



ANTIOXIDANT HERBAL SUPPLEMENTS FOR HEMORRHOIDS

Developing a new formula

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INTRODUCTION

Haemorrhoids are one of the most common gastrointestinal disorders seen by general practitioners. It has been estimated that they can occur at any age and can affect both women and men. The natural evolution of haemorrhoids is benign but haemorrhoids tend to get worse over time, and disease should be treated as soon as it occurs (1). The best treatment is always prevention (2), i.e. despite the scarcity of clinical evidence, a diet high in fibre and bulk is considered the basis of both prevention and treatment of haemorrhoids. Several clinical studies report the beneficial effects of oral treatment with *Aesculus hippocastanum*, *Ruscus aculeatus*, *Centella asiatica*, *Hamamelis virginiana*, and bioflavonoids to reduce symptoms associated with hemorrhoids. The main effects of the orally administered herbal treatments are: influence of vessel-wall tone, decrease of capillary permeability, circulation improvement, decrease of oedema and blockage of inflammatory mediators. Recent findings have proven contribution of free radicals to the pathogenesis of varicose veins and hemorrhoids, the latter may be considered as varicose veins that occur around the rectum, suggesting that the administration of safe antioxidants (vitamin C and E, flavonoids) may have beneficial outcomes (3,4). Pavon et al (5) have very recently demonstrated that oxidative stress in patients with varicocele or subclinical varicocele

was greater than in healthy subjects. Etiologically, varicocele and haemorrhoids are related, because they are both genital manifestations of a pelvic venous disease (6).

On the basis of these considerations, we report the rational approach sustaining the development of the new herbal multi-component formula developed by us, and characterized by antioxidant ingredients, such are tocotrienols, bioflavonoids and ascorbic acid, whose properties we widely investigated in previous studies (7-9).

The herbal formula was administered as soft gel capsules that contained a *tocotrienols enriched extract* (70% titred in tocotrienols), *Rosa canina* (70% titred in ascorbic acid), *Ginkgo biloba* (20%, titred in flavonoids), *Ruscus aculeatus*, and *Centella asiatica* (10%, titred in asiaticoside), *Aesculus ippocastanum* (20%, titred in escin) and *Silybum marianum* (30%, titred in silybin) (Table 1). The goal of this botanical formula were to reduce the pain, anal discomfort, soiling and bleeding present in acute phase.

Key words

Hemorrhoids

Antioxidant capacity

Tocotrienols

Photochemiluminescence

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To the best of our knowledge, the efficacy of an oral supplementation with synergistic antioxidants, in particular based on vitamin E-related compounds, was never evaluated on haemorrhoidal symptoms. In view of the well-established relationship between the aetiology of a plethora of diseases and reactive oxygen species (ROS), and continuing our studies on claim substantiation of antioxidant supplements, we have carried out the present work to verify the antioxidant capacity of the herbal formula before submitting the product, to the clinical trial. The antioxidant capacity of the finished product was tested by means of photochemiluminescence (PCL). These latter results will be reported in details together with the preliminary considerations emerging from a double-blind, placebo controlled clinical study, conducted to evaluate the efficacy and safety of this herbal supplement.

Specific botanicals for prevention and treatment of hemorrhoids

A major approach to a safe and effective treatment for both varicose veins and hemorrhoids is represented by the use of botanical products and nutrients. Thus, different ingredients have been selected on the basis of literature and clinical evidence supporting properties useful in the treatment and prevention of haemorrhoids, such as antioxidant, anti-inflammatory, anti-edema and hepatoprotective. The synergistic antioxidant phytoteraphic mixture included tocotrienols, bioflavonoids, polyphenols, and ascorbic acid. The formula (placebo and treatment) was delivered into soft-gel capsules whose composition is described in *Table 1*. The properties of each component included in the above-mentioned formula are briefly described in *Appendix 1*.

