2.13 Cellulite Severity Scale (CSS)**

The CSS consists of 20 photographs, and by comparison with the volunteer, the morphological characteristics are classified (A, B, C, D and E) and scored from 0 to 3, as follows: A) Number of evident depressions, classified into: 0 : no depression, 1: small amount (1-4 depressions), 2: moderate amount (5-9 depressions), 3: large amount (10 or more depressions); B) Depth of depressions, classified as: 0: no depression, classified as: 1: superficial, 2: medium, 3: deep; C) Morphological aspect of skin surface alterations, classified as: 0: no protuberances, 1: 'Orange peel', 2: 'Requeijão', 3: 'Mattress'; D) Degree of sagging of the skin, classified as: 0: no sagging, 1: mild, 2: moderate, 3: severe; and E) Nürnberger and Müller Scale, classified as: 0: zero, 1: one degree, 2: two degrees, 3: three degrees. The final sum of the evaluation varies from 1 to 15 and classifies cellulite as: medium severity (1-5 points), moderate severity (6-10 points) and severe (11-15 points) (Hexsel, Dal'Forno & Hexsel, 2009).

### **2.14 Quality of Life Index in Dermatology (DLQI-BRA)**

The DLQI-BRA is composed of 10 questions that consider aspects such as symptoms and feelings, daily activities, leisure, work and school, personal relationships and treatment. Each question contains four answer alternatives, which correspond to 0: nothing/no relevance; 1: a little; 2: quite; and 3: really a lot. The result generates a score that varies from 0 to 30, resulting in: 0-1 (no impact), 2-5 (slight impact), 6-10 (moderate impact), 11-20 (severe) and finally 21- 30 (very severe) (Ferraz *et al.*, 2006, Basra *et al.*, 2008).

### **2.15 Photographic Documentation**

The volunteers were photographed with a DMC-FX100 Lumix 12 megapixel Panasonic digital camera; Kenko Tripod ST-3 tripod, without the use of zoom and flash, and positioned standing, with their backs to the camera, wearing underwear,



with a black fabric as a background. 4 photos were taken of each volunteer, 2 before and 2 after treatment, one with the gluteal region at rest and the other with gluteal contraction as demonstrated in the study of Rawlings (2006). The images were used for demonstrative and/or comparative data, for observation of the clinical aspect before and after treatment.

### 2.16 Statistical analysis

Categorical data were analyzed using Fisher's test. Continuous variables were checked for normality and homoscedasticity using the Kolmogorov-Smirnov and Mild tests, respectively. Due to the non-parametric distribution of the data, non-parametric statistical tests were applied. For dependent data, the Wilcoxon test (intragroup changes) was applied. The Mann-Whitney test was performed to compare baseline values and differences between treatment groups (intergroup changes). Quantitative data such as mean and standard deviation (SD), minimum and maximum values; and categorical data were expressed as absolute number (n) and percentage and data. All statistical analyzes were performed using IBM SPSS, version 26 (IBM, Armonk, NY, USA). The tests were two-tailed and  $\alpha = 0.05$  indicated statistically significant results. The sample size was evaluated to determine statistical differences of at least 10% in defined parameters. The G\*Power 3.1.9.2 for Windows program (<http://www.gpower.hhu.de/>) was used to calculate the sample size.

## 3. Results and Discussion

### 3.1 Determination of caffeine

The determination of caffeine content and total chlorogenic acids and antioxidant activity by ABTS of the GCE showed presented of 9.55% ( $\pm 0.73$ ) ( $\mu\text{g.ml}^{-1}$ ) of caffeine content and 1.23% ( $\pm 0.21$ ) ( $\mu\text{g.ml}^{-1}$ ) of 5-caffeoylquinic acid. The antioxidant activity of GCE evaluated by ABTS was 45.4 g of Trolox/100 g. Thus, the high antioxidant activity values of the GCE are highlighted, attributed to the active ingredients in the *C. canephora* and process used (lyophilized) that contributed to the maintenance of the bioactives. A study carried out by Boger *et al.* (2022) also confirms these results.

