


# Evaluation of the Use of Compressive Stockings Impregnated With Hesperetin-Based Nanocapsules in the Healing of Venous Ulcers: A Case Report

Clinical Medicine Insights: Case Reports  
Volume 12: 1–6  
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DOI: 10.1177/1179547619858977



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**ABSTRACT:** Venous ulcers are a more severe complication of chronic venous insufficiency, significantly compromising patient quality of life (QoL). Compressive stockings are still the gold standard treatment method with alternative therapies currently being evaluated. In this perspective, we investigate the influence of compressive stockings impregnated with hesperetin-based nanocapsules in the healing process of venous ulcers. Compressive stockings impregnated with hesperetin-based nanocapsules were applied to a consenting patient for 6 months following all relevant ethical principles for patient studies. The patient was evaluated at baseline (T0), 3 months (T3), and 6 months (T6), using photographic register (healing) probes to measure skin melanin, erythema and hydration parameters, and venous diameters, followed by questionnaires regarding QoL and pain perception. Healing was observed at the 3-month time point and with 91.6% and 93.1% of retraction area in larger ulcers of the right leg and lateral portion of the left leg, respectively. The deepest ulcer in a medial portion of the left leg healed 47.3%. A reduction of all measured skin parameters was observed, indicating a possible hesperetin effect. The scores of QoL and pain were, respectively, in the ranges of 91.6 to 31.2 and 7 to 0. Reduction in venous diameters also indicates healing function. These preliminary findings suggest that compressive stockings impregnated with hesperetin nanocapsules enhance venous ulcer healing. Further clinical trial controlled by placebo, involving a greater number of patients, is required to confirm the findings of this case report.

**KEYWORDS:** Compression, nanotechnology, stockings, varicose ulcer, venous insufficiency

**RECEIVED:** May 22, 2019. **ACCEPTED:** May 24, 2019.

**TYPE:** Case Report

**FUNDING:** The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: We acknowledge funding from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq-MCTI/CNPQ N° 01/2016—Universal), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES—fellowships), and Fundação de Apoio à Pesquisa e a Inovação Tecnológica do Estado de Sergipe (FAPITEC-SE—fellowships), all from Brazil.

**DECLARATION OF CONFLICTING INTERESTS:** The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

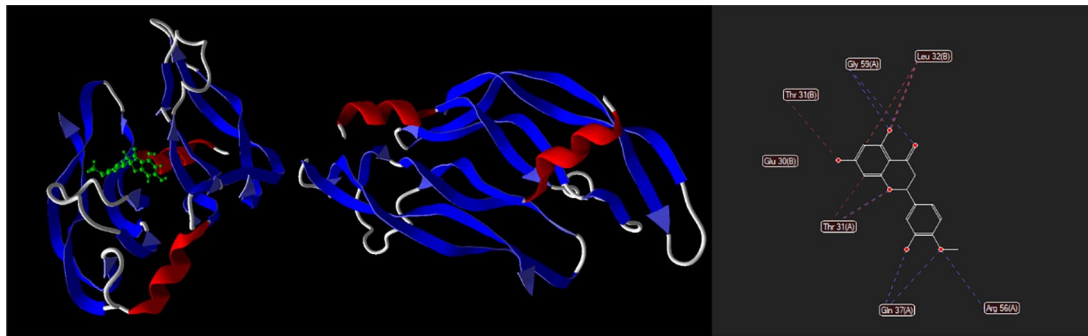
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## Introduction

Chronic venous insufficiency (CVI) is a debilitating and highly prevalent clinical condition that affects millions of individuals mainly in western countries.<sup>1,2</sup> This condition is attributed to several factors such as genetic predisposition and imbalance of protein synthesis (collagen type I and type III), caused by matrix metalloproteinases (MMPs), leading to an abnormal remodeling and distensibility of the veins associated with reduced elasticity.<sup>3</sup> Thus, the disease manifests with a variety of signs and symptoms that are caused by valve incompetence or reflux.<sup>4</sup> The CVI can result in varicose veins, edema, eczema, lipodermatosclerosis, or venous ulceration, the most advanced clinical manifestation of the disease, which is strongly associated with reflux.<sup>5</sup> Most hospital admissions in the United States are due to venous ulcers, occurring in approximately 20550 patients each year, which generate a treatment cost of US\$3 billion/year.<sup>6</sup> The first-choice clinical treatment of CVI is worldwide recognized as a compression stocking consisting of initial external pressures of 20 to 30 mm Hg.<sup>7</sup> Noteworthy, studies have shown the positive effect of flavonoids (as a

