Assessment report on *Fucus vesiculosus* L., thallus

Based on Article 16d(1), Article 16f and Article 16h of Directive 2001/83/EC as amended (traditional use)

Draft

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Fucus vesiculosus* L. , thallus
Herbal preparation(s)	Powdered herbal substance
Pharmaceutical form(s)	Herbal preparations in solid dosage form for oral use
Rapporteur	
Assessor(s)	

Note: This draft assessment report is published to support the release for public consultation of the draft Community herbal monograph on *Fucus vesiculosus* L., thallus. It should be noted that this document is a working document, not yet fully edited, and which shall be further developed after the release for consultation of the monograph. Interested parties are welcome to submit comments to the HMPC secretariat, which the Rapporteur and the MLWP will take into consideration but no 'overview of comments received during the public consultation' will be prepared in relation to the comments that will be received on this assessment report. The publication of this <u>draft</u> assessment report has been agreed to facilitate the understanding by Interested Parties of the assessment that has been carried out so far and led to the preparation of the draft monograph.

Table of contents

1. Introduction

1.1. Description of the herbal substance(s), herbal preparation(s) or combinations thereof

- Herbal substance(s)

Fucus vesiculosus L. (bladderwrack) grows on rocky shores in areas with cold and temperate climate. Mostly at North American and Western European shores of the North Atlantic and the Pacific Ocean (Verhelst 2010). *Fucus vesiculosus* is a small brown seaweed measuring 20 to 100 centimetres in length. Their flat thallus branches dichotomously. Oval with air filled bubbles in the membraneous parts make the seaweed float vertically. This seaweed is harvested at the start of summer (Verhelst 2010, De Smet *et al.* 1997).

- Minerals: iodine (mostly bound in organic substances), with a minimum of 0.03 and a maximum 0.2 per cent of total iodine determined on the dried drug. (European Pharmacopoeia 7.0, Delfosse 1998, Williamson 2009) Other minerals present are bromide (Van Hellemont 1985), sodium, potassium, calcium, magnesium, iron, phosphor, sulphates, copper, chrome, chloride, zinc, manganese, silicon and selenium (Verhelst 2010, De Smet *et al.* 1997, British Herbal Compendium 1992).

- Polysaccharides: laminarin (Hänsel *et al.* 1993), alginic acid and fucoidan (Delfosse 1998, Wichtl 1994, Van Hellemont 1985, Verhelst 2010). The content of alginic acid is estimated at 12%. Alginic acid is a linear polymer with various sequences of beta-(1-4)-D-mannuronic acid and alpha-(1-4)-L-guluronic acid residues; fucans of varying structure such as fucoidancomposed mainly of alpha-(1-2)-L-fructose-4-sulphate residues (British Herbal Compendium 1992).
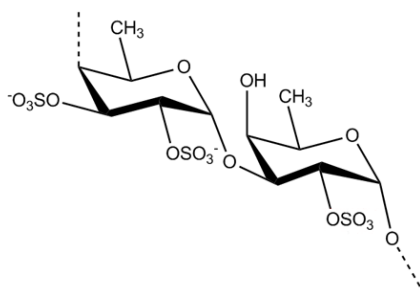
Figure 1: Fucoidan (site glycomix)

- Polyphenols: ca. 15%, composed of phloroglucinol units. Most are high in molecular weight (25% greater than 10,000), phlorotannins consisting of carbon-carbon or ether linked phloroglucinol units in linear chains with numerous side branches. Lower molecular weight polyphenols with 4 to 7 phloroglucinol units, such as fucols (carbon-carbon linked) and fucophlorethols (one carbon-carbon and one or more ether links) have been isolated as well as free phloroglucinol (British Herbal Compendium 1992).

- Lipids: glycosyldiacylglycerids, phosphatidylethalolamin, phosphatidylcholin, eicosapentaeenacid (EPA), arachidonic acid (AA) (Verhelst 2010, De Smet *et al.*1997, Hänsel *et al.* 1993, Delfosse 1998)

- Sterols: fucosterol, β-sitosterol (Verhelst 2010, De Smet *et al.*1997, Hänsel *et al.* 1993)

- Polyphenols: phlorotanin (Hänsel *et al.* 1993, Verhelst 2010, De Smet *et al.* 1997)

- Pigments: fucoxanthin, zeaxanthin (Verhelst G. 2010, De Smet P.A.G.M. et al. 1997) lutein, violaxanthin, neoxanthin, fucoxanthinol, β-carotene, squalene (Hänsel *et al.* 1993)

- vitamins: C (Baines J. 2007) B1, B2, B3, B6, folic acid, choline (Verhelst 2010, De Smet *et al.* 1997), vit K (Williamson 2009)

- Other constituents: pectin-like membrane slime, ethereal oil (Van Hellemont 1985, Verhelst 2010, De Smet *et al.* 1997), phloroglucinol, mannitol, sorbitol, aminoacids, proteins, bromophenols, acrylic acid (Verhelst 2010, De Smet *et al.* 1997)

- Possibly contaminated with heavy metals (Williamson 2009).

- Herbal preparation(s)

The monograph describes the uses of the powdered herbal substance.

- Combinations of herbal substance(s) and/or herbal preparation(s) including a description of vitamin(s) and/or mineral(s) as ingredients of traditional combination herbal medicinal products assessed, where applicable.

Not applicable.

1.2. Information about products on the market in the Member States

Regulatory status overview

Member State	Regulatory Status				Comments
Austria	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Only in homeopathic products
Belgium	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Only in combined preparations (see 2.3.)
Bulgaria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No registered products
Cyprus	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Czech Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Denmark	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No registered products
Estonia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No registered products
Finland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
France	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	Registered product
Germany	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No registered products
Greece	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No registered products
Hungary	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No registered products
Iceland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Ireland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Italy	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Latvia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Liechtenstein	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Lithuania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Luxemburg	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Malta	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	

Member State	Regulatory Status				Comments
The Netherlands	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No registered products
Norway	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Poland	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Combined products
Portugal	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No registered products
Romania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No registered products
Slovak Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Slovenia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Spain	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No registered products
Sweden	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No registered products
United Kingdom	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	Registered products

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'

This regulatory overview is not legally binding and does not necessarily reflect the legal status of the products in the MSs concerned.