The caffeine content of the formulations was performed in triplicate by HPLC and the results obtained were the Nat/GCE1 formulation showed 0.02% ( $\pm 0.01$ ) ( $\mu\text{g.ml}^{-1}$ ) of caffeine content, the Nat/GCE3 formulation resulted in 0.10  $\pm$  0.01% ( $\mu\text{g.ml}^{-1}$ ), the formulation Lec/GCE1 presented 0.44%  $\pm$  0.01% ( $\mu\text{g.ml}^{-1}$ ) and the formulation presented Lec/GCE3 0.60%  $\pm$  0.01% ( $\mu\text{g.ml}^{-1}$ ). The Lec/GCE3 formulation was selected for further characterization and testing as it had the highest caffeine content and was consequently used in the clinical study.

### 3.2 Preparation and characterization of formulations

Twelve semi-solid formulations were developed and prepared, using the polymers: carbomer (Carbopol® 940), copolymer of sodium acrylates and lecithin (Lecigel®), hydroxethylcellulose (Natrosol® 250HHR) and poloxamer 407 (Pluronic® F127). These polymers were chosen for their availability in the cosmetics market, for their skin compatibility and for enabling the formation of gel, a fundamental property for the application of phonophoresis (Oberli *et al.*, 2014). The selection of formulations for the in vivo stage of the study took place through the process of eliminating the formulations developed in the in vitro study during analysis and characterization.

The results of the centrifugation tests, organoleptic analyzes (appearance, color, odor) and pH were shown in Table 2. The Carb/GCE1 and Carb/GCE3 formulations were not submitted to the centrifugation test, as they presented instability to GCE incorporation, losing the gel characteristic, being eliminated from the study, along with Carb. The Plu/GCE1 and Plu/GCE3 formulations showed phase separation after centrifugation, and together with the Plu, they were also excluded from the study. The other formulations, on the other hand, did not show modifications or instabilities such as phase separation, compact sediment formation and coalescence, so were considered stable (ANVISA, 2008).

The organoleptic tests (Table 2) showed that the formulations containing the polymer Lecigel and Natrosol did not show signs of instability such as creaming and phase separation, remaining normal, without alteration, and with a pH between 4.5-6.5 which is recommended for dermocosmetics (ANVISA, 2004).

**Table 2** - Result of the analysis of the twelve formulations regarding organoleptic analysis (appearance, color, smell), pH value, and centrifugation test.

Formulation	Organoleptic characteristics				
	Appearance	Color	Smell	pH value	Centrifugation test
Base Carb	Homogeneous	translucent	odorless	5.98±0.20	No change
Carb/GCE1	Heterogeneous	light amber	coffee	5.41±0.15	Separation
Carb/GCE3	Heterogeneous	dark amber	coffee	5.45±0.20	Separation
Base Lec	Homogeneous	white	odorless	5.50±0.10	No change
Lec/GCE1	Homogeneous	light amber	coffee	5.78±0.32	No change
Lec/GCE3	Homogeneous	dark amber	coffee	5.45±0.22	No change
Base Nat	Homogeneous	translucent	odorless	5.81±0.18	No change
Nat/GCE1	Homogeneous	light amber	coffee	5.59±0.26	No change
Nat/GCE3	Homogeneous	dark amber	coffee	5.65±0.30	No change
Base Plu	Homogeneous	white	odorless	5.84±0.19	No change
Plu/GCE1	Homogeneous	light amber	coffee	5.61±0.25	Separation
Plu/GCE3	Homogeneous	dark amber	coffee	5.63±0.2	Separation

Source: Authors.