miconized purified flavonoid fraction (MPFF)—association of diosmin and hesperidin) on ulcer healing.<sup>8</sup> These therapeutic alternatives have been prescribed isolated, demonstrating low oral bioavailability and side effects including gastric disorders.<sup>9</sup> In light of these considerations, our research group has recently published a new and efficient method to impregnate hesperetin-based nanocapsules in textile and fiber for compression stocking fabrication. Our results clearly demonstrated the controlled drug release with subsequent permeation/penetration of these nanocapsules from textile to dermis while maintaining particles adhered to the textile during several washes.<sup>10</sup> This novel technology may be an excellent topical delivery route, with promising applications in the treatment of venous ulcers. Hesperetin is a natural product derived from hesperidin, a safe flavonoid that is commercially available, with antioxidant, anti-inflammatory, and vasoprotective properties already reported in the scientific literature, and shows promise in maintaining vascular health.<sup>11</sup> To understand the role of hesperetin in the ulcer healing process, we performed docking investigations between this drug and vascular endothelial





**Figure 1.** Docking results of hesperetin against VEGFR-2 membrane receptor. The flavonoid makes steric interactions with Thr 31 of chain A, and both Leu 32 and Thr 31 of chain B. Also, hydrogen bonds were made between Gly 59, Thr 31, Gln 37, and Arg of chain A. Arg indicates arginine; Gln, glutamine; Gly, glycine; Leu, leucine; Thr, threonine; VEGFR-2, vascular endothelial growth factor receptor 2.

growth factor receptor 2 (VEGFR-2), a receptor involved in the pathology of CVI (unpublished data). Noteworthy, hesperetin ( $-51$  kcal/mol) is theoretically able to bind with the receptor VEGFR-2, which is usually cleaved by MMPs in CVI, restoring the endothelial viability<sup>12</sup> (Figure 1). The drug interacts with the receptor preventing its degradation by MMPs and could contribute in the healing process. Therefore, this study reports the results of a patient with recurrent and unhealed ulcers of large extension, in which these innovative compression stockings are applied to simultaneously improve quality of life (QoL) and better understand the efficacy of this technology on venous ulcer healing.

### Case Report

The patient (EGJ; GTP8\_747231) was a 50-year-old man with a body mass index (BMI) of 34.1 and with symmetric unhealed venous ulcers in both legs, since 2014. The patient was evaluated by a vascular clinician in the Vascular Surgery Ambulatory of the School Hospital from Federal University of Sergipe, Brazil. The patient was previously treated with Unna boot, *Melilotus officinalis* L., and benzathine benzylpenicillin (6 months prior to the therapy described in this study), without any success. He then received and accepted (by consent form) the proposal to participate in this study (which was approved by the Ethics Committee on Human Research of the Federal University of Sergipe: CAAE—91428318.7.0000.5546, registered at Brazilian Register of Clinical Trials: RBR-5n9cmf under Universal Trial No. U1111-1217-7510). During the protocol, the patient used compressive stockings (3/4, 20–30 mm Hg) impregnated with hesperetin nanocapsules for 6 months (from January to June 2017), on top of wound dressings (without any pharmacologic agent), daily after waking up. Also, the patient washed his compressive stockings at least 5 times per month, due to the adhesive properties of this current technology.<sup>10</sup> Every month, we replaced his compressive stockings with other re-impregnated pairs.