Table 1 Formula of tested soft-gel capsules

INGREDIENTS	Treatment mg/caps	Placebo mg/caps
<i>Aesculus hippocastanum</i> d.e. (20% escin)	100	
Tocotrienols	50	
<i>Centella asiatica</i> d.e (10% asiaticosides)	40	
<i>Ruscus aculeatus</i> d.e 20%	20	
Sterolic Heterosides 20%		
<i>Ginkgo biloba</i> d.e. (25% flavonoids)	40	
<i>Rosa canina</i> (70% ascorbic acid)	20	
<i>Silybum marianum</i> 70% (30% silybin)	150	
Soybean oil	440	440
Glycerylmonostearate	30	30
Lecithin	19	19

Appendix 1

PROPERTIES OF THE BOTANICALS USED IN THE FORMULA

TOCOTRIENOLS

Tocotrienols are found in high concentrations in palm oil and rice bran oil. Enriched extracts have been extensively investigated for their nutritional and health properties, including antioxidant activity, cholesterol lowering, anti-cancer effects and protection against atherosclerosis (10,12). Collectively known as vitamin E, tocotrienols are identical in structure to tocopherols except for the degree of saturation in their side chain. It is of particular interest that the slight structural differences between tocopherol and tocotrienol can account for the greater and unique physiological activities found in tocotrienol. Indeed, vitamin-E related compounds are well known for their antioxidant properties (13). A number of mechanisms were shown to contribute to their higher antioxidant activity, as compared to α -tocopherol itself, including: a) a more uniform distribution in the membrane lipid bilayer, (14) b) a more efficient interaction of the chromanyl ring with lipid radicals, and c) a higher recycling efficiency from chromanoxyl radicals (15). The evidence that tocotrienol has superior antioxidant activity may have important clinical implication, and to date, to the best of our knowledge, its benefits have never been investigated on venous related pathology, such as haemorrhoids. The dosage of tocotrienols present in the developed formula is 50-100 mg/die, which is considered effective and safe (16,17).

Table 2 Grade of prolapse severity before treatment
The percentage refers to the frequency of cases for each grade monitored at the beginning of the clinical trial

Grade of Prolapse	Placebo (%)	Treatment (%)
I (No prolapse)	0	0
II (Spontaneous reduction)	55	46
III (Manual reduction)	35	45
IV (Incarcerated, reduction impossible)	10	9
TOTAL	100	100

Table 3 Grade of prolapse severity after treatment
The percentage refers to the frequency of the cases for each grade monitored at the end of the clinical trial $p=0.00854$

Grade of Prolapse	Placebo (%)	Treated (%)
I (No prolapse)	17.6	68.8
II (Spontaneous reduction)	52.9	18.8
III (Manual reduction)	23.5	9.4
IV (Incarcerated reduction impossible)	5.9	3.1
TOTAL	100	100

GINGKO BILOBA

Bioflavonoids, oligomeric proanthocyanidin complexes (OPCs), and hesperidin, are potent antioxidants and have demonstrated efficacy in the treatment of haemorrhoids and varicose veins.

These bioflavonoids exhibit phlebotonic activity, vasculoprotective effects, and antagonism of the biochemical mediators of inflammation. OPCs, diosmin, and hesperidin have been the subject of numerous clinical trials on efficacy and safety in the treatment of varicose veins and haemorrhoids (18).

Several randomized controlled studies have established their efficacy in the treatment of varicose veins and haemorrhoids. The safety of these flavonoids has been established through animal studies, and confirmed clinically in long-term trials. Ginkgo is generally well tolerated, but it can increase the risk of bleeding if used in combination with warfarin, antiplatelet agents, and certain other herbal medications (19).

Moreover, taking into account the reported possible effects in the onset of infant leukaemia, Ginkgo is contraindicated while trying to get pregnant and during pregnancy and lactation (20). The dosage suggested of *Ginkgo biloba* titred extract is 40-80 mg, and literature data confirm that this quantity is well tolerated (21) and has no contraindications even if used at higher dosage (22).

ROSA CANINA

The synergistic antioxidant phyto-therapeutic mixture was completed with *Rosa canina* fruits extract, one of the principal medicinal plants, well known to contain a large amount of vitamin C, which together with tocotrienols, polyphenolic compounds and Silymarin, contained in the other herbal extract here considered,

is responsible of the antioxidant effects of the present finished product (23).

