European Medicines Agency
Evaluation of Medicines for Human Use

London, 4 September 2008
Doc. Ref. EMEA/HMPC/261939/2007

ASSESSMENT REPORT ON

RUSCUS ACULEATUS L., RHIZOMA

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ABBREVIATIONS

Afssaps	Agence Française de Sécurité Sanitaire des Produits de Santé (French Health Products Safety Agency)
ATP	Adenosine Tri-Phosphate
AUB	Area Under Baseline
CVD	Chronic Venous Disease
CVI	Chronic Venous Insufficiency
DOMA	3,4-dihydroxymandelic acid
DOPEG	3,4-dihydroxyphenylglycol
EMEA	European Medicines Agency
ESCOP	European Scientific Cooperative On Phytotherapy
ETDRS	Early Treatment Diabetic Retinopathy Study
FLQA	Freiburger Life Quality Assessment
HPLC	High Pressure Liquid Chromatography
HUVEC	Human Umbilical Vein Endothelial Cell
IV	Intravenous
LD50	Lethal Dose 50% (dose that kills 50% of experimental animals)
LOCF	Last Observation Carried Forward
LSC	Liquid Scintillation Counting
MOPEG	3-methoxy-4-hydroxyphenylglycol
NMN	Normetanephrine
PGF2α	Prostaglandin F2α
PLA2	Phospholipase A2
PSUR	Periodic Safety Update Report
Pt	Total protein concentration
PTS	Post-Thrombotic Syndrome
THMC	Trimethylhesperidin chalcone
TLC	Thin Layer Chromatography
VAD	Vaso-Active Drug
VAS	Visual Analog Scale
VMA	3-methoxy-4- hydroxymandelic acid
WBA	Whole Body Autoradiography

I. REGULATORY STATUS OVERVIEW [1]

MA: Marketing Authorisation;
TRAD: Traditional Use Registration;
Other TRAD: Other national Traditional systems of registration;
Other: If known, it should be specified or otherwise add 'Not Known'

Member State	Regulatory Status				Comments[2]
Austria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Belgium	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Bulgaria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Cyprus	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Czech Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Denmark	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Estonia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Finland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
France	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	
Germany	☐ MA	☒TRAD	☐ Other TRAD	☐ Other Specify:	
Greece	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Hungary	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
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Sweden	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
United Kingdom	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	

[1] This regulatory overview is not legally binding and does not necessarily reflect the legal status of the products in the MSs concerned.
[2] Not mandatory field

II. ASSESSMENT REPORT FOR HERBAL SUBSTANCE(S), HERBAL PREPARATION(S) OR COMBINATIONS THEREOF WITH WELL-ESTABLISHED USE AND/OR TRADITIONAL USE

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Ruscus aculeatus* L., rhizoma
Herbal preparation(s)	Traditional use Dried powdered root Dry extract (2.5-6.5 : 1 ; water) Dry extract (5-8.5 : 1 ; 80% V/V ethanol) Dry extract (6-9 : 1 ; primary solvent 96 % V/V ethanol followed by water.) Dry extract (15-20 : 1 ; 60% V/V methanol)
Pharmaceutical forms	Solid forms for oral use
Rapporteurs	Antoine Sawaya Jacqueline Viguet Poupelloz

II.1 INTRODUCTION

The aim of this report is to assess the preclinical and clinical available data on *Ruscus aculeatus* for preparing a Community herbal monograph. This report is based on the documentation provided by the European Medicines Agency (EMEA) completed by additional researches and information taken from recently revised monographs on *Ruscus aculeatus* (Commission E Monographs, 2001; ESCOP, 2003). Recent investigations on the efficacy and safety of *Ruscus aculeatus* have also been published in reviews specializing in phytotherapy and alternative medicines (Commission E Monographs, 2001; Bone, 2003). This report takes up parts of these works. However, as far as *Ruscus aculeatus* alone is concerned with the monograph, except for relevant safety data, the studies performed with *Ruscus aculeatus* combinations such as Cyclo 3® or Phlebodril® are not discussed in this report.

This report focuses on findings with preparations based on aqueous-alcoholic extracts of the rhizome since clinical experience has been collected with these types of extracts, and they were used in most preclinical and clinical trials. The extracts used in the trials are specified as far as possible. Unfortunately, correct specifications of solvent and drug-extract ratio are missing in most of the publications. No detail can be given if the extract could not be identified.

II.1.1 DESCRIPTION

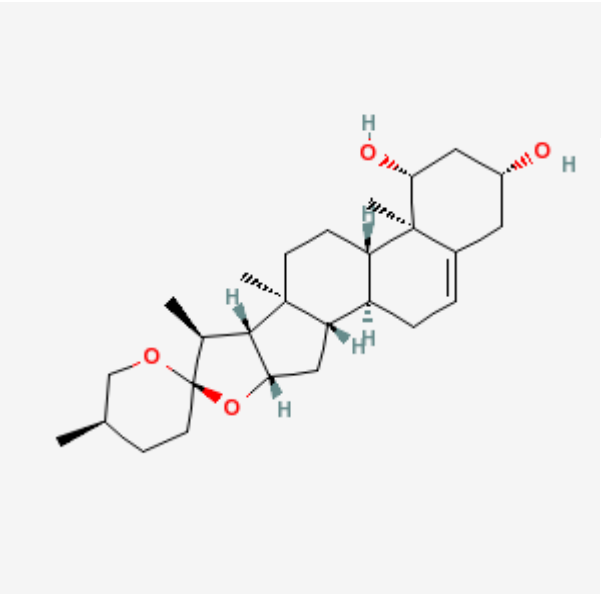
- **Herbal substance**

Ruscus aculeatus L. *(Liliaceae)* is a widely distributed European plant native from Western Europe. This plant has numerous appellations. The English name - Butcher's Broom - derives from the use by the European butchers of the stems to clean their cutting board not only because of their stiffness and solidity, but also because of the essential oil which was credited with antibacterial properties. *Ruscus aculeatus* is a small, clump-forming shrub with erect shoots bearing stiff, ovate, leaf-like phylloclade. Tiny green flowers appear in late winter and spring on the phylloclade. Both root and stem are used in preparations.

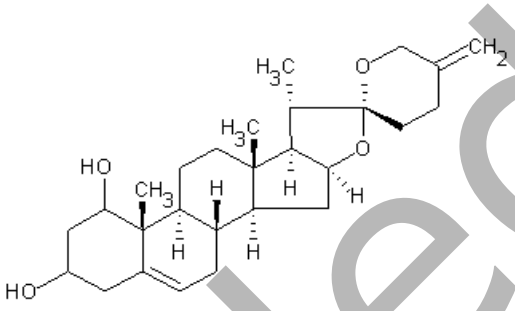
The European Pharmacopoeia prescribes not less than 1.0 per cent of total sapogenins expressed as ruscogenins (mixture of neoruscogenin and ruscogenin).

- **Herbal preparations**

It is around the middle of the twentieth century that steroidal saponins (ruscogenin and neoruscogenin) were isolated from the rhizome. Besides primary steroidal saponins the extract of the rhizome contains also several other minor compounds such as other steroidal sapogenins and saponins, sterols, triterpenes, flavonoïds, coumarins, sparteïne, tyramine and glycolic acid (Dunouau *et al.*, 1996; Mimaki *et al.*, 1998). Both the above ground and below ground parts of the plant contain ruscogenins, although concentrations are higher in the below ground parts of the plant (Nikolov *et al.*, 1976). In the present time, preparations are mainly based on aqueous and aqueous-alcoholic extracts of the rhizome. The dried powdered root is also on the market. *Ruscus aculeatus* extracts are also used in commercial products combined with other products such as trimethylhesperidin chalcone (TMHC), ascorbic acid or Melilotus officinalis extract.



Ruscogenin



Neoruscogenin

II.1.2 INFORMATION ON PERIOD OF MEDICINAL USE IN THE COMMUNITY REGARDING THE SPECIFIED INDICATION

This plant was used during the ancient times as a diuretic or to relieve menstrual pains and water retention discomfort. *Ruscus aculeatus* was also used throughout Europe for the treatment of constipation, urinary disorders and abdominal pains. *Ruscus aculeatus* sinks into oblivion in the early 1900s. The reported vasoconstrictive effect of the ruscogenin and neoruscogenin (see paragraph II.2 Non-clinical data) accounted for its current use in chronic venous insufficiency, haemorrhoids and varicose veins.

Ruscus aculeatus has been used over a decade as herbal substance or herbal preparations notably in France or Germany, since 1986 and 1978, respectively. At present, with regard to the different available monographs, *Ruscus aculeatus* is listed as follows:

- French Monograph : cahiers de l'Agence n° 3 (Afssaps, 1998)
  Adults recommendations given for dried powered root and dry aqueous extracts in capsules for oral administration corresponding to a daily amount of total ruscogenins around 10 mg.
  "Traditionally used in subjective symptoms of chronic insufficiency such as sensation of heavy legs and in haemorrhoids symptoms".

- ESCOP Monograph (ESCOP, 2003)
  Adults recommendations given for solid or liquid extracts in amounts corresponding to 7-11 mg of total ruscogenins for oral administration taken once a day.
  "Supportive therapy for symptoms of chronic venous insufficiency, such as painful, tired and heavy legs, tingling and swelling.
  Supportive therapy for symptoms of haemorrhoids, such as itching and burning."

- It has to be noted that the ESCOP Monograph takes only into account the available data on *Ruscus aculeatus* alone.

- Commission E Monograph (Commission E Monographs, 2001)
  Adults' recommendations given for alcoholic extracts of the whole plant or standardized for ruscogenins as determined by the total amount of ruscogenin and neoruscogenin (ranges 7-11 mg) in capsules for oral administration.

"Supportive therapy for discomforts of chronic venous insufficiency, such as pain and heaviness, as well as cramps in the legs, itching and swelling.
Supportive therapy for complaints of haemorrhoids, such as itching and burning. "

- It has to be noted that the Commission E Monograph takes into account the whole data available on *Ruscus aculeatus* including non-clinical and clinical data referring to combinations.

➢ Bfarm (BfArM information, 2007)
Adults recommendations given for dry extracts for oral administration:
Ethanolic 80 % (V/V); (5-8.5 : 1) in coated tablets and Ethanolic 96 % (V/V); (6-9 : 1) in soft capsules, respectively.
"Traditional herbal medicinal product to ease/soothe the feeling /sensation of heavy legs. The product is a traditional herbal product for use in specified indications exclusively based on long-standing use."

- Daily dosage and corresponding daily amount of total ruscogenins are not given.

II.2 NON-CLINICAL DATA

Non clinical data presented in this assessment report should be taken into account according to the kind of preparations registered in the European Union. Request of information concerning *Ruscus aculeatus* showed that in France and Germany, preparations consist of hydro-alcoholic extracts, aqueous extracts, and plant powder.

Assessor's comment

The non clinical assessment report was mainly based on bibliographic data submitted by the European Medicines Agency (EMEA). Some references were occasionally added by the non clinical assessor on specific points.

In general, the characteristics of the extract used (mode of extraction, content in active substances, etc.) were not provided. Such a lack of data does not allow an adequate assessment of non clinical studies, and clear conclusion on efficacy and safety of the extract
In most of the studies, the extract was provided by Pierre Fabre Médicament (Castres, France) and/or the authors were employed by this company. ***Therefore, we will consider that the extract produced by Pierre Fabre Médicament was used, unless otherwise indicated.*** *It should be noted that no precision on this extract is given by any author (mode of extraction, content in active substances, etc.)*

II.2.1 PHARMACOLOGY

II.2.1.1 PRIMARY PHARMACODYNAMICS

Ruscus aculeatus being reported to be used in against venous insufficiency-related feeling/sensation of heavy leg, several *in vitro*/*in vivo* studies aimed at demonstrating its contractile properties on veins or lymphatic vessels as well as its capacity to improve vascular permeability.

Assessor's comment

It should be noted that studies conducted with the combination used in Cyclo 3® (*Ruscus* extract and hesperidine methylchalcone) were not taken into consideration. Indeed, the potential beneficial effect of the flavonoids or of the combination should not be attributed to *Ruscus* extract taken separately.

II.2.1.1.1 *CONTRACTILE EFFECT ON VEINS*

A. In vitro studies

- **Studies performed on rings of canine saphenous veins**

This model was first described by Marcelon *et al* (Marcelon *et al.*, 1983b; Marcelon and Vanhoutte, 1984). Rings of canine saphenous veins were mounted in organ chambers filled with a physiological salt solution and connected to a force transducer for continuous recording of isometric tension. Prior to experimentation with *Ruscus* extract, the segments were placed at the optimal point of their length-tension relationship using standard electrical stimulation. Preparations were then allowed to equilibrate at their optimal length for 90 minutes. Thereafter, *Ruscus* extract was applied and the tension measured. It showed that at concentrations $\geq 3.10^{-5}$ g/ml, *Ruscus* extract caused a dose-dependent increase in tension.

The effects of the following various pharmacological agents on the contractile effect induced by *Ruscus* were studied in an attempt to determine the mechanism of action underlying this contractile effect:

- Phentolamine (3.10^{-6} M), an α-adrenolytic agent, nearly abolished the contractile response to *Ruscus*;
- Cocaine (3.10^{-5} M), a blocker or the recapture of norepinephrine in the inter-synaptic gap, reduced the contractile response to *Ruscus*;

- 6-hydroxydopamine (10^{-6} M), inducing chemical denervation, reduced the contractile response to *Ruscus* similarly to cocaine;
- Tetrodotoxin (10^{-7} M), atropine (10^{-8} M), methysergide (10^{-7} M), indomethacin (3.10^{-5} M) did not impact on the contractile response to *Ruscus*;
- Adenosine (2.10^{-5} M) and verapamil (2.10^{-6} M) produced relaxation of the rings contracted by *Ruscus* (-37% and -50%, respectively);
- Acetylcholine (10^{-7}M, 10^{-6}M) caused further increase in tension (+23% and +37%, respectively).

In parallel, the same authors examined the effect of *Ruscus* extract on helical strips of canine Saphenous veins connected to a force transducer and incubated in a moist tunnel-shaped chamber superfused with a Krebs-Ringer solution containing 7-1-[^{3}H]-norepinephrine. *Ruscus* extract (5.10^{-4} g/ml) caused an increase in tension. In addition, the release of intact [^{3}H]-norepinephrine and the overflow of all metabolites (DOPEG, DOMA, MOPEG, NMN) except VMA were increased.