This aim of this study was to quantitatively and qualitatively understand improvements in the patient QoL and to report any benefit on ulcer healing during the treatment. To achieve this, the patient answered the Aberdeen Varicose Vein Questionnaire

(AVVQ) and Visual Analog Scale (VAS), to determine QoL and pain perception, respectively, at baseline (T0), 3 months (T3), and 6 months (T6) of patient use of experimental compressive stockings. Then, his legs were evaluated through macroscopic register (Nikon D3400 digital camera). These pictures were processed on ImageJ software to measure the healing percentage through the retraction index of the lesion (centimeters), according to equation (1)

$$\text{Healing} = \frac{T0 - T6}{T0} \times 100 \quad (1)$$

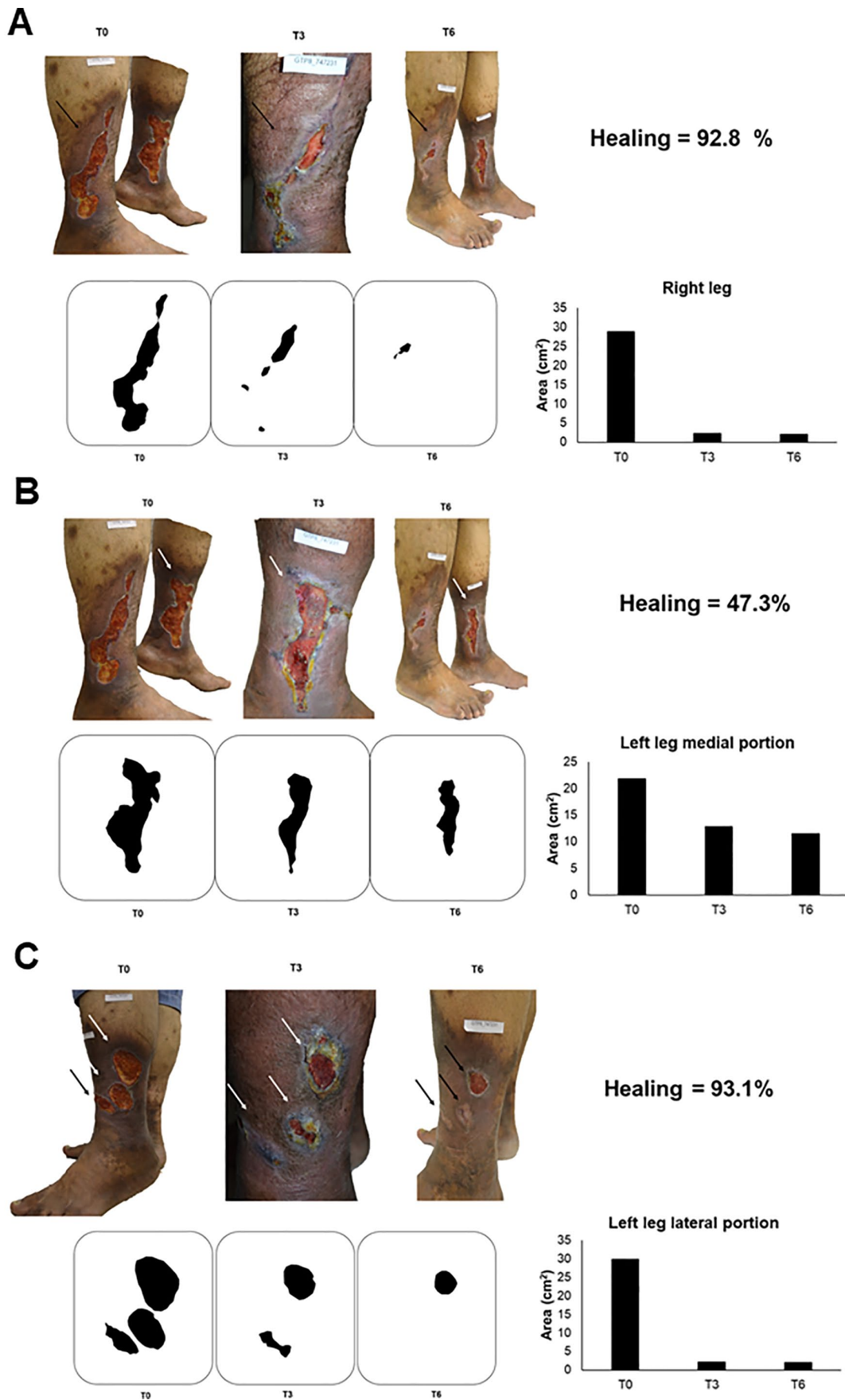
where Healing represents the retraction of the lesion (centimeters), T0 represents day 0, and T6 represents 6 months.