1.3. Search and assessment methodology

LIMO: (*Fucus vesiculosus*) AND (supplement OR medicine) AND human
Food Science and Technology Abstracts: (*Fucus vesiculosus* OR kelp) AND (medicine OR supplement) AND human (no related terms included)
Biosis Previews: (*Fucus vesiculosus* OR kelp) AND (medicine OR supplement)
Up to date: *Fucus vesiculosus*
Web of Science: (*Fucus vesiculosus* OR kelp) AND (medicine OR supplement)
PubMed: (*Fucus vesiculosus* OR kelp) AND (medicine OR supplement)
EMBASE: (*Fucus vesiculosus* OR kelp) AND (supplement OR medicine)
CINAHL: *Fucus vesiculosus* OR kelp
International Pharmaceutical Abstracts: (*Fucus vesiculosus* OR kelp) AND (supplement OR medicine) {no related terms}
PsycInfo: (*Fucus vesiculosus* OR kelp) AND (supplement OR medicine) {No Related Terms}

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┌─────────────────────────────────────────────┐
│ Search Results March 2012                     │
│ n=675                                         │
│ LIMO (n= 194)                                 │
│ Food Science and Technology Abstracts (n=103) │
│ Biosis previews (n=87)                        │
│ Up to date (n=1)                              │
│ Web of science (n=67)                         │
│ PubMed (n=41)                                 │
│ EMBASE (n=96)                                 │
│ CINAHL (n=23)                                 │
│ International Pharmaceutical Abstracts (n=17)  │
│ PsycInfo (n=46)                               │
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┌──────────────────────────────────────────────────┐
│ Studies excluded after title and abstract screening │
│ n=584                                              │
│ LIMO (n=180) not entirely accessible               │
│ Food Science and Technology Abstracts (n = 100)    │
│ Biosis Previews (n = 67)                           │
│ Up to date (n=0)                                   │
│ Web of Science (n = 48)                            │
│ PubMed (n=24)                                      │
│ EMBASE (n=90)                                      │
│ CINAHL (n=17)                                      │
│ International Pharmaceutical Abstracts (n=14)       │
│ PsycInfo (n=44)                                    │
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┌────────────────────────────────────────────────┐
│ Studies retrieved for detailed evaluation:       │
│ n= 91                                            │
│ LIMO (n = 14)                                    │
│ Food Science and Technology Abstracts (n =3)     │
│ Biosis Previews (n=20)                           │
│ Up to date (n=1)                                 │
│ Web of Science (n = 19)                          │
│ PubMed (n=17)                                    │
│ EMBASE (n=6)                                     │
│ CINAHL (n=6)                                     │
│ International Pharmaceutical Abstracts (n=3)      │
│ PsycInfo (n=2)                                   │
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┌──────────────────────────────────────────────┐
│ Studies to be included:                         │
│ n=30                                           │
│ LIMO (n = 7)                                    │
│ Food science and technology abstracts (n=0)    │
│ Biosis previews (n=2)                          │
│ Up to date (n=1)                               │
│ Web of science (n=3)                           │
│ PubMed (n=17)                                   │
│ EMBASE (n=0)                                    │
│ CINAHL (n=0)                                    │
│ International Pharmaceutical Abstracts (n=0)    │
│ PsycInfo (n=0)                                  │
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┌──────────────────────────────────┐
│ Studies included after checking    │
│ references of other studies:       │
│ LIMO: 4                            │
│ Pubmed 4                           │
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┌────────────────────────────────┐
│ Total number of studies included │
│ n = 38                          │
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2. Historical data on medicinal use

2.1. Information on period of medicinal use in the Community

Fucus vesiculosus was already known by the Romans, in those times it was used against joint complaints. From the 16th century on, *Fucus vesiculosus* was used in China to treat goitre caused by iodine deficit. In the 17th century, *Fucus vesiculosus* was used in France to treat goitre and other thyroid complaints. This was also the case for the United Kingdom with the additional indication of corpulency treatment. In the United States, it was also indicated for psoriasis and as a strengthening agent. During the 18th century, *Fucus vesiculosus* was used to treat asthma, goitre and skin diseases (Morel *et al.* 2005, Verhelst 2010). Other applications were treatment of rheumatism and slimming baths, but the latter is questionable (Delfosse 1998).

2.2. Information on traditional/current indications and specified substances/preparations

Nowadays *Fucus vesiculosus* is administered orally and topically. Oral uses include auxiliary measure for weight loss, treatment of gastritis, pyrosis, reflux oesophagitis and hiatus hernia, the prevention of atherosclerosis, viscous blood and hypercholesterolemia, the management of constipation, colitis, asthenia, fatigue, mineral deficit, anemia, hair loss and leg cramps, an adjuvant for menopausal complaints, fibrocystic breasts, prostate complaints, growth deprivation, arthritis, arthrosis, gout and lymph edema. External uses are treatment of wounds, an adjuvant in the therapy for cellulites and obesity and an aid for rheumatism and arthritis (Verhelst 2010, Van Hellemont 1985, Delfosse , Barnes *et al.* 2007). In cosmetics, *Fucus vesiculosus* is applied because of its iodine and oligoelement content (Delfosse 1998).

Fucus vesiculosus is used as a natural source of iodine. The iodine content gives some plausibility to a possible stimulating effect on the thyroid gland. There is the connotation of an increased burning of fat (Weiss and Fintelmann 1999). Some sources are warning against latent hyperthyroidism if *Fucus* preparations are administered for a long time (Verhelst 2010). It has even been mentioned that iodine in *Fucus* can cause a thyreotoxic crisis and hypersensitivity reactions (Weiss and Fintelmann 1999, Van Hellemont 1985). However it should be noted that the iodine content is variable in seaweeds (Tyler 1993, De Smet *et al.* 1997). This variability as well as the daily intake of iodine make predictions difficult.

2.3. Specified strength/posology/route of administration/duration of use for relevant preparations and indications

Austria

In Austria *Fucus* is only present in homeopathic medicinal products.

Belgium

As far as medicinal products are concerned, the substance was included in a range of "ancient" herbal teas of varying composition. It was also included in various combination products used as laxatives.

Herewith an overview of combined preparations.

Tisane: Equisetum arvense 72.85 mg/g, Glycyrrhizae (radix) 101.42 mg/g, Iceland moss 123.57 mg/g, Chondrus crispus (carragaheen) 72.85 mg/g, Althaea flos et radix 247.85 mg/g, **Fucus vesiculosus** (extracta fluidum et siccum) 190.71 mg/g, Theobroma 130 mg/g, Quercus iles cortex 30 mg/g

Tisane: Senna (leaf) 308 mg/g, Peppermint leaf 81.33 mg/g, Pterocarpus santalinus vel indicus (lignum) 22 mg/g, *Fucus vesiculosus* (extracta fluidum et siccum) 111.33 mg/g, Spiraea ulmaria (flores) 81.33 mg/g, Birch tar 222 mg/g, Phaseoli fructus sine semine 44.66 mg/g, Parsley sativum 22 mg/g, Ononis spinosa (bugrane) 81.33 mg/g

Tisane: Senna (leaf) 35.33 mg/g, Valerianae officinalis (radix) 41.33 mg/g, Equisetum arvense 155.33 mg/g, Achillea millefolium (herba) 41.33 mg/g, Crataegus oxyacantha 200 mg/g, *Fucus vesiculosus* (extracta fluidum et siccum) 155.33 mg/g, Mistletoe 155.33 mg/g, Birch tar 143.33 mg/g, Phaseoli fructus sine semine 41.33 mg/g

As far as pharmacovigilance is concerned: one case was reported.

folnum	specianame	reaction	year	indication	relation	age	sex	initials	outcome
16793	fucus vesic.	hyperthyroidism	2000	e66.9	6	31	f	l.v.	f

Upon request, a list of 453 food supplements was received as a result of a query ("*Fucus vesiculosus*") done by the National Competent Authority for food supplements in Belgium (FAVV). The information is not very conclusive as the herbal substance/preparation is not always (almost never) mentioned on the list and can therefore not be further characterised. An analysis of the list indicates that the substance is mostly used in food supplements with claims refering to "minceur", "silhouette", "detox". Notifications only go back to 1990 in Belgium.