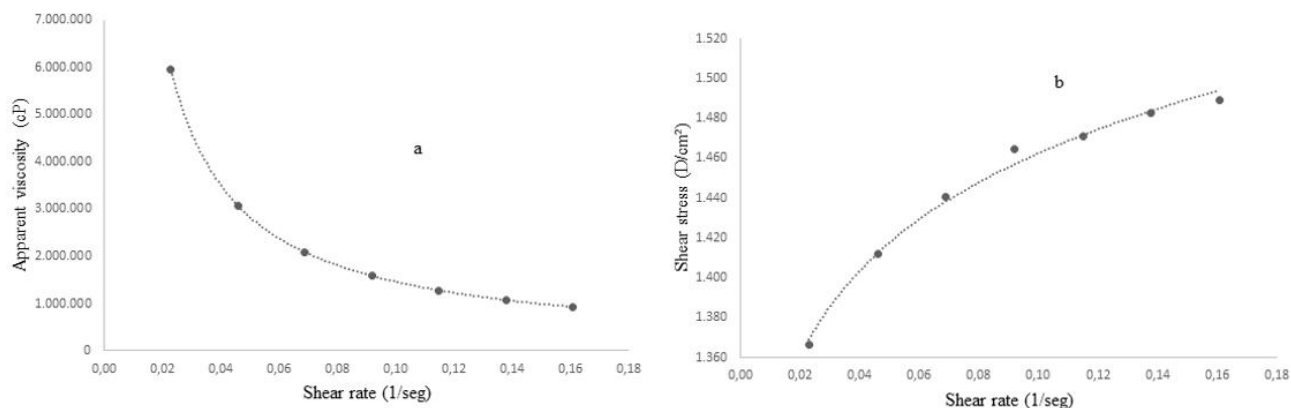
The spreadability analysis of the Lec/GCE3 formulation showed an increase in the spreadability profile up to a certain weight used 27.82 (mm<sup>2</sup>), when compared to Deuschle *et al.* (2015) that had 21.09 (mm<sup>2</sup>), and from this weight on, it remained practically constant, which suggests good spreadability and high yield (Basra *et al.*, 2008).

### 3.2.1 Rheological characterization

Rheological studies can allow the understanding of the interactions among the components of the formulation, as well as their structuring, allowing the selection of the improved systems for topical applications (Jones, Brown & Woolfson, 2001; Tonon, Grosso & Hubinger, 2011). The Lec/GCE3 formulation presented non-Newtonian or shear-thinning behavior (Figure 1), with a pseudoplastic behavior (Higuchi, 1963) at controlled room temperature (20 ± 5 °C), in which the apparent viscosity decreases with the increase of the shear rate and following the power law.



**Figure 1.** Flow rheograms of Lec/GCE3 formulations, at controlled room temperature ( $20 \pm 5$  °C), using a Brookfield Viscometer spindle T-F, specified as a) Apparent viscosity and b) Shear stress. The coefficient of variation of replicate analysis was less than 10%.



Source: Authors.

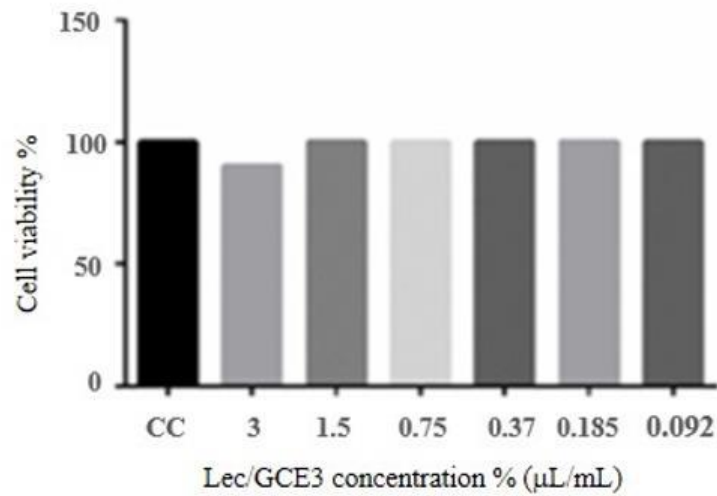
### 3.3 Preliminary stability study

The result of the preliminary stability tests carried out after 15 days, it is observed that after the centrifugation test, physical-chemical tests (pH, spreadability), organoleptic evaluation (aspect, color and odor) (ANVISA, 2004), the formulation Lec/GCE3 showed no modifications or alterations during the entire period evaluated. Lecigel is a gelling agent with emulsifying properties, used to increase the stability and viscosity of formulations, has high compatibility with the compounds and can be used on any type of skin, providing hydration, a soft, non-sticky touch, without the need for neutralization (Miot, Paixão & Paschoal, 2006).

