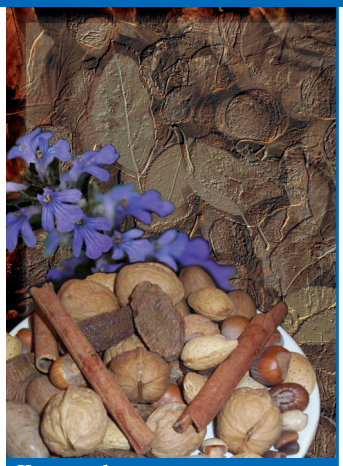

A preparation of procyanidol oligomers, triterpenes and anthocyanosides*

In Chronic Venous Insufficiency and Haemorrhoids

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Key words

Procyanidol Oligomers
Triterpenes
Anthocyanosides
Chronic Venous Insufficiency
Centella asiatica
Vitis vinifera
Vaccinium myrtillus

SUMMARY

Chronic Venous Insufficiency (CVI) of the lower limbs and/or causing haemorrhoidal disease, affects almost 50% of the population over 50. In this study, a time-dependent controlled-release, multilayered, solid preparation for oral administration containing procyanidol oligomers, anthocyanosides and terpenes from *Centella asiatica* was tested for clinical efficacy in CVI. 50 patients with a diagnosis of CVI were enrolled, 38 of whom also had chronic haemorrhoidal signs and symptoms. The patients showed a reduction of about 80% in the CVI and haemorrhoidal symptoms by 90 days and 45 days, respectively. The new preparation may be considered a safe and effective tool in terms of tolerance, compliance and side effects in CVI even when complicated by chronic haemorrhoidal disease.

INTRODUCTION

Among angiological diseases, disorders linked to conditions of peripheral chronic venous insufficiency (CVI) are extremely common, and the number of patients applying to an angiologist or their family doctors is ever increasing. Two of the most telling signs of CVI are varicose veins and haemorrhoids. The prevalence of these conditions is apparent from their number. Recent population studies mention an incidence amounting to 10-15% in males and 20-26% in females. Population studies that also consider the age-adjusted incidence parameter, report incidence rates between 48% and 58% in either sex, and in western populations, it has been estimated that about 50% of the over-50 usually resort to medicine or surgery for disorders linked to CVI (**1, 2**).

CVI is a pathological condition in which the venous system is typically unable to carry on its return circulation function. This disease is caused by a varicose condition or deep venous thrombosis. In either case, it becomes clinically apparent with the occurrence of tissue decompensation and typical stasis events. The latter may be distinguished between intravasal and extravasal events. This situation also includes the role played by mastocytes - quite numerous in perivasal tissues of limbs affected by CVI - which release mediators such as histamine and thus cause vasodilatation and increased permeability. These processes cause plasma extravasation with the consequent formation of an edema, which is the common denominator in CVI.

Increased permeability favours the increase in the concentration of fibrogen a high molecular weight protein unable to cross the vessel walls thus producing increased osmolarity. This, in turn, exacerbates microvascular hemorrheologic events and contributes to the onset of thrombotic disorders by promoting red blood cell stiffening (**3-5**).

Among the causes that bring about CVI, a major role is played by the trophic conditions of the connective framework; prolonged venous hypertension causes a state of suffering of the connective tissue in the venous wall and results in fibromuscular atrophy and connective sclerosis, which worsen venous hypertension with consequent anatomic/functional disorders. In this sense, varicose veins may both be the cause and consequence of a CVI condition. In fact, veins are rather delicate structures, and any defects in their walls may result in dilatations that may even damage the valves. When the valves are damaged, static pressure increases to the point of wearing out the structure, thus causing the onset of varices.

Finally, a hint to the lymphatic system, which is also frequently involved in CVI, especially when CVI events appear as a pathologic picture in the lower limbs. Some disorders affecting the venous tree may be the consequence of damage to lymphatic vessels, whose tasks also include tissue

Emospid®, slow-release preparation containing *Leucoselect*™, *Fitosoma*®, *Mirtoselect*® and *triterpenes selected* from *Centella asiatica* provided by *Indena* (MI), developed by *Velleja Research*, Pontenure (PC), manufactured by SIIT, Trezzano S/N (MI) and marketed by *PharmExtracta*, Pontenure (PC).