Bulgaria

There are no products containing *Fucus vesiculosus* with marketing authorisation or registration in Bulgaria. No information on food supplements is transmitted.

Denmark

One product containing *Fucus vesiculosus* is listed in the Danish Food and Veterinary Agency list of food supplements. The product is a combination product with Lucerne (*Medicago sativa* L.) and is sold as a iodine supplement.

Estonia

There are no authorised medicinal products on the market in Estonia. Other products containing this seaweed are probably classified as food supplements, under notification at the Veterinary and Food Board.

Finland

Fucus has not been registered as traditional herbal product in Finland, and there is no marketing authorisation as WEU medicinal product either. As the food supplement market is vast and changes, it is difficult to know if currently there is Fucus on market as food supplements. According to the food supplement list from November 2011, there is one food supplement containing, among other ingredients, *Fucus vesiculosus*.

France

Galenic form: hard capsules with 130 mg powder of *Fucus vesiculosus*.
Posology for adults only: 1 capsule 2 times daily.
Therapeutic indication: traditionally used as an adjuvant to slimming diets.
No pharmacovigilance actions were taken towards this product. On the market since 1981.

Greece

There are not any marketed products contaning Fucus as simple ingredients and/or in combinations.

The Netherlands

There are no WEU authorised/TU registered herbal medicinal products in the Netherlands containing *Fucus vesiculosus* as a single active ingredient, neither combination products. No data on the use of this seaweed in food supplements are available.

Poland

There are two combination products for oral use containing:
Menthae piperitae herba, Hyperici herba, Rosae fructus, Rhei radix, Frangulae cortex and
Fucus vesiculosus, marketed since 1989 (herbal tea) and 1999 (herbal tea in bag).

In adolescents over 12 years of age and adults, the single dose (=daily dose) is 1.7 – 2.5 g (51 – 75 mg of *Fucus vesiculosus*) before sleep. The daily dose should not be exceeded. The use in children under 12 years of age is contraindicated.
Use more frequently than 2 to 3 times weekly is not recommended. The duration of use should not exceed 1 to 2 weeks.
Indications: As a laxative for short-term use in constipation.
Risks (adverse drug effects, literature):
Abdominal pains, abdominal cramps, watery stool, especially in patients with irritable colon syndrome. On prolonged use: electrolyte and water balance disorders which potentiate the action of cardiac glycosides, medicinal products inducing reversion to sinus rhythm (e.g. quinidine) and medicinal products inducing QT prolongation; albuminuria and hematuria. Pseudomelanosis coli and pH-dependent red-brown or yellow discoloration of urine may occur. In hypersensitive individuals, photoallergy manifesting with rush and erythema may occur due to the content of St. John's Wort. In patients with gastroesophageal reflux, heartburn may increase.

Portugal

There are no authorised products on the market. INFARMED, I.P. is the national authority only for medicines and healthcare products. No information can be given about the existence in the market of food supplements with this seaweed.

Romania

Regarding Fucus there are no products authorised by National Agency for Medicines and Medical Devices.

Spain

AEMPS is responsible for medicinal products only; hence no information can be given about the existence on the market of food supplements with *Fucus vesiculosus*.

Sweden

There are no authorised products on the market in Sweden.

United Kingdom

Since 1968 there are a number of authorised products containing Fucus on the market in UK. These will all be transferred soon to the traditional category and will have indications along the lines of: "A traditional herbal medicinal product used as an aid to slimming as part of a calorie controlled diet, based on traditional use only". All preparations have to be considered as already being on the market before 1980.

The composition of the different combination products are given below:

Product (capsule) containing

        Powdered Fucus (*Fucus vesiculosus* L.) 100 mg

        Alginic acid 200 mg

        Hypromellose capsule 75 mg

Indication: traditional herbal remedy used as an adjuvant to slimming diets to help weight loss.

Posology: 1 capsule, 1 to 3 times daily during 3 weeks to be taken with a glass of water before meals. The treatment should be limited to 3 weeks.

Not recommended for children under 12 years of age.

Date of first authorisation (renewal): 29 August 1997.

Product (tablet) containing

        Powdered Fucus   150 mg

        Powdered Extract Fucus 5:1   20 mg

Indication: A traditional herbal remedy to help in the treatment of obesity and the symptomatic relief of rheumatic pain.

Posology: Adults: 2 tablets to be taken orally morning and evening. Not recommended for children under 12 years of age-. Elderly patients: normal adult dose.

Date of first authorisation: 20 March 1991.

Product (tablet) containing

        Fucus Dry Extract 5:1   120 mg

Indication: an aid to slimming.

Posology: to be taken by mouth. Adults (16 years and above) and elderly: 1 tablet 3 times a day. This product should be taken as part of a calorie-controlled diet.

Date of first authorisation: 20 May 1999.

Product (tablet) containing

        Fucus Aqueous Powdered Extract 5:1   120 mg

Indication: a herbal remedy traditionally used as an aid to slimming.

Posology: to be taken orally. Elderly, adults and adolescents over 16 years of age: 2 tablets to be taken with water 3 times a day.

Not recommended in children and adolescents under 16 years of age.

Date of first authorisation: 30 March 2009.

Product (tablet) containing

        Fucus vesiculosus BHP 500 mg

Indication: a herbal remedy traditionally used as an adjunct with calorie control for weight reduction.

Posology: for oral administration. Adults and Elderly: 2 to 3 tablets to be taken with a tumbler of water, half an hour before meals.

Not recommended for children.

Date of first authorisation: 1 July 2004.

Note

The **British Herbal Pharmacopoeia** (1983) contains a monograph on Fucus (bladderwrack). The herbal substance is described as containing*... Small variable amounts of iodine...*, referring to the Martindale 25th edition (Todd 1967) with an upper limit for iodine of 0.2%.

