



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration

Memorandum

Date: JUN 16 2000
From: (Acting) Division Director
Division of Standards and Labeling Regulations, HFS-820
Subject: 75-Day Premarket Notification for New Dietary Ingredients
To: Dockets Management Branch, HFS-305

New Dietary Ingredient:	Diosmin
Firm:	Nutraceutical, Inc.
Date Received by FDA:	April 5, 2000
90-Day Date:	July 2, 2000

In accordance with the requirements of section 413(a) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day premarket notification for the aforementioned new dietary ingredient should be placed on public display in docket number 95S-0316 after July 2, 2000.

Felicia Satchell
Felicia Satchell

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95S-0316

RPT 68



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Washington, DC 20204

JUN 16 2000

Carl Germano, RD, CNS, LDN
NutraTech, Incorporated
East Coast Office
208 Passaic Avenue
Fairfield, New Jersey 07004

Dear Mr. Germano:

This is in response to your letter to the Food and Drug Administration (FDA) dated April 5, 2000, making a submission for a new dietary ingredient pursuant to 21 U.S.C. 350b(a)(2) (section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act (the Act)). Your letter notified FDA of the intent of NutraTech, Inc. to market a dietary supplement containing a new dietary ingredient, diosmin.

21 U.S.C. 350b(a)(2) requires that a manufacturer or distributor of a dietary supplement that contains a new dietary ingredient submit to FDA, at least 75 days before the dietary ingredient is introduced or delivered for introduction into interstate commerce, information that is the basis on which the manufacturer or distributor has concluded that a dietary supplement containing such new dietary ingredient will reasonably be expected to be safe. FDA reviews this information to determine whether it provides an adequate basis for such a conclusion. Under section 350b(a)(2), there must be a history of use or other evidence of safety establishing that the dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe. If this requirement is not met, the dietary supplement is deemed to be adulterated under 21 U.S.C. 342(f)(1)(B) because there is inadequate information to provide reasonable assurance that the new dietary ingredient does not present a significant or unreasonable risk of illness or injury.

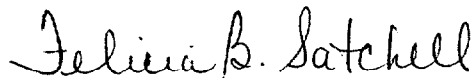
FDA has carefully considered the information in your submission, and the agency has significant concerns about the evidence on which you rely to support your conclusion that a dietary supplement containing diosmin will reasonably be expected to be safe. Your submission does not provide the basis to reasonably determine or support the safety of diosmin as a dietary supplement for humans. Studies using diosmin alone were not included in your submission. Instead, the studies in your submission used Daflon, a diosmin hesperidin mixture, micronized to increase absorption. Moreover, the submission contains no explanation or information that provides a valid basis to conclude that studies of Daflon are suitable to establish the safety of a dietary supplement containing a different substance, namely, diosmin. For this reason the information in your submission does not provide an adequate basis to conclude that

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diosmin will reasonably be expected to be safe. Therefore, your product may be adulterated under 21 U.S.C. 342(f)(1)(B) as a dietary supplement that contains a new dietary ingredient for which there is inadequate information to provide reasonable assurance that such ingredient does not present a significant or unreasonable risk of illness or injury. Introduction of such product into interstate commerce is prohibited under 21 U.S.C. 331(a) and (v).

Please contact us if you have any questions concerning this matter.

Sincerely yours,



Felicia B. Satchell
(Acting) Division Director
Division of Standards
and Labeling Regulations
Office of Nutritional Products, Labeling
and Dietary Supplements

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cc:

HFA-305 (Docket No. 95S-0316)

HFS-22 (CCO)

HFS-315 (Cichowicz)

HFS-605 (field programs)

HFS-800 (Lewis)

HFS-810 (Moore)

HFS-820 (Satchell)

HFS-821 (Strauss, Powers)

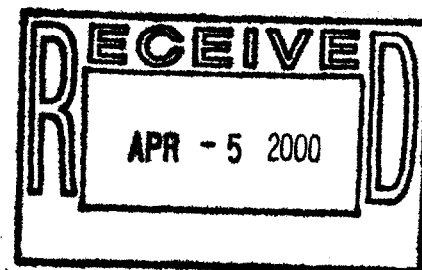
HFR- MAR-PA140 (District Compliance)

