DIOSVEIN™ brand of DIOSMIN (CAS 520-27-4)

The following new dietary ingredient submission for diosmin (brand name: Diosvein™) is submitted to the FDA by Exquim,S.A. Pursuant to 21 C.F.R. § 190.6, Exquim,S.A provides the following information:

Address:	Edifici L'Illa Av. Diagonal, 549 5th floor 08029 Barcelona, SPAIN
Name of New Dietary Ingredient:	Diosmin (brand name: Diosvein™)
Recommended Conditions of Use:	500 mg/day Maximum recommended duration of use: 3 months. Not recommended for use by children or pregnant or nursing women.
History of Use Establishing Safety:	See detailed discussions below.

Basis for Concluding Diosvein™ is Reasonably Expected to be Safe

This document presents a New Dietary Ingredient (NDI) submission for Exquim,S.A (hereinafter referred as Exquim) Diosvein™ (hereinafter referred as Diosvein).

This product, which is a bioflavanoid derivative of the food ingredient Hesperidin, is the same as products which have been marketed worldwide, and the subject of studies since at least 1971. Specific evidence of marketing in the United States prior to 1994, however, has not been found. Accordingly, notwithstanding the strong presumption based on the marketing and literature data described herein that such marketing in the United States occurred, pursuant to Section 413(a)(2), Exquim,S.A makes this 75-day pre-market notification to demonstrate the safety of Diosmin and hence, its acceptability for marketing.

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In addition, this product is the same in formulation as another diosmin product previously submitted to FDA in a 75-day notification without FDA objection, and which is therefore on the market in the United States.

In April 2000, Nutratech, Inc. (Nutratech) submitted an NDI to the Food and Drug Administration for its Diosmin Complex, a formulation consisting of —, Diosmin and — Hesperidin. Following receipt of a response letter from the Agency, Nutratech resubmitted its NDI with additional data to support!a finding that Diosmin Complex was reasonably expected to be safe. This NDI was resubmitted in August 2000 without comment from the FDA and, to our knowledge, Nutratech's —— Diosmin Complex has been subsequently marketed in the United States.

Diosvein is reasonably expected to be safe because it is virtually identical to the Diosmin present in Nutratech's —— Diosmin Complex, which the Agency has already reviewed. Minor differences between the two formulations indicate that Diosvein should have at least as favourable a safety profile as the Nutratech 90/10 Diosmin Complex the FDA already reviewed. In addition, Diosvein complies with the specification established for Diosmin in the European Pharmacopoeia.

Exquim's product consists of a minimum of —— and a maximum of —— of Diosmin, being the content of Hesperidin — maximum. Furthermore, Diosvein is recommended for the same three-month duration of use as the Nutratech formulation the Agency already reviewed. Moreover, as Nutratech's formulation, Diosvein is not intended for use by children or pregnant or nursing women and will be so labelled.

Similarly, the proposed daily dosage for Diosvein is entirely consistent with the dosage of the safety studies presented in Nutratech —— Diosmin formulation submission. Specifically, Exquim's proposed daily dosage for its Diosvein is which is identical to Nutratech's daily dose, being the daily dose used in Europe 1000 mg. Both dosages levels are well within the levels tested repeatedly in the studies that the Agency has already reviewed as part of Nutratech's submission. In fact, as noted herein, most of the over twenty clinical trials addressing the safety of Diosmin-related complexes were conducted on formulations containing a minimum daily dose of 1,000 mg Diosmin Complex, being the maximum daily dose in some of the safety studies 3,000 mg (see table 2 entitled "Diosmin Dose and Safety" attached in this dossier). In addition, the safety of Diosmin is also demonstrated by the millions of tablets that have been sold and ingested worldwide since 1971 (see table 1 entitled "History of Use" attached in this dossier).

- This NDI submission contains the same comprehensive studies that the FDA has already reviewed in the context of Nutratech's 2000 NDI submission. These include clinical trials, animal studies and *in vitro* studies, which have demonstrated that diosmin complex formulations have an excellent safety profile at dosages ———— and for durations of use as long as 1 year[21].

In addition to the studies contained in the Nutratech —— Diosmin Complex NDI submission, this NDI submission for Diosvein also contains additional studies

conducted after the Nutratech submission was filed that further support!the safety profile of Diosvein. All this information clearly supports the favourable safety profile of this ingredient.

Based upon the extensive similarities to the Nutratech —— NDI submission the FDA has already reviewed, as well as the additional information Exquim has provided in support!of ingredient safety, the Agency should find that the safety profile of Diosvein is equivalent to that of the —— Diosmin Complex already reviewed by the FDA. The FDA should, therefore, find that Diosvein is reasonably expected to be safe under the conditions of use recommended or suggested in its labelling.

INTRODUCTION

Diosmin is a naturally occurring flavonoid glycoside that can be isolated from various plant sources or derived from the flavonoid hesperidin[1]. Diosmin was first isolated in 1925 from *Scrophularia nodosa*, and first introduced as a therapeutic agent in 1969. Diosmin is considered to be a vascular-protecting agent used to help improving chronic venous insufficiency (CVI), haemorrhoids, lymphedema, and varicose veins. As a flavonoid, diosmin also exhibits anti-inflammatory, free-radical scavenging, and antimutagenic properties.
Diosmin differs molecularly from hesperidin by the presence of a double bond between two carbon atoms in diosmin's central carbon ring. Diosmin can be manufactured by extracting hesperidin from citrus rinds, followed by conversion of hesperidin to diosmin, as it is shown below. Diosmin has been used for more than 30 years as a phlebotonic and vascular-protecting agent, and has recently begun to be investigated for other therapeutic purposes, including cancer, premenstrual syndrome, colitis, and diabetes.