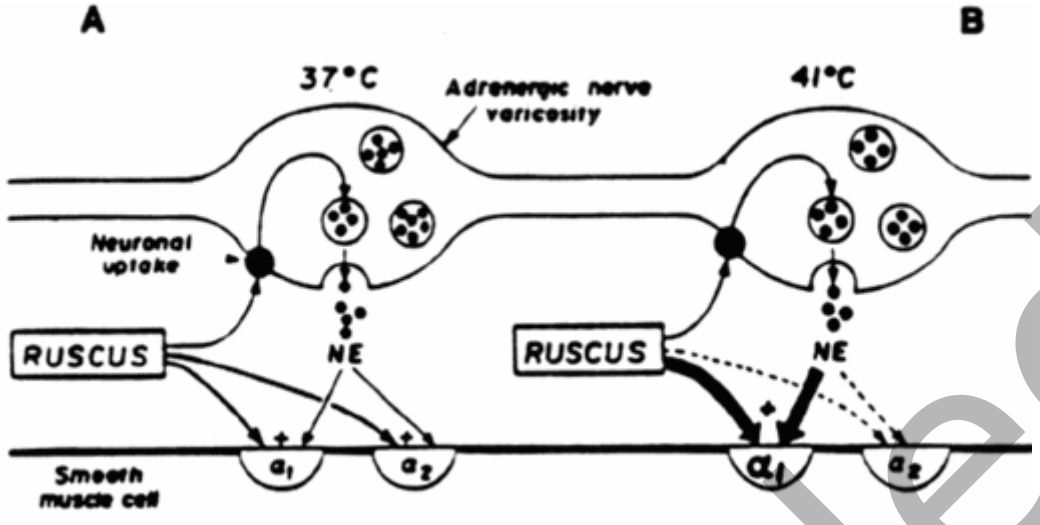
From these findings, it was concluded that the venoconstrictor response to *Ruscus* extract is not due to the activation of cholinergic, serotoninergic, or prostaglandinergic receptors. The effect being inhibited by phentolamine but persisting after chemical denervation with 6-hydroxodopamine, a direct effect on postjunctional α-adrenergic receptors of the smooth muscle cells is suggested. However, it seems that the release of norepinephrine stored in adrenergic nerve endings is also involved in the contractile effect of *Ruscus* extract in view of the responses obtained with 6-hydroxodopamine, and cocaine, and in view of the increased overflow of [^{3}H]-norepinephrine in veins previously incubated with this neurotransmitter.

The release of norepinephrine from adrenergic nerve endings does not involve the initiation of spike electrogenesis, since tetrodotoxin had no influence on the effect provoked by *Ruscus* extract. The authors considered that the effects of adenosine, verapamil and acetylcholine reflected interference with the postjunctional effect rather than with its action on the adrenergic nerve endings. However, the effect by acetylcholine may be artificially biased by damaging of the endothelium in the experimental model; in a non de-endothelialised vessel a relaxing effect by NO release would be expected.

In another study, Rubanyi *et al* confirmed that *Ruscus* extract could provoke the contraction of rings from canine saphenous veins. The tension increased as *Ruscus* extract concentration increased from 10^{-4} to 10^{-3} g/ml; the maximal contraction averaged 80% of the response to 10^{-4} M norepinephrine. It should be mentioned that a factor 10 within a dose relationship from basic tension to maximum has to be considered as very narrow in pharmacological terms. However, the contractile effect of *Ruscus* was depressed by prazosin or rauwolscine (α1- and α2-antagonist, respectively) from 5.10^{-8} M and in a dose-dependent fashion, while it was abolished when both substances were used concomitantly. The same response was observed when phentolamine, a non selective α-antagonist was used in the experiment described above (Marcelon *et al.*, 1983b; Marcelon and Vanhoutte, 1984). This suggests that the contractile response of *Ruscus* in this model is due to α-adrenergic activation only (Rubanyi *et al.*, 1984).

At 5.10^{-6} M, the inhibitory effect of rauwolscine was higher than that of prazosin. This difference was not observed when prazosin or rauwolscine was used with cocaine, both combinations depressing the contractile activity of *Ruscus* in the same extent. According to the authors, this suggests that norepinephrine released from adrenergic nerve endings activates preferentially postjunctional α2-adrenoceptors (Rubanyi *et al.*, 1984).

The same authors reiterated the study using *Ruscus* extract (2.10^{-4} g/ml), prazosin and rauwolscine (5.10^{-6} M) at 24°C, 37°C, and 41°C. Compared to the results obtained at 37°C, cooling decreased while warming increased the contractile response to *Ruscus* extract in rings of canine saphenous veins. These results were explicated in details by other authors. They acknowledged that these differences are due to the preferential modulation by temperature of the α1-adrenergic component of the response. At 37°C, the α1- and α2-adrenergic components of the response to *Ruscus* are equivalent (Rubanyi *et al.*, 1984; Marcelon and Vanhoutte, 1988).



Abbreviations: U1=neuronal uptake; NE=norepinephrine; +=activation

Figure 1: proposed mechanism of action for Ruscus and effect of temperature (Marcelon and Vanhoutte, 1988)

To study the influence of the hormonal status of the animals on the contractile effect obtained with *Ruscus* extract, Miller *et al* treated ovariectomized female dogs with subcutaneous pellets containing a carrier substance (untreated), 17β-estradiol, progesterone, or 17β-estradiol and progesterone for 16-25 days. After that treatment period, the animals underwent surgery. The serum levels of hormones were measured and the carotid arteries and lateral saphenous veins removed and cut into rings after having been cleaned of connective tissue. The endothelium was removed in all rings. Thereafter, isometric tension was determined in absence or presence of a combination of prazosin or rauwolscine (10^{-7} M) following *in vitro* exposure to norepinephrine (10^{-8} to 10^{-4} M), tyramine (10^{-8} to 10^{-4} M), or *Ruscus* extract (10^{-6} to 10^{-3} g/ml) (Miller *et al.*, 1991a). Serum hormone levels are reported in the table below.

Group	Estrogen level (ng/100 mL)	Progesterone level (ng/100 mL)
Untreated	1	80
Estrogen	30	400
Progesterone	–	750
Estrogen+Progesterone	30	2300

Table 1 : estrogen and progesterone measured in serum levels of ovariectomised dogs, according to their hormonal supplementation (Miller *et al.*, 1991a)

The contractile effect induced by electrical stimulation, norepinephrine and tyramine (indirect sympathomimetic substance, releases norepinephrine from adrenergic nerve endings) were not influenced by hormonal treatment. On the contrary, the contractile effect observed with *Ruscus* tended to be augmented in the progesterone group compared to the untreated group, the difference being significant compared to the group treated with estrogen and progesterone. Subsequent assays showed no effect of adrenergic blockade (prazosin plus rauwolscine) on the contractile effect of *Ruscus* in rings from untreated animals, whereas this effect was decreased in progesterone group and increased in estrogen and estrogen plus progesterone groups (Miller *et al.*, 1991a).

The same authors published further work performed on the same animals, which consisted in studying the influence of hormonal status and endothelium on the contractile effect of *Ruscus* (Miller *et al.*, 1991b).

In coronary arteries, *Ruscus* extract was able to initiate the release of endothelium-derived factors whose action is inhibitory to contractions initiated by the extract itself, PGF2α and norepinephrine. The release of this factor could involve the stimulation of muscarinic receptors because the relaxing effect was

inhibited by atropine. Considering the nature of this relaxing factor, it is not probably prostacyclin because the experiment was performed in presence of indomethacin in the medium. Similarities with nitric oxide are evoked because the relaxing effect attributed to this relaxing factor is inhibited by haemoglobin (which inactivates NO) and by methylene blue (which inactivates guanylate cyclase). The hormonal status of the animals had no effect on the results obtained.

In femoral veins, the constrictor effect of *Ruscus* was influenced by the integrity of the endothelial cells and by the hormonal status of the animals. Indeed, the contractile effect obtained with *Ruscus* was greater in veins without endothelium, and greater in rings from animals receiving oestrogen plus progesterone. This result is contradictory to previous results obtained by these authors and described above [see (Miller *et al.*, 1991a)]. In varicose veins, the endothelium may be dysfunctional. Therefore, excitatory effect of the extract would prevail in this situation. Elevated oestrogen and progesterone serum levels would potentate this effect (Miller *et al.*, 1991b).

- **Study performed on rings of saphenous veins taken from rabbits**

The influence of the hormonal status of female rabbits on the contractile effect obtained with *Ruscus* extract, Harker *et al.* treated ovariectomized rabbits with either placebo or 17β-oestradiol (subcutaneous implants) for 14 to 21 days. After that treatment period, the animals underwent surgery. The serum level of oestradiol was measured and the lateral saphenous veins removed and cut into rings after having been cleaned of connective tissue. The endothelium was removed in all rings which were then suspended in organ chambers to measure isometric tension after application of various agents. The effect of α-adrenergic antagonists and of temperature (24°C, 37°C, 41°C) on the contractile effect of *Ruscus* extract was also studied (Harker *et al.*, 1988).

The serum oestradiol level reported by the authors amounted to 1.0 ng/ml or less in control animals, while it reached 54 ng/dl in estradiol treated animals. It should be noted that these figures may be wrong as 1.0 ng/ml is equivalent to 100 ng/dl.

In both groups, a dose-dependent contractile effect of *Ruscus* was observed. The involvement of postjunctional adrenoceptors was dependent upon the hormonal status of the animals. Indeed, contractions were not affected by adrenergic blockade in control animals, and partially inhibited by prazosin and rauwolscine in estradiol-treated animals suggesting that hormone permits the expression of postjunctional α-adrenergic effect of *Ruscus*.

Cooling increased tension mediated by α2-adrenoceptors (rauwolscine) after application of *Ruscus* extract on rings of control rabbits, but not on rings of estradiol-treated animals. Moreover, it was concluded that warming did not affect the contractile response to *Ruscus* extract (Harker *et al.*, 1988).

- **Studies performed on rings of human saphenous and varicose veins**

The contractile effect of *Ruscus* extract was investigated on rings of human varicose veins collected from females undergoing varicectomy (Marcelon *et al.*, 1988b). To study the influence of the hormonal status, three groups were considered:

- Rings from women at the end of menstrual cycle (14th to 25th day) : n=3;
- Rings from women at the beginning of cycle (1st to 6th day): n=4;
- Rings from post-menopausal women: n=6.

As previously described, isometric tension was measured in organ chambers (at 37°C).The contractile effect of *Ruscus* extract (10^{-5} to 10^{-3} g/ml) was compared to that obtained with noradrenaline (10^{-4} M). In these conditions, the variation of hormone levels occurring during the menstrual cycle or in post-menopausal women did not influence the contractile effect of *Ruscus* extract which reached 43 to 52% of the contractile effect obtained with norepinephrine (Marcelon *et al.*, 1988b).

In another study, the role of endothelium in the contractile effect of *Ruscus* extract (10^{-6} to 10^{-3} g/ml) was evaluated on rings of varicose and saphenous veins taken from patients undergoing surgery for primary varicose veins (Miller *et al.*, 1994). In this study, *Ruscus* caused a concentration-dependent contractile

effect to which varicose veins were more sensitive than saphenous veins. Additionally, contractions to *Ruscus* were not affected by removal of the endothelium. It was also confirmed in human veins that a major component of the contraction results from activation of adrenergic receptors because the blockade of adrenergic receptors significantly reduced that contractile effect. It is unlikely that *Ruscus* extract stimulates contraction by endothelin-A receptors in veins of these patients as the selective antagonist of these receptors (BQ-123) did not reduce the contractions either in absence or presence of adrenergic blockade. It is also unlikely that endothelin-B receptors are stimulated by *Ruscus* extract as varicose tributaries do not contract to sarafotoxin S6c (selective endothelin-B agonist). Therefore, the authors concluded that contractions to *Ruscus* in human varicose veins are independent of the endothelium and mediated by activation of adrenergic (but not endothelin-A) receptors on the smooth muscle (Miller *et al.*, 1994).

- **Studies performed on segments of canine and human saphenous/varicose veins**

Branco and Osswald (1988) studied the influence of *Ruscus* extract on the uptake and metabolism of norepinephrine in segments of canine lateral saphenous veins, and of human varicose/saphenous veins. In these tissues, the endogenous norepinephrine and dihydroxymandelic acid (DOMA) contents were determined. Thereafter, removal, accumulation and metabolism of [^{3}H]-norepinephrine were studied without and with application of *Ruscus* extract (10^{-5}, 10^{-4}, 10^{-3} g/ml).

Compared to the normal canine vein, the normal human veins appeared sparsely innervated by the sympathetic system as shown by 10-fold lower norepinephrine content. However, it should not be concluded that it is not endowed with efficient adrenergic mechanism. Indeed, its capacity to metabolize norepinephrine is rather high and its high reactivity to adrenergic agonists is well known; in this study, the capacity of human vein to remove, accumulate and metabolize norepinephrine was one-half of that exhibited by the canine vein. Normal canine and human veins also differed in the pattern in which noradrenaline was metabolized. In human veins, O-methylation by Catechol-O-methyltransferase (COMT) was of lesser importance than in canine veins. From this finding, the authors concluded that in what concerns disposition of noradrenaline, extrapolation from vessels of experimental animals to those of humans is not permissible.

The comparison of human normal and varicose veins showed that varicose veins contain and accumulate less norepinephrine. They have a high endogenous content and a raised rate of formation of DOMA.

Concerning the effect of *Ruscus* extract in human normal veins, it produced a concentration-dependent reduction of [^{3}H]-norepinephrine accumulation (-50% at 10^{-3} g/ml, compared to control). Changes were less marked in the varicose veins (-50% at 10^{-3} g/ml, compared to control). The authors concluded that the highest concentration of *Ruscus* extract only may have released norepinephrine. However, all three concentrations affected the metabolism of norepinephrine: a concentration-dependent reduction of the formation of all norepinephrine metabolites was observed, and was similar in normal and varicose veins (DOMA formation was more affected in varicose veins).

Overall, quantitative but not qualitative differences were noted between human normal and varicose veins concerning the effect of *Ruscus*. It provoked a depression of the metabolism of norepinephrine and reduced its accumulation. This led to an increase in the concentration of norepinephrine in the biophase. This could explain the potentiation of the action of norepinephrine (Branco and Osswald, 1988).

Assessor's comments

The **contractile effect** of *Ruscus* extract was investigated on rings of saphenous veins taken from dogs and rabbits, and on human saphenous and varicose veins by recording of the isometric tension. Studies on the canine model showed that a concentration-dependent contractile effect was obtained within the concentration range of 10^{-5}-10^{-3} g/ml for *Ruscus* extract. This was confirmed in the human model.

To determine the **mechanism** underlying this effect, further experimentations with various pharmacological agents and a study of tritiated norepinephrine metabolism were performed. They showed that in the canine venous rings, this effect was mediated by direct activation of postjunctional α1- and α2-

adrenergic receptors, and by stimulation of the release of norepinephrine form adrenergic nerve endings. It should be noted that α-adrenergic activation only was involved. The involvement of adrenergic receptors in the contractile effect of *Ruscus* extract was confirmed in human veins (saphenous, varicose).

These results are in accordance with those obtained on segments of human normal and varicose veins, where *Ruscus* extract was shown to reduce norepinephrine accumulation and metabolism. Although less marked in varicose veins, the reduction of norepinephrine accumulation was significant from 10^{-4} g/ml, a concentration compatible with those inducing a contractile effect on venous rings.

The influence of **temperature** was evaluated. In canine venous rings, cooling decreased while warming increased the contractile response to *Ruscus* extract obtained at 37°C. It was hypothesized that this differences is due to the preferential modulation by temperature of the α1-adrenergic component of the response. However, in venous rings taken from rabbits, cooling increased the contraction induced by *Ruscus* depending on the hormonal treatment, while warming had no effect.

Regarding the influence of the **hormonal status** on the contractile effect induced by *Ruscus* extract, contradictory results were obtained in animals. In one study performed with rings of varicose veins collected from women, the hormonal status did not impact on the contractile effect obtained. However, the number of samples tested was too low and the grade of varicosis of the veins was not studied, so that no definitive conclusion can be drawn. Similarly, conflicting results were obtained about a possible role of **endothelium** in the *Ruscus* effect in canine venous rings. In a human model, it was shown that "contractions to *Ruscus* in human varicose veins are independent of the endothelium and mediated by activation of adrenergic […] receptors on the smooth muscle" (Miller *et al.*, 1994).