The following therapeutic indications are mentioned: myxedema, lymphadenoid goiter, obesity, rheumatism and rheumatoid arthritis. A specific indication is mentioned: obesity associated with hypothyroidism.

> Preparation and posology (trice daily):
>
> Dried thallus. Dose: 5-10 g OR by infusion.
> Liquid extract in 25% ethanol. Dose: 4 to 8 ml.

The **Martindale** describes the following (Todd 1967):

> Preparation and posology:
>
> Soft extract prepared with ethanol (45%). Dose: 200 to 600 mg.
> Liquid extract prepared with ethanol (45%). Dose: 4 to 8 ml.

The number of daily intakes is not mentioned.

The **British Herbal Compendium** (1992) describes *Fuci thallus* as an anti-obesic, a thyroactive, an antirheumatic and a demulgent. The therapeutic indications are: (1) obesity associated with iodine deficiency and hypothyroidism and (2) lymphadenoid goitre.

> Preparation and posology (daily):
>
> Dried thallus. Dose: 0.8 to 2 g
> Liquid extract (1:1, ethanol 25%). Dose: 1-2 ml
> Tincture (1:5, ethanol 25%). Dose: 4-10 ml.

Product (tablet) containing

Bladderwrack extract 5:1	32 mg
Bladderwrack	200 mg

Indication: A herbal remedy traditionally used for the treatment of obesity.
Posology: to be taken orally. One tablet after meals 3 times daily.
Elderly patients: normal adult dose.
Not recommended for children.
Date of first authorisation: 1 August 2004.

Product (oral liquid) containing

> Each 1 ml of oral liquid contains 1 ml of liquid extract from dried bladderwrack
> (*Fucus vesiculosus* L.) thallus (1:1). Extraction solvent: ethanol 21% v/v.

Indication: A traditional herbal medicinal product used as an aid to slimming as part of a calorie controlled diet, based on traditional use only.
Posology: For oral use only. Adults (18 years and above)*:* 5ml twice a day with water.
The use in children and adolescents under 18 years of age and in the elderly is not recommended.
Date of first authorisation: 1 March 2011.

Summary

The powder of *Fucus vesiculosus* L., thallus meets the requirement for 30-year medicinal use.

Table 1: Posologies for preparations meeting the criteria for traditional use

Posology	Source
1 hard capsule with 130 mg powdered herbal substance; to be taken orally, 2 times daily	**France**: on the market since 1981
1 tablet containing 500 mg powdered herbal substance; 2-3 tablets to be taken with a tumbler of water half an hour before meals	**UK**: on the market since 2004, but referred to the BHP of 1983

The posology as reported by France for the traditional use of the powder has been chosen for the draft monograph, in relation to the recommendations for the upper daily limit for iodine intake.

3. Non-Clinical Data

3.1. Overview of available pharmacological data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

In order to get an overview of the different activities, a summary table is included. Most of the activities are not related to the therapeutic indication of the monograph. Their value should be relativated. In the more detailed approach of preclinical data, a difference is made between the properties related to the therapeutic indication of the monograph and those which are of a more general interest.

Summary table of *in vitro* experiments

| Reference	Outcome
Thring *et al.* (2009)	Anti-collagenase, anti-elastase and anti-oxidative activity of *Fucus vesiculosus* extract.
Fujimura *et al.* (2000)	Fucoidan from *Fucus vesiculosus* significantly stimulates gel contraction, this effect would arise from a significant increase in integrin $α_2β_1$ expression on fibroblasts.
Aksenov *et al.* (2007)	Significant decrease in trans-sialidase activity caused by extracts of *Fucus vesiculosus*. Decrease in enzyme suggests less intracellular cholesterol accumulation.
Rupérez *et al.* (2002)	Fucoidan fraction of *Fucus vesiculosus* extract showed the highest antioxidant capacity.
Diaz-Rubio *et al.* (2009)	Major component of *Fucus vesiculosus* is fucose. Antioxidant capacity found which correlates with the total polyphenol content.
O'Sullivan *et al.* (2011)	Extracts from *Fucus vesiculosus* show significant anti-oxidant activity *in vitro*.
Parys *et al.* (2010)	Fucophlorethols from *Fucus vesiculosus* have radical scavenging activity that is comparable to that of phloroglucinol.
Skibola *et al.* (2005)	50 and 75 µmol/l *Fucus vesiculosus* extracts significantly reduce 17β-estradiol levels in human granulosa cells and *Fucus vesiculosus* also competes with estradiol and progesterone for binding to their receptors.

Parys *et al.* (2010)	Fucophlorethols from *Fucus vesiculosus* inhibit aromatase.
Roy *et al.* (2011)	Phlorotannins from *Fucus vesiculosus* inhibit α-amylase and α-glucosidase.
Trento *et al.* (2001)	Fucansulfate from *Fucus vesiculosus* significantly inhibits the production of thrombin and significantly inhibits thrombin-induced platelet aggregation.
de Azevedo *et al.* (2009)	Extracts from *Fucus vesiculosus* significantly prolong the activated partial thromboplastin and prothrombin time.
Kwak *et al.* (2010)	Fucoidan from *Fucus vesiculosus* inhibits ADP-induced platelet aggregation and the activities of both thrombin and factor X_a. It prolongs the activated partial thromboplastin time.
Dürig *et al.* (1997)	High molecular weight fucoidan from *Fucus vesiculosus* increases platelet aggregation significantly more than low molecular weight fucoidan. If the molecular weight of the fucoidan remains constant and the sulfate level increases, the activated partial thromboplastin time increases, whereas the α2-antiplasmin inhibitor activity and plasminogen activator inhibitor activities decrease.
Cumashi *et al.* (2007)	The anticoagulant activity of 1 mg fucoidan from *Fucus vesiculosus* is equal to that of approximately 9.4 U heparin and fucoidan doesn't inhibit the platelet aggregation induced by thrombin significantly.
Queiroz *et al.* (2008)	Galactofucan, fucoidan and fucan B from *Fucus vesiculosus* inhibit reverse transcriptase.
Oomizu *et al.* (2006)	Fucoidan from *Fucus vesiculosus* significantly inhibits the release of IgE by B cells.
Price *et al.* (2002)	*Fucus vesiculosus* extracts inhibit histamine release from mast cells.
Choi *et al.* (2005)	When macrophages and lymphocytes are exposed to fucoidan from *Fucus vesiculosus* their viability increases significantly.
Kim and Joo (2008)	Fucoidan from *Fucus vesiculosus* increases the viability of dendritic cells significantly. The results also suggest that fucoidan stimulates the immune system and the maturation of dendritic cells.
Kwak *et al.* (2010)	Fucoidan from *Fucus vesiculosus* decreases the production of several inflammatory cytokines and inhibits the proliferation, migration and adhesion of rat aortic smooth muscle cells.
Cumashi *et al.* (2007)	Fucoidan from *Fucus vesiculosus* doesn't significantly inhibit the adhesion of polymorphonuclear leukocytes.
Ritter *et al.* (1998)	Potential anticoagulant activity of fucoidan.
Cumashi *et al.* (2007)	Fucoidan from *Fucus vesiculosus* significantly inhibits tumour-platelet interactions.
Byun *et al.* (2008)	Fucoidan from *Fucus vesiculosus* protects granulocytes from cell death that is due to irradiation and deprevation of cytokines. It also significantly increases the number of granulocytes and the production of IL-12 and TNF-α.
Rhee and Lee (2011)	Fucoidan from *Fucus vesiculosus* significantly increases the viability of γ-irradiated cells.
Angulo and Lomonte (2003)	Fucoidan from *Fucus vesiculosus* reduces the toxicity of snake venom to skeletal muscle cells by forming insoluble complexes with the phospholipases A_2.
Parys *et al.* (2010)	Fucophlorethols from *Fucus vesiculosus* inhibit CYP1A activity.
Aisa *et al.* (2005)	Fucoidan from *Fucus vesiculosus* decreases the proliferation of