CHEMISTRY. PRODUCTION PROCESS

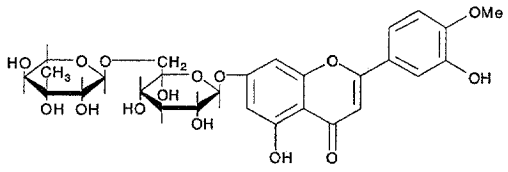
Description

Pale yellow needles, melting point 278-280ºC. Difficulty soluble or insoluble in organic solvents. Diosmin dissolves in concentrated H_2SO_4 with a slight fluorescence. It crystallizes from pyridine solution by adding much water. Hydrolysed by heating in a fused tube to 130-140ºC or slowly in concentrated H_2SO_4 on a water bath. Acetate, pale yellow crystalline substance which sinters at about 120ºC and begins to melt at 140ºC.

Molecular Formula

$C_{28}H_{32}O_{15}$

Structural Formula



Relative Molecular Mass

608.55

Physical Form

Odourless greyish-yellow or light yellow hygroscopic powder.

Solubility

The following table shows diosmin solubility in several solvents:

SOLVENT	Solubility (g/100 ml) at 25ºC	Description
NaOH 1 M	20.4	Freely soluble
DMF	1.7	Sparingly soluble
DMSO	11.9	Freely soluble

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Production Process (CONFIDENTIAL INFORMATION)

<u>Characteristics of Exquim's Diosvein</u>

Exquim's Diosvein dietary supplement:

- **contains a minimum of — and a maximum of — of Diosmin, being the content of Hesperidin — maximum;**

- **is substantially similar to Nutratech's Diosmin Complex product[1] and to the Diosmin and Hesperidin formulations studied in the clinical trials presented with this submission;**
- **complies with all the specifications of the current European Pharmacopoeia.**

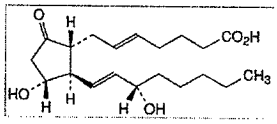
SPECIFICATION

Diosmin is included in the European Pharmacopoeia (see attached copy of the Monograph).
Adopted specification includes all parameters and limits included in the Ph.Eur. Monograph plus limits related to residual solvents content, in accordance with the criteria established by the ICH (guideline *Note for Guidance on Impurities: Residual Solvents*, CPMP/ICH/ 283/95).

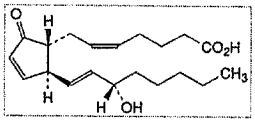
Besides microbiological specifications have also been defined for Diosmin. These are those established by the European Pharmacopoeia for preparations for oral administration (Category 3.B) [for further details see Section *5.1.4. Microbiological Quality of Pharmaceutical Preparations* in European Pharmacopoeia].

Complete specifications for Diosmin are described next (after Monograph).

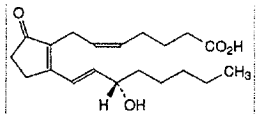
[1] Nutratech's Diosmin Complex was submitted to the FDA in a 75-Day Premarket Notification, and is currently on the market in the United States;



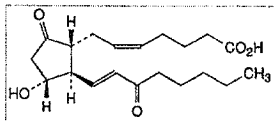
C. (*E*)-7-[(1*R*,2*R*,3*R*)-3-hydroxy-2-[(*E*)-(3*S*)-3-hydroxyoct-1-enyl]-5-oxocyclopentyl]hept-5-enoic acid (5-*trans*-PGE_2; (5*E*)-PGE_2),



D. (*Z*)-7-[(1*R*,2*S*)-2-[(*E*)-(3*S*)-3-hydroxyoct-1-enyl]-5-oxocyclopent-3-enyl]hept-5-enoic acid (PGA_2),



E. (*Z*)-7-[2-[(*E*)-(3*S*)-3-hydroxyoct-1-enyl]-5-oxocyclopent-1-enyl]hept-5-enoic acid (PGB_2),



F. (*Z*)-7-[(1*R*,2*R*,3*R*)-3-hydroxy-2-[(*E*)-3-oxo-oct-1-enyl]-5-oxocyclopentyl]hept-5-enoic acid (15-oxo-PGE_2; 15-keto-PGE_2).

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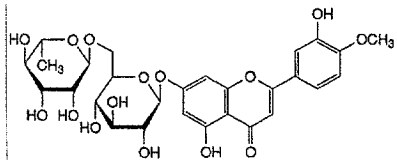
DIOSMIN

Diosminum



$C_{28}H_{32}O_{15}$ M_r 609

DEFINITION

7-[[6-*O*-(6-Deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4*H*-1-benzopyran-4-one.

Substance obtained through iodine-assisted oxidation of (2*S*)-7-[[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-2,3-dihydro-4*H*-1-benzopyran-4-one (hesperidin) of natural origin.

Content: 90.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: greyish-yellow or light yellow hygroscopic powder.

Solubility: practically insoluble in water, soluble in dimethyl sulphoxide, practically insoluble in alcohol. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *diosmin CRS*.

B. Examine the chromatograms obtained in the assay.
Results: the principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Iodine: maximum 0.1 per cent.

Determine the total content of iodine by potentiometry, using an iodide-selective electrode (*2.2.36*), after oxygen combustion (*2.5.10*).

Test solution. Wrap 0.100 g of the substance to be examined in a piece of filter paper and place it in a sample carrier. Introduce into the flask 50 ml of a 0.2 g/l solution of *hydrazine R*. Flush the flask with oxygen for 10 min. Ignite the filter paper. Stir the contents of the flask immediately after the end of the combustion to dissolve completely the combustion products. Continue stirring for 1 h.

Reference solution. Dilute 2.0 ml of a 16.6 g/l solution of *potassium iodide R* to 100.0 ml with *water R*. Dilute 10.0 ml of the solution to 100.0 ml with *water R*.

Introduce into a beaker 30 ml of a 200 g/l solution of *potassium nitrate R* in *0.1 M nitric acid*. Immerse the electrodes and stir for 10 min. The potential of the solution (nT_1) must remain stable. Add 1 ml of the test solution and measure the potential (nT_2).

Introduce into a beaker 30 ml of a 200 g/l solution of *potassium nitrate R* in *0.1 M nitric acid*. Immerse the electrodes and stir for 10 min. The potential of the solution must remain stable (nR_1). Add 80 µl of the reference solution and measure the potential (nR_2).