Rings of human varicose veins were more sensitive than saphenous veins to the contractile effect obtained with *Ruscus* extract.

B. *In vivo* studies

- **Studies performed in dogs**

To confirm *in vivo* the adrenergic mechanism of action of *Ruscus* extract, this drug was administered intravenously to anaesthetised dogs at doses ranging from 1 to 10 mg/kg (Marcelon *et al.*, 1983a). The lateral saphenous vein was transilluminated and its diameter measured by a photoelectric cell. *Ruscus* caused a dose-dependent constriction of the saphenous vein and potentiated the dose-response curve to local norepinephrine. These responses were antagonized by phentolamine. In the conditions of the study, the venoconstriction caused by *Ruscus* was the result of α-adrenergic activation.

- **Studies performed on the hamster cheek pouch**

A team of the University of Lund, Sweden, aimed at examining the effects of *Ruscus* extract on the microcirculation of the hamster cheek pouch and at gaining knowledge about the mechanism(s) underlying these effects. They recognized that "the usefulness of the healthy hamster cheek pouch preparation to study chronic venous insufficiency is debatable, but *in vivo* animal models for such study are clearly lacking" (Bouskela *et al.*, 1994).

In the first experiment, six male hamsters were treated with *Ruscus* extract solution for 28 days by oral route at 0 and 150 mg/kg/day. At the end of the treatment period, animals were anaesthetised and an area of the cheek pouch isolated for measurement of vascular diameter (arterioles and venules)[3] by means of a

[3] Anesthesia was induced by an intraperitoneal injection of 0.1-0.2 ml of sodium pentobarbital and maintained with a-chloralose (100 mgjkg) administered intravenously. The femoral artery and vein were cannulated for pressure measurements, anesthetic and *Ruscus* extract injections. Throughout the surgery and subsequent experiment, the animal rested on a heating pad controlled by a rectal thermistor and body temperature was maintained at 36.5°C. A tracheal tube was inserted to facilitate spontaneous breathing. The hamster was placed on a stage containing a chamber with a silicon rubber ring surrounding a transillumination window. This chamber was preceded by another one which pre-heated the superfusion solution. Both chambers were mounted with Peltier elements for temperature control, allowing easy change and regulation of the superfusate's temperature. The cheek pouch was carefully everted with the aid of a moist cotton stick and the distal, non-muscular, part of it identified and pinned to a silicon ring. Dissection was performed under a stereomicroscope: a crescent-shaped incision was made in the top layer, the flap was pinned to the side and the areolar connective tissue removed to expose the bottom layer vasculature for microscopic observations. During the

videotape recording device. The measurements were made on the same region in every animal. In these conditions, no significant difference was noted between control and treated animals regarding the body weight and mean arterial blood pressure. In the group receiving *Ruscus* extract, constriction of venules (diameter decreased by 30% compared to controls) and dilation of arterioles (diameter increased by 37% compared to controls) were observed (Bouskela, 1991).

IV injection (5 mg/kg) of *Ruscus* extract caused a venular constriction but did not significantly affect either the arteriolar diameter or the mean arterial blood pressure. It should be noted that the measurement of vascular diameters were made before and after the injection of *Ruscus* extract. When the extract was added to the superfusate (topical application), venular constriction and arteriolar dilation were reported. Cooling to 25°C induced dilation of both types of vessels, whereas warming to 40°C produced the opposite effect (constriction) at 50.10^{-3} mg/ml and higher (Bouskela, 1991).

In a similar study, arteriolar and venular diameters were measured before (3 times, separated by 10 min intervals) and after (every 10 min for 60 min) intravenous injection of *Ruscus* extract at the dose of 5 mg/kg to 7 male hamsters. No effect on mean arterial blood pressure was detected. In these animals, venular but not arteriolar injection was observed at the cheek pouch level. When applied topically to 18 male hamsters, the *Ruscus* extract produced venular constriction and arteriolar dilation at the cheek pouch level. The effect of temperature was similar to what was reported above by Bouskela (1991) (Bouskela *et al.*, 1993b).

In an attempt to further characterize the mechanism of action of *Ruscus* extract on the hamster cheek pouch, another study was performed with male animals. *Ruscus* extract was used at concentrations ranging from 5 to 1000 µg/ml/min applied topically (in the superfusate). At 50 µg/ml/min and above, *Ruscus* extract application induced venular but not arteriolar constriction. The venular constriction amounted to approximately 10%. At the concentration of 0.2 mg/ml/min, the venular constriction remained for 120 min (determined every 10 min) whereas the internal diameter of arterioles was not modified. Therefore, the concentration of 0.2 mg/ml/min was used in further explorations. The venular constriction evoked by *Ruscus* was blocked by low concentrations (10^{-9}M) of prazosin and diltiazem (these drugs did not induce significant venular dilation at this concentration), and only by high concentrations (10^{-6}M) of rauwolscine. The authors postulated that at high concentration, rauwolscine may not be selective only for α2-adrenoceptors. It was concluded that venular constriction in the hamster cheek pouch was mediated by calcium and preferentially by α1-adrenoreceptors. Additionally, the authors hypothesized that the lack of effect on arterioles could be explained by augmented liberation of endothelium-derived relaxing factors on the arteriolar side which probably overrides its constrictor properties (Bouskela and Cyrino, 1994; Bouskela *et al.*, 1994).

- **Complementary mechanistic studies**

A team of the Faculdade de Medicina, Porto tested the effect of *Ruscus* extract on segments of veins.

They compared at the microscopic level human varicose and normal veins, saphenous vein taken from dogs aged 4 months to 7 years and above, and canine saphenous vein obtained after surgical denervation. Human varicose vein and canine denervated saphenous veins shared common characteristics: thickening of the vessel wall due to increase in extracellular material and smooth muscle cells hypertrophy, abnormalities of smooth muscle cells (signs of increased protein synthesis). In dogs, impact of surgical sympathetic denervation on the structure of the vein was independent of the age of the animals. Additionally, the [^{3}H]-norepinephrine content was determined in vessels of dogs (4 months to 7 years and above), and in human varicose/normal veins. The authors showed that sympathetic innervation was lowered in veins taken from aged dogs, and in human varicose veins (compared to normal veins). Therefore, it was concluded that human varicose vein behaves like a partially denervated vessel and many of its structural and biochemical peculiarities appear to be linked to the reduced sympathetic supply of the

preparation and throughout the experiment, the cheek pouch was constantly superfused with a bicarbonate buffered saline solution at a rate of 4.6 ml/min.

vein. The authors considered that the denervated canine vein represents an interesting model for the study of the human varicose vein (Azevedo *et al.*, 1991).

Texeira and Osswald (1988) produced denervation of lateral saphenous vein in anaesthetized dogs by using artery clamps applied down- and upstream of the segment for 5 min. During the surgical intervention, osmotic minipumps were implanted in the subcutaneous tissue of dogs' leg and connected by a catheter to the plantar branch of the denervated vein. These minipumps allowed IV injection of saline (containing Na2-EDTA + heparin) ± *Ruscus* extract (50 µg/kg/h). Animals recovered for 5 days and were then re-operated to remove the segment of the denervated vein and of the contralateral vein (to serve as control). This allowed to study the effect of denervation (injection of saline), of *Ruscus* on denervated vein (injection of *Ruscus* and saline). For the morphological study of the vein, the dimension of smooth muscle cells was taken as the most important criterion of morphological alterations caused by denervation (at the extraneuronal level). In collected segments, the endogenous norepinephrine content was measured by HPLC; denervation was considered successful and complete when the norepinephrine content was decreased by 95%. Additionally, the O-methylating capacity of these tissues was reflected by the total amount of O-methylated metabolite (OMI), determined after incubation of vein strips with ^{3}H-7-(±)-isoprenaline for 30 minutes (Texeira and Osswald, 1988).

This study showed that intravenous infusion of *Ruscus* extract protected the extraneuronal component of the venous tissue against the deleterious consequences of denervation. Indeed, it induced prevention of increase in smooth muscle cell diameter, and partial prevention of the impairment of O-methylating capacity of tissues. However, *Ruscus* extract administration had no effect on the denervation process itself (Nad depletion of at least 95% in denervated veins) (Texeira and Osswald, 1988).

Assessor's comments

The venoconstricting property of *Ruscus* extract shown *in vitro* was confirmed in *in vivo* models. In anaesthetised dogs, a dose-dependent contractile effect was shown for *Ruscus* extract doses ranging from 1 to 10 mg/kg given intravenously, and attributed to result of α-adrenergic activation only.

The IV administration of *Ruscus* extract (5 mg/kg) to hamsters induced venular constriction at the level of cheek pouch microcirculation, without any impact on the diameter of arterioles or mean arterial blood pressure. However, topical application of the extract resulted in venular constriction and arteriolar dilation. Cooling was shown to induce dilation of both types of vessels, whereas warming produced their constriction. Studies conducted with pharmacological agents showed that venular constriction in the hamster cheek pouch was mediated by calcium and preferentially by α1-adrenoreceptors.The dilation of arterioles was considered to result from augmented liberation of endothelium-derived relaxing factors on the arteriolar side.
The oral route was also investigated in this experimental model; the dose of 150 mg/kg/day for 28 days induced venular constriction (internal diameter decreased by 30%) and arteriolar dilation (internal diameter increased by 37%) which was also attributed to liberation of endothelium-derived relaxing factors on the arteriolar side.

Briefly, it can be concluded that the contractile effect of *Ruscus* extract on veins was shown in two *in vivo* experimental models by intravenous route (anaesthetised models, hamster cheek pouch), topical application (hamster cheek pouch), and oral route (hamster cheek pouch, 150 mg/kg/day). The involvement of the α-adrenergic system was confirmed. Arteriolar dilation was noted in some of these studies, and particularly when *Ruscus* extract was administered orally, but no effect on the mean arterial pressure was detected. It could result from induction of liberation of endothelium-derived relaxing factors on the arteriolar side. Additionally, in a canine model of human varicose vein, IV injection of *Ruscus* extract for 5 days prevented against the occurrence morphological alteration of smooth muscle cells, and against the impairment of O-methylating capacity of tissues.

The animal models used to demonstrate the venoconstricting activity of *Ruscus* extract are acceptable, taking into consideration the lack of experimental models reproducing the physiopathological complexity of the chronic venous insufficiency. These models are those commonly used for the evaluation of drugs belonging to this therapeutic class, even if the use of functional exploration methods (e.g. Doppler) could have been used to complete the weight of evidence.

II.2.1.1.2 *CONTRACTILE EFFECT ON LYMPHATIC VESSELS*

A. *In vitro* studies

- **Study performed on rings of canine lymphatic vessels**

Thoracic ducts were removed from dogs, cleaned of connective tissue, and cut into rings. Those rings were put in organ chambers filled with a physiological salt solution and connected to a force transducer for continuous recording of isometric tension. The substances tested, directly added to the bath solution, were norepinephrine (10^{-8} to 10^{-4} M) and *Ruscus* extract (10^{-5} to 2.10^{-3} g/ml). To clarify the mechanism of action of *Ruscus* extract, some α-adrenergic antagonists were used: phentolamine (3.10^{-6} M), prazosin (3.10^{-7} M) and rauwolscine (3.10^{-7} M) (Marcelon *et al.*, 1988a).

In these experimental conditions, it was shown that both norepinephrine and *Ruscus* extract caused a concentration-dependent contraction of the lymphatic thoracic duct rings. The contractile activity of *Ruscus* was partially inhibited by prazosin or rauwolscine, and completely eliminated by phentolamine. Taking into account that venous smooth muscle cells have been isolated in mesenteric and thoracic lymphatics, the importance of the presence of adrenergic nerve endings at this level for the control of lymphatic pumping, and the results obtained in this study, the authors concluded that *Ruscus* causes a similar adrenergic activation of both lymphatic collectors and cutaneous veins. The contractile effect obtained on lymphatic rings was obtained at similar concentrations than those inducing contraction of venous rings (Marcelon *et al.*, 1988a).

- **Study performed on bovine lymphatic vessels**

In order to determine the mechanism of noradrenaline action on lymphatic vessels, various studies were performed using electric stimulation on bovine mesenteric lymphatic vessels. Among those studies, one was performed to gain knowledge about the desensitization of adrenoceptors after application of the endogenous transmitter (norepinephrine) or *Ruscus* extract (McHale, 1991).

While application of norepinephrine (10^{-6} M) induced an increase of contraction frequency which returned rapidly to control values as the result of receptors desensitization, application of *Ruscus* extract (30 µg/ml, i.e. 3.10^{-5} g/ml) had similar excitatory effect which was sustained for the duration of drug perfusion. According to the author, this might suggest that *Ruscus* is acting on different receptors in these vessels from those through which norepinephrine is acting (McHale, 1991).

Assessor's comment

Ruscus extract was shown to exert contractile effect *in vitro* on dog thoracic and bovine mesenteric lymphatic vessels. The results concerning the mechanism involved are contradictory between both experiments. Indeed, results obtained with canine tissues suggest that the mechanism of action is similar to the one described at the venous level, i.e. α-adrenergic activation. According to its authors, the study performed on bovine tissue suggests that pathways involved differ from those of norepinephrine. However, they did not further discuss on the role of chemical stability to explain their results (norepinephrine is probably less chemically stable than *Ruscus*).

B. *In vivo* studies

A study was conducted in anaesthetised dogs, after isolation of a lymph vessel parallel to the saphenous vein at the ankle level and ligature of other vessels (Pouget *et al.*, 1991). After *Ruscus* extract intravenous injection, the total protein concentration (Pt) was measured by UV-spectrometry in lymph collected at 10 min intervals. This allowed the calculation of oncontic pressure (Ponc) using the following formula: Ponc = 0.45 Pt + 0.078 Pt. The doses of *Ruscus* extract administered were 1, 2 and 5 mg/kg because they were in the range of the doses reported as veinotonic [see (Marcelon *et al.*, 1983a)].
In some animals, the activity of *Ruscus* was studied in presence of calcium antagonism by nifedipine. *Ruscus* extract (5 mg/kg) was administered and its activity followed for 1 hour. Then, nifedipine was administered intravenously (0.1 mg/kg, the activity of this dose was maintained for more than 1 hour). After 30 minutes, a second injection of *Ruscus* extract was performed, and its activity again followed for 1 hour.

At 2 and 5 mg/kg, *Ruscus* extract caused a rise in lymph flow without affecting lymph pressure. This reflected the augmentation of the contractility of lymph vessels. This is in agreement with the results of an *in vitro* study performed on peripheral lymphatic vessels [see (McHale, 1991)]. It was also shown that *Ruscus* caused contraction of the isolated thoracic duct of the dog *in vitro* [see (Marcelon *et al.*, 1988a)]. This adds weight to the hypothesis of an enhancement of the "pumping system" suggested by the results obtained *in vivo* in this study, with enhanced propulsion of peripheral lymph toward the heart.

Ruscus also caused a rise in oncotic pressure which suggests that it induces a favourable effect on edema.

The activity of *Ruscus* extract remained unchanged after nifedipine injection. This suggests that the mechanism underlying the effect of *Ruscus* on peripheral lymphatics does not involve the opening of voltage operated calcium channels.