### 3.4 Cytotoxicity assay

The analysis of the result presented through the colorimetric test by MTT, of Lec/GCE3 (Figure 2), it was observed that the concentrations of 1.5, 0.75, 0.37, 0.185 and 0.092 ( $\mu\text{g}/\text{mL}$ ) did not show cytotoxicity, keeping the viability of cells in Hep-2 cell culture at 100%. This result was similar to cell control group (group that was only treated with culture medium without having been exposed to Lec/GCE3), and the concentration of 3.0  $\text{mg}/\text{mL}$  obtained the cell viability rate at 90%, that demonstrated that this formulation does not present relative cytotoxicity and is considered safe for human use and can be tested in vivo stage of the study (Martó *et al.*, 2018). The Lec/GCE3 formulation was tested at different concentrations and showed no toxicity in 100% of the cells.

**Figure 2** - The MTT cytotoxicity assay performed with Hep-2 cell culture using control cell (cc) and different concentrations of Lec/GCE3 at 1.5, 0.75, 0.37, 0.185 and 0.092 ( $\mu\text{L}/\text{mL}$ ).



Source: Authors.

### 3.5 Clinical Protocol

In the evaluation of CSS before treatment, the two groups (CG and TG) showed similar characteristics regarding cellulite severity ( $p=0.174$ ), in relation to the number of depressions ( $p=0.512$ ), depth of depressions ( $p=0.267$ ), morphological appearance ( $p=0.148$ ), sagging ( $p=0.806$ ) and the Nürnberger and Müller scale ( $p=0.595$ ). The clinical protocol consisted of DLQI-BRA and CSS. The formulation chosen to follow the clinical protocol was Lec for CG and Lec/GCE3 for TG, applied by phonophoresis.

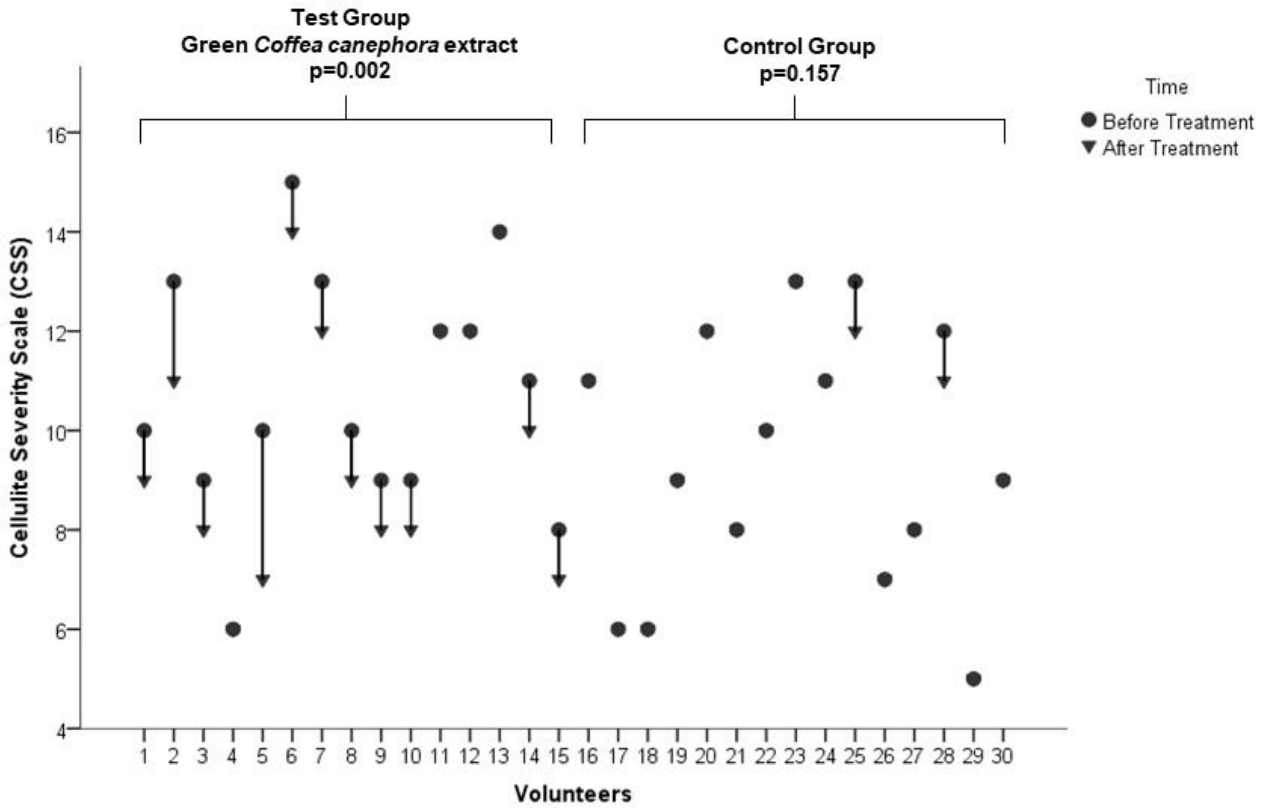
The results of other study by Quessada *et al.* (2021) showed that the use of caffeine gel alone and caffeine gel associated with iontophoresis is efficient on reduction of subcutaneous tissue thickness in woman with GLD imafter 10 sessions when assessed by ultrasound. The results demonstrated the efficacy of caffeine used alone or associated with iontophoresis on lipolysis of fat layer, being effective in lipolytic activity and reducing lipogenesis.