The absolute value $|nT_2 - nT_1|$ is not higher than the absolute value $|nR_2 - nR_1|$.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 25.0 mg of the substance to be examined in *dimethyl sulphoxide R* and dilute to 25.0 ml with the same solvent.

Reference solution (a). Dissolve 25.0 mg of *diosmin CRS* in *dimethyl sulphoxide R* and dilute to 25.0 ml with the same solvent.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 100.0 ml with *dimethyl sulphoxide R*.

Reference solution (c). Dissolve 5.0 mg of *diosmin for system suitability CRS* in *dimethyl sulphoxide R* and dilute to 5.0 ml with the same solvent.

Column:
- *size*: *l* = 0.10 m, Ø = 4.6 mm,
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm),
- *temperature*: 40 °C.

Mobile phase: *acetonitrile R, glacial acetic acid R, methanol R, water R* (2:6:28:66 *V/V/V/V*).

Flow rate: 1.5 ml/min.

Detection: spectrophotometer at 275 nm.

Injection: 10 µl loop injector; inject the test solution and reference solutions (b) and (c).

Run time: 6 times the retention time of diosmin.

Relative retention with reference to diosmin (retention time = about 4.6 min): impurity A = about 0.5, impurity B = about 0.6, impurity C = about 0.8, impurity D = about 2.2, impurity E = about 2.6, impurity F = about 4.5.

System suitability: reference solution (c):

- *resolution*: minimum of 2.5 between the peaks due to impurities B and C.

Limits:

- *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.38; impurity F = 0.61,
- *impurity A*: not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent),
- *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (5 per cent),
- *impurity C*: not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3 per cent),
- *impurity E*: not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3 per cent),
- *impurity F*: not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3 per cent),
- *any other impurity*: not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent),
- *total of other impurities and impurity A*: not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent),
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (10 per cent),
- *disregard limit*: 0.02 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Heavy metals (*2.4.8*): maximum 20 ppm.

2.0 g complies with limit test C. Prepare the standard using 4.0 ml of *lead standard solution (10 ppm Pb) R*.

Water (*2.5.12*): maximum 6.0 per cent, determined on 0.300 g.

Sulphated ash (*2.4.14*): maximum 0.2 per cent, determined on 1.0 g.

ASSAY

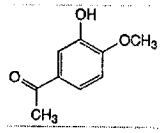
Liquid chromatography (*2.2.29*), as described in the test for related substances.

Injection: test solution and reference solution (a).

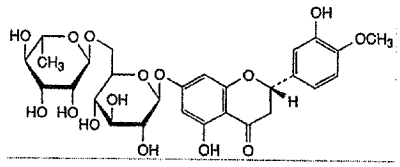
STORAGE

In an airtight container.

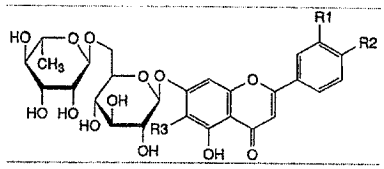
IMPURITIES



A. 1-(3-hydroxy-4-methoxyphenyl)ethanone (acetoisovanillone),



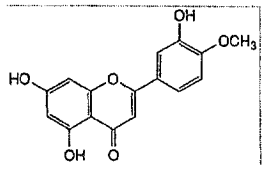
B. (2*S*)-7-[[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-2,3-dihydro-4*H*-1-benzopyran-4-one (hesperidin),



C. R1 = R3 = H, R2 = OH: 7-[[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one (isorhoifin),

D. R1 = OH, R2 = OCH_3, R3 = I: 7-[[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-6-iodo-4*H*-1-benzopyran-4-one (6-iododiosmin),

E. R1 = R3 = H, R2 = OCH_3: 7-[[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-2-(4-methoxyphenyl)-4*H*-1-benzopyran-4-one (linarin),



F. 5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-4*H*-1-benzopyran-4-one (diosmetin).

Monographs D-E

Description: Greyish – yellow or light yellow hygroscopic powder. Practically insoluble in water, soluble in dimethyl sulphoxide, practically insoluble in alcohol. It dissolves in dilute solutions of alkali hydroxides

ANALYSIS	LIMIT	METHOD
Identification		
Infrared absorption spectrophotometry	conform	current Ph.Eur.
HPLC	conform	current Ph.Eur.
Tests		
Iodine	0.1 % max	current Ph.Eur.
Related substances (HPLC)		current Ph.Eur.
Acetoisovanillone (A)	1 % max	
Hesperidin (B)	5 % max	
Isorhoifolin (C)	3 % max	
Linarin (E)	3 % max	
Diosmetin (F)	3 % max	
Any other impurity	1 % max	
Total of other impurities and A	1 % max	
Total	10 % max	
Heavy metals	20 ppm max	current Ph.Eur.
Water	6.0 % max	current Ph.Eur.
Sulphated ash	0.2 % max	current Ph.Eur.
Assay		
Content (on anhydrous substance)	90.0 to 102.0%	current Ph.Eur.

ANALYSIS	LIMIT	METHOD
Residual solvents		Ph.Eur. Test 5.4
1-Butanol	3000 ppm max	
Butyl acetate	3000 ppm max	
Microbiological purity		Ph.Eur. 2.6.12/13
Total plate count	10.000 cfu/g max	
Moulds and Yeasts	100 cfu/g max	
Enterobacteria	100 cfu/g max	
Salmonella	absence in 10 g	
Escherichia coli	absence in 1 g	
Staphylococcus aureus	absence in 1 g	

SAFETY, EFFICACY & METABOLISM

To demonstrate the safety profile of Diosmin, the following section contains the articles cited in Nutratech's submission, as well as other articles, all of which confirm the safe use of Diosmin.