Skin parameters in the periulcer area were measured by Mexameter MX 18 (index of melanin and erythema) and Sebumeter SM 81 (hydration-sebum) (Courage and Khazaka, Cologne, Germany) probes at time points T0, T3, and T6. In addition, the venous diameters of different segments (arch, proximal, knee, and distal segments) of the great saphenous vein (GSV) were evaluated by duplex ultrasound linear probe (Toshiba Nemio XG) at 10 Hz. The examination began with the patient in a supine position where patency was initially tested followed by reflux maneuvers with Valsalva and proximal compressions.

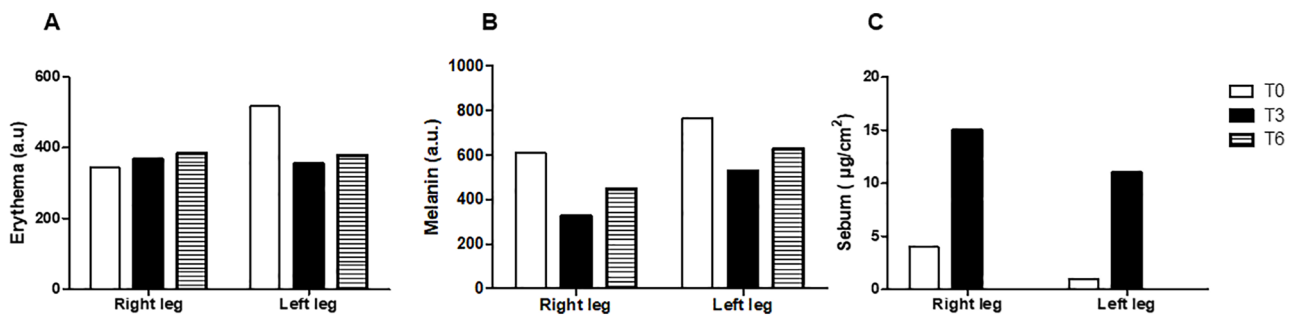
### Results and Discussion

We investigated a case of improvement in ulcer healing, whereas the patient was submitted for new therapy using compressive stockings impregnated with nanocapsules of hesperetin. Macroscopically, the healing process was observable after 3 months of treatment. And 6 months later, we observed a great percentage (92.8%; Figure 2A, and 93.1%; Figure 2C) of lesion area retraction in the superficial ulcers. However, in a deeper ulcer (Figure 2B), the lesion retracted 47.3% after 6 months of treatment.

The skin conditions on periulcer areas such as hyperpigmentation, dehydration, and erythema (Figure 3) are associated with disease's chronicity and nonhealing.<sup>13</sup> The skin pigmentation changes during the course of CVI become more severe as the disease advances. However, this mechanism is not



**Figure 2.** Macroscopic view of the healing process and quantification of lesion retraction: (A) right leg; (B) left leg medial portion; and (C) left leg lateral portion at time 0 (T0), after 3 months (T3), and after 6 months (T6).



**Figure 3.** Skin parameters in the periulcer area assessed using Mexameter MX 18 (A, B) and Sebometer SM 815 (C). The measurements were performed immediately after removing the dressing and cleansing the ulcer and surrounding skin area. All measurements were performed under standard room conditions (25°C, 55% RH), and 3 consecutive readings were taken at the same site. The results are given as mean values. RH indicates relative humidity.

**Table 1.** Skin parameters in the periulcer area assessed using Mexameter MX 18, Tewameter TM 300, and Sebometer SM 815 at time 0 (T0), after 3 months (T3), and after 6 months (T6).

TIME POINT (MONTHS)	RIGHT LEG			LEFT LEG		
	MELANIN (A.U.)	ERYTHEMA (A.U.)	SEBUM (µG/CM²)	MELANIN (A.U.)	ERYTHEMA (A.U.)	SEBUM (µG/CM²)
0	608	345	4	768	517	1
3	324	367	15	529	354	11
6	448	385	0	629	379	0