drainage and lymph conduction and transportation, and which may become "flooded" and run into small wall lesions that are often the chief cause of CVI-induced disorders (**6, 7**).
On the other hand, haemorrhoids are masses of areolar tissue in the form of small vascular pads, which in particular situations may undergo enlargement and prolapse and cause rather intense pain. When these areolar pads are located above the dentate line they are called internal haemorrhoids; on the contrary, when the areolar tissue dilatation occurs below the dentate line, they are called external haemorrhoids (**8**).
One of the most feared symptoms of haemorrhoids is undoubtedly pain; this is not caused, as it is mistakenly thought, by the constriction of the anal sphincter, but by a true vascular thrombosis caused by the closure of the vein through which blood flows from haemorrhoids. This closure prevents venous reflux, thus generating a greater increase in internal pressure and still more serious breakdown of the connective structures in the vessel walls (**9,10**).
In consideration of the etiological multifactoriality of CVI and its different pathological manifestations, a time-dependent controlled-release, multilayered, solid preparation for oral administration has been developed and its clinical efficacy was tested in CVI.

MATERIALS AND METHODS

The tested product

The preparation (Emospid®, developed by *Velleja Research*, Pontenure (PC), Italy and manufactured by *SIIT*, Trezzano S/N (MI), Italy) consists of a three-layered tablet with each layer containing an active ingredient and excipients required to ensure the desired time-dependent release. The first layer, normal-release, which dissolves in about 45 min, contains 100 mg *Leucoselect™ Fitosoma®* (Indena, Milan, Italy). This is a highly standardised (100%) mix of procyanidol oligomers (PCO) whose molecular weight falls between the catechin monomer and the catechin heptameter esterified with 3 residues of gallic acid. The PCO mix obtained from *Vitis vinifera* seeds is complexed with soybean (*Glycine max*) phospholipids (forming the so called Leucoselect Phytosome) to optimise its oral bioavailability. The second layer, controlled-release, which dissolves in about 8 h, contains 30 mg selected and purified triterpenes obtained from aerial part of *Centella asiatica* (*asiatic acid: madecassic acid: asiaticoside* in a 30:30:40 ratio) (Indena, Milan, Italy).
The third layer, normal-release, which dissolves in about 45 min, contains 80 mg of *Mirtoselect®* (*Indena*, Milan, Italy), a highly standardised (36%) mix of 15 anthocyanosides, which are in turn the result of different glycosidations of 5 anthocyanins extracted from *Vaccinium myrtillus* fruits.

Clinical Study

Between June, 2007 and November, 2008, 50 individuals of either sex (36 females and 14 males), aged between 37 and 60 years, with diagnosed CVI were selected. Thirty-eight of the subjects also presented with first or second-degree chronic haemorrhoids. All were enrolled, upon written consent, to ascertain the anti-symptomatic efficacy of the slow release botanical preparation (SRBP) (Emospid®). The subjects were all healthy (except for the diagnosed CVI and haemorrhoids) and had not been under any treatment for at least one month the trial and did not take any preparations (whether drugs or supplements) other than the test preparation. The planned treatment duration was 3 months. The subjects took 3 tablets a day for the first 30 days and 2 tablets a day for the following days (60 days for CVI and 15 days for haemorrhoids).
The exclusion criteria from enrolment were as follows: age not included between 18 and 70 years, renal failure; ascertained congestive heart failure; and a concomitant other drug therapy. Symptoms were assessed using a visual analogue scale, according to the Scott-Huskisson model, which goes from 0 to 10 with the assigned values rising with the symptom severity. The symptoms considered for CVI events in the lower limbs were as follows: pain, heaviness, itch, heat, motor restlessness, night cramps and evening sub-oedema. Ankle circumference was the objective measurement. The symptoms considered to assess concomitant haemorrhoids were itch, pain and a sensation of heaviness. Tolerance, therapeutic compliance and the onset of any side effects were also assessed in the patients. Prior to each measurement, the clinicians made sure that the patient had not been standing for more than 1 h or had not been sitting for more than 2 h. Otherwise, the individual was asked to walk for at least 15 min before the parameters were measured.
Events in the lower limbs were assessed at t = 0, 45 and 90 days; the symptoms linked to the presence of haemorrhoids were assessed at t = 0 and 45 days.
Statistical analysis was performed using the unpaired t test.