R/D:HFS-821:RPowers:6/11/00:202-401-9858:70312

Reviewed:HFS-811:RJMoore:6/12/00:202-205-4605:

HFS-820:FSatchell::6/16/00:202-260-0545

F/T:bls:6/16/00



April 3, 2000

Office of Nutritional Products, Labeling and Dietary Supplements (HFS-820)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, SW
Washington, DC 20204

Dear Sir or Madam,

Pursuant to Section 8 of the Dietary Supplement Health and Education Act of 1994, on its own behalf, Nutraceutical, Inc. wishes to notify the Food and Drug Administration that it will market a new dietary ingredient, DIOSMIN, a bioflavonoid derived from hesperidin, which is found in plants or citrus rinds. Accordingly, enclosed are an original and two copies of this notification.

As a dietary supplement, DIOSMIN will be put into a capsule, tablet, powder, or bar that will be suggested to be taken at an oral dose of 500 mg twice daily. **Diosmin is not intended for use by pregnant women and will be so labeled.**

Attached are a summary and reports of the safety studies and other information establishing that this dietary ingredient, when used as set forth above, is reasonably expected to be safe. These supporting studies include:

- A DIOSMIN safety profile with references,
- Preclinical pharmacology and toxicology journal articles,
- Clinical trial journal articles, and
- A selection of product information and package inserts from DIOSMIN formulations marketed internationally.

Yours truly,

Carl Germano, RD, CNS, LDN
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DIOSMIN (CAS 520-27-4)

Basis for Concluding Diosmin is Reasonably Expected to be Safe

INTRODUCTION

Diosmin* is a bioflavonoid derived from hesperidin, found in citrus rinds and in some plants. Diosmin is available under many brand names throughout the world (Table 1). Daflon® (diosmin+h), composed of 90% diosmin and 10% hesperidin, was the first therapeutic application of diosmin. It was launched commercially in France in 1971 for the treatment of venous insufficiency of the lower limbs and hemorrhoids, and, as of 1992, was being marketed in 57 countries, including 8 in Western Europe. Today, its application has been extended to include many other venocapillary disorders, such as varicose veins, venous stasis ulcers, subconjunctival and retinal hemorrhage, and gingival bleeding.¹ In the United States, diosmin is used in dietary supplement formulas.

Diosmin has been the subject of more than 160 clinical trials, animal studies, and *in vitro* studies. Clinical trials have been conducted using doses of 1000 mg to 3000 mg per day orally for up to 1 year. According to the results of animal studies, transplacental passage of the drug involves only traces, and its elimination in breast milk is minimal, hence avoiding any toxic risk for the fetus or newborn. In all clinical trials, diosmin has been well tolerated. Only rarely have participants experienced mild, transitory adverse events (Table 2), the incidence and nature of which were similar to a placebo. During the trials, hemodynamic and laboratory parameters were unaffected by long-term treatment. There was no evidence of any contraindication to the therapeutic use of diosmin. Diosmin demonstrated no photosensitizing action, caused no drug interactions and its safety of use was unmodified in the elderly.

***Systemic name:** 4H-1-Benzopyran-4-one, 7-((6-O-(6-deoxy- α -L-mannopyranosyl)- β -D-glucopyranosyl)ox- y)-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-

Synonym: 3',5,7-Trihydroxy-4'-methoxyflavone-7-rutinoside

Molecular formula: C₂₈H₃₂O₁₅

TOXICITY STUDIES IN ANIMALS

Animal toxicology studies and studies evaluating the effect of diosmin+h on the digestive tract, reproductive function, and lactation demonstrated an excellent safety profile. In toxicity studies conducted by Heusser & Osswald on rats, diosmin 200 mg/kg per day orally for 50 days produced no toxicity or abnormality in blood count, GOT, GPT, urea, histology, or weight development. In mice, diosmin 60 and 620 mg/kg per day orally for 196 days showed no toxicity or abnormality in blood count, GOT, GPT, urea, histology, or weight development. In minipigs, diosmin in doses of 50 and 250 mg/kg per day orally for 180 days produced no systematic abnormalities in clinical, biochemical, or hematological values pointing to a toxic effect.³