Moreover, the anti-inflammatory properties of rose hip herbal remedies has been clinically observed. Rose hip has a higher proportion of vitamin C than any other commonly available fruit or vegetable, and extracts are sometimes also enriched in this vitamin to increase efficacy. The extract included in this formula is titred in ascorbic acid (70%). The safety of this extract is quite high.

CENTELLA ASIATICA

Centella asiatica is a tropical medicinal plant with a long history of therapeutic use. An important active constituent of *Centella asiatica*, asiaticoside, was isolated and purified in 1940 and the first systematic clinical studies were carried out in 1945.

Centella preparations are titrated for the pentacyclic triterpene derivatives asiatic acid, madecassic acid, and asiaticoside (24). Rigorous clinical investigation on *Centella asiatica* has been conducted on chronic venous insufficiency and varicose veins (25,26).

The *Centella* extract was shown to reduce serum levels of lysosomal enzymes involved in the degradation of mucopolysaccharides.

AESCULUS HIPPOCASTANUM

Horse chestnut seed extracts (HCSE) are used clinically to relieve the subjective symptoms and reduce the objective signs of chronic venous insufficiency (27,28). The active component of the extract is thought to be the triterpenic saponin, aescin. The pharmacological profile of aescin has received significant contributions in recent years. At least four types of pharmacodynamic actions have been attributed to aescin: i) anti-oedematous properties; ii) anti-inflammatory activities; iii) veno-tonic properties; iv) free-radical scavenging properties. These properties make HCSE useful for the treatment of both varicose veins and hemorrhoids. The formula considered has an daily aescin intake of 40 mg. A recently conducted study to assess the safety and tolerability of *Aesculus hippocastanum* with 50 mg *Aesculus hippocastanum* tablet, twice daily showed it to be safe and well-tolerated (29).

***RUSCUS ACULEATUS* (BUTCHER'S BROOM)**

Extract of *Ruscus aculeatus* is effective in increasing venous tone because of its anti-inflammatory and astringent properties. The active biochemical constituent is proposed to be the saponin glycoside ruscogenin (30). Herbalists of various cultures have historically used *Ruscus aculeatus* for the treatment of varicose veins and hemorrhoids. There is an increasing body of scientific literature and clinical evidence to support these traditional folk medicine uses (31,32). Butcher's broom has been shown to have a significant effect on patients with hemorrhoids in an open trial (33).

Ruscus extract has also shown *in vivo* inhibition of elastase, part of the enzyme system involved in degrading perivascular structural components (34). Several clinical trials have highlighted the safety and efficacy of ruscus aculeatus extracts, on maintaining venous tone and improving venous emptying in comparison to placebo-treated patients (32).

***SILYBUM MARIANUM* (MILK THISTLE)**

Silybum marianum is currently the most well studied plant in the treatment of liver disease (35). The relationship between liver functionality and haemorrhoids is well established, and, in order to promote

hepatic functionality, this latter ingredient has been included in the formula (36). The active constituents of milk thistle are flavonolignans including silybin, silydianin, and silychristine, collectively known as silymarin. Silybin is the component with the greatest degree of biological activity, and milk thistle extracts are usually standardized to contain 70-80 percent silybin. Silymarin is found in the entire plant but is concentrated in the fruit and seeds. Silybum seeds also contain betaine (a proven hepatoprotector) and essential fatty acids, which may contribute to silymarin's anti-inflammatory effects. The mechanisms which provide silymarin's hepatoprotective effects are many and varied, and include antioxidation, (37) anti-lipid peroxidation, enhanced detoxification (38), and protection against glutathione depletion. Silymarin has a good safety record and only rare case reports of gastrointestinal disturbances and allergic skin rashes have been published (39).