RESULTS

As seen in ***Table 1***, the symptoms linked to CVI of the lower limbs decreased considerably already after 45 days of treatment. The subjective symptoms were reduced by 40-80%, depending on the parameter considered. Ankle circumference showed a good (albeit non-significant) decrease as early as the 45th day. Even greater evidence of progressive symptom reduction was obtained after 90 days, when reduction in ankle circumference reached statistical significance.
The symptoms of the haemorrhoid condition (***Table 2***) were also significantly reduced to lower clinical values already after 45 treatment days. In addition, according to the evaluation given by both the patient and clinicians in charge (**Table 3**), treatment was well tolerated (in a scale from 'bad' to 'excellent', tolerance was assessed as 'excellent' in 80% of the cases; therapeutic compliance was satisfactory (in the same scale, compliance was assessed as 'good' in 50% of the cases and 'excellent' in the remaining 50%); with an absence of side effects apart from gastric burning (3 cases), headache (5 cases) and nausea (1 case) reported during the 90-day study where anyway no interruption of treatment or drop-outs was needed.

CONCLUSIONS

The preparation used in this pilot study is a product developed in multilayer tablets, containing highly standardised derivatives of *Vitis vinifera* seeds, *Centella asiatica* and *Vaccinium myrtillus*. The individual preparations - all of them obtained through extraction - correspond chemically to some proprietary medicines, but in some cases differ from the latter in the food grade of their manufacturing process.

This multilayer and multicomponent preparation was developed on a galenical basis in an attempt both to optimise the oral bioavailability of its active ingredients (through a phytosome process) and to obtain plasma rates that could be kept as constant as possible in a 24-h time span (controlled-release forms to 8 h).

Table 1 *Clinical efficacy of SRBP on CVI symptoms of the lower limbs over time (days)*

	CVI Score (days)		
Symptom	***0***	***45***	***90***
Pain	6.6 ± 1.4	1.3 ± 0.7°	0.2 ± 0.1*
Heaviness	7.8 ± 2.1	1.6 ± 0.6°	0.3 ± 0.3*
Itch/Heat	5.5 ± 1.2	0.8 ± 0.4°	0.6 ± 0.4*
Motor restlessness	7.5 ± 2.0	2.6 ± 1.1°	0.5 ± 0.1*
Night cramps	3.6 ± 0.9	1.5 ± 0.8°	0.5 ± 0.3*
Oedema	3.5 ± 0.8	0.9 ± 0.4°	0.6 ± 0.3*
Ankle circumference (cm)	26.8 ± 3.4	24.5 ± 2.5	23.0 ± 2.2°

CVI scores (mean scores ± SD; n=50) were determined by the Scott-Huskisson visual analogue scale (1-10 as the expression of the symptom seriousness), with the exception of the measurement of ankle circumference;° $p<0.05$ and * $p<0.01$ *vs* day 0 Statistical analysis was performed using the unpaired t test.

Table 2 *Clinical efficacy of SRBP on symptoms related to the presence of haemorrhoids*

	Symptom Score (days)	
Symptom	***t=0***	***t=45***
Pain	8.6 ± 1.8	0.3 ± 0.2*
Heaviness	7.5 ± 2.1	0.2 ± 0.2*
Itch/Heat	4.5 ± 1.6	0*

Symptom scores (mean scores ± SD; n=38) were determined by the Scott-Huskisson visual analogue scale (1-10 as the expression of the symptom seriousness); *$p<0.01$ vs day0

Table 3 *Tolerancey, compliance, side effects and drop-out during treatment with SRBP*

Tolerance	Excellent (39)	Good (11)	Bad (0)
Compliance	Excellent (25)	Good (25)	Bad (0)
Side effects	Gastric pain (3)	Headache (5)	Nausea (1)
Drop-outs	(0)		

In brackets, No of patients

Finally, the choice - in terms of quality - of the three active ingredients was suggested by the abundant available documentation on the preparations and the different capabilities exhibited by these ingredients in terms of their mechanisms of action.

The above clinical data - albeit highly preliminary inasmuch as they were assessed on a modest sample size (n=50) - highlights the qualities of the product. In some ways these qualities confirm those expected on the basis of the well-known properties of the individual active ingredients found in the formulation. The preparation is substantially effective in reducing the symptoms of disorders linked to diagnosed CVI whether occurring in the lower limbs or perineal area.

In the light of these results, other clinical studies are being planned which will test this preparation in comparison with chemically different standard products (for example, diosmin) on larger sample sizes, in double blind, randomized conditions and on subjects on concomitant therapy (heparinoids, anti-hypertensives, etc.) and on individuals diagnosed with acute haemorrhoidal crisis.

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