In our study, no intergroup difference was found in relation to the quality of life verified by the CELLUQOL questionnaire. Future studies are needed to clarify the effects in quality of life in use of caffeine gel alone and associated with iontophoresis in GLD treatment.

#### 3.5.1 Quality of Life Index in Dermatology (DLQI-BRA) and Cellulite Severity Scale (CSS)

Both groups showed positive and significant changes in DLQI-BRA after GC ( $p=0.001$ ) and TG ( $p=0.001$ ) treatment. The study volunteers did not know which group they were placed in (randomized), however, the fact that they received treatment for cellulite even though they were in the CG, made them show an improvement in their quality of life. And according to the CSS analysis before and after treatment (Figure 3), 11 of the 15 TG volunteers showed improvement after treatment with GCE ( $p=0.002$ ), but only 2 of the 15 CG volunteers showed improvement after treatment, while the others started and finished the study without presenting changes ( $p=0.157$ ), this demonstrates the efficiency of Lec/GCE3 in the treatment of GL.

**Figure 3** - The Cellulite Severity Scale (CSS) score chart before and after treatment: Test Group (TG) that used the Lec/GCE3 formulation, composed of 15 volunteers numbered 1-15; and Control Group (CG) that used the Lec formulation, composed of 15 volunteers numbered 16-30.



Analysis of Wilcoxon. Source: Authors.