MATERIALS AND METHODS

Determination of antioxidant capacity

The photochemiluminescence (PCL) assay was used because it presents some advantages over other methods (TEAC I-III, TRAP, DPPH, DMPD) measuring antioxidant activity: it does not require high temperatures to generate radicals and it is more sensitive in measuring, in few minutes, the scavenging activity of antioxidants against the superoxide radical ($O_2^{\bullet-}$), one of the most dangerous reactive oxygen species (ROS) occurring in the human body. (Please see 40 for further details). The PCL method was carried out as described by

Popov and Lewin (41) using the ACL kit (AnalytikJena) which measures lipid soluble antioxidants (i.e. tocopherols, tocotrienols, carotenoids, etc.) and several water soluble antioxidants: 2.3 ml of Reagent 1 (sample solvent and detection reagent), 200 μ l Reagent 2 (buffer solution, pH 10.5), 25 μ l Reagent 3 (Photo sensitizer and detection reagent) and sample solution, mixed and measured. In this manner, the conditions were standardised to give comparable results. ACL calibration and measurements were performed according to the standard kit protocol. The measurements were done by Photochem®.

The *in vitro* antioxidant efficacy of the finished product was comparatively evaluated, by means of a photochemiluminescence method (PCL), on treatment vs placebo, composed of soybean oil. The antioxidant capacity is reported as nmol equivalents of Trolox per gram of product under examination. Because each capsule weighed about 1 gram, the activity may also be referred to nmol equivalents of Trolox per capsule.

Figure 1 shows that the soft gel capsules containing the herbal formula exhibited an antioxidant capacity of 132 nmol equivalent of Trolox per capsule while that of the placebo was practically nil. It may be considered that the antioxidant property of this complex herbal product is attributable to the different phytoextracts acting synergistically.

Sample preparation

The softgel capsules (placebo and finished capsules) were dissolved and extracted in 10 ml of methanol. The resulting solutions were centrifuged for 10 min. Samples were diluted (1:100) with R1 solution (ACL-kit, Analytik Jena). Measurements were accomplished using 10 μ l volumes of the sample. Samples were taken in duplicate and measurement were repeated three times.

Ingredients

Ingredients were supplied by Polichimica, Bologna Italy and Giellepi Chemicals, Milano. Soft gel capsules were prepared by Gelfipharma International, Lodi Italy.

Design of the study

The clinical efficacy, compliance and safety of the multi-herbal formula was investigated in a double-blind randomised placebo controlled clinical study, carried out on hospital outpatients with several degrees of haemorrhoids.

The study involved 96 well-documented adult patients, with first-fourth degree of haemorrhoids. Both symptoms and haemorrhoids degree were monitored at T0 and two weeks after treatment (T2).

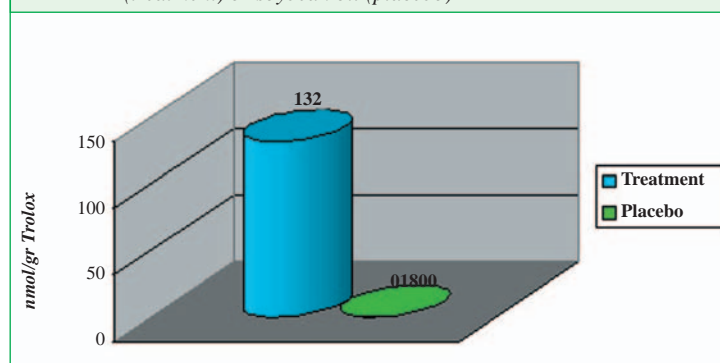
The dosage of dietary supplements was 1 soft gel capsule twice a day for the first 2 weeks. If the treatment was successful, the treatment was stopped.

If the clinical signs or symptoms still persisted, the treatment was continued for another two weeks using the same dosage and re-evaluated at the end of the fourth week after initial treatment.

Safety and efficacy

The parameters for efficacy were symptoms (pain, soiling, bleeding, exudation and pruritus) and the objective signs on proctoscopy (bleeding, inflammation and dilatation of the haemorrhoidal venous plexus).