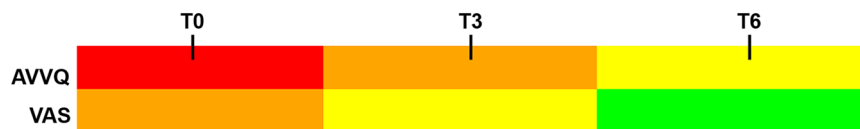
completely elucidated. The abnormality of melanin (dermal and epidermal) metabolism plays an important role in skin dyschromia.<sup>14</sup> In our study, we observed a reduction (Figure 3A) of detectable melanin in the first 3 months of treatment, which coincided with ulcerous healing (Figure 2). This was observed primarily in the right leg, which had less extensive lesions. In general, delayed and nonhealing venous ulcers have a high score of erythema or redness and dehydration due to associated inflammation and infection. We then observed a reduction in erythema (Table 1 and Figure 3B) and an increase in sebum scores (Table 1 and Figure 3C). Tsai et al<sup>15</sup> evaluated the in vivo efficacy of a microemulsion containing hesperetin and reported that the drug could be used as an effective whitening agent due to its antioxidant and anti-inflammatory properties. Together, these results seem to suggest better perfusion and restoration of skin barrier function, typical of the ulcer healing process (Figure 2).<sup>16</sup>

Recent reports have demonstrated the negative impact on QoL for patients with venous ulcers.<sup>17,18</sup> That variable has a notable personal impact, mainly due to intense pain, compromised sleep, difficulty walking, as well as impaired social and labor activities.<sup>17</sup> The AVVQ was validated in Brazil and has been used as an important instrument to measure the patient-related outcomes specifically for CVI interventions.<sup>19</sup> It is scored from 0 to 100, where 0 means the best QoL and 100 the most severe condition.<sup>20</sup> As can be observed from Figure 4, both AVVQ and VAS, the instruments widely used to measure pain intensity (0-10 scale of pain),<sup>21</sup> agree with the abovementioned results. An improvement was reported the patient's QoL (AVVQ score: 91.6-31.2) which was directly associated

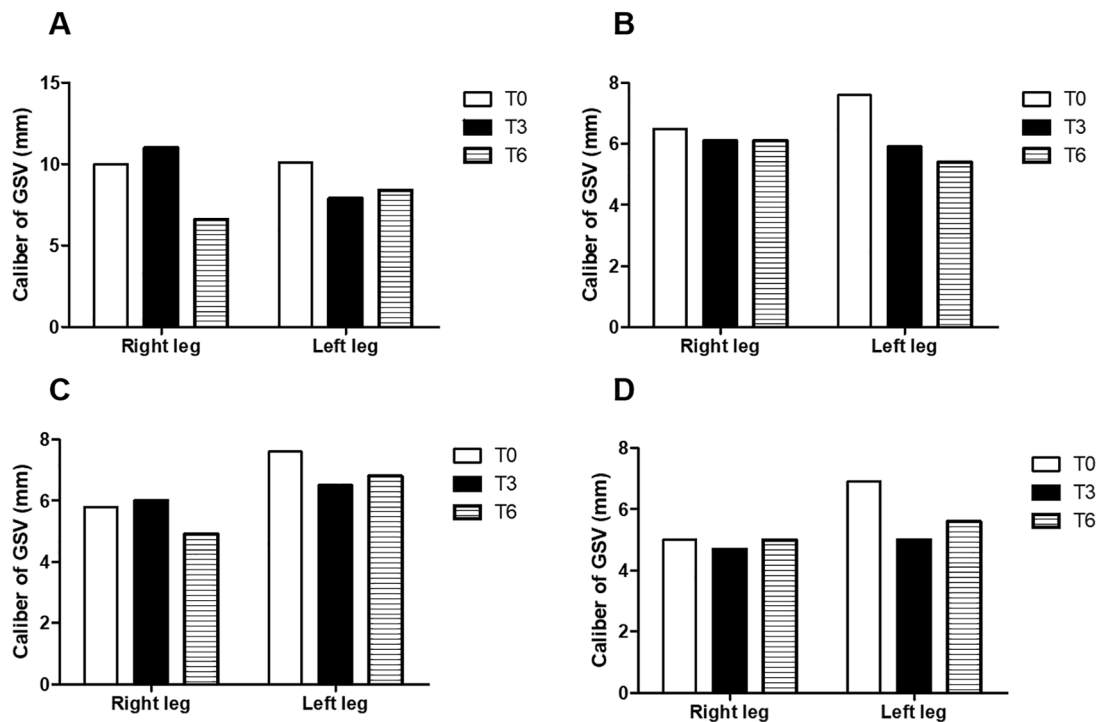
with the decrease of pain (VAS score: 7-0), probably resulting in ulcer healing.<sup>22</sup> Moreover, the patient did not report any gastrointestinal disorder or other adverse events.