In Guilhou's study of diosmin+h for the treatment of venous ulcers, 107 men and women were enrolled in a multicenter, double-blind, randomised, placebo-controlled trial and received a 2-month treatment with diosmin+h 1000 mg daily. Ninety-nine patients completed the protocol. Six patients withdrew from the study for reasons other than ulcer healing. In the diosmin group 2 withdrew because of phlebitis and 1 because of noncompliance. In the placebo group, 3 individuals withdrew due to mild cutaneous eruptions and 1 for personal reasons. Treatment was well tolerated. Two venous thromboses were diagnosed in the diosmin group but investigators thought that they were unrelated to treatment. Other adverse events were eczema (2), urticaria (1), puritis of the scalp (1), and local pain (1) in the placebo group; and skin changes around ulcer (1), asthemia (1), headaches (1), and exacerbation of chronic colopathy (1) in the diosmin+h group[26].

Guillot and colleagues carried out a study to confirm the long term therapeutic efficiency of diosmin + hesperidin (Daflon 500 mg) and the safety of this agent in one year of continuous administration. They concluded that a long term treatment with diosmin+h was beneficial to patients suffering from chronic functional venous insufficiency. Two hundred and fifteen out-patients (28 males and 187 females) aged between 19 and 81 years were included in the study. All patients suffered from functional symptoms of venous insufficiency like leg heaviness, cramps, paresthesia, evening edema. Exclusion criteria included pregnancy and some others. The disease had been present for 1 to 3 years in 28.3%, 4 to 10 years in 42.3%, more than 10 years in 29.3%. Each patient was given diosmin+h, 2 tablets daily for a year with regular controls every two months. The efficacy was clearly demonstrated with a rapid improvement of functional signs in the first months, being maintained till the end of the study. The reduction of ankle and calf circumferences is probably linked to a decrease in plasma filtration through capillary walls. This fact confirms the positive role of the drug on tissue microcirculation. The safety was demonstrated by the rareness and mildness of clinical side effects: gastralgia in 7 patients, dizziness in 4, gynaecological signs in 7 and cutaneous eruption in two cases. The side effects led to treatment withdrawal in 4 patients: nausea and gastralgia in two cases and an increase of the body weight in two others but probably not in relation with the treatment. No abnormal changes in laboratory parameters were observed[2].

In a 1995 review article in *Drugs of Today*, Godeberge assesses studies conducted by Cospite, Copé, and Delmont that enrolled a total of 299 patients to test diosmin+h as a treatment for hemorrhoids. In all trials, diosmin+h was well tolerated. The side effects, generally transient and mild, were anxiety, shivering, oppressive feeling across the chest, and epigastric pain. The frecuency of side

effects was similar in both treated and control groups and never required specific treatment. There was no evidence of drug interaction in any of the studies[15].

Diosmin+h 1000 mg was given daily for two months to 174 women and 26 men with either organic CVI (83) or functional CVI (117) in 2 double-blind randomised trials, placebo-controlled trials. Results showed that variations in blood parameters were within accepted physiological limits. There were no allergic reactions or drug interaction seen. Side effects were of the same type and frequency in both groups. In the diosmin group, 1 patient experienced hypotension, 4 patients complained of nausea, 1 of headache, 2 of gastric pain, 1 of insomnia. Only 3 patients dropped out: 1 in the diosmin group for epigastric pain and 2 in the control group for nausea and hypotension. In the placebo group, 1 patient experienced hypotension, 4 patients complained of nausea, 4 of headache, 2 of gastric pain, 1 of insomnia, 1 of metrorrhagia[27].

Tsouderos presented the results of a study that included 20 patients who had been suffering from CVI for at least one year. This study evaluated the activity of 1000 mg Diosmin Complex as a single dose, compared to a placebo. The results showed that there was no significant change in cardiac index, capillary filtration index, blood pressure, cardiac or respiratory rate. Tsouderos also reported the results of a double blind randomised controlled trial of the effect of Diosmin Complex (1000 mg/day) compared to placebo, over a two-month treatment period. Eighteen patients with functional venous insufficiency were examined in each group. Assessments were undertaken for capillary filtration, arterial output, respiratory and cardiac rates and systolic and diastolic blood pressure. There was no statistically significant difference between Diosmin Complex and placebo groups[22].

Cesarone *et al.* reported the results of a pilot study in which 43 patients with venous hypertension were administered Diosmin Complex at a daily dose of 1500 mg or 1000 mg. In addition, ten healthy subjects were also administered Diosmin Complex at a daily dose of 1500 mg. The duration of administration was 4 weeks. After treatment with Diosmin Complex, a dose-related decrease in capillary filtration was observed. The treatments were well tolerated and the patients did not report!any unwanted effects[23].

Meyer conducted a review in the use of diosmin+h (Daflon 500 mg) in which they point out that clinical trials fulfil international scientific requirements and have collected more than 2850 patients treated with diosmin+h at the dosage of two tablets per day for six weeks to one year. The proportion of patients with side effects (10% of those treated), essentially of a gastrointestinal or autonomic nature and leading to a rare of only 1.1% trial dropouts, is less than described in 225 patients given a placebo (13.9%) in controlled trials. Satisfactory clinical acceptability already confirmed in the short term was equally found in long term treatment. Hemodynamic parameters as well as laboratory parameters were uninfluenced even by prolonged treatment for one year at the dosage of two tablets

per day. No contraindications have been found, even in the elderly and in pregnant women[3].

Belcaro and colleagues reported the results of a three-month double-blind randomised study, which allocated patients into three groups with different daily doses of Diosmin Complex: 500 mg (n=34), 1000 mg (n=33) or 2000 mg (n=37). All 104 patients included in the trial were affected with mild CVI. Fourteen patients dropped out of the study: nine for reasons not related to treatment, 2 lost to follow-up and 3 because of an adverse event. Side effects leading to withdrawal occurred in one patient in group 1 (inguinal pain) and in 2 patients in group 3 (gastralgia and cystitis). The treatment was discontinued and the adverse events disappeared. For all patients, haematological and biochemical parameters remained stable over the study period[24].

In a safety and efficacy study of micronized diosmin+h for the treatment of internal haemorrhoids of pregnancy, Buckshee and co-workers showed that treatment was safe, acceptable, and effective in the treatment of haemorrhoids of pregnancy. In an open study on hospital outpatients, they studied therapy with diosmin+h for a median of 8 weeks before delivery and 4 weeks after delivery, in 50 women with acute haemorrhoids. The outcome measures were symptoms and signs of haemorrhoids, adverse effects, and acceptability of treatment. 66% of the patients had relief from acute symptoms by the fourth day, 53.6% fewer patients had relapse in the antenatal period. Treatment was well accepted, and did not affect pregnancy, fetal development, birth weight, infant growth and feeding[4].