In a teratogenicity study, Heusser & Osswald discovered no pathology in 126 skeletons of the fetuses of mother mice given sodium salt of diosmin 50 mg/kg per day from the 4th to the 12th postcoital days. Postnatal mortality (20 days) was 19% in the control group and 26% in the diosmin group in another group of mother mice given the same dose of diosmin and allowed to deliver spontaneously. Weight gain, length development, gross behavior, growth of hair and opening of the eyes were identical in the both groups. Organ weights, macroscopical and histological findings did not differ significantly in the diosmin and control animals.³ There were no abnormalities in the skeletons of the fetus of mother rats given sodium salt of diosmin 100 mg/kg per day from the 4th to the 12th postcoital days. After spontaneous delivery, the average number of animals per litter was 12.9 in the control group and 11.6 in the diosmin group. There were no significant differences between the 2 groups in respect to weight gain, length, gross behavior, hair growth, opening of the eyes, organ weight, macroscopic and histologic examinations.³

In an article published in the *International Journal of Gynecology & Obstetrics*, Buckshee quotes Bromont's 1985 toxicological dossier, which states that there was no toxicity and no change in fertility or reproductive function in mice at doses 35 times greater than the therapeutic dose during 26 weeks of treatment.⁴ Tests on bacteria, human lymphocytes, mice bone marrow and DNA in HeLa cells revealed no mutagenic effects, and the embryology, peri- and postnatal development of rat offspring born to treated parents were not affected.⁴ Additionally, Meyer wrote that there was no genetic toxicity in the bacteria gene mutation test, in an analysis of metaphases in human lymphocytes in culture, in a DNA repair test in a leukocyte system gene mutations test, or in an *in vivo* clastogenic lesions test.² Buckshee also cites an unpublished report by Bromet and colleagues discussing a study in which the accumulation of diosmin in the uterus of Wistar rats was 0.02%, the transplacental passage was 0.003 %, and passage in breast milk was 1%. Lastly, Buckshee mentions an international marketing survey published in the French Medical Index, based on data obtained in France between August 1995 and August 1996, which reported that 1.4% of all prescriptions of micronized diosmin+h were issued to women who had a normal pregnancy.⁴

CLINICAL TRIALS

Results of a Review of 12 Clinical Trials

A review by Meyer** published in 1994 in *Angiology: The Journal of Vascular Diseases*, analyzed data on 3075 patients participating in 12 mid- and long- term trials.^{a,b,c,d,e,f,g,h,i,j,k,l} Participants were treated with 1 of 2 formulations of diosmin+h, or placebo taken twice daily for 6 weeks to 1 year. The nature and incidence of the side effects, which was

found in 10% of all patients treated, were similar in all groups and involved mostly gastrointestinal and autonomic disorders. Of the patients treated with diosmin+h, 6.9% experienced either abdominal pain, gastric discomfort, epigastric pain, nausea, dyspepsia, vomiting, or diarrhea; 1.7% of the patients treated with diosmin+h experienced either insomnia, drowsiness, vertigo, headache, tiredness, anxiety, cramps, palpitations, or hypotension. Other side effects reported were 1 case of pruritus and 2 cases of menometrorrhagia in the placebo group and 1 case of epistaxis and 1 case of menometrorrhagia in the diosmin group. There was also 1 case of an eczematiform rash and 1 case of pityriasis rosea not attributable to treatment.²

Regardless of the length of the trial, approximately 10% of the patients in the diosmin+h (micronized formulation) groups, 13% in the diosmin+h (unmicronized formulation) groups, and 13.9% in the placebo groups developed side effects. The percent of patients treated who dropped out of trials because of side effects was 1.1% in the diosmin+h (micronized formulation) groups, 4.8% in the diosmin+h (micronized formulation) groups and 3.2% in the placebo group. Additionally, the 12-study diosmin+h review showed:

- No evidence of any change in hemodynamic parameters with diosmin+h 1000 mg in a 1-year multicenter trial that enrolled 215 patients.
- Side effects in participants 70 years and older was 16.3% in diosmin+h groups and 15.9% in placebo groups, without being significantly different from the total population.
- The incidence of side effects did not differ significantly in diosmin+h and placebo groups in patients with hypertension, atherosclerosis, diabetes, neurologic disorders, psychiatric disorders, or alcoholism.
- When diosmin+h was combined with other drugs used to treat concomitant disorders, there was no evidence of any drug incompatibility or interaction in any of the 12 trials.
- Side effects in trials lasting 6 weeks to 2 months were equivalent to those in trials lasting from 6 months to 1 year.
- No side effects were seen in 18 patients treated at the daily dosage of 3000 mg for 28 days, in 10 patients treated at the daily dosage of diosmin 2000 mg for one month^a or in 18 patients treated once with 2000 mg of diosmin+h.⁵
- There was no change in the laboratory values of 437 patients treated with diosmin+h or placebo in 4 trials^{b,c,d,e} lasting between 2 and 6 months.
- When photosensitivity was evaluated in 40 high-risk patients (elderly, past history of allergy or, iatrogenic photosensitization), there was no evidence of a photosensitizing effect with diosmin+h.^f

Blood count, hemoglobin, packed-cell volume, prothrombin, creatinine, urea, albumin, fasting blood glucose, total cholesterol, HDL and LDL-cholesterol, HDL/LDL cholesterol ratio, triglycerides, uric acid, calcium, phosphorus, magnesium, transaminases, GGT,

and alkaline phosphatase were not modified by treatment with Daflon in a 1-year multicenter trial conducted by Pointel and cited by Meyer. There was a slight decrease in plasma creatinine in 65.5% of patients that was significant during treatment. There was also a regular but nonsignificant fall in fibrinogen levels in 65.2% of patients. Both of these parameters, however, remained within the normal physiologic range.²

Clinical Trial Safety Data

In a safety and efficacy study of micronized diosmin+h for the treatment of internal hemorrhoids of pregnancy, Buckshee and colleagues concluded that treatment was well accepted, and did not affect pregnancy, fetal development, birth weight, infant growth and feeding. Fifty women with acute hemorrhoids were enrolled in an open study on hospital outpatients, for a median of 8 weeks before delivery and 4 weeks after delivery. Treatment was divided into 3 phases. In the first phase, to assess the response of acute symptoms, a loading dose of 3000 mg per day was given for 4 days and 2000 mg per day was given for 3 days. In the second and third phases, to assess relapse in the ante- and post- natal periods respectively, a maintenance dose of 1000 per day was given in a divided dose after lunch and dinner for 30 days. Among those recruited, 47 patients completed the loading treatment phase of 7 days; 44 the antenatal maintenance treatment phase of 8 weeks; and 41 the post natal maintenance treatment phase of 30 days. Four patients withdrew: 1 due to nausea and diarrhea in the loading phase and 2 for reasons unrelated to treatment. Five patients were lost to follow up. Five patients complained of nausea and diarrhea (4 in the loading phase and 1 in the maintenance), but it did not lead to withdrawal. Hemodynamic and biochemical variables showed no significant change with treatment during pregnancy, and were normal at the end of the study. No ultrasonographic fetal abnormality was detected during the study. One intrauterine death occurred due to a cord around the neck of the fetus. At delivery, gross placental insufficiency was detected in 6 (13.6%) patients. The median maturity of the infant at birth was 39 weeks and weight 2.9 kg. One infant had a single umbilical artery. At the end of postpartum treatment, 38 infants were breast fed or supplemented artificially and the median weight gain was 1 kg.⁴

Cospite conducted a study of 100 patients undergoing an acute hemorrhoidal attack who were treated with either diosmin+h or placebo. Diosmin+h was given for 7 days at the dosage of 6 tablets daily during the first 4 days and 4 tablets daily during the following 3 days. One patient in the diosmin group and 5 in the placebo group withdrew from the treatment because of dissatisfaction with the therapeutic results. Four patients in the diosmin group and 3 in the placebo 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. In the diosmin group, 3 patients experienced gastralgia, 2 diarrhea, 1 abdominal pain, and 1 headache. In the placebo group, 1 patient experienced gastralgia, 1 dyspepsia, and 1 nausea.⁶

**Clinical Trials Reviewed by Meyer: ^aLacombe, ^bFrileux, ^cDelmont, ^dCope, ^ePointel, ^fOrtonne, ^gAmiel, ^hGalley, ⁱLagrue, ^jCospite 1998, ^kPeker, ^lElbaz, ^mVicari