	lymphoma cells by increasing the expression of caspase-3 and decreasing the expression of Rh123, GSK and ERK.
Hyun *et al.* (2009)	Fucoidan from *Fucus vesiculosus* inhibits the tumor cell proliferation significantly by downregulating Bcl-2 and increasing the concentrations of Bax and active ERK, p38 kinase, caspase-3 and caspase-9.
Ale *et al.* (2011)	Fucoidan from *Fucus vesiculosus* significantly reduces the viability of melanoma B16 cells by significantly increasing apoptosis and caspase-3 activity.
Choi *et al.* (2005)	Addition of fucoidan from *Fucus vesiculosus* to macrophages and lymphocytes significantly increases their ability to kill melanoma and lymphoma cells
Liu and Gu (2012)	Phlorotannins from *Fucus vesiculosus* significantly inhibit protein glycation significantly ($p<0.05$) in a mixture of albumin and methylglyoxal or glucose by significantly decreasing the methylglyoxal content.
Cumashi *et al.* (2007)	Fucoidan from *Fucus vesiculosus* does not inhibit angiogenesis.
Parys *et al.* (2010)	Fucophlorethols from *Fucus vesiculosus* inhibit Cyclo-oxygenase-1.

bw= body weight

Fv= Fucus vesiculosus

Building of thyroid hormones
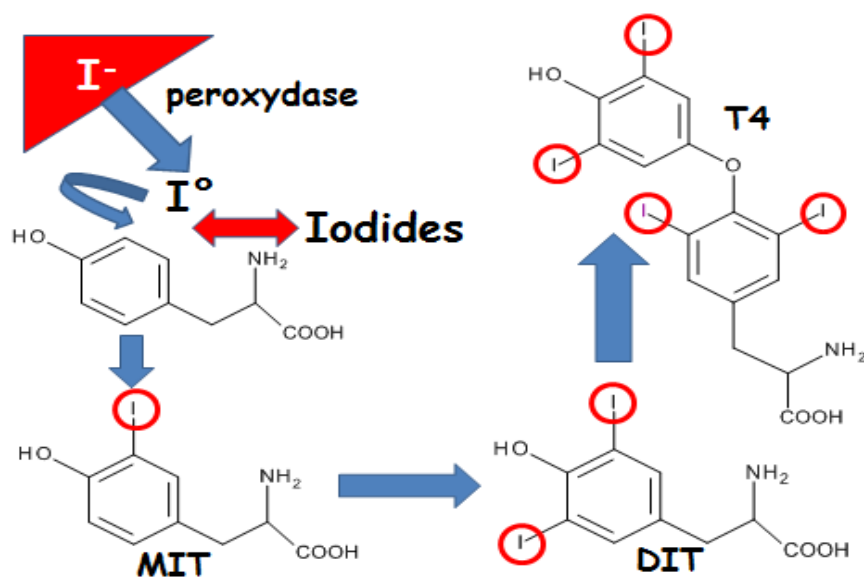
Figure 2: Iodine is transported from the blood compartment to the thyroid gland, to be reduced by peroxidase and subsequently incorporated into the amino acid thyrosine. This process is inhibited by iodides. Mono-iodothyroxine (MIT) and di-iodothyroxine (DIT) are are formed. Two units of DIT are fused to tetra-iodothyroxine (T4) or thyroxine (Katzung 2004, DiPiro *et al*. 2005).
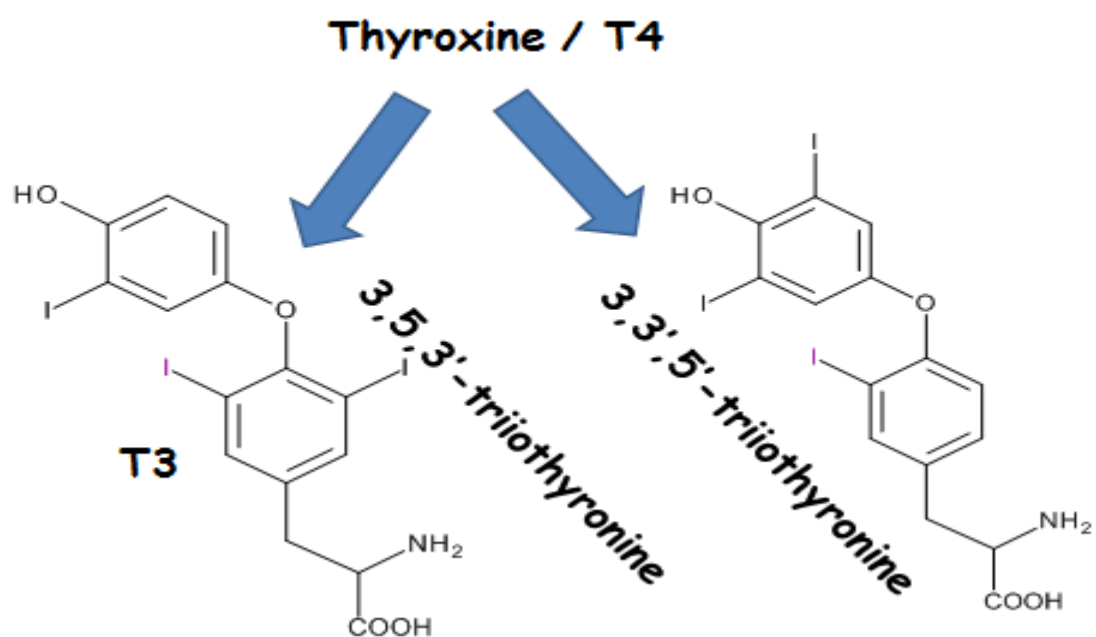
Figure 3: Thyroxine (T4) can be activated peripherally to 3,5,3'-triiodothyronine or T3, which is 3 to 4 times more potent than T4. It can also be deactivated to 3,3',5'-triiodothyronine (reverse T3) (Katzung 2004).