Assessor's comment
In anaesthetised dogs, administration of *Ruscus* extract improved the contractility of peripheral lymph vessels. Taken together with *in vitro* results, this suggests that *Ruscus* extract enhances the lymphatic pumping system thus favouring a better return of peripheral lymph to the heart. Additionally, a rise in oncotic pressure was detected, which could suggest a favourable effect on edema.
However, it should be noted that no study was conducted by the intended therapeutic route of administration, *i.e.* the oral route. It is questionable whether active substance(s) involved in this effect would 1) be absorbed by oral route and 2) undergo significant hepatic metabolism impairing its(their) activity at the lymphatic level.

II.2.1.1.3 *EFFECT ON VASCULAR PERMEABILITY*

A. *In vitro* studies

An experiment was conducted using segment of the lateral ear vein of the pig canulated on both sides and put into an organ bath of Krebs solution. *Ruscus* extract (0.05%) was applied intraluminally for 15 minutes. Then, ethacrynic acid (0.1%) was added to damage the endothelium (2.5 minutes). Finally, the permeability to low- and high molecular substances was measured. To quantify the water edema, an aqueous solution of the dye Evansblue was applied, and the protein edema was measured with bovine serum marked by the dye, each being applied under a pressure of 30 cm H_2O for 30 minutes. The content of Evansblue was then determined by photometry (Hönig and Felix, 1989).

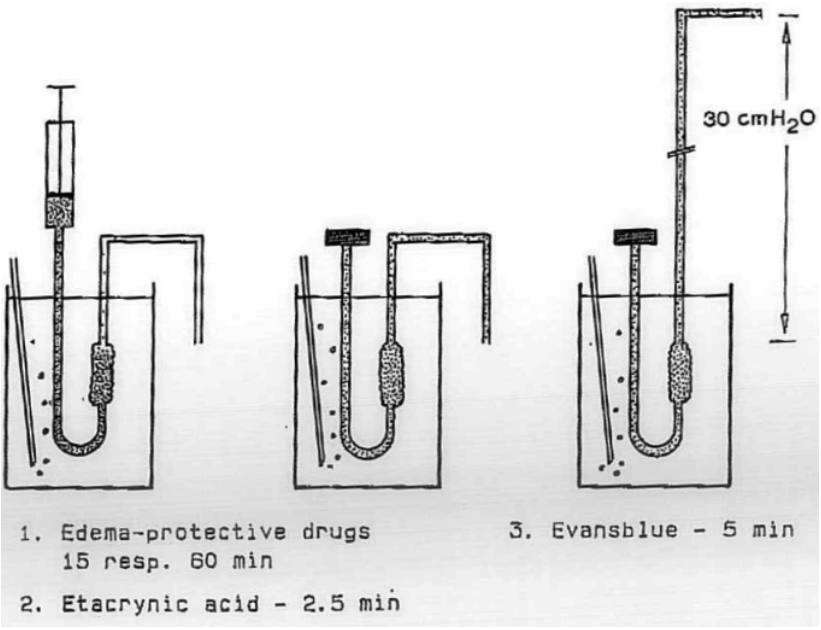


Figure 2: schematic representation of the experiment (Hönig and Felix, 1989)

The results obtained showed that *Ruscus* extract reduced the water- and protein permeability induced by ethacrynic acid. The drug had to be present when the damaging agent was applied; if it was washed 1 min. before, no protective effect was observed. The authors also showed that no chemical binding occurred between the drug and the damaging agent.

To elucidate this edema-protective mechanism, saponins from *Ruscus* were applied for 15 minutes and then washed. The permeability of these vessels was increased compared to controls. According to the authors, the surface-active saponins so strongly bind to the endothelium that they leave holes when being removed.

Assessor's comment
In vitro on ear vein of pig, *Ruscus* extract had protective effects against ethacrynic-induced edema. Saponins may play an important role by anchoring in the endothelium and taking part to the membrane structure.

B. *In vivo* studies

- **Study performed on the hamster cheek pouch**

The number of leakage sites on hamster cheek pouch[4] was measured by fluorescence microscopy after IV injection of fluorescein-labelled dextran. The number of leakage sites (diameter of the fluorescent spot > 100 µm) was determined 2, 5, 7, 10, 15, 20, and 20 minutes after the beginning of each topical application of histamine. Each histamine application lasted 5 minutes with a minimum interval of 40 minutes between each application. The first application of histamine, after injection of FITC-dextran, was made prior to any drug treatment and thus served as a positive control. *Ruscus* extract was then applied topically in the

[4] Experiments were performed on the cheek pouch of male hamsters 7 to 10 weeks old. Anesthesia of the animals was induced by intraperitoneal injection of 0.1-0.2 ml of sodium pentobarbital and maintained with α-chloralose (100 mg/kg) administered through the femoral vein. The femoral artery was cannulated for pressure measurements, anesthetic and *Ruscus* extract injections. Throughout the surgery and subsequent experiment, the animal rested on a heating pad controlled by a rectal thermistor and body temperature was maintained at 37.5°C. A tracheal tube was inserted to facilitate spontaneous breathing. The hamster was placed on a microscope stage. The cheek pouch was gently everted and pinned with 4-5 needles into a circular well filled with silicone rubber to give a plane bottom layer, thus avoiding stretching of the tissue but preventing shrinkage. In this position, the pouch was submerged in a superfusion solution that continuously flushed the pool of the microscope stage. Before the pouch was pinned, large arterioles and venules were located with the aid of a Zeiss binocular microscope. Fashioning of a single layer preparation started with incision of the upper layer to swing a triangular flap to one side. The exposed area was dissected and the fibrous, almost avascular, connective tissue covering the vessels was removed. The dissected part of the pouch was 120-150 µm thick.

following concentrations: 0.002, 0.02, 0.2 and 2 mg/ml/min before the subsequent application of histamine. In the group dosed at 0.2 mg/ml/min, the influence of prazosin, rauwolscine and diltiazem (10^{-9}M to 10^{-5}M) on *Ruscus* effect was studied (Bouskela and Cyrino, 1994).

In this experimental model, histamine increased the number of fluorescent vascular leakage sites from postcapillary venules, which evidence an increase in macromolecular permeability (quantified as the number of leaky sites in the prepared area). The topical application of *Ruscus* extract induced a dose-dependent inhibition of the macromolecular permeability-increasing effect of histamine (-25% at 0.002 mg/ml/min, almost -50% at 0.2 mg/ml/min).This effect was blocked by prazosin and diltiazem, but not by rauwolscine. Overall, it was concluded that *Ruscus* extract inhibited the microvascular permeability induced by histamine. This effect would be mediated by calcium and preferentially by α1-adrenoreceptors (Bouskela and Cyrino, 1994).

These findings confirm the results previously obtained in a similar study which showed that topical application of *Ruscus* extract has a protective effect against leakage of FITC-dextran in the cheek pouch of hamsters after administration of various permeability-increasing substances, *i.e.* bradykinin, histamine, and leukotriene B4 (Bouskela *et al.*, 1993a).

- **Study performed on a feline model of edema**

Edema was induced in anaesthetised cats by IV injection of ethacrynic acid. *Ruscus* extract was administered one hour before edema induction. Its antiedema effect was evaluated in hindlegs by measuring the water and protein content of the edema (Felix *et al.*, 1984).

Compared to control animals, the protein content of the edema was decreased in *Ruscus* pre-treated cats. Water content was not affected, which would suggest that *Ruscus* extract only influences the passage of plasma protein in the interstitium. However, in *Ruscus* pre-treated animals, it was demonstrated that waters flows into the tissues more slowly than in control animals. Indeed, the capillary filtration coefficient increased to a smaller degree in *Ruscus* pretreated animals, compared to control ones. Therefore, it was concluded that *Ruscus* extract inhibited the destruction of the endothelium by ethacrynic acid without totally suppressing it.

The effective dosage was 20 mg/kg by IV route, 10-20 times higher by oral route (administration 4 hours before induction of edema). By oral route, the effective dose decreased after subchronic administration and reached 20-40 mg/kg (4-6 days).

It is also reported that a protective effect was observed with ruscogenin at 4 mg/kg by IV route. This effect was weaker than the effect reported with the whole extract at 20 mg/kg. This last contained 2.5% ruscogenin, leading to a ruscogenin dose of 0.5 mg/kg (Felix *et al.*, 1984).

Assessor's comment

The antiedema effect reported for *Ruscus* extract *in vitro* was confirmed in two *in vivo* studies. After IV administration, *Ruscus* extract inhibited the microvascular permeability induced by histamine on hamster cheek pouch, an effect suggested to be mediated by calcium and preferentially by α1-adrenoreceptors. In a feline model of ethacrynic acid-induced edema, IV administration of 20 mg *Ruscus* extract/kg decreased the protein content of the edema and slowed the water flow into the tissues. This is in agreement with the increase in oncotic pressure reported by Pouget *et al* (1991) in lymphatic vessels of anaesthetised dogs administered 2 and 5 mg/kg *Ruscus* extract intravenously (see II.1.2.2. above).

However, none of these studies was performed by the oral route. In the study performed by Felix *et al.* (1984), the anti-edema effect of *Ruscus* extract was investigated by oral route. It can be concluded that the oral effective dose approximates 200-400 mg/kg, or 20-40 mg/kg/day after repeated administrations for 4-6 days (the criteria used by the authors to decrease the dose were not detailed). Additionally, it should also be noted that this effect is not only due to ruscogenin, but also to other components of the extract.

II.2.1.1.4 *EFFECT ON HYPOXIA-INDUCED ACTIVATION OF ENDOTHELIAL CELLS*

Endothelium plays a role in the development of varicose veins. Hypoxic conditions which develop during blood stasis are able to activate endothelial cells to release inflammatory mediators and growth factors. Inflammatory mediators induce neutrophil adherence and activation. Those activated neutrophils may then infiltrate and induce damage in the subendothelial layers. On the other hand, the growth factos trigger smooth muscle cell proliferation, leading to a thickening and dizorganisation of the media as observed in the wall of varicose veins. Therefore, a study was performed on human umbilical vein endothelial cells (HUVEC) to determine whether *Ruscus* extract could prevent endothelial cell activation by hypoxia (Bouaziz *et al.*, 1999).

Ruscus extract was shown to prevent the activation of endothelial cells (HUVEC) incubated in hypoxia conditions for 2 hours. This was demonstrated by measuring the ATP content of control and treated cells. The hypoxia-induced decrease in ATP was concentration-dependently inhibited by *Ruscus* extract, the effect being maximal at 50 µg/ml.

Hypoxia strongly increased Phospholipase A2 (PLA2) activity, more than 2-fold the value obtained in normoxic conditions (↓ ATP → cytosolic calcium → PLA2 activation). When HUVEC cells were incubated with *Ruscus* extract under hypoxia, PLA2 activation was inhibited (50% inhibition at 0.05 µg/ml). Additionally, *Ruscus* extract inhibited neutrophil adherence to HUVEC (PLA2 activity → prostaglandin, platelet activating factor → ↑ in HUVEC adhesiveness for neutrophils).

II.2.1.2 SAFETY PHARMACOLOGY STUDIES

No study available.

Assessor's comment

Considering the pharmacological profile of *Ruscus* extract, i.e. stimulation of α-adrenergic system, the lack of a safety pharmacology study evaluating its potential effects on the cardiovascular function gives cause for concern. No toxicology study evaluating this endpoint is available.

In the studies performed on the hamster cheek pouch, the mean arterial blood pressure was not modified after IV administration of 5 mg/kg *Ruscus* extract, and oral administration at the dose of 150 mg/kg. The need of such a safety pharmacology study addressing cardiovascular aspects should be discussed in light of clinical safety data.

II.2.1.3 PHARMACODYNAMIC INTERACTIONS

No study available.

Assessor's comment

Ruscus extract contains substances having α-adrenergic stimulating properties. Moreover, it was shown to displace norepinephrine from adrenergic nerve endings. Therefore, pharmacodynamic drug-drug interactions could occur with:

- any drug potentiating the α-adrenergic system;
- any drug antagonizing the α-adrenergic system.

As a precaution measure, it may be relevant to include a warning for patients treated with any of those drugs.

II.2.1.4 ASSESSOR'S OVERALL CONCLUSIONS ON PHARMACOLOGY

Primary pharmacodynamics studies performed *in vitro* and *in vivo* using various experimental models showed that *Ruscus* extract possess a **contractile activity on veins**. This activity is mediated by stimulation of the α-adrenergic system. *In vitro* mechanistic studies showed that direct activation of postjunctional α1-and α2-adrenergic receptors, and stimulation of the release of norepinephrine from adrenergic nerve endings were involved. Although this effect does not appear to be clearly influenced by the hormonal status (estrogens, progesterone), it seems potentiated by temperature.
In *in vivo* studies, this venoconstricting activity was shown after intravenous and oral routes; in the hamster cheek pouch model, local application of the extract (*i.e*. in the superfusate) was also effective. It should be noted that only one study was conducted by the oral route: at the level of hamster cheek pouch microcirculation, the dose of 150 mg/kg/day administered for 28 days induced venular constriction (internal diameter decreased by 30%) and arteriolar dilation (internal diameter increased by 37%) without any impact on the mean arterial blood pressure, the latter effect being attributed to liberation of endothelium-derived relaxing factors on the arteriolar side.

Similarly, other primary pharmacodynamics studies showed that *Ruscus* extract exerts a **contractile effect on lymphatic vessels** in anaesthetised dogs at 2 and 5 mg/kg administered intravenously. A rise in oncontic pressure suggested a **favourable effect on edema**. This was confirmed in a feline model of ethacrynic acid-induced edema. The effective dosage amounted to 20 mg/kg by intravenous route, and 10-20 times higher by oral route. However, after subchronic administration (4-6 days), the oral effective dosage decreased to reach 20-40 mg/kg/day. The same study showed that ruscogenin was also effective, but that other components of the extract were involved to obtain maximal activity.

Due to the mechanisms underlying the effect of *Ruscus* extract, **pharmacodynamic drug-drug interactions** could occur with any drug potentiating or antagonizing the α-adrenergic system. As a precaution measure, it may be relevant to include a warning for patients treated with any of those drugs.

Considering the pharmacological profile of *Ruscus* extract, i.e. stimulation of α-adrenergic system, the lack of a **safety pharmacology** study evaluating its potential effects on the cardiovascular function gives cause for concern. No toxicology study evaluating this endpoint is available. In the studies performed in the hamster cheek pouch model, the mean arterial blood pressure was not modified after IV administration of 5 mg/kg *Ruscus* extract, and oral administration at the dose of 150 mg/kg. Therefore, the need of a safety pharmacology study addressing cardiovascular aspects should be discussed in light of clinical safety data.

II.2.2 PHARMACOKINETICS

II.2.2.1 PREPARATION OF THE EXTRACTS

Three studies were performed in rats and dogs to gain knowledge in the pharmacokinetics of *Ruscus* extract (Chanal *et al.*, 1978; Chanal *et al.*, 1981; Bernard *et al.*, 1985).

In each study, the extract was tritiated according to the Wilzbach technique. Briefly, this labelling method involved an incubation period with tritium gas (5-7 days) followed by the elimination of labile tritium. Analysis by TLC was then performed and showed each time that most of the radioactivity was associated with the sapogenins (80 to 94%, depending on the study).

According to the authors, other labelling methods could not be applied:

- Carbon 14 labelling was not appropriate because the extract contains a multiplicity of sapogenins;
- Labelling by biosynthesis would have required years of growth (the extract is made from the rhizome);
- These sapogenins cannot be chemically synthesized because of the lack of any precursor.