Venous Doppler ultrasonography is the gold standard diagnostic modality for evaluation and monitoring response after the therapy of CVI.<sup>23</sup> The venous diameter of different segments from the GSV (superficial vein) observed in our study and shows a reduction (Figure 5) during the healing process. According to previous reports, the normal caliber limit of GSV is 4 mm.<sup>24,25</sup> Venous reflux in the lower extremities is a manifestation of a degenerative process in the venous wall, typical of this chronic disease.<sup>24</sup> Our findings highlight reflux in the distal portion of superficial veins in both legs, and the reflux was still present in the left leg after 3 months of treatment due to its greater compromised starting state.

In contrast to the Cochrane review, which did not indicate evidence about the ulcer healing with phlebotonics,<sup>26</sup> other studies including systematic reviews and meta-analyses supported effectiveness of MPFF, containing the precursors of hesperetin, in the venous ulcer healing process.<sup>27</sup> Coleridge-Smith et al<sup>28</sup> reported that the oral treatment with MPFF associated to standard compression and wound management accelerates venous leg ulcer healing in larger ulcers (5-10 cm<sup>2</sup>) of 6- to 12-month duration. However, there are no studies involving the topical application of these drugs, and the evidence of moderate quality already reported was related to the oral route, where the drug bioavailability is limited by enzymes such as  $\beta$ -glucosidase. Furthermore, hesperetin is recognized by its higher bioavailability compared with other phlebotonics, due to its better membrane interaction.<sup>29</sup> This case report is the first of its kind, giving us an insight



**Figure 4.** Scores of Aberdeen Varicose Vein questionnaire (AVVQ)—quality of life perception—and Visual Analog Scale (VAS) pain intensity, respectively, both from the patient's perspective, from baseline to final assessment. Note that the red color represents severe; orange, moderate; yellow, mild; and green represents none.



**Figure 5.** Reduction of great saphenous vein (GSV) diameter in the (A) arch, (B) proximal, (C) knee, and (D) distal segments associated without the observed reflux in the healing. T0 represents the beginning of the study; T3, evaluation after 3 months; and T6 represents evaluation after 6 months.

toward understanding the possible association of compression factor, which was already demonstrated in the literature, with the topical effects of hesperetin (vasoprotective, anti-inflammatory, and antioxidant) that could be observed together. These factors may contribute to improving the ulcer healing process, but we still need to deeply investigate the variables involved in this mechanism through robust clinical trials involving a greater number of patients.

#### Study limitations

This study provides an overall picture of a new method to manage venous ulcers. However, it is limited by the small sample size, which can weaken the power of conclusion.

#### Conclusions

To the best of our knowledge, this is the first report on a new compressive stocking impregnated with flavonoid-based nanocapsules, which indicated some benefits as an adjuvant for ulcer healing. All the evaluated parameters suggested that hesperetin delivery combined with compression improved the QoL as a possible result of ulcer healing and reduction of

venous hypertension. These clinical findings are expected in a successful treatment of the disease. However, there are opportunities for further research to elucidate the biological mechanism of the healing process, the way that the drug is acting (vasoprotective, anti-inflammatory, or antioxidant) and small molecules are involved in this process. Our research group is still working on a clinical trial involving a larger number of patients to answer these questions.

#### Acknowledgements

The authors thank Sigvaris-Brazil for donation of compression stockings. They also thank Prof Paulo Cesar de Lima Nogueira for the use of the rotaevaporator to obtain the nanocapsules and Prof Luciana Scotti for help on the docking analysis. In addition, they are grateful to Anthony Tahayeri for reviewing the paper for English language.

#### Author Contributions

PdPM conceived the idea, took the images, and wrote the paper. CVCG performed the clinical evaluation and conducted venous Doppler ultrasonography. YMBGdC impregnated the compressive stockings. NGLS and AMSO evaluated the skin

parameters. VMA applied the questionnaires. CMdL and AAdSA reviewed the paper.

### Informed Consent

The patient was informed about this research protocol and provided written consent to publish this case study.

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