In vitro experiments related to the therapeutic indication of the monograph

- Effect on skin models

Reference	Experimental model	Methods	Outcome
Thring *et al.* (2009)	Screening of 23 plant extracts (final concentration 10 mg/ml). *Fucus vesiculosus* (Bladderwrack) obtained from Neal's Yard remedies Ltd., extracted with boiling water, tested on: (1) Anti-collagenase activity • Positive control: EGCG, 250 µM (0.114 mg/mL) • Negative control: water. (2) Anti-elastase activity • Positive control: EGCG, 250 µM (0.114 mg/mL) • Negative control: water. (3) Total phenolic content (4) Anti-oxidant capacity (5) Superoxide Dismutase (SOD) activity • Control: 3 mL aqueous NTB solution with 30 µl distilled water. All other reagents from Sigma-Aldrich Ltd., UK.	(1) Measurement of absorbance at 335 nm for 20 minutes after adding: • N-[3-(2-furyl)acrylouyl]-Leu-Gly-Pro-Ala) • collagenase (from Clostridium histolyticum). (2) Absorbance measured between 381 and 402 nm for 20 minutes. Reagents: • procine pancreatic elastase • N-succinyl-Ala-Ala-Ala-p-nitroanilide (3) The phenolic content was measured using the Folin-Coiocalteu method with a gallic acid standard curve. (4) To verify the anti-oxidant capacity the 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS$^+$) diammonium salt free radical assay was used with Trolox standards at 730 nm. (5) SOD activity was measured indirectly with a modified nitroblue tetrazolium (NTB) assay at 550 nm.	Calculation for assays 1 and 2: enyme inhibiton activity (%) = $[(OD_{control} - OD_{sample})/OD_{control}]$ x 100 (1) 50.2% (2) 24.52% (3) Ca. 0.1 mg/ml gallic acid equivalents (4) 4.59 µM Trolox (5) <5% (= no activity) (no p-values provided)
Fujimura *et al.* (2000)	Fucoidan from *Fucus vesiculosus*: Sigma Chemical Co. Extracts from *Fucus vesiculosus*: Ichimaru Pharcos Co. Fibroblast-populated collagen gel was treated with *Fucus vesiculosus* fractions to determine which component increases α2β1 expression on the surface of fibroblasts, there would be a relation between this increased expression and gel contraction.	Gel contraction assay During 5 days the gel was treated with: (1) separated fractions of *Fucus vesiculosus* extracts (2) fucoidan from *Fucus vesiculosus* After addition of the extracts and fucoidan the volume of the collagen gel was measured to determine whether the collagen contracted. Integrin expression assay To fibroblasts the following was added: (1) separated fractions of *Fucus vesiculosus* extracts (2) fucoidan from *Fucus vesiculosus* After that to each of the above the following was added: (1) mouse IgG (=control) (2) mouse anti-human integrin	Gel contraction assay Low polar fractions didn't stimulate gel contraction significantly. High polar fractions stimulated gel contraction significantly (p<0.01). The high polar fraction from *Fucus vesiculosus* consisted amongst others of fucoidan. Fucoidan itself also significantly (p<0.01) promoted gel contraction. Fucose, sodium alginates and fucosterol (other constituents of *Fucus vesiculosus*) at concentrations between 1 to 100 µM had no effect. Integrin expression assay The high molecular weight (MW> 10000) extracts and fucoidan from *Fucus vesiculosus* significantly (p-value not mentioned) increase integrin $α_2β_1$ expression on fibroblasts.

The results of the study of Thring *et al.* (2009) show an anti-collagenase activity by *Fucus vesiculosus*. Its anti-elastase activity is mild and it has slightly anti-oxidative properties. These effects could be useful, but it is not known whether *Fucus vesiculosus* extracts are able to penetrate in sufficiently high concentrations through the skin. As a consequence, the clinical relevance of this study is limited.

In the study by Fujimara *et al.* (2000), the Student t-test was performed to test whether the results are significant ($p < 0.05$) and every experiment was conducted at least twice. The results of the experiment show that the high polar fraction and fucoidan from *Fucus vesiculosus* significantly ($p < 0.05$) stimulate gel contraction and increase the integrin $\alpha_2\beta_1$ expression on fibroblasts compared to control. The results suggest that the increase in gel contraction arises from an increased integrin $\alpha_2\beta_1$ expression and that the high polar fraction of *Fucus vesiculosus* contains fucoidan. It needs to be noted that the exact chemical structure of the active fucoidan remains unknown. A drawback of this study (because it has been performed *in vitro*) is that an *in vitro* model of dermal tissue is used, the consequence is that it is unknown to what extent the extracts penetrate the epidermis, therefore it is unclear to what extent the extracts reach the dermis in clinical conditions. This limits the clinical relevance of the study.

- α-amylase and α-glucosidase activity

Reference	Experimental model	Methods	Outcome
Roy *et al.* (2011)	Phlorotannin extract from *Fucus vesiculosus*: InnoVactiv Inc. (tradename InSea[2])	2.1 ml 0.83 µg/ml α-amylase solution was mixed with (1) 1 ml of 47.6 µg/ml soluble starch and 100 µl water (control) (2) 1 ml of 47.6 µg/ml soluble starch and 100 µl phlorotannin solution 0.2 U α-glucosidase was mixed with (1) 0.8 mM p-nitrophenol-α-D-glucopyranoside and 100 µl water (control) (2) 0.8 mM p-nitrophenol-α-D-glucopyranoside and 100 µl phlorotannin solution	The IC_{50} values for α-amylase and α-glucosidase were 2.8 µg/ml and 5 µg/ml phlorotannins, respectively. The inhibition constants of the phlorotannin solution for inhibiting α-amylase and α-glucosidase were 6×10^{-8} and 7×10^{-8}, respectively.

The study by Roy *et al.* (2011) shows that phlorotannins from *Fucus vesiculosus* have low IC_{50} values and high maximum inhibition rates of α-amylase and α-glucosidase *in vitro*. This demonstrates that phlorotannins from *Fucus vesiculosus* are potent inhibitors of both α-amylase and α-glucosidase *in vitro*. Because α-amylase and α-glucosidase are of great importance in the breakdown of carbohydrates, these results suggest that phlorotannins may be useful in modulating plasma glucose and insulin levels after a meal if phlorotannins also show these effects *in vivo*. This could be plausible mechanism of action to justify clinical studies with (pre)diabetic patients.