Assessor's comment

As indicated by other authors, the Wilzbach method used to label the extract is controversial (Bernard *et al.*, 1985). Tritium labelling of a complex mixture such as the *Ruscus* extract used in this study presents some disadvantages which preclude a full confidence in the results obtained. In particular, the stability of the labelling is not justified (possible ^{3}H-^{1}H exchanges after the labelling, etc.).

Additionally, no quantification of $^{3}H_2O$ was performed in any sample of the studies. After ^{3}H-^{1}H exchange or metabolism, $^{3}H_2O$ mix with the body water pool. The elimination of tritiated water from the organism being particularly slow, the monitoring of ^{3}H radioactivity to study the pharmacokinetics of *Ruscus* extract's compounds is likely to produce biased results. Overall, **these studies should not be taken into account for regulatory purposes (however main results will be described below)**.

II.2.2.2 ABSORPTION

Blood kinetics of radioactivity were determined in a study involving 2 male Wistar rats administered the labelled-extract orally (Chanal *et al.*, 1978). Blood samples were collected at 0.25, 0.5, 1, 2, 3.5, 5, 6, 8 and 24 hours after administration. Radioactivity level, expressed as per cent radioactivity per 10 ml of blood in relation to the dose administered, was measured by Liquid Scintillation Counting (LSC).

In both rats, blood radioactivity reached a level close to the maximum 2 hours after the administration of the extract. Thereafter, radioactivity level remained practically constant over the remaining 22 hours (see figure 3). The authors concluded that the half-lives of the tritiated compounds were relatively long, on the order of several days.

In another study involving oral administration of the labelled extract to 6 male Wistar rats, blood samples were collected at 0.25, 0.75, 1.75, 2.25, 3.5, 5, 6.5, 8, 24 and 48 hours after administration (Chanal *et al.*, 1981). Radioactivity level was expressed as per cent radioactivity per 10 ml of blood in relation to the dose administered, and the figures given are the mean for the 6 rats (see figure 4). Relatively high radioactivity levels were reached from 3.5 hours and the Tmax amounted to 8 hours. Blood levels declined slowly and remained high after 24 hours.

Figure 3: blood kinetics of tritiated compounds of Ruscus extract in 2 rats after oral administration (from Chanal *et al*., 1978)

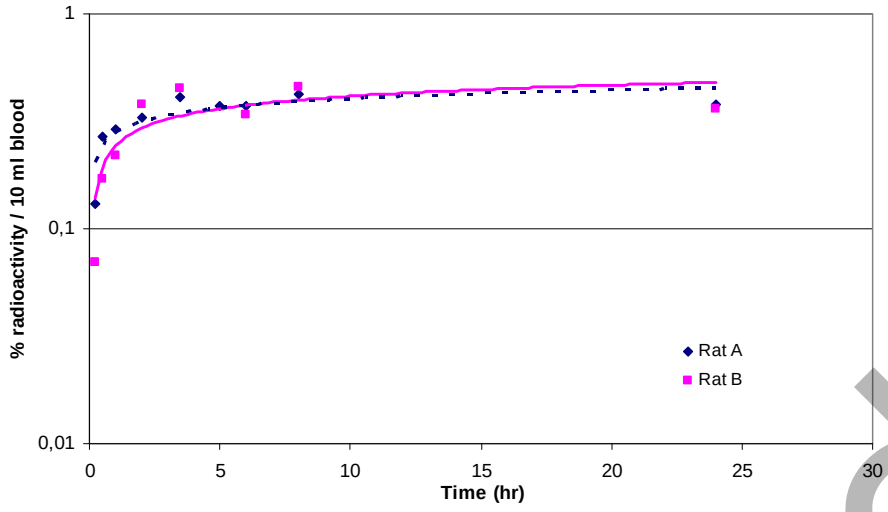
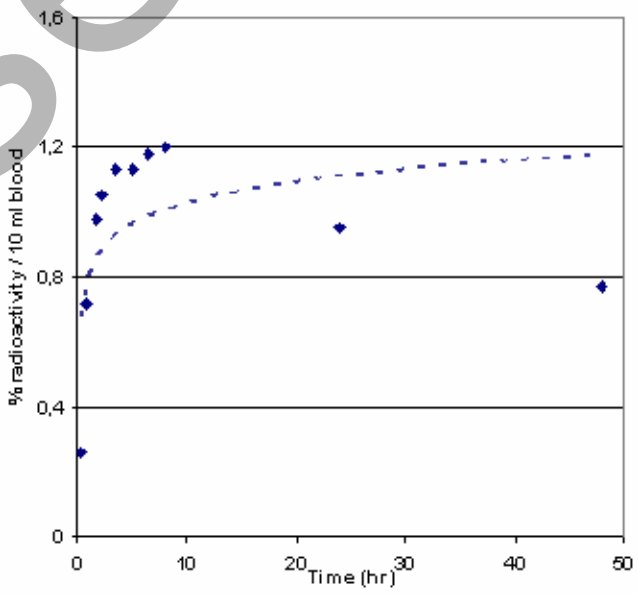


Figure 4: radioactivity levels measured in blood of Wistar rats after oral administration of a ^{3}H-labelled Ruscus extract (graph comprising a logarithmic trend curve elaborated from data of Chanal *et al*, 1981)

Time	Mean percentage of radioactivity for 6 rats
15 min	0.26 ± 0.03
45 min	0.72 ± 0.05
1 h 45 min	0.98 ± 0.1
2 h 15 min	1.05 ± 0.05
3 h 30 min	1.13 ± 0.02
5 h	1.13 ± 0.1
6 h 30 min	1.18 ± 0.1
8 h	1.2 ± 0.1
24 h	0.95 ± 0.1
48 h	0.77 ± 0.2



Assessor's comment

Two studies performed on a limited number of rats after oral administration of a labelled *Ruscus* extract showed that radioactivity was detected in the blood for more than 24 hours. The authors suggest that the tritiated compounds of the extract would have relatively long half-lives. However, such a conclusion is speculative because the tritiated water, which is slowly eliminated, was not quantified in the samples collected. Therefore, no conclusion can be drawn from these studies.

It should be noted that the results obtained in the studies performed by Chanal *et al* in 1978 and in 1981 are contradictory; in the first study, blood radioactivity remained stable over 25 hours, whereas in the second one, a decrease of blood radioactivity starts after 8 hours.

II.2.2.3 DISTRIBUTION

Tissue distribution was investigated by whole body autoradiography (WBA) in macaca monkeys administered a labelled extract of *Ruscus* by either intravenous (n=1, extract dissolved in sodium chloride 0.9%), or oral (n=4, extract dissolved in the content of one Cyclo 3 oral ampoule) routes (Bernard *et al.*, 1985).

WBA performed 24 hours after IV administration showed intense labelling in the bile, the contents of the distal digestive tract and to a lesser extent the urine. Moderate label was found in the circulating blood, and relatively substantial label in the renal and hepatic parenchyma, spleen, bone marrow, and adrenals (cortex). The intense binding of radioactivity at the hepatic and renal levels was confirmed by LSC.

Two hours after oral administration, highest radioactivity levels were detected in the bile, digestive and urinary contents; liver and kidney were labelled uniformly. Blood activity was slightly lower than after IV administration. The radioactivity levels decreased the following 5 hours except for digestive organs (urine, bile, feces). After 24 hours, non negligible radioactivity levels persisted in circulating blood, renal and hepatic parenchyma, bone marrow, spleen, adrenal cortex, contents of the distal digestive tract, bile and urine.

Assessor's comment

Radioactivity was mainly found at the hepatic and renal levels 24 hours after both intravenous and oral administration of the labelled *Ruscus* extract. It should be noted that intense radioactivity was found in the bile. Again, it remains unknown if the radioactivity arises from any of the compounds of the extract, or from tritiated water. Therefore, no conclusion can be drawn.

However, deep distribution of radioactivity in the bone marrow after IV and oral administrations was shown, thus underlying the need of genotoxicity studies.

II.2.2.4 METABOLISM

To determine the nature of plasma radioactivity, blood samples collected 2 hours after oral administration of a radio-labelled extract to 2 rats were extracted with methanol, and analysed by TLC (Thin Layer Chromatography). Results indicate that 39% of the radioactivity deposited corresponded to the sapogenin spot. The authors conclude that there was a "considerable fraction of the unchanged product" in the plasma after 2 hours (Chanal *et al*., 1978).

Assessor's comment

This experiment does not allow drawing any conclusion on the metabolism of *Ruscus* extract due to concerns already expressed on labelling method and experimental procedures, and to the poor separating capacity of the method employed (TLC).

II.2.2.5 ELIMINATION

Ninety six hours after oral administration of the labelled extract to 2 rats, approximately 18% and 29% of radioactivity was recovered in the urine and feces, respectively. It should be noted that excretion occurred mainly during the first 24 hours. A non negligible part of the fraction recovered in the faeces arised from notable biliary excretion (approx. 10% of the administered radioactivity). (Chanal *et al.*, 1978).

Urinary excretion amounted to 32-35% in Wistar and Atrichis rats administered a labelled extract orally, while the corresponding figure for faecal excretion was slightly higher, *i.e.* 39-45%. Most of the radioactivity was excreted within the first 24 hours. The authors report that the existence of an enterohepatic cycle was confirmed in a study conducted by intravenous administration (one third of total radioactivity eliminated in the faeces)(Chanal *et al.*, 1981).

In monkeys, radioactivity was excreted in the urine (26%) and in the feces (6.5%) 24 hours after IV administration (n=1). Those figures amounted to 20% and 23% for urine and feces, respectively, 24 hours after oral administration. The authors suggested the existence of an enterohepatic cycle considering the extent of faecal elimination and bile activity (Bernard *et al.*, 1985).

Assessor's comment

First, it should be noted that these figures were obtained from a limited number of animals. Additionally, it seems that the amount of radioactivity recovered from urine and faeces appears to be insufficient in all studies (less than 50% to 80% of the administered dose). Taking into consideration the relatively long half-lives of the labelled compounds which was hypothesised from blood kinetics, an incomplete radioactivity collection after either 24 hours or 96 hours seems coherent.
However, as most of the studies showed that excretion occurred mainly during the first 24 hours, and in view of the stable blood radioactivity levels after 24 hours, accumulation of radioactivity could occur after repeated administrations.

Radioactivity was excreted in urine and faeces, with faecal elimination slightly above. The authors suggest that *Ruscus* extract labelled components undergo an enterohepatic cycle. However, the excretion of a compound in the bile and a high amount of faecal elimination are not sufficient to prove the existence of an enterohepatic cycle. This suggestion should be rather regarded as a hypothesis to be further investigated.

This experiment does not allow to draw any conclusion on the metabolism of *Ruscus* extract due to concerns already expressed on labelling method and experimental procedures.

II.2.2.6 ASSESSOR'S OVERALL CONCLUSIONS ON PHARMACOKINETICS

The pharmacokinetics of *Ruscus* extract was investigated in rat (2 studies) and in monkey (1 study). In each study, the extract was tritiated according to the Wilzbach technique. Briefly, this labelling method involved an incubation period with tritium gas (5-7 days) followed by the elimination of labile tritium. Afterwards, the extract could be administered to animals. However, this labelling method presents some disadvantages. For example, the stability of the labelling remains unknown (possible ^{3}H-^{1}H exchanges after the labelling, etc.).

Additionally, the authors did not perform a quantification of tritiated water in the samples collected. This represents a major bias because the radioactivity measured in samples cannot be attributed without any doubt to the compounds of the *Ruscus* extract. Indeed, $^{3}H_2O$ can mix with the body water pool after ^{3}H-^{1}H exchange or metabolism, and the elimination of tritiated water from the organism is particularly slow.

Overall, it is concluded that **these studies should not be taken into account for regulatory purposes** because they are endowed with major bias precluding a full confidence in the results obtained.

One concern arises from the monkey distribution study, where in-depth distribution of radioactivity was reported at the bone marrow level. While it remains unknown if radioactivity is due to any component of the extract or to tritiated water, the worst-case scenario should be considered. Therefore, this finding strongly underlies the need of genotoxicity studies with *Ruscus* extract.

II.2.3 TOXICOLOGY

II.2.3.1 ACUTE TOXICITY

The acute toxicity of an ethanolic extract of *Ruscus* was investigated in dogs and guinea pigs (Caujolle *et al.*, 1953; ESCOP, 2003):

– In 6 male and female dogs, death occurred within 1 hour following intravenous infusion of the extract at doses ranging from 0.83 g/kg to 1.8 g/kg. The frequency of cardiac contractions was progressively decreased but was not attributed by the authors to a toxic effect on the myocardium because the hearts of treated dogs could react normally to epinephrine. Additionally, the blood pressure was decreased. The monitoring of respiratory function showed that at toxic doses, tachypnea occurred and was sometimes associated with rhythm perturbation. At lethal doses, hyperventilation was followed by fatal apnea. The authors considered that at high doses, the *Ruscus* ethanolic extract that they administered to dogs produced cardiovascular and respiratory reactions. The respiratory centres were deeply affected and apnea always preceded the cardiac arrest so that death was attributed to respiratory alteration. Moreover, at high doses, hyperglycemia was reported (1.82 to 2.84 g/l).

– In 8 male guinea pigs, the intraperitoneal injection of the ethanolic extract induced no toxic symptoms at doses lower than 1.5 g/kg. Animals receiving 2g/kg and above died.

Estimation of the oral and intraperitoneal LD50 values of an ethanolic fluid extract of *Ruscus* in rats and mice revealed differences depending on the harvest time of the plant, the route of administration, and the use of roots or rhizomes. The oral LD50 of the rhizome extract could not be determined in rats because administration of doses inducing 100% mortality could not be reached. In mice, it amounted to 24.69-33.73 ml/kg in mice. After intraperitoneal administration, the DL50 of the rhizome extract reached 1.15-1.70 ml/kg in mice, and 2.07-2.39 ml/kg in rats. Root extract was found to be more toxic than rhizome extract in both species. The observed symptoms of intoxication were convulsion, paralysis and gastro-intestinal inflammation with dysentery. Animals died following respiratory failure. Autopsies revealed pronounced irritation of the mucosa and strong visceral congestion (Boucard *et al.*, 1967; ESCOP, 2003).

Assessor's comment

Extracts used in both studies differed from the extract produced by the company Pierre Fabre Médicament used in pharmacology and pharmacokinetic studies. They were obtained by ethanolic extraction but contents in ruscogenin and neoruscogenin remain unknown.

The first study in dogs reported a mean LD0 of 1.20 g/kg (0.83 – 1.8 g/kg) by intravenous route. The authors attribute the cardiovascular findings observed at high dose (decreased frequency of cardiac contractions, decreased blood pressure) to be secondary to alteration of respiratory centres. However, this is not sufficiently established, particularly if the α-adrenergic activity of *Ruscus* components is taken into consideration. In the second study, the oral LD50 of mice reached 25-34 ml/kg. Death occurred by respiratory failure in rats and mice treated by oral and intraperitoneal routes.

As described in section II.2.1.2. no safety pharmacology study is available on cardiovascular and respiratory systems. The need of such studies should be discussed in light of clinical safety data.

II.2.3.2 REPEAT-DOSE TOXICITY

The ESCOP monograph reports the findings of a 26-week toxicity studies performed in male rabbits by administration in the diet (Roux, 1969; ESCOP, 2003). A *Ruscus* extract was administered to 17 animals at 2 g/kg, and to 19 animals at 5 g/kg. Five animals served as controls. It is stated that body weight and blood counts did not reveal any difference between treated animals and controls.