In Guilhou's study of diosmin+h for the treatment of venous ulcers, 107 men and women were enrolled in a multicenter, double-blind, randomized, 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), asthenia (1), headaches (1), and exacerbation of chronic colopathy (1) in the diosmin+ h group.⁷

Guillot and colleagues investigated the safety of diosmin+h 500 mg taken twice daily for 1 year for the treatment of chronic venous insufficiency (CVI). Of the 250 outpatients who received diosmin+h, 170 completed the study. Laboratory parameters remained constant during the 12 months. Side effects were mainly gastralgia (n = 7). Four patients were excluded from the final analysis because of side effects, 10 for non-compliance, 19 for dropping out, and 12 for causes unrelated to the trial. Clinical side effects were rare and seen in only 20 patients: gastralgia in 7 patients, dizziness in 4, gynecological signs in 7, and cutaneous eruption in 2. Side effects led to treatment withdrawal in 4 patients: nausea and gastralgia in 2 cases and an increase of body weight in 2 others but probably not related with the treatment. Blood pressure measured before and after treatment showed a slight decrease of the systolic (125.6 vs 129.9) and diastolic values (74.4 vs 76.3). Laboratory parameters remained in normal ranges. RBC, WBC, Hg remained unchanged within the trial SGPT, SGOT, and GGT, alkaline phosphatase, quick test and fibrin showed no modifications. Blood urea varied between 0.32 and 0.34 g/l and creatinine decreased from 87.7 ± 1.6 to 84.0 ± 1.8 $\mu\text{mol/l}$. Lipid fluctuations remained in normal range and so did glucose, magnesium, phosphate, and calcium levels.⁸

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 frequency 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.⁹

Diosmin+h 1000 mg was given daily for 2 months to 174 women and 26 men with either organic CVI (83) or functional CVI (117) in 2 double-blind randomized 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.¹⁰

DOSE CONSIDERATIONS

Doses for diosmin used as a dietary supplement have been calculated after an assessment of animal and human clinical trial data. The usual dose for adults is 500 mg twice daily, and loading doses of 3000 mg per day for 4 days have been given without incident (Table 2). Diosmin has been used in numerous clinical trials lasting from 2 months to 1 year.

Diosmin is not intended for use by pregnant women and will be so labeled. The safe use of diosmin for the treatment of pregnant or nursing women with hemorrhoidal disease or venous insufficiency has not been established in large-scale clinical trials. However, no deleterious effects have been reported in pregnant women or their offspring after administration during pregnancy.²

PHARMACOKINETICS & METABOLISM

In a pharmacokinetic study conducted by Cova and colleagues, the mean plasma concentration of diosmetin, the aglycone form of diosmin, was assessed following the oral administration of diosmin to healthy volunteers (Table 3). Diosmetin was identified by HPLC and LC-MS techniques. No parent compound was present in the plasma at 20 ng/ml, only the aglycone diosmetin with a retention time of 3.4 minutes. The peak plasma level of diosmetin, 417 ng/ml, was reached after 1 hour. Drug levels in the plasma started to decrease slowly after 2 hours, constantly after 24 hours, and were still detectable after 48 hours. The drug was rapidly absorbed, and diosmetin had a plasma elimination half-life ranging from 26 to 43 hours. After oral ingestion of diosmin, there was no urinary elimination of either diosmin or diosmetin. Its minor metabolites were eliminated in the urine, mainly as glucuronic acid conjugates. The presence of degradation products such as alkyl-phenolic acids confirmed a metabolic pattern similar to other flavonoids. The prolonged presence of diosmetin in the blood suggested an enterohepatic circulation, which is known to have the effect of slowing the complete elimination of drugs. Investigators speculated that (1) the high value of the volume of distribution accounted for the low plasma levels compared with the administered dose of diosmin, (2) the apparent volume of distribution of approximately 62.1 liters pointed to an extensive uptake of the compound by the tissues, and (3) the value of the total body clearance accounts for an active metabolism that can occur in the lumen of the gastrointestinal tract or in the liver, before its elimination in the urine, where it cannot be found in unmetabolized forms.¹¹