- Effect on advanced glycation end product accumulation

Reference	Extract	Methods	Outcome
Liu and Gu (2012)	Dry *Fucus vesiculosus*: Maine Seaweed Co. Phlorotannin was extracted and fractionated. The acetone fraction was used in the experiment. Features of phlorotannin *Fucus vesiculosus* acetone extract (in mg/g equivalents) - total phenolic content: 286.6 ± 20 mg/g gallic acid - total phlorotannin content: 42.29 ± 0.59 mg/g phloroglucinol - total antioxidant capacity: 3214.5 ± 161.9 mg/g Trolox	To test the formation of advanced glycation end products (AGEs), bovine serum albumin in phosphate buffer was mixed with (1) glucose (2) methylglyoxal Addition to these mixtures of (1) phosphate buffer (=control) (2) *Fucus vesiculosus* acetone extract (3) Aminoguanidine (=positive control) (4) Phloroglucinol (=positive control)	EC_{50} of phlorotannin *Fucus vesiculosus* acetone extract in the inhibition of AGEs formation (1) glucose solution: 0.338 ± 0.0146 mg/ml (2) methylglyoxal solution: 0.393 ± 0.0127 mg/ml Compared to control the *Fucus vesiculosus* acetone extract significantly ($p < 0.05$) decreased protein glycation in the presence of glucose as well as of methylglyoxal. EC_{50} = half inhibition concentration
		Decrease of methylglyoxal content compared to control	Decrease of methylglyoxal content compared to control

		To test how many methylglyoxal the *Fucus vesiculosus* acetone extract can scavenge, methylglyoxal was dissolved in phosphate buffer. This solution was incubated for 120 minutes with (1) phosphate buffer (=control) (2) phlorotannin *Fucus vesiculosus* acetone extract (3) Aminoguanidine (=positive control) (4) Phloroglucinol (=positive control)	(2) Circa 20% (3) 82.6% (4) 77.2% Compared to control there was a significant (p<0.05) decrease in the methylglyoxal content after incubation with the phlorotannin *Fucus vesiculosus* acetone extract.

In the study by Liu and Gu (2012), the statistical significance (p<0.05) of the results is determined using one way ANOVA and the Tukey-Kramer HSD test. This *in vitro* study shows that addition of phlorotannins from *Fucus vesiculosus* to a mixture consisting of albumin and glucose or methylglyoxal inhibits protein glycation significantly (p<0.05). Glucose and methylglyoxal are a substrate and an active intermediate in protein glycation, respectively. Furthermore the study shows that phlorotannins from *Fucus vesiculosus* significantly (p<0.05) decrease the methylglyoxal content *in vitro*. These results suggest that the mechanism by which phlorotannins from *Fucus vesiculosus* inhibit the formation of advanced glycation end products (AGEs) is by scavenging reactive carbonyls that otherwise would glycate proteins. It needs to be noted that in both experiments the activity of the phlorotannins is low compared to that of phloroglucinol or aminoguanidine. Although qualitatively in concordance with the treatment of overweight, the clinical relevance of the effect is unclear.

In vitro experiments not directly related to the therapeutic indication of the monograph

- Effect on blood cholesterol

Reference	Experimental model	Methods	Outcome
Aksenov *et al.* (2007)	On human blood plasma from fasting donors (number not reported) a 70% ethanol extract of *Fucus vesiculosus* (from Krasnogorsklek-sredstva) was tested for the effects on trans-sialidase activity.	Volunteers were given perorally a dose of plant preparations. Afterwards blood was taken and trans-sialidase activity was measured (*in vitro*) with the method of Lowry.	The extracts of *Fucus vesiculosus* with trans-sialidase activity • 0 µg/ml: 100% • 1 µg/ml: 90% (p>0.005) • 10 µg/ml: 81% (p<0.005) • 100 µg/ml: 75% (p<0.005) • 1000 µg/ml: 65% (p<0.005) There was a correlation found between the decrease in enzyme activity (induced by garlic powder, Allicor, INAT-Farma) and intracellular cholesterol accumulation. (p<0.05, compared to basal level in patients with high basal enzyme activity)

The study of Aksenov *et al.* (2007) shows that a reduction in trans-sialidase activity may play a role in the intracellular cholesterol accumulation. The therapeutic outcome is based upon a biochemical reasoning and analogy with garlic. The correlation between the marked decrease of trans-sialidase activity and cholesterol levels in blood has to be confirmed for *Fucus vesiculosus* extracts. The extracts did reduce the enzyme activity significantly. As the results are obtained by a mixed approach ex vivo and *in vitro*, it is difficult to translate them to therapeutic practice.

- Antioxidative capacity

Reference	Experimental model	Methods	Outcome
Rupérez *et al.* (2002)	*Fucus vesiculosus* freeze-dried and milled (Algamar C. B., Redondela, Pontevedra, Spain)	Extractions based on the different solubilities of the polysaccharides giving four different soluble fractions. water at 22°C water at 60°C with 0.1 M HCl 37°C 2 M KOH at 37°C	The third fraction with fucoidan as main component showed the highest antioxidant power (no p-values provided). See Table 3 for detailed results.