Assessor's comment

The lack of information available on this (unpublished) study precludes its use in safety evaluation. Indeed, no precision on the extract administered to the animals is given (mode of extraction, content in active substances, etc.), and the choice of the rabbit as a toxicology species is amazing. Usually, rat or rabbit are used as rodent, and dog or monkey as non-rodent. The parameters monitored in treated animals are not indicated, except body weight and blood count which is insufficient.

II.2.3.3 GENOTOXIC AND CARCINOGENIC POTENTIALS

No information available.

Assessor's comment

The lack of genotoxicity study precludes the listing of *Ruscus aculeatus*. Additionally, as no long-term study is available, the carcinogenic risk cannot be appreciated.

II.2.3.4 REPRODUCTIVE TOXICITY

The ESCOP monograph reports the findings of an unpublished study conducted in female pregnant rat by administration of a preparation containing ethanolic *Ruscus* extract, trimethylhesperidin methylchalcone, methyl-4-esculetol and acorbic acid (Labie, ; ESCOP, 2003). Twenty female rats received a daily dose of 2.4 ml of the preparation corresponding to 0.24 ml of *Ruscus* extract and equivalent to 25 times the recommended dose for humans. Twenty animals served as control. Treatment started one week before conception and continued until delivery. No sign of intoxication was noted in treated animals. The fertility of females in the treatment group was comparable to that in the control group and this offspring did not show any teratogenic sign.

Assessor's comment

The lack of information available on this (unpublished) study precludes its use in safety evaluation (route of administration, number of resorptions, viable foetuses, number of foetuses examined for evaluation of visceral/skeletal abnormalities, etc.). Only one dose level was tested, which is not acceptable. The study was performed on an association of a mixture (*Ruscus* extract) with other components; a conclusion on the teratogenic risk of *Ruscus* extract only cannot be drawn.

It is considered that the embryo-fetotoxic risk of *Ruscus* extract is unknown; adequately conducted reproduction toxicity studies are lacking.

II.2.3.5 ASSESSOR'S OVERALL CONCLUSIONS ON TOXICOLOGY

Extracts used in two acute toxicity studies were obtained by ethanolic extraction. Their characteristics (content in various compounds, e.g. ruscogenin and neoruscogenin) remain unknown. Potential variability compared to the extract(s) intended for therapeutic cannot be evaluated. In dogs, a mean intravenous LD0 of 1.20 g/kg was measured, while the oral LD50 of mice reached 25-34 ml/kg with another extract. The authors attribute the cardiovascular findings observed in dogs at high doses (decreased frequency of cardiac contractions, decreased blood pressure) to be secondary to alteration of respiratory centres. In rats and mice, death occurred by respiratory failure too. **No safety pharmacology study is available on**

cardiovascular and respiratory systems. Considering the α-adrenergic activity of Ruscus components, the need of such studies should be discussed in light of clinical safety data.

No other toxicity studies are available. The ESCOP monograph reports repeat-dose toxicity and reprotoxicity studies performed in rabbits and rats, respectively. However, these studies remain unpublished so that the information available is very sparse. Therefore, they cannot be taken into consideration for safety evaluation.

The lack of adequately conducted genotoxicity and embryo-fetal toxicity studies precludes the listing of Ruscus aculeatus. Additionally, as no long-term study is available, the carcinogenic risk cannot be appreciated.

II.3 CLINICAL DATA

Clinical data on efficacy and safety of *Ruscus aculeatus* alone are very limited.

II.3.1 CLINICAL PHARMACOLOGY

Clinical pharmacology on *Ruscus aculeatus* is not well documented. Two publications have been identified.

II.3.1.1 PHARMACODYNAMICS

II.3.1.1.1 *OVERVIEW OF AVAILABLE DATA*

In a randomized, placebo-controlled, double-blind, crossover, 4-arm study, 20 healthy volunteers (11 men and 9 women aged between 20 and 43 years) took a single dose of four different treatments immediately before the first measurements: 450 mg *Ruscus* extract, 450 mg trimethylhesperidin chalcone (TMHC), 900 mg of a combination of the two substances or a placebo. A 1-week wash-out phase was provided between the treatments. The venous function was determined by plethysmography. Volumetric measurements were performed in orthostatic conditions with normal blood flow (foot volume) and after a pronounced ischemia (tissue volume). The difference between foot volume and tissue volume corresponds to the blood volume. Heamodynamic and volumetric reactions were monitored before intake and after 70, 90, 120 and 150 minutes. *Ruscus* extract caused a significant decrease in venous capacity and venous outflow. *Ruscus* extract also significantly reduced tissue volume compared to placebo whereas the decrease in blood volume was not significant (Rudofsky, 1991).

Assessor's comments:
The characteristics of the Ruscus extract are unknown.

A long term study was also performed with 141 patients, with chronic venous insufficiency (CVI), who were recruited to a randomized, double-blind, multicentre study. The cause of late CVI was either primary varicosis or post-thrombotic syndrome (PTS). After a two weeks washout, they were given 4 weeks of treatment with 3 x 2 and then 8 weeks with 2 x 2 capsules of *Ruscus* extract or placebo.

The patients venous pump function during toe-stand exercises were also investigated by plethysmography.

In CVI patients there was a continuous decrease in the foot and ankle volume after a 12-week treatment with active substance whereas the volume increased under placebo. The tissue volume was reduced by the same degree as the foot volume in PTS patients. In varicosis patients, the reduction in leg swelling was due to a decrease in tissue volume and volume of blood stored in the veins during orthostasis (Rudofsky, 1991).

Assessor's comments:
The characteristics of the Ruscus extract are unknown.

II.3.1.1.2 *ASSESSOR'S OVERALL CONCLUSIONS ON PHARMACODYNAMICS*

On the basis of publications, the quality of the two available pharmacodynamics studies cannot be evaluated. For example, the characteristics of the patients are incomplete as well as the design of the studies. The statistical analysis is not given. Moreover, the characteristics of the *Ruscus* extract are not specified. However, the findings corroborate the preclinical pharmacological properties described in section II.2.1 that acknowledge to *Ruscus* extract (manufactured by Pierre Fabre) an alpha-adrenergic effect and thus a venous vasoconstrictive effect that account for a reduction in volume of blood stored in the veins and for a stimulating effect on the lymphatic drainage. These two pharmacological properties assume a positive effect in patients suffering from chronic venous insufficiency.

Dose-effect studies are missing which preclude from justifying the dosage regimen used in the clinical studies.

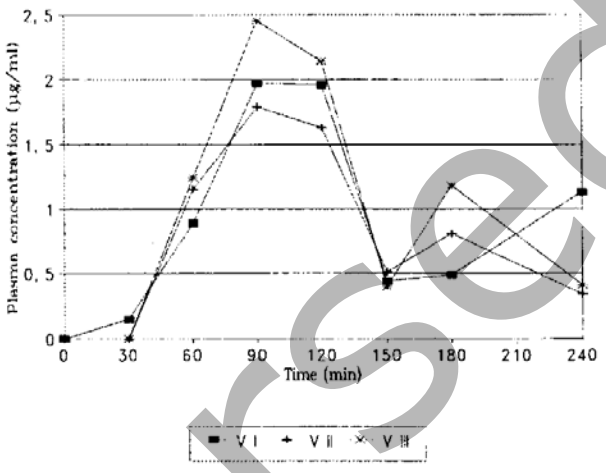
Other methods of functional exploration could have been used to evaluate the effect on veins (e.g. Doppler).

II.3.1.2 PHARMACOKINETICS

II.3.1.2.1 *OVERVIEW OF AVAILABLE DATA*

During a pilot study involving three volunteers, the major spirostanol glycosides of *Ruscus aculeatus* (degluconeoruscin and deglucoruscin) were detected in human plasma after an oral administration of 1g of *Ruscus* extract (Rauwald and Grunwidl, 1991) (see figure 5 below).

Figure 5: time course of plasma concentration of degluconeoruscin after oral ingestion of 1 g Ruscus extract by 3 human volunteers (Rauwald and Grunwidl, 1991)



Assessor's comments:
The only conclusion that can be drawn from this study is that degluconeoruscin and deglucoruscin seems to be absorbed after Ruscus extract oral administration. Of note, the characteristics of the extract are not given. The publication wasn't detailed enough to determine the grade of CVI, the dose administered, the statistical analysis and the Doppler procedure.

II.3.1.2.2 *ASSESSOR'S OVERALL CONCLUSIONS ON PHARMACOKINETICS*

Available pharmacokinetics data are too scarce. The pharmacokinetics of *Ruscus* extract should further studied.

II.3.2 CLINICAL EFFICACY

II.3.2.1 DOSE RESPONSE STUDIES

According to the provided literature no dose-finding studies have been conducted with *Ruscus* extract alone neither in patients with chronic venous insufficiency nor in patients suffering from haemorrhoids. Monographs dosage recommendations are empirical.

With respect to the ESCOP and the Commission E monographs an adult daily dose should be equivalent to an amount of 7 to 11 mg of total ruscogenins. The French herbal preparations containing *Ruscus aculeatus* alone recommend a daily dose equivalent to an amount of around 10 mg of total ruscogenins with regard to a traditional use in subjective symptoms of chronic insufficiency such as sensation of heavy legs.

II.3.2.2 CLINICAL STUDIES (CASE STUDIES AND CLINICAL TRIALS)

The efficacy of *Ruscus aculeatus* has been proposed in the different following indications. A review of the literature for each indication has been performed.

1. Chronic venous insufficiency

Chronic Venous Insufficiency (CVI): symptoms and therapeutic measures Chronic venous insufficiency is a common disease which is characterised by symptoms due to disorders of the venous return in the lower leg and in foot. The situation leads to an increased pressure in the veins and lack of blood flow to the legs and feet. The main symptoms of chronic venous insufficiency are: leg heaviness, aching, dilated or unsightly veins and oedema (swelling). In the more severe cases, patients can have skin colour changes, recurrent skin infections and chronic ulcers.

Two main options to treat symptoms due to chronic venous insufficiency are firstly compression therapy which has demonstrated an efficacy to reduce leg volume, to hinder progression and to reduce symptoms. This therapy can be completed or replaced by the use of systemic veno-active drugs. Physical activity such as for example walking or swimming can also be suitable.

Clinical efficacy and safety data:

Study from Vanscheidt and al. (2002) (submitted during the first step of the procedure)

Only one clinical study, with *Ruscus* extract alone, performed in patients with chronic venous insufficiency (CVI) and involving *Ruscus aculeatus* alone has been found in the literature.

Methodology:

This multicenter double-blind, randomized, placebo-controlled clinical study has been recently performed with a *Ruscus* extract alone and published (Vanscheidt *et al.*, 2002). The aim of this trial was to confirm the efficacy and safety of a *Ruscus* extract (Fagorutin® *Ruscus* Kapseln) according to the latest scientific standards.

Design: The study enrolled women suffering from chronic venous insufficiency (Widmer classification grades I and II, CEAP 3-5) and was conducted at 10 different centres in Germany. Randomisation was carried out after a 2-week placebo run-in phase. The treatment phase lasted 12 weeks. Checkpoints were scheduled after 4, 8, and 12 weeks of treatment.

Treatments: Active treatment and placebo were taken twice daily (morning and evening) orally with some fluid. One capsule active treatment contained 36.0-37.5 mg dry extract from *Ruscus aculeatus* rhizome with a drug extract ratio of 15-20 : 1 (excipient methanol 60%) corresponding to an amount of around 4.5 mg of total ruscogenins.

Outcomes:

- The primary variable of the study was the change in foot and lower leg volume, measured as the area under the curve of volume changes over the 12-weeks treatment-time of (AUB_{0-12}, area under baseline).

- Among the secondary variables "changes in leg volume" and "changes in subjective symptoms" were evaluated after 12 weeks of treatments.

All measurements were carried out at the same time of the respective days in the late afternoon or early evening. Before the measurements the patients underwent a 45-min temperature and cardiovascular equilibrium period in a sitting position. The leg volume was determined by water displacement. The ankle and lower leg circumference were measured using a measuring tape. The measurements were carried out at the lateral and medial ankle and the middle of the middle of the lower leg.

The subjective symptoms: tiredness and heaviness, sensation of tension, tingling sensation, and pain were assessed at each visit before the volume measurements. The subjective symptoms were evaluated by a 10 cm Visual Analog Scale (VAS) where 0 was equivalent to "no complaints" and 10 to "strongest complaints".

The quality of life was investigated at visits 1 and 5 by a disease specific and validated questionnaire on the quality of life which included subjective symptoms and life style judgement (Launois *et al.*, 1996). It ca be compared with FLQA (Freiburger Life Quality Assessment). Global efficacy was assessed by the investigator with a four point scale (very good, good, moderate and bad).

Results: Overall, 167 patients were screened, of whom 166 were randomised and included in the study. Eighteen had insufficient data and were excluded from the efficacy analysis.

Efficacy on leg volume: For the AUB_{0-12}, the median values (min/max) were – 656 [ml x day] (- 13972/5908) for the *Ruscus* extract and 175 [ml x day] (-22795/4970) for placebo which reflected a decrease of leg volume in the *Ruscus* group but an increase in the placebo group. Statistical analysis revealed a significant treatment contrast ($p < 0.001$). Moreover, the median values of all parameters showed a decrease over time reflecting an increasing reduction of leg volume, ankle circumference, lower leg circumference, and subjective symptoms in the *Ruscus* extract group. With the placebo treatment generally, the baseline values were maintained for all parameters. Significant differences between the treatment groups were seen for the volume changes of the lower leg after 8 and 12 weeks, ankle circumference after 4, 8 and 12 weeks, lower leg circumference for 8 and 12 weeks, and finally after 12 weeks for the subjective symptom 1 (heaviness and tiredness) and subjective symptom 2 (sensation of tension).

Evaluation of subjective symptoms : The global efficacy of the *Ruscus* extract was evaluated by the investigator as very good and good more frequently, whereas placebo was more frequently assessed as moderate and bad ($p = 0.0498$). A significantly positive correlation coefficient was calculated for the changes of each of the subjective symptoms 1 (heaviness and tiredness), 2 (sensation of tension) and 3 (tingling tension) and leg volume changes.

Quality of life: The Quality of life did not reveal any changes after 12 weeks of treatment for both groups.

Assessor's comments:

The study of Vanscheidt and al is deserving of being the first and the only one clinical study performed with a Ruscus extract alone. . Nevertheless, it is always rather difficult to appreciate the real quality of a study through a publication. Several methodological insufficiencies remain unsolved and information is missing such as: lack of complete protocol with modalities of randomisation, sample calculation and power of the study.

Moreover, the respect of the double-bind procedure can not be evaluated.

18 patients have been excluded from the analysis for insufficient data of which no information is given. At 12 weeks, all missing data have been replaced by a LOCF value (last observation carried forward). However, we do not know how many data are missing.

Lastly, the study has been conducted in 10 centres in Germany; however, the homogeneity between centres has not been evaluated in terms of recruitment or clinical practices. Taking into account these overall comments, efficacy results should be interpreted with caution.

According to the author, the study was designed in accordance with the guidelines for testing drugs for chronic venous insufficiency (CVI), i.e. study design with oedema reduction as the primary variable. It has to be noted that these guidelines were written by the author himself and published in "Phlebologie" after the beginning of this study i.e. April 1999 (Vanscheidt et al., 2000) and that oedema is not considered as a cardiovascular risk factor in the recommendations made by the European Society of Hypertension and the European Society of Cardiology in 2007.