In a double-blind, placebo-controlled trial of a flavonoid fraction (consisting of 90% diosmin and 10% hesperidin) in the treatment of symptomatic capillary fragility, Galley andThiollet concluded that the flavonoid fraction increased to a large extent the capillary resistance in patients with abnormal capillary fragility without significant side-effects. The treatment was performed in 100 patients with symptomatic capillary fragility in a double-blind, randomised, placebo-controlled trial. Treatment lasted 6 weeks and consists of 2 daily tablets of either diosmin+h or placebo. Patients were examined at weeks 0, 2, 4 and 6. Compared to placebo, capillary resistance, assessed by the negative suction cup method, was significantly higher in the diosmin+h group at week 4 and week 6. This resulted in a significant improvement of symptoms of capillary fragility in the treated patients. The drug was well tolerated. The rate of side-effects spontaneously volunteered by the patients was similar in both groups[6].

Jean and Bodinier studied the mediators involved in inflammation and the effects of diosmin + hesperidin on their release. They concluded that the anti-inflammatory effects of these drugs have been observed in a variety of in vivo models. Diosmin + hesperidin inhibit the formation of arachidonic acid derivatives and free radicals. This results in a decrease of membrane permeability and then in a protection against the development of the edema. These properties may explain, at least in part, the clinical activity of diosmin + h and justify its therapeutic use[8].

Cospite conducted a study of 100 patients undergoing an acute hemorrhoidal attack who were treated with either diosmin + hesperidin and placebo. Diosmin + h was given for 7 days at the dosage of six tablets daily during the first four days and four tablets daily during the following three days. One patient in the diosmin group and five in the placebo one withdrew from the treatment because of dissatisfaction with the therapeutic results. 4 patients in the diosmin group and three in the placebo group experienced mild digestive side effects. No patient stopped due to major side effects. Blood pressure remained normal and showed no modification attributable to treatment. There was no statistically significant difference between groups. A significant improvement of signs and symptoms was demonstrated in the diosmin group. Moreover, a reduction in duration and intensity of attacks and a improvement of the local anatomic lesions were observed, both of which were more important in the diosmin group than in the placebo group[9].

Ho and colleagues performed a prospective randomised controlled trial on the effects of a flavonoid fraction containing 450 mg diosmin and 50 mg hesperidin on bleeding after haemorrhoidectomy. In all, 228 consecutive patients with prolapsed irreducible piles were recruited. Elective haemorrhoidectomy was performed with a standardized diathermy excision method. Some 114 patients were randomised to receive diosmin + h for 1 week after operation (group one), and there were 114 controls (group two). Postoperative analgesia and laxative prescription as well as hospital stay were otherwise the same. One patient from group one and seven from the placebo group had postoperative bleeding. All bleeding occurred from 6 to 15 days after haemorrhoidectomy. There were no side-effects from the use of diosmin + h. The risk of secondary bleeding from haemorrhoidectomy is reduced with postoperative diosmin + hesperidin[10].

Ramelet studied the clinical benefits of a flavonoid fraction (diosmin + hesperidin) in the most severe stages of CVI, and concluded that this fraction, thanks to its comprehensive mode of action on the veins, lymphatics, and microcirculation, is the method of choice not only in the early stages of CVI treatment, but also in the severe stages of this condition, in combination with compression treatment, sclerotherapy, and surgery if appropriate[11].

Geroulakos and colleagues performed three controlled studies in CVI using Daflon 500 mg (diosmin + hesperidin). This substance could play a greater role in the management on functional venous insufficiency. Patients with this condition have no clinically detectable anatomic abnormality in their veins, but they complain of intermittent functional symptoms such as leg heaviness, cramps, paraesthesia, and evening edema. Approximately 5% of the patients who present in the varicose clinic of their hospital have functional venous insufficiency. Diosmin + h has been demonstrated to significantly improve the symptoms and the plethysmographic parameters in these patients. It also decreases the ankle circumference, indicating an effect on the fluid leakage rate from the capillaries. In these patients, treatment with diosmin + h is far more acceptable than elastic compression[12].

Cova and colleagues performed a pharmacokinetic studies of diosmin after oral administration to healthy volunteers. Diosmin and its aglycone, diosmetin, were determined using different analytical techniques. Diosmin was not present in the plasma but only diosmetin. Analysis of the pharmacokinetic parameters showed that the drug was rapidly absorbed. Diosmetin presents a long plasma elimination half-life ranging from 26 to 43 hours (which suggests an enterohepatic circulation which is known to have the effect of slowing down the complete elimination of the drugs). Their data showed the total absence of urinary elimination for both diosmin and diosmetin, while its minor metabolites are eliminated in the urine, mainly as glucoronic acid conjugates. The presence of degradation products such as alkyl-phenolic acids confirms a metabolic pattern similar to other flavonoids[5].

PHARMACOLOGY

Elimination

In a study conducted by Oustrin and colleagues, ^{3}H-labelled diosmin was administered both i.v. and orally to Wistar rats. Absorption by the GI track was rapid, and the peak plasma concentration was between 1 and 2 hours. Of the organs examined, almost all had 0.1% to 0.2% of the original activity after 48 hours, only the liver had a 1% concentration. Elimination took place in the urine and in the faeces. After i.v. administration, elimination was predominantly in the urine, while after oral administration it was eliminated almost equally in the urine and in the faeces, during the first 24-hour period. In the following 24-hour periods, the faeces carried the greater portion of diosmin or its metabolites. Binding to the vascular wall was relatively late[28].