Table 3. Pharmacokinetic parameters (mean \pm SD) after a single oral administration of diosmin

Parameters	Mean \pm SD
Maximum plasma concentration (ng/ml)	417 \pm 94.1
Half life (h)	31.5 \pm 8.6
Mean residence time (h)	36.6 h \pm 9.9
Area under plasma concentration-time curve (ng/ml h)	5,617.1 \pm 1,518.4
Total body clearance (l/h)	1.32 \pm 0.42
Volume of distribution (l)	62.1 \pm 7.9

PHARMACOLOGY

Elimination

In a study conducted by Oustrin and colleagues, ^3H -labelled diosmin was administered both IV 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 feces. After IV administration, elimination was predominantly in the urine, while after oral administration it was eliminated almost equally in the urine and in the feces, during the first 24-hour period. In the following 24-hour periods, the feces carried the greater portion of diosmin or its metabolites. Binding to the vascular wall was relatively late.¹²

Mechanism of Action

Inflammatory reactions are triggered by chemical and biological mediators, such as arachidonic acid derivatives (prostaglandins, leukotrienes, or thromboxanes), vasoactive amines (histamine or serotonin), and oxygen free radicals (superoxide ion, O_2^- , or hydrogen peroxide, H_2O_2). In venous inflammation, histamine causes vasodilatation; and PGE_2 , 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 E_2 and F_2 (PGE_2 , PGF_2), thromboxane B_2 (TXB_2), and oxygen free radicals.⁵

Diosmin reinforces venous tone by prolonging the activity of parietal 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.³ 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 an *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.¹³

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 $\mu\text{mol/l}$ was shifted from 31 to 70 ng/ml in a concentration dependent manner.¹⁴ 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-1.¹⁵

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 IV injection of D (12.500 mg/kg-1) 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.¹⁶

Daflon is a strong inhibitor of $\text{Cu}(2+)$ -induced arachidonic acid peroxidation, as revealed by the inhibition of thiobarbituric acid- reactive substance formation in mixed liposomes of phosphatidylcholine and arachidonic acid. Diosmin is a good complexant of Cu^{2+} ions but not of Fe^{2+} ions. The Cu^{2+} complex formation 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 involve metal ions.¹⁷

Table 3.

Diosmin Dose and Safety

Author/Study	Dose	Duration	Patients/DZ	Safety Results
Thanapongsathorn 1992¹ Clinical trial of oral diosmin (Daflon) in the treatment of hemorrhoids	Daflon 12 tablets in 3 divided doses for the first 4 days, 2 tablets twice daily for the following 10 days	14 days	100 patients internal hemorrhoids	No side effects detected
Meyer 1994² Safety and security of Daflon 500 mg in venous insufficiency and in hemorrhoidal disease	Daflon 500 mg 2 tablets daily	6 weeks to 1 year	2850 patients CVI and hemorrhoidal disease	Treatment did not effect pregnancy and infant feeding. No major side effects were found. Acceptability was equal in short- and long- term treatments
Buckshee 1997⁴ Micronized flavonoid therapy in internal hemorrhoids of pregnancy	Daflon 500 mg twice daily	8 weeks before delivery and 4 weeks after delivery.	50 women acute hemorrhoids	Treatment did not affect pregnancy, fetal development, birth weight, infant growth and feeding
Cospite 1994⁶ 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 first 4 days, 2 tablets daily the following 3 days	7 days	100 patients hemorrhoidal disease	Acceptability good with no major side effects
Guilhou 1997⁷ Efficacy of Daflon 500 mg in venous leg ulcer healing: a double-blind, randomized, controlled versus placebo trial in 107 patients	Daflon 500 mg 2 tablets daily	1 year	215 patients CVI	Laboratory parameters remained constant during the 12 months. Side effects were essentially gastralgia
Laurent 1988¹⁰ Clinical evaluation of a venotropic drug in man. Example of Daflon 500 mg	Daflon 500 mg 2 tablets daily or placebo	2 months	200 men and women CVI	Good acceptability

Author/Study	Dose	Duration	Patients/DZ	Safety Results
Tsouderos 1991¹⁸ 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
Boccalon 1997¹⁹ 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

Daflon 500 mg = 540 mg diosmin and 50 mg hesperidin

References

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