Although we can agree with most of the proposed design of this study (e.g. inclusion and exclusion criteria, duration of the study), ***the clinical relevance of the primary criterion is debatable****. Even if oedema reduction as primary variable can be considered a reliable quantitative primary end-point to evaluate one of the pharmacological effects of Ruscus aculeatus, the clinical relevance of this primary variable is questionable. According to the assessor's opinion,, improvement in subjective symptoms such as sensation of heaviness or tiredness, tingling or pain should be of more clinical relevance. As stated by*

the author himself, any reduction of oedema is only regarded as clinically relevant if it is accompanied by an improvement in patient's quality of life.

Thus, despite significant results regarding the primary variable and the positive correlation shown between leg oedema and all the subjective symptoms except pain, the clinical relevance of these results remains questionable. Indeed, the positive effect relative to the subjective symptoms is very limited. The difference between both groups for the subjective symptoms "tingling sensation" and "pain" are not statistically significant and the significativity of the difference for the two other subjective symptoms "heaviness and tiredness" and "sensation of tension" is debatable taking into account the multiplicity of the analyses. Moreover, the treatment response measured by the disease specific questionnaire on the quality of life appeared negative at the end of this study as results on this questionnaire did not reveal any changes in both arms).

In conclusion, the credit that we can attribute to this study is to have tested a Ruscus extract alone. The posology of the extract used in the study is in adequacy with the one recommended by the available monographs i.e. a daily dose equivalent to an amount of 7 to 11 mg of total ruscogenins. However, the level of evidence of Ruscus extract efficacy in relieving symptoms of chronic venous insufficiency given by this study is low. It has to be noted that the efficacy was not evaluated in men. Another study to confirm these results is deemed necessary. Evaluation of a sustained efficacy over a longer period (up to 1 year) has not been studied.

Other Clinical Efficacy Data (submitted in response to the list of Questions by the AESGP)

- Human pharmacological studies. Rudofsky G and al.Wirkung eines Kombinationspräparates auf die Venenkapazität [Effect of a drug combination on venous capacity]. Fortschr Med 1982; 100: 1217-20. Rudofsky G. and al Die Wirkung der Kombination aus *Ruscus*-Extrakt und Trimethylhesperidinchalkon bei gesunden Probanden unter Wärmebelastung [Improving venous tone and capillary sealing. Effect of a combination of *Ruscus* extract and hesperidine methyl chalcone in healthy probands in heat stress]. Fortschr Med 1989; 107: 52, 55-2, 58.

Additional studies were provided in addition to the only clinical study to sustain efficacy results. We are of the opinion that these results, despite the fact that they demonstrate an increase of the tonus of the venous wall, are of limited interest as *Ruscus aculeatus* extract was not tested alone but in combination with other substances. It is therefore difficult to differentiate a specific effect of *Ruscus aculeatus* extract from the effect of the combinations tested.

- Meta-analysis. Boyle P and al. Meta-analysis of clinical trials of Cyclo 3 Fort in the treatment of chronic venous insufficiency. *Int Angiol* 2003; 22: 250-62.

Clinical data for *Ruscus aculeatus* have also been published in a meta-analysis that included 20 placebo-controlled randomised double-blind studies. However, the conclusions of the meta-analysis by *Boyle and al* cannot be taken into account. Indeed, this publication is related to Cyclo 3 which contains *Ruscus aculeatus* extract (150 mg per capsule), hesperidine chalcone (150 mg) and ascorbic acid (100 mg) and not to *Ruscus* extract alone; this demonstration of the clinical efficacy cannot be attributed to *Ruscus*.

- International Consensus Symposium. Ramelet AA, and al Veno-active drugs in the management of chronic venous disease. An international consensus statement: current medical position, prospective views and final resolution. *Clin Hemorheol Microcirc* 2005; 33: 309-19.

The International Consensus symposium, held during the 13th Congress of European Society for Clinical Hemorheology (ESCH) in Siena (Italy), 2005, concluded that vaso-active drugs (VAD) are effective and may be applied in chronic venous disease (CVD), when symptomatic, at any class of CEAP. The Consensus statement of the international experts also declares, that "in some cases VAD may replace compression and/or complement its effects". The experts classified, based on the available data, both the horse chestnut extract and *Ruscus* extract to "Grade B" of their recommendation explained in the statement published by *Ramelet and al*. Despite this conclusion, we still consider that the efficacy of

Ruscus aculeatus to relieve symptoms of chronic venous insufficiency is not demonstrated due to the lack of relevance, for this procedure, of the study and the meta-analysis.

- Comparative study with another herbal: Horse Chestnut Extract Diehm C and al Comparison of leg compression stocking and oral horse-chestnut seed extract therapy in patients with chronic venous insufficiency. Lancet 1996; 347: 292-4.

Comparison with horse chestnut extract *Diehm and al* study "*Comparison of leg compression stocking and oral horse-chestnut seed extract therapy in patients with chronic venous insufficiency*".

Results of a randomised placebo-controlled three-armed study performed with another herbal substance: oral horse chestnut extract, were provided. This study demonstrated that on the criterion oedema protection (leg volume reduction), results are comparable between horse chestnut extract, and leg compression stockings.

However, this extrapolation of results to *Ruscus aculeatus* extract is not acceptable. This indirect comparison is hazardous and cannot be accepted as no direct comparison has been performed between *Ruscus* Aculeati Extract and leg compression stockings.

2. Haemorrhoids

No clinical studies are available in the treatment of haemorrhoids with *Ruscus aculeatus* alone. Most of the studies are performed with a *Ruscus* combination with various flavonoïds. One can not rule out a positive synergistic effect of the combinations. Thus the proper efficacy of *Ruscus aculeatus* is difficult to assess (Abascal and Yarnall, 2005).

Experiments on single cases: patients treated with a 10% hydro-alcoholic (alcohol 30°) *Ruscu*s extract given per os were reported (Caujolle *et al.*, 1953). Over 11 cases followed during a few months, 10 cases showed an improvement of the symptoms whereas in one case *Ruscus* extract was ineffective. However, this later patient was suffering from haemorrhoids associated with haemorrhage. Another successful case corresponding to a pregnant women treated locally with suppository was reported.

In addition, the author reported 13 out of 15 observations from rural physicians corresponding to patients treated either per os or locally using suppository.

Assessor's comments:
Despite the identified Ruscus aculeatus pharmacological effects i.e. enhancement effect on venotonicity, no well-conducted studies are available with Ruscus aculeatus alone.
Even if there is evidence to suggest that Ruscus extract is effective in relieving symptoms of haemorrhoids, this evidence is of very low level. Further researches in that field are needed. However, due to the long-term use of ruscus extract-containing products in this indication, Ruscus aculeatus can be considered as traditional herbal medicinal product for symptomatic relief of itching and burning associated with haemorrhoids.

3. Orthostatic hypotension

One case of profound refractory orthostatic hypotension treated for longer than 2 years has been reported (Redman, 2000). The author recommends one "standard" 470 mg capsule of *Ruscus aculeatus* every hour from waking till evening until blood pressure is high enough that it is no longer needed. This corresponds to doubling the normal "recommended" dose of two capsules three times daily. It has to be noted that the author recommends even so combining the intake of *Ruscus* with non pharmacological measures and other natural products medicines.

Assessor's comments:
According to the author, with regard to its identified pharmacological properties Ruscus application and extension to the treatment of orthostatic hypotension is obvious. In the assessor's opinion this application should be clearly better documented. To date, with regard to the available data, no recommendation can be given. Further researches in that field are needed.

4. Diabetic retinopathy

One study involving *Ruscus aculeatus* alone has been found in the literature (Archimowicz-Cyrylowska *et al.*, 1996).

The study was carried out with 60 patients (32 women, 28 men), aged from 20-75 years, mostly suffering from non-insulin dependent diabetes mellitus (type II) for 1-27 years, characterized by non-proliferative diabetic retinopathy. They were randomly assigned to three equal groups: Group I - treated with troxerutin (Venoruton Zygma, GmbH) 1 tablet containing 0.5g of 0-(beta-hydroxyethyl)-rutoside 2 times a day; Group II - treated with 1 capsule containing 0.0375g of *Ruscus* extract (Fagorutin-*Ruscus*, Fink GmbH) 2 times a day, and Group III – treated with 2 tablets of pressed buckwheat herb (each tablet containing 0.5g Fagopyrum esculentum herb and 0.03 troxerutin (Fagorutin Buchweisen tabl., Fink GmbH)) 3 times a day. During the study period of 3 months, all subjects remained on stable diabetic diet, unchanged hypoglycaemic medication for the period of treatment. If administered earlier, any hypolipaemic medication was withdrawn at least 4 weeks before the onset of the study.

At the beginning, as well as on the last day of the investigation each patient was subjected to an ophtalmological examination and clinical biochemistry. The oscillating potentials of the electroretinogram were also recorded.

→ Results:

Group I, medicated with troxerutin, was characterized by a decrease in amplitude of oscillating potentials by 21 % considering both eyes. In contrast, in group II and group III, treated with *Ruscus* and buckwheat herb, an increase in amplitude of oscillating potentials (by 15 % and by 18 % respectively) was observed. The changes in the amplitude detected were statistically insignificant when compared with the initial values.

In all patients treated for 3 months with troxerutin, *Ruscus* and buckwheat herb preparations, a slight statistically insignificant increase in visual acuity was observed.

Examination of the anterior segment of the eyeball after 3 months of pharmacotherapy did not show any differences when compared with the initial picture in all the groups evaluated.

A regression of changes located in fundus of eye was demonstrated in 27.8% while a progression in 5.6 % of patients treated with troxerutin was observed. Evaluation of the fundus of the eye in group II (*Ruscus* extract) revealed a quite distinct improvement in 23.1 % of patients and no cases with progression, while in patients receiving buckwheat herb (group III) an improvement was demonstrated in 26.7 % and a deterioration in 3.3 % of the examined diabetics.

Mean blood serum concentrations of glucose significantly decreased by 12.7% in the troxerutin treated group, by 10.6 % in the *Ruscus* group and by 15.1 % in subjects medicated with buckwheat herb. Similarly, concentrations of glycosylated haemoglobin were lower after the 3-month period of treatment in all groups studied.

Assessor's comments:

The design of the study is not acceptable for many reasons, such as:

- *The patients are not adequately defined: the stage of the non-proliferative diabetic retinopathy is not given; no reference is made to an approved European diabetic retinopathy classification such as the ETDRS classification; neither baseline blood pressures nor baseline glycosylated haemoglobin concentrations are given.*
- *With regard to the pathology, the duration of the study is too short.*
- *Only 20 patients were enrolled in each group of treatment.*
- *With regard to the pathology, the choice of the comparators is not justified and cannot be considered as relevant. Moreover, there is no comparison with a placebo.*

Thus, the design of this study is not relevant and cannot be taken into account.

II.3.2.3 CLINICAL STUDIES IN SPECIAL POPULATION (E.G. ELDERLY AND CHILDREN)

Elderly
According to the provided literature, no clinical studies have been conducted with *Ruscus* extract alone in elderly.

Children
According to the provided literature, no clinical studies have been conducted with *Ruscus* extract alone in children.

Pregnancy
The use of *Ruscus* extract alone has not been evaluated in pregnant women.

II.3.2.4 ASSESSOR'S OVERALL CONCLUSIONS ON CLINICAL EFFICACY

To date, the clinical data on *Ruscus aculeatus* extract alone that can be taken into consideration are limited to only one publication of a randomized placebo-controlled study performed in patients with chronic venous insufficiency (Vanscheidt *et al.*, 2002).

The results obtained from this clinical study suggest an efficacy in the relief of symptoms such as "heaviness and tiredness" and "sensation of tension" in patients suffering from chronic venous insufficiency. However, in the opinion of the assessor, the provided evidence is insufficient to implement the *Ruscus aculeatus* monograph for a well-established use in relieving symptoms of chronic venous insufficiency.

In the treatment of haemorrhoids, no clinical data are available with *Ruscus aculeatus* alone; only pharmacological effects, data provided by studies with *Ruscus* in combination and the long-term use suggest that *Ruscus* extract is effective to relieve symptoms of haemorrhoids.

There is no sufficient data to sustain the indication of *Ruscus aculeatus* extract in orthostatic hypotension and in diabetic retinopathy.

Based on the available data, **the monograph information should remain limited to the traditional use**:

- for relief of symptoms of heavy legs.
- for symptomatic relief of itching and burning associated with haemorrhoids.

The use *Ruscus* extract should be limited to adults. Of note, no data are available in men.

The use of *Ruscus* extract alone has not been evaluated in pregnant women.

II.3.3 CLINICAL SAFETY/PHARMACOVIGILANCE

II.3.3.1 PATIENT EXPOSURE

According to the provided literature no data are available.

II.3.3.2 ADVERSE EVENTS

Results from the study published by Vanscheidt and al (2002)

This study is a multi-centre, double-blind, randomized, placebo-controlled trial with women suffering from chronic venous insufficiency to investigate the efficacy and safety of an extract of *Ruscus aculeatus* rhizome. Randomization was carried out after a 2-week run-in phase at visit 2. During the run-in period, all patients received placebo. The following treatment phase with either *Ruscus* extract or placebo in the two parallel groups lasted 12 weeks. Checkpoints were scheduled after 4, 8 and 12 weeks of treatment.

The daily dosage of the *Ruscus* extract (72-75 mg dry extract from butcher's broom rhizome) was chosen according to the German monograph.

166 patients from 30 to 89 years-old were included. 37 patients experienced one or more treatment emergent adverse events:

- 17 patients experienced 22 adverse events in the *Ruscus* extract group, including 2 cases of calf cramps;
- 20 patients experienced 26 adverse events in the placebo group, including 4 cases of calf cramps.

The tolerability was assessed as very good in 76.8% in the *Ruscus* extract group versus 78.8% in the placebo group, good in 23.2% versus 20.0% and moderate in 0% versus 1.3%.

Assessor's comments:
37 patients experienced adverse effects, of whom 17 were in the Ruscus extract group. No information is available on these cases regarding the nature and the seriousness, excepted for six of them (2 in the Ruscus extract group and 4 in the placebo group), which were calf cramps. No conclusion with regards to the safety of this extract can be drawn from these data.

Data from the literature

Three publications and one abstract presented during a French conference regarding the safety of *Ruscus aculeatus* have been identified.

(Valnet-Rabier *et al.*, 2004)

The abstract reports one case of collagenous colitis which occurred in one 52 years-old female patient. This patient initiated *Ruscus aculeatus* therapy about 10 months before the diagnosis, which has been histologically confirmed. At the onset of the colitis, other fluid extracts were taken by the patient, but none had a known colorectal toxicity.

Assessor's comments:
It is of note that Cyclo 3®, a Ruscus aculeatus containing medicinal product has been associated with cases of lymphocytic colitis and diarrhea. From 1991 to 2003, several publications reported such cases in the literature.

(Landa *et al.*, 1990)

This first publication reports one case of papulo-erythematous eruption of both legs, that spread within a few days to the entire skin, with oedema of the eyelids in a 30 years-old pregnant female patient, after the application of a vasoconstrictor cream for the treatment of varices. Patch tests revealed positive results to *Ruscus aculeatus* and thimoresal, 2 ingredients of the cream.