Mechanism of Action

Inflammatory reactions are trigged by chemical and biological mediators, such as arachidonic acid derivatives (prostaglandins, leukotrienes, or tromboxanes), vasoactive amines (histamine or serotonin), and oxygen free radicals (superoxide ion, O^{2-}, or hydrogen peroxide). In venous inflammation, histamine causes vasodilatation; and PGE2, histamine, and free radicals increase membrane permeability. Diosmin acts by inhibiting the enzyme phosphodiesterase, increasing intracellular cyclic adenosine monophosphate (cAMP) and consequently reducing the level of the main biochemical mediators of inflammation prostaglandin E2 and F2 (PGE2, PGF2), thromboxane B2 (TXB2), and oxygen free radicals[36].

Diosmin reinforces venous tone by prolonging the activity of pariental norepinephrine. In experiments on the saphenous vein strips of dog, conducted by Heusser & Osswald, diosmin blocked the inactivation of exogenous noradrenaline and caused a slow and gradual contractile response of an oil-immersed strip, which was not attributable to the release of noradrenaline[21]. Diosmin exerts a significant potentiation toward NE in both normal and varicose veins under acidotic conditions. Local acidosis depresses reactivity of vascular smooth muscle, especially the response of human isolated saphenous veins to exogenous

norepinephrine. In a *in vitro* study, Juteau and colleagues used isolated varicose veins to test the effect of diosmin+h and norepinephrine on human rings of veins under acidosis conditions. Results showed that the diosmin+h combination induced a shift to the left of the concentration-response curves for norepinephrine. This potentiation was significant in both normal and varicose veins and was increased in proportion with the pathological status of the venous rings[29].

In an *in vitro* study, the cytotoxic effect of lipopolysaccharide (LPS) on cultivated bovine aortic endothelial cells was attenuated by diosmin. Melzig and Loose speculated that the inhibition of LPS-induced cytotoxicity in bovine aortic endothelial cell cultures by diosmin may be mediated via inhibition of tyrosine kinases. Study data showed that the IC50-value of LPS in the combination with diosmin 8 µmol/l was shifted from 31 to 70 ng/ml in a concentration dependent manner[30]. In another *in vitro* study conducted by Korthuis, diosmin prevented ischemia and reperfusion-induced leukocyte adhesion in skeletal muscle. This anti-adhesive effect appeared to be mediated, in part, by inhibition of induced expression of ICAM-1[31].

When the lymphatic activity diosmin+h was tested in dogs and rats, diosmin induced a lymphatic flow increase that was correlated with the administered doses. The maximal 10 minute period flow after i.v. injection of D (12.500 mg/kg) was 191% higher than the corresponding one in the control group. A correlation between lymphatic flow increase and pulsatility was demonstrated. Infusion of 14C-labelled-D evidenced a clear blood-lymph transfer of the drug: an active transport!into the lymph was suggested during a 15 minute to 100 minute period from the concentration curves[32].

Daflon is a strong inhibitor of Cu(2+)-induced arachidonic acid peroxidation, as revealed by the inhibition of thiobarbituric acid- reactive substance formulation in mixed liposomes of phosphatidylcholine and arachidonic acid. Diosmin is a good complexant of Cu2+ ions but not of Fe2+ ions. The Cu2+ complex formulation may thus explain part of the antioxidant effect. However, Daflon is also a good quencher of the singlet oxygen-induced arachidonic acid peroxidation that does not involved metal ions[33].

Struckmann and colleagues carried out a review of the pharmacology and therapeutic efficacy of Daflon 500 mg (diosmin + hesperidin) in patients with CVI and related disorders. The major pathologic event in CVI, whether it is due to obstruction, reflux, or both, is an inability to reduce venous pressure during ambulation. This results in abolition of the arteriovenous reflex, and extravasation of plasma proteins, which accumulate in the pericapillary space and which may take part in the pathogenic mechanism of venous ulceration. White blood cells marginate in the capillary bed, where they liberate cytotoxic substances that may induce an inflammatory reaction, cell death, and ulceration. Animal experiments have provided evidence of an effect of diosmin+h on venous tone, prostaglandin synthesis, free radicals, complement activity, and lymphatic drainage. These findings may explain, at the microvascular level, the observed clinical findings in

placebo-controlled, double-blind trials, which have shown significant effects on edema reduction and improvement in subjective parameters coincident with reductions in venous capacitance, distensibility, and emptying times[7].

US REVIEW ARTICLES

In this Section we would like to show two examples of review articles published in an US Journal by US scientists.

Douglas MacKay carried out a review article entitled "Hemorrhoids and Varicose Veins: A Review of Treatment Options", in which he states that several botanical extracts have been shown to improve microcirculation, capillary flow, and vascular tone, and to strengthen the connective tissue of the perivascular amorphous substrate. Oral supplementation with bioflavonoids and some others may prevent time-consuming, painful, and expensive complications of varicose veins and hemorrhoids[13].

Bergan and colleagues performed a therapeutic approach to CVI and its complications: place of Daflon 500 mg. The drug acts favourably in venous ulcer treatment by inhibiting the synthesis of prostaglandins and free radicals. It decreases bradykinin-induced microvascular leakage and may act favourably to inhibit leukocyte activation, trapping, and migration. Clinically, edema is reduced, ulcer healing is accelerated, and leukocyte trapping diminished. The action of diosmin + h is beginning to be better understood, and better pharmacologic control of CVI is a tantalizing promise[14].

TOXICITY STUDIES IN ANIMALS

Toxicity studies evaluating the effect of diosmin/hesperidin formulations in animals have documented an excellent safety profile.

Heusser and Oswald conducted several studies examining the subchronic, chronic and teratogenic toxicities of diosmin. In a first study, oral treatment with diosmin, at 200 mg/kg daily, for 50 days, was assessed in 20 white rats. A second study also examined chronic toxicity in 22 white mice. Oral treatment with diosmin was administered at a dose of 620 mg/kg daily for 196 days. The observations from both experiments indicated that there were no toxic effects. These findings were confirmed by the blood count, the macroscopic and histological assessment of the organs, the weight development and biochemical examination. In another experiment, diosmin oral treatment (doses between 50 and 250 mg/kg) was given to male and female mini-pigs for 180 days. There were no systematic deviations in clinical, biochemical or haematological values, suggesting that there was no toxicological effect of diosmin[21].