Figure 1 Antioxidant capacity of softgel capsules tested in the clinical trial. The capsules contained the finished herbal formula (treatment) or soybean oil (placebo)



RESULTS

The preliminary results of the study, accounts for a significant effect for the tested product compared to placebo, and the treatment was well accepted and safe. The study revealed improvement of symptoms in the study group which was better than in the control group after 2 weeks of treatment, but the clinical signs were not different. After a further 2 weeks of treatment, the result showed improvement of both clinical signs and symptoms in this study. The main result was the reduction of severity of haemorrhoids. Most patients (90%) included in the trial presented grade 2 and grade 3 haemorrhoids, and after 4 weeks the treatment revealed a decrease of the degrees of haemorrhoids in comparison to the placebo group (Pearson's test, $p=0.00854$).

As reported in the Tables 2 and 3, before the treatment, 0% of patients were included in the first degree group, while after the treatment the efficacy was demonstrated by a significant increase of 68.8% of cases that shifted to first degree, judged to be less severe because of the absence of prolapse. A logistic regression was constructed to investigate the relation between the dependent variable, such as the decrease in the degree of haemorrhoids, and the independent covariates, such as sex, age and treatment. The analysis showed a decrease in the degree of haemorrhoids to be statistically significant for the treatment group (Odd Ratio (OD)=16.58, $p=0.0015$).

The efficacy of the studied formula was expressed by the OD value of 16.58 which indicates the probability of having a decrease of haemorrhoid degree in comparison to placebo group.

DISCUSSION AND CONCLUSIONS

A specific nutraceutical formula for the treatment of haemorrhoids was designed taking into account the potential role of oxidative processes in the onset and development of the disease. Herbal ingredients were selected considering: 1, their different complementary antioxidant activities with respect to the mechanisms and oxidative species encountered; 2, their synergistic positive effects deriving from the different complementary physiological activities involved with the onset and progression of the pathology. Tocotrienols and tocopherols, among the most important antioxidants in lipids, act by at least two different mechanisms: as direct ROS scavengers (i.e alkoxy radicals, lipid peroxy radicals and alkyl radicals derived from PUFA oxidation) and as up-regulators of antioxidant enzymes (tocopherols). Vitamin C, the most important antioxidant present in hydrophilic compartments, mainly acts by scavenging ROS directly, and among these species probably the most important are superoxide and peroxynitrite. Moreover, it is well-known that ascorbate also acts, in a cooperative manner, by recycling tocopheroxyl and tocotrienoxyl radicals (TO^{\bullet}), restoring them to their reduced and active forms.

The polyphenolic structure of bioflavonoids confer on them the ability to either scavenge free radicals or chelate transition metals, a basis for their potent antioxidant abilities. The forecasted antioxidant properties of the preparation were analyzed in vitro by means of PCL, using Trolox as a reference compound, in comparison to placebo and the capsules were than administered to a panel of selected patients. The preliminary considerations emerging from the clinical study indicate that the rationale behind the formula was correct and that the properties of the selected extracts are most probably responsible for the observed effects, thus encouraging further studies to assess the possible extension of the approach to other antioxidant ingredients.

SUMMARY

Hemorrhoids are one of the most common gastrointestinal disorders.

The uncomfortable nature of treatment options often leads a patient to postpone evaluation until invasive intervention is necessary. Thus, the oral supplementation is an attractive strategy to the tra-

ditional treatment/prevention of haemorrhoids. Several clinical studies report the beneficial effects of oral supplementation with *Aesculus hippocastanum*, *Ruscus aculeatus*, *Centella asiatica*, *Hamamelis virginiana*, and bioflavonoids to reduce symptoms associated with hemorrhoids. On the basis of recent findings suggesting that administration of safe antioxidants (vitamin C and E, flavonoids) may have beneficial effects, the present study was devised with the aim to develop herbal supplements for the treatment of symptomatology related to hemorrhoids based on antioxidant ingredients, such as tocotrienols, bioflavonoids, ascorbic acid and other synergistic phytoextracts.

The antioxidant capacity of the formula was tested by photochemiluminescence, and *in vivo* efficacy and safety were investigated in a double-blind, placebo controlled-clinical trial of 96 patients. The results showed improvement in the overall uncomfortable symptoms and a marked improvement in the severity of prolapse.

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