(Elbadir *et al.*, 1998)

In this second publication, 8 cases of contact allergy were collected from 1986 to 1995, in patients receiving ruscogenins containing medications. Ruscogenins are components of *Ruscus aculeatus.* These 8 cases involve 6 women and 2 men aged from 28 to 55 years-old who experienced eczema at the application site, after the use of a topic medication (7 cases) or a cosmetic cream (1 case). Prick tests or patch tests were performed in 7 patients and all were positive for ruscogenins or *Ruscus.*

***Assessor's comments*:**
One of these cases is described in the publication by N. Landa.

(Ramirez-Hernandez, 2006)

The last publication, and the most recent one, reports one case of perianal eczema in one 34 years-old patient following the local application of an antihaemorrhoidal cream. The outcome was favourable after therapy withdrawal. Several months later, the patient developed a generalized eczematous cutaneous

eruption one day after the application of an anticellulitis product on the lower limbs. Patch tests for both creams revealed a positive reaction to ruscogenins.

***Assessor's comments*:**
According to these data, the local application of Ruscus aculeatus or ruscogenins has been associated with allergic reactions, mainly represented by contact eczema. In one case, the event spread to the entire skin. In all cases, the outcome was favourable after therapy withdrawal and administration of corticoids.

Other data

The review of the PSURs regarding *Ruscus aculeatus* medicinal products (topics and capsules) did not allow to identify other safety issues, but confirms that *Ruscus* containing creams may potentially be associated with allergic reactions, mainly eczemas, and that the capsules may cause diarrhoea and lymphocytic colitis.

II.3.3.3 SERIOUS ADVERSE EVENTS AND DEATHS

According to the provided literature no data are available.

II.3.3.4 LABORATORY FINDINGS

According to the provided literature no data are available.

II.3.3.5 SAFETY IN SPECIAL POPULATIONS AND SITUATIONS

II.3.3.5.1 *INTRINSIC (INCLUDING ELDERLY AND CHILDREN) / EXTRINSIC FACTORS*

II.3.3.5.2 *DRUG INTERACTIONS*

According to the provided literature no data are available.

II.3.3.5.3 *USE IN PREGNANCY AND LACTATION*

In an open study involving 9 pregnant women, 3 of the pregnant women applied 2 to 3 grams of a *Ruscus* containing cream (100 mg of cream contains 1.6 grams *Ruscus* extract and 1.6 g Melilot extract) twice daily during the 3rd trimester of pregnancy. No embryotoxic effects were noted by the author (Berg, 1991).

Assessor's comments:
The study was performed with a combination of Ruscus extract and another product, thus a conclusion on the safety of the Ruscus extract alone cannot be drawn. Furthermore, as the combination has been administered during the third trimester of pregnancy, the embryotoxicity cannot be ruled out, only the foetotoxicity is addressed.

In an open study, 20 pregnant women have been enrolled (Baudet *et al.*, 1991). The women have taken two capsules per day of Cyclo 3 Fort (a combination of *Ruscus* extract 150 mg, trimethylhesperidin chalcone 150 mg and ascorbic acid 100 mg) after 21 to 24 weeks of amenorrhea. Fetal development was measured through the pulse Doppler method of the cord. The authors conclude that this test shows "an absolute harmlessness for the infant, assessed with the usual clinical and ultrasonographic criteria of pregnancy surveillance, with Doppler's velocimetry at the level of the umbilical artery and with the state of the infant and the anatomopathologic aspect of the placenta after birth."

Assessor's comments:
As for the prior study, this study was performed with a combination of Ruscus extract and other products. The combination administered from the second trimester of pregnancy has shown neither foetotoxic effect nor harmful effect for the new born.

In a review of the botanical treatments for haemorrhoids, some authors concluded that the available studies in pregnant women treated with a *Ruscus* combination do not establish the safety of *Ruscus* in pregnancy conclusively (Abascal and Yarnall, 2005).

Assessor's comments:
No data are available with Ruscus extract alone. Even considering Ruscus combinations, data are too limited to allow any recommendations.

Lactation
As there are no clinical or animal data available on the use of *Ruscus* extract during lactation and due to the potential harmful effect on the breast fed new born, particularly with regard to gastrointestinal disorders, the use of *Ruscus* extract should be avoided during the lactation.

II.3.3.5.4 *OVERDOSE*

According to the provided literature no data are available.

II.3.3.5.5 *DRUG ABUSE*

According to the provided literature no data are available.

II.3.3.5.6 *WITHDRAWAL AND REBOUND*

According to the provided literature no data are available.

II.3.3.5.7 *EFFECTS ON ABILITY TO DRIVE OR OPERATE MACHINERY OR IMPAIRMENT OF MENTAL ABILITY*

According to the provided literature no data are available.

II.3.3.6 ASSESSOR'S OVERALL CONCLUSIONS ON CLINICAL SAFETY

No conclusion with regards to the safety of this extract can be drawn from the study published by W Vanscheidt *et al*. The data are too scarce and insufficient. No information is available on the adverse effects regarding the nature and the seriousness, excepted for six of them (2 in the *Ruscus* extract group and 4 in the placebo group), which were calf cramps.

Data from the literature highlight two kinds of adverse effects which have been associated with the intake of *Ruscus aculeatus* or ruscogenins containing products. The topical forms have been associated with contact dermatitis. Although the patient received medicinal products containing multiple substances, prick tests and patch tests allowed confirming, for the topical use, the responsibility of *Ruscus*/ruscogenins in the occurrence of the allergic reaction. The second well identified risk concerns the oral route and the administration of capsules. Therapy with *Ruscus aculeatus* containing capsules has been associated with diarrhea/lymphocytic colitis. The literature data seem to be supported by spontaneous reports regarding different medicinal products and collected data in the PSURs.

From the available studies, a conclusion on the safety of the use of *Ruscus* extract alone during pregnancy cannot be drawn. The women were exposed to a mixture containing R*uscus* extract from the second trimester of pregnancy. Thus, the only conclusions which can be drawn relates to the foetotoxicity or the new born effects. For a very limited number of pregnant women (23) no foetotoxic effect appeared to date but complementary data are necessary to conclusively establish the safety of the *Ruscus* extract alone during the latter pregnancy. No data on exposure during the first trimester of pregnancy are available. So, no conclusion can be drawn about the teratogenic potential of the *Ruscus* extract alone.

As there are no clinical or animal data available on the use of *Ruscus* extract during lactation and due to the potential harmful effect on the breast fed new born, particularly with regard to gastrointestinal disorders, the use of *Ruscus* extract during breast feeding should be avoided.

II.4 ASSESSOR'S OVERALL CONCLUSIONS

Non-clinical aspects

Pharmacology

Primary pharmacodynamics studies performed *in vitro* and *in vivo* using various experimental models showed that *Ruscus* extract possess a **contractile activity on veins**. This activity is mediated by stimulation of the α-adrenergic system. *In vitro* mechanistic studies showed that direct activation of postjunctional α1-and α2-adrenergic receptors, and stimulation of the release of norepinephrine form adrenergic nerve endings were involved. Although this effect does not appear to be clearly influenced by the hormonal status (estrogens, progesterone), it seems potentiated by temperature.

In *in vivo* studies, this venoconstricting activity was shown after intravenous and oral routes; in the hamster cheek pouch model, local application of the extract (*i.e*. in the superfusate) was also effective. It should be noted that only one study was conducted by the oral route: at the level of hamster cheek pouch microcirculation, the dose of 150 mg/kg/day administered for 28 days induced venular constriction (internal diameter decreased by 30%) and arteriolar dilation (internal diameter increased by 37%) without any impact on the mean arterial blood pressure, the latter effect being attributed to liberation of endothelium-derived relaxing factors on the arteriolar side.

Similarly, other primary pharmacodynamics studies showed that *Ruscus* extract exerts a **contractile effect on lymphatic vessels** in anaesthetised dogs at 2 and 5 mg/kg administered intravenously. A rise in oncontic pressure suggested a **favourable effect on edema**. This was confirmed in a feline model of ethacrynic acid-induced edema. The effective dosage amounted to 20 mg/kg by intravenous route, and 10-20 times higher by oral route. However, after subchronic administration (4-6 days), the oral effective dosage decreased to reach 20-40 mg/kg/day. The same study showed that ruscogenin was also effective, but that other components of the extract were involved to obtain maximal activity.

Due to the mechanisms underlying the effect of *Ruscus* extract, **pharmacodynamic drug-drug interactions** could occur with any drug potentiating or antagonizing the α-adrenergic system. As a precaution measure, it may be relevant to include a warning for patients treated with any of those drugs.

Considering the pharmacological profile of *Ruscus* extract, i.e. stimulation of α-adrenergic system, the lack of a **safety pharmacology** study evaluating its potential effects on the cardiovascular function gives cause for concern. No toxicology study evaluating this endpoint is available. In the studies performed in the hamster cheek pouch model, the mean arterial blood pressure was not modified after IV administration of 5 mg/kg *Ruscus* extract, and oral administration at the dose of 150 mg/kg. Therefore, **the need of a safety pharmacology study addressing cardiovascular aspects should be discussed in light of clinical safety data.**

Pharmacokinetics

Available pharmacokinetics studies should not be taken into account for regulatory purposes because they are endowed with major bias precluding a full confidence in the results obtained.

Toxicology

Extracts used in two acute toxicity studies were obtained by ethanolic extraction. Their characteristics (content in various compounds, e.g. ruscogenin and neoruscogenin) remain unknown. Potential variability compared to the extract(s) intended for therapeutic cannot be evaluated. In dogs, a mean intravenous LD0 of 1.20 g/kg was measured, while the oral LD50 of mice reached 25-34 ml/kg with another extract. The authors attribute the cardiovascular findings observed in dogs at high doses (decreased frequency of cardiac contractions, decreased blood pressure) to be secondary to alteration of respiratory centres. In rats and mice, death occurred by respiratory failure too. No safety pharmacology study is available on cardiovascular and respiratory systems. As previously indicated, the need of such studies should be discussed in light of clinical safety data.

No other toxicity studies are available. The ESCOP monograph reports repeat-dose toxicity and reprotoxicity studies performed in rabbits and rats, respectively. However, these studies remain unpublished so that the information available is very sparse. Therefore, they cannot be taken into consideration for safety evaluation.

The lack of adequately conducted genotoxicity and embryo-fetal toxicity studies precludes the listing of Ruscus aculeatus. Additionally, as no long-term study is available, the carcinogenic risk cannot be appreciated.

Clinical aspects

Pharmacology

On the basis of publications, the quality of the two available pharmacodynamics studies cannot be evaluated. For example, the characteristics of the patients are incomplete as well as the design of the studies. The statistical analysis is not given. Moreover, the characteristics of the *Ruscus* extract are not specified. However, the findings corroborate the preclinical pharmacological properties that acknowledge to *Ruscus* extract (manufactured by Pierre Fabre) an alpha-adrenergic effect and thus a venous vasoconstrictive effect that account for a reduction in volume of blood stored in the veins and for a stimulating effect on the lymphatic drainage. These two pharmacological properties assume a positive effect in patients suffering from chronic venous insufficiency.

Dose-effect studies are missing which preclude from justifying the dosage regimen used in the clinical studies.

Other methods of functional exploration could have been used to evaluate the effect on veins (e.g. Doppler).

Pharmacokinetics

Available pharmacokinetics data are too scarce.

Efficacy

To date, the clinical data on *Ruscus aculeatus* extract alone that can be taken into consideration are limited to only one publication of a randomized placebo-controlled study performed in patients with chronic venous insufficiency (Vanscheidt *et al.*, 2002).

The results obtained from this clinical study suggest an efficacy in the relief of symptoms such as “heaviness and tiredness” and “sensation of tension” in patients suffering from chronic venous insufficiency. As stated by the author himself, any reduction of oedema is only regarded as clinically relevant if it is accompanied by an improvement in patient’s quality of life. However, the treatment response measured by the disease specific questionnaire on the quality of life appeared negative at the end of this study., Finally, the provided evidence is insufficient to implement the *Ruscus aculeatus* monograph for a well-established use in relieving symptoms of chronic venous insufficiency.

Moreover, the French National Authority for Health had recently reassessed the benefice of all veinotonics in the treatment of chronic venous insufficiency. All the veinonotics with marketing authorization in France such as Cyclo 3, Diosmin, Troxerutin had been studied. The conclusions of the Authority were that the efficacy of all the medicines was minor and the proofs given to demonstrate the efficacy were poor.

In the treatment of haemorrhoids, no clinical data are available with *Ruscus aculeatus* alone; only pharmacological effects, data provided by studies with *Ruscus* in combination and the long-term use suggest that *Ruscus* extract is effective to relieve symptoms of haemorrhoids.

Based on the available data, the monograph information should remain limited to the traditional use in subjective symptoms of chronic venous insufficiency such as sensation of heavy legs and in symptomatic relief of itching and burning associated with haemorrhoids. The ESCOP and Commission E recommended daily dosage could be considered as acceptable as it is in line with the one use in the randomized placebo-controlled study performed in patients with chronic venous

insufficiency (Vanscheidt *et al.*, 2002) and with the daily dosage recommendations for the Ruscus aculeatus containing products already on the market.

The use *Ruscus* extract should be limited to adults. Of note, no data are available in children.

The use of *Ruscus* extract alone has not been evaluated in pregnant women.

Safety

No conclusion with regards to the safety of this extract can be drawn from the study published by W Vanscheidt *et al*. The data are too scarce and insufficient. No information is available on the adverse effects regarding the nature and the seriousness, excepted for six of them (2 in the *Ruscus* extract group and 4 in the placebo group), which were calf cramps.

Data from the literature highlight two kinds of adverse effects which have been associated with the intake of *Ruscus aculeatus* or ruscogenins containing products. The topical forms have been associated with **contact dermatitis**. Although the patient received medicinal products containing multiple substances, prick tests and patch tests allowed confirming, for the topical use, the responsibility of *Ruscus*/ruscogenins in the occurrence of the allergic reaction. The second well identified risk concerns the oral route and the administration of capsules. Therapy with *Ruscus aculeatus* containing capsules has been associated with **diarrhea/lymphocytic colitis**. The literature data seem to be supported by spontaneous reports regarding different medicinal products and collected data in the PSURs.

The relevance of preclinical data on the cardiovascular system has not been confirmed by clinical data.

Form the available studies, a conclusion on the safety of the use of *Ruscus* extract alone during pregnancy cannot be drawn. The women were exposed to a mixture containing R*uscus* extract from the second trimester of pregnancy. Thus, the only conclusions which can be drawn relates to the foetotoxicity or the new born effects. For a very limited number of pregnant women (23) no foetotoxic effect appeared to date but **complementary data are necessary to conclusively establish the safety of the *Ruscus* extract alone during the latter pregnancy**. No data on exposure during the first trimester of pregnancy are available. So, **no conclusion can be drawn about the teratogenic potential of the *Ruscus* extract alone. The use of *Ruscus* extract should be not recommended during pregnancy.**

As there are no clinical or animal data available on the use of *Ruscus* extract during lactation and due to the potential harmful effect on the breast fed new born, particularly with regard to gastrointestinal disorders, **the use of *Ruscus* extract should be avoided during the lactation.**

ANNEXES

COMMUNITY HERBAL MONOGRAPH ON *RUSCUS ACULEATUS* L., RHIZOMA

LITERATURE REFERENCES