In a teratogenicity study conducted by Heusser and Oswald, mother white mice were given diosmin at a daily dose of 50 mg/kg from the 4th to the 12th postcoital day. The fetuses were delivered by laparotomy on the 19th day after conception.

After laparotomy, the mean number of animals per litter was 12.3 in the control group and 11.5 in the diosmin treated group. 126 fetuses in each group were assessed and their skeletons were also examined. No pathological findings were observed[21]. In another group of mice, diosmin was administered in the same dose and fetuses were delivered by spontaneous birth. The fetuses were examined macroscopically and the development of the animals was carefully monitored. Postnatal mortality (20 days) was 19% in the control group and 26% in the diosmin group. Weight gain, length development, gross behaviour, hair growth and opening of the eyes were similar in both groups. No significant differences between groups in organ weights, macroscopic and histological findings were observed[21]. In a similar study, mother white rats were administered a daily dose of 100 mg/kg of sodium salt from the 4th to the 14th postcoital day. The fetuses were delivered by laparotomy on the 21st day after fertilization. The mean number of animals per litter was 13.9 in both the control and diosmin groups. The average weight was 3.56 and 3.17 g respectively. After examination of their skeletons, no pathological findings were observed in the fetuses[21]. In another group of rats, fetuses were delivered by spontaneous birth. The average number of animals per litter was 12.9 in the control group and 11.6 in the diosmin group. Mortality within the 20 postnatal days was respectively 11% and 20%. There was no significant difference between the two groups in terms of weight gain, length development, gross behaviour, hair growth, opening of eyes, organ weights, macroscopic and histological examinations[21]. Heusser and Oswald concluded from their toxicological experiments that diosmin is well tolerated, even at very high doses, by the different animal species studied.

Animal toxicity studies of diosmin have also been carefully reviewed and summarised by Meyer[3] and Buckshee *et al.*[4]. The major findings are detailed below. Diosmin Complex (90% diosmin and 10% hesperidin) was administered to mice, rats and primates as single oral dosing, as well as repeated oral dosing (13 weeks and 26 weeks). These represent respectively 180 times and 35 times the recommended daily dose in humans. No toxic or lethal effect could be observed. The 50% Lethal Dose (LD_{50}) was impossible to determine both in the mouse and in the rat, because of the excessively low toxicity; it was assessed as greater than 3000 mg/kg for the active principle[3,4].

The good gastrointestinal acceptability was confirmed in the Wistar rat, at oral doses representing 12, 24 and 48 times the recommended daily dose. No impairment of the reproductive function was found in the rat after administration of an oral dose, representing 37 times the recommended daily dose. Fertility, embryotoxicity, perinatal, and postnatal development of the generation born from treated parents were not affected. The absence of genetic toxicity was shown by the following tests: bacteria gene mutation, analysis of metaphases in human lymphocytes in culture, in vitro eukaryote system gene mutation, in vivo clastogenic lesions, and DNA repair.

Transplacental passage in the rat was assessed for a single dose of 10 mg/kg of diosmin and was minimal (0.003% per fetus, of the dose administered to the mother). Passage into breast milk was assessed as 1% of the dose administered

to the mother. Accumulation in the uterus was 0.02%. Autoradiography of pregnant females showed that the compound was distributed essentially in the intestine and secondarily in the kidneys. However, there was no uptake of the compound by the genital organs of the mother[4].

Meyer concluded that these studies, evaluating the possible toxicity on the digestive tract, the lactation and the reproductive function, demonstrated the excellent safety of Diosmin Complex in animals.

Hitzenberger also described several toxicology studies of Daflon 500 mg. Acute symptoms were studied in both mice and rats (oral administration up to a maximum of 3000 mg/kg). However, LD_{50} could not be determined. No deaths were observed during the 15-day observation period and no substance-related changes were detected during the autopsy[25]. Macaca monkeys were administered an oral dose of 4500 mg/kg. No relevant toxicity symptoms were observed. In addition, subchronic toxicity was tested on rats for a period of 13 weeks. Maximum dosage of 600 mg/kg per day was given without any toxicological symptoms[25]. Chronic toxicity was studied on rats for a period of 26 weeks, at a dosage of 600 mg/kg per day. No substance-related changes were observed[25]. A similar administration schedule was also used in cynomolgus monkeys, with the same overall results[25]. Mutagenicity was examined with various tests and no effect could be shown. Reproduction toxicological test on rats and rabbits, as well as peri- and post-natal toxicity studies and further teratogenic studies on rabbits were negative. Furthermore, fertility was not impaired[25].

In conclusion, chronic toxicity, teratogenicity, mutagenicity, fertility and embryotoxicity studies have clearly demonstrated that diosmin has an excellent tolerability profile in animals at dose ranges superior to the recommended dosage regimen in humans.

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Table 1: HISTORY OF USE

Diosmin is available under many brand names throughout the world. The following table summarizes the preparations in which diosmin is the principle active ingredient as a pharmaceutical substance in several countries and the launching year:

		TRADE NAME	LAUNCH YEAR
Europe	Austria	Daflon	1996
	Belgium	Daflon	1972
		Ven-Detrex	1979
	Czech Republic	Detralex	1993
	Denmark	Capiven	1990
	France	Daflon	1971
		Diovenor	1978
		Diosmil	1983
		Flebosmil	1988
		Endium	1992
		Dio	1992
		Mediveine	1995
		Venirene	1996
		Diosmine Ratioph.	1996
	Germany	Daflon	1977
		Tovene	1980
	Greece	Daflon	1991
	Hungary	Detralex	1993
	Italy	Daflon	1976
		Venosmine	1980
		Arvenum	1984
		Doven	1989
		Diosven	1990
		Diosmin	1993
		Alven	1996
	Luxembourg	Flebosmil	1988
	Poland	Detralex	1992
		Daflon	1993
	Portugal	Daflon	1974
		Venex	1976
		Veno V	1977
	Slovak Republic	Detralex	1994
	Spain	Diosminil	1975
		Insuven	1977
		Daflon	1980