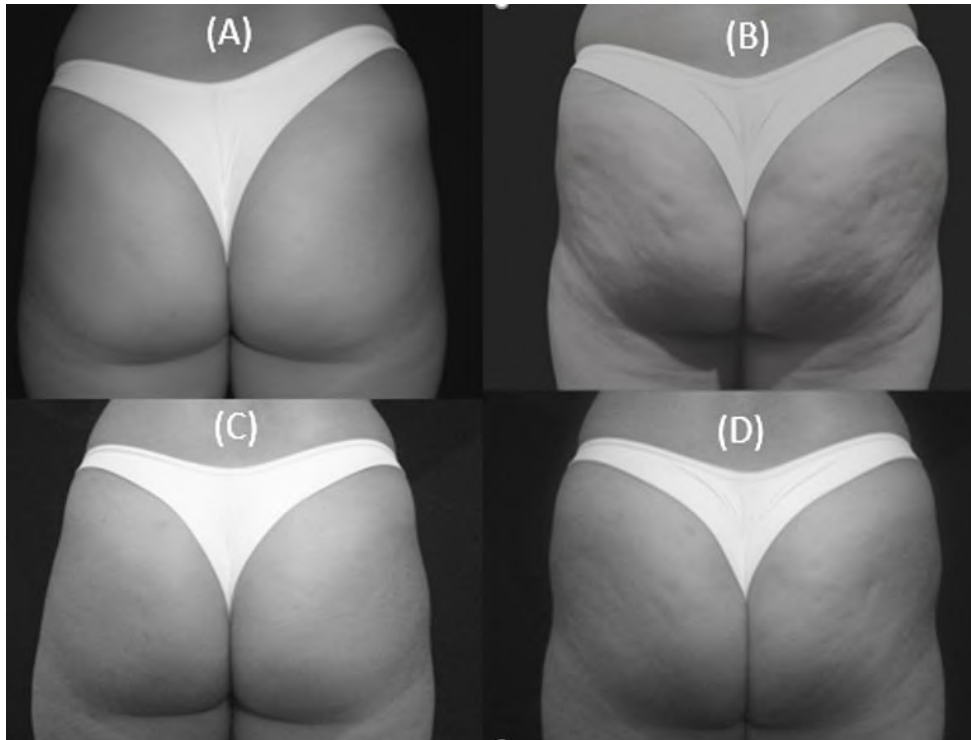
The treatment method applied in the TG showed effective, non-invasive responses and evidenced an improvement in the GL in the gluteal region and a reduction in the thickness of the subcutaneous tissue after 5 treatment sessions, with a real condition of use of the product and corroborate with studies by Teaima and collaborators (2018) and Quessada and collaborators (2021).

Volunteer number 05 from the TG (Figure 4) showed a notable clinical difference in the photographic documentation and showed the greatest improvement in CSS. It is suggested that caffeine stimulates lipolysis and reduces lipogenesis, reducing adipose tissue, with USG-proven results, consequently improving the clinical appearance of the GL.

The dermocosmetic developed in the present study can be safely applied in clinical practice, together with the care protocol, contributing to increase the arsenal of therapies available in the treatment of GL in young women. Even so, further studies are needed that seek to evaluate the effectiveness of dermocosmetics containing phytoactives, the use of phonophoresis in the treatment of GL and its associations with validated evaluation parameters.

The GL is a multifactorial pathology that requires complex treatment, taking into account its chronic and progressive nature. The strategy of using caffeine-containing cosmetics is intended to minimize this condition and prevent its progression, and it is essential to adopt lifestyle changes, such as physical exercise, adequate water intake, balanced diets, among others. However, regardless of the method used, there is, to date, no definitive treatment for GL.

**Figure 4** - Voluntary photographic documentation image from TG group (A) before Lec/GCE3 treatment, musculature at rest, (B) before treatment, in muscle contraction, (C) after treatment, musculature at rest, and (D) after treatment, in muscle contraction.



Source: Authors.

#### 4. Conclusion

The lyophilized green extract of *Coffea canephora* was added in the semi-solid formulations. The formulations were prepared, characterized and evaluated for organoleptic characteristics, centrifugation, pH determination, caffeine content, rheological characterization, spreadability test, and, preliminary stability study. The semi-solid formulation using copolymer of sodium acrylates and lecithin plus 3.0% (w/w) of *Coffea canephora* extract (Lec/GCE3) did not show cytotoxicity demonstrated by the MTT assay and was chosen to be tested in the young women with gynoid lipodystrophy.

The Lec/GCE3 formulation applied by phonophoresis showed effective results in reducing gynoid lipodystrophy in young women after 5 sessions when evaluated by Cellulite Severity Scale. In our study, both the GT and the CG showed an improvement in quality of life verified by the Quality of Life Index in Dermatology associated with the phonophoresis treatment of gynoid lipodystrophy.

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