		Daflon 500	1995
	Switzerland	Ven Detrex	1975
		Daflon	1977
		Hemerven	1984
	Turkey	Daflon	1994
America	Argentina	Flebotropin	1977
		Volpino	1986
		Flebofarm	1988
		Rupediol	1990
		Flebon	1990
		Flebon 500	1994
		Daflon 500	1994
	Brazil	Daflon	1980
	Central America	Daflon	1975
	Chile	Flebopex	1981
		Insuven	1990
		Daflon	1992
	Colombia	Daflon 500	1994
	Ecuador	Daflon	1994
	Mexico	Daflon	1977
		Variton	1994
	Peru	Insuven	1991
		Daflon	1991
		Daflon 500	1996
	Venezuela	Daflon	1992
Africa	Egypt	Daflon	1983
		Diosed	1990
		Dioven	1990
		Varicotone	1995
	French W. Africa	Daflon	1971
		Diovenor	1978
	Morocco	Daflon	1978
		Flebosmil	1989
		Diovenor	1991
	Saudi Arabia	Daflon	1982
		Diovenor	1989
	Tunisia	Diosmil	1987
		Flebosmil	1992
Asia	Hong Kong	Daflon	1975
	Jordan	Daflon	1996
	Korea	Dismin	1984
		Pranol DGI	1985
		Venoron	1985
		Daromin	1987
		Zasmide	1988

	Venex	1989
	Diosmin CH/	1993
	Venitol	1995
Malaysia	Daflon	1987
Pakistan	Daflon	1981
Thailand	Heteroid	1988
	Daflon 500	1992
	Heroid	1997

Table 2: DIOSMIN DOSE AND SAFETY

Author/Study	Dose	Duration	Patients/DZ	Safety Results
Tsouderos 1991[34] Venous tone: are the phlebotonic properties predictive of a therapeutic benefit? A comprehensive view of our experience with Daflon 500 mg	Daflon 500 mg or placebo	NA	20 patients post-thrombotic syndrome 10 women CVI	Hemodynamic parameters remained constant.
Galley 1993[6] A double-blind, placebo-trial of a new veno-active flavonoid fraction (S 5682) in the treatment of symptomatic capillary fragility.	S 5682 (500 mg diosmin) 2 tablets daily	6 weeks	100 patients abnormal capillary fragility	Well tolerated. Side-effects (no significant) reported were similar in placebo and S 5682 groups.
Cesarone 1993[23] Capillary filtration and ankle edema in patients with venous hypertension: effects of Daflon.	Daflon 500 mg group 1: 2 tablets daily; group 2: 3 tablets daily; group 3: 3 tablets daily	4 weeks	53 patients group 1 & 2 (43): venous hypertension group 3 (10): healthy subjects	Treatments well tolerated and patients did not report!any unwanted effects.
Cospite 1994[9] Double-blind, placebo-controlled evaluation of clinical activity and safety of Daflon 500 mg in the treatment of acute hemorrhoids	Daflon 500 mg 3 tablets daily the 1st 4 days, 2 tablets daily the following 3 days	7 days	100 patients hemorrhoidal disease	Good acceptability with no major side effects.
Godeberge 1994[15]	Daflon 500 mg	2 months	120 patients	No significant side effects were

Daflon 500 mg in the treatment of hemorrhoidal disease: a demonstrated efficacy in comparison with placebo.	2 tablets daily		treatment of chronic and acute symptoms of hemorrhoidal disease	noted.
Ho 1995[10] Prospective randomised controlled trial of a micronized flavonidic fraction to reduce bleeding after haemorrhoidectomy.	Daflon 500 mg 2 tablets 3 times daily for 3 days, 1 tablet 3 times daily the next 4 days	1 week	228 patients bleeding after haemorrhoidectomy	There were no side-effects encountered with this regimen.
Belcaro 1995[24] Laser Doppler and transcutaneous oximetry: modern investigations to assess drug efficacy in chronic venous insufficiency	Daflon 500 mg group 1: 1 tablet daily; group 2: 2 tablets daily; group 3: 4 tablets daily	3 months	104 patients patients affected by mild CVI	Haematological and biochemical parameters remained stable over the study period.
Buckshee 1997[4] Micronized flavonoid therapy in internal hemorrhoids of pregnancy.	(450 mg diosmin + 50 mg hesperidin) 6 tablets daily for 4 days, 4 tablets daily for 3 days, and 2 tablets daily till 30 days after delivery.	A median of 12 weeks	50 patients acute hemorrhoids of pregnancy	It is safe to use in the last trimester of pregnancy.

Boccalon 1997[35] Characteristics of chronic venous insufficiency in 895 patients followed in general practice.	Daflon 1000 mg daily	2 months	895 patients CVI	Safety information not available.
Diana 2000[17] Activity of purified diosmin in the treatment of hemorrhoids.	Diosmin 450 mg 2 tablets for the 1st 7 days, and 1 tablet for up to 2 months	2 months	66 patients treatment of hemorrhoids	Diosmin tolerability was excellent.
Jantet 2002[16] Chronic venous insufficiency: worldwide results of the RELIEF study	Daflon 500 mg or equivalent 2 tablets daily	6 months	3174 patients evolutions of symptoms and signs of CVI	Continuous improvement in quality of life scores over the study period.
Roztocil 2003[18] Efficacy of a 6-month treatment with Daflon 500 mg in patients with venous leg ulcers associated with chronic venous insufficiency.	Daflon 500 mg 2 tablets daily	6 months	150 patients venous leg ulcers associated with CVI	No treatment-related side effects were reported.
Smith 2005[19] Daflon 500 mg and venous leg ulcer: new results from a meta-analysis.	Daflon 500 mg 2 tablets daily	6 months	723 patients leg ulcer healing	Safety information not available.

Mlakar 2005[20] Flavonoids to reduce bleeding and pain after stapled hemorrhoidopexy: a randomised controlled trial	Detralex 500 mg 2 tablets 3 times daily	5 days	63 patients	There were no side effects from Detralex treatment.