		The antioxidant power was estimated with the ferric reducing antioxidant power (FRAP) assay. (no control group) See Table 2 for composition of the different fractions.	
Diaz-Rubio *et al.* (2009)	Comparison of raw Fucus vesiculosus with commercial nutraceuticals developed from Fucus vesiculosus. Tested products: Raw *Fucus vesiculosus* collected at the coast of Ribadeo (Galicia, Spain), immediately freeze-dried and milled. Fucoidan (99%) from Fucus vesiculosus (Sigma-Aldrich Química S.A., Madrid, Spain). Brown seaweed powder extract (containing 85% fucoidan) from Fucus vesiculosus (Shanghai Herbsea Nutraceuticals Inc, Shanghai, China). Commercial pills: Arkocapsulas (Arkochim S.A., Madrid, Spain) containing dried *Fucus vesiculosus*. A methanolic liquid commercial extract (Laboratorios Haussman, San Adrés de la Barca, Barcelona, Spain)	Tests performed: Dietary fiber: Samples were treated with pepsin, α-amylase and amyloglucosidase solution. Residue after centrifugation was the insoluble dietary (sum of neutral sugars, uronic acids and klason lignin). The supernatant was the soluble dietary fiber (neutral sugars and uronic acids). Antioxidant capacity: Aqueous-organic extracts were used to test on antioxidant capacity and polyphenol content. Following assays were carried out: a ferric reducing/antioxidant capacity assay (FRAP), an ABTS assay and an oxygen radical absorbance capacity (ORAC) assay. Polyphenol content: The Folin-Ciocalteau procedure to assess the antioxidant capacity.	Composition: as major component fucose (except in the commercial pills Arkocapsulas), then glucose in raw *Fucus vesiculosus*. In fucoidan (99% and 85%) this was rhamnose. Total dietary fiber (insoluble + soluble) in raw *Fucus vesiculosus* 59.1 ±1.00, in fucoidan (99%) 57.20 ±1.30, fucoidan (85%) 46.73 ±1.80, commercial pills Arkocapsulas 44.74 ±1.80. The highest values for antioxidant capacity were found in raw *Fucus vesiculosus* powder, except in the FRAP assay were the commercial pills Arkocapsulas had a higher antioxidant capacity. The extract of raw *Fucus vesiculosus* however had the highest antioxidant capacity (no p-values provided). See Table 4. A correlation was found between the antioxidant capacity (ABTS and ORAC assay) and the total polyphenol content. (R^2=0.992 and 0.991, respectively and $p<0.01$).
O'Sullivan *et al.* (2011)	*Fucus vesiculosus* harvested in spring at the shore of Galway. The methanol extracts were prepared using the method of Connan, Goulard, Stiger and Deslandes. Ferric reducing anti-oxidant assay Fe^{3+} Radical scavenging assay 3.9 ml 2,2-diphenyl-2-picylhydrazyl hydrate (DPPH) in methanol solution. β-carotene bleaching assay (this is caused by peroxyl free radicals) Oxygenated water, β-	Ferric reducing anti-oxidant assay To the test solution following solutions were added: Ascorbic acid (=standard) *Fucus vesiculosus* extract Radical scavenging assay Addition of DPPH solution to methanol (=control) *Fucus vesiculosus* extracts in methanol with concentrations ranging from 0.2 to 5 mg/ml Trolox in methanol with concentrations ranging from 0.01 to 0.27 mg/ml (=standard) ascorbic acid in methanol with concentrations ranging from 0.04 to 0.9 mg/ml (=standard) β-carotene bleaching assay 200 μl of this mixture was added to: 50 μl of 10 mg/ml fucoidan	Ferric reducing anti-oxidant assay *Fucus vesiculosus* had a ferric reducing antioxidant activity of 109.8 ± 17.7 μM ascorbic acid equivalents per gram dry weight of sample. Radical scavenging assay Percentage of DPPH that was scavenged compared to control 31.2 ± 3.2% ($p<0.05$) 37 to 94% for concentrations ranging from 0.11 to 0.27 mg/ml, respectively > 90% at concentrations ranging from 0.04 to 0.9 mg/ml β-carotene bleaching assay Compared to control the percentage of β-carotene bleaching was 28.8 ± 3.8% ($p<0.05$)

		carotene, Tween-40 and linoleic acid.	50 µl of Trolox with concentrations ranging from 0.05 to 0.53 mg/ml (=standard) methanol (=control)	33 to 0% for concentrations ranging from 0.05 to 0.53 mg/ml
		Catalase and superoxide dismutase activity, cell viability, reduced glutathione activity and DNA damage assay 1 x 105 human colon adenocarcinoma Caco-2 cells/ml	Catalase, superoxide dismutase activity and cell viability assay These carcinomacells were incubated for 24 h with (1) 100 µg/ml *Fucus vesiculosus*. After incubation 200 µM H_2O_2 was added to induce oxidative stress. (2) 100 µg/ml *Fucus vesiculosus* (=control)	Catalase and superoxide dismutase activity assay Catalase and superoxide dismutase are both antioxidative enzymes. Catalase activity compared to control: (1) 131 ± 3.0% (not significant) Superoxide dismutase activity compared to control: (1) 89.0 ± 2.7% (not significant)
				Cell viability assay The human colon adenocarcinoma Caco-2 cells remained viable for concentrations of up to 2 mg/ml *Fucus vesiculosus*.
			Reduced gluthatione activity assay To these carcinomacells a mixture of reagents (method of Hissin and Hilf) and 100 µl *Fucus vesiculosus* were added.	Reduced gluthatione activity assay *Fucus vesiculosus* caused an increase of reduced gluthatione activity in human colon adenocarcinoma Caco-2 cells of 27.3 ±1.0%. This is significant ($p<0.05$).
			DNA damage assay The Caco-2 cells were incubated for 24 h with (1) 100 µg/ml *Fucus vesiculosus* in methanol (2) Methanol (=control) 50 µM of H_2O_2 was added. After this an electrophoresis was performed.	DNA damage assay Compared to control 100 µg/ml *Fucus vesiculosus* decreased DNA damage caused by H_2O_2 by 10.5%, this is significant ($p<0.05$).
Parys *et al.* (2010)	*Fucus vesiculosus* harvested in February 2003 at Armorique, St. Efflam, France was extracted with ethanol. Three fucophlorethols were isolated from the ethanol extract. Radical scavenging assay 1,1-diphenyl-2-picrylhydrazyl (DPPH=stable radical) was used to detect if the fucophlorethols scavenge radicals.		Radical scavenging assay Following solutions were added to DPPH in concentrations ranging from 1 to 50 µg/ml: (1) Fucophlorethol consisting of 5 units of phloroglucinol (2) Fucophlorethol consisting of 6 units of phloroglucinol (3) Fucophlorethol consisting of 7 units of phloroglucinol (4) Phloroglucinol (=positive control)	Radical scavenging assay IC_{50} values of fucophlorethols and phloroglucinol: (1) 14.4 ± 2.0 µg/ml (2) 13.8 ± 1.3 µg/ml (3) 10.0 ± 0.6 µg/ml (4) 13.2 ± 0.8 µg/ml IC_{50} = half maximum inhibitory concentration

Table 2: Chemical composition of polysaccharide fractions from *Fucus vesiculosus* (g/100 g dry weight) (Rupérez *et al.* 2002)

fraction	yield (%)	total carbohydrate		sulfate	protein	polyphenols (GAE)
		neutral sugar	uronic acids[b]			
F1	10.1	33.3 ± 2.6	39.1 ± 1.2	6.4 ± 0.7	1.4 ± 0.2	1.5 ± 0.0
F2	3.2	25.3 ± 1.8	36.8 ± 1.5	5.1 ± 0.9	4.2 ± 0.0	2.7 ± 0.8
F3	3.4	48.4 ± 1.2	8.8 ± 0.5	11.5 ± 0.1	trace	0.1 ± 0.0
F4	25.7	18.9 ± 0.3	52.8 ± 1.8	2.4 ± 0.0	6.1 ± 0.4	1.1 ± 0.0
F5	14.8	7.1 ± 0.0	5.1 ± 0.2	1.7 ± 0.8	2.6 ± 0.1	nd

[a] Data are mean value of triplicate determinations ± standard deviation; F1, soluble in water at 22 °C; F2, soluble in water at 60 °C; F3, soluble in 0.1 M HCl at 37 °C; F4, soluble in 2 M KOH at 37 °C; F5, insoluble residue. Neutral sugar and uronic acids in sulfuric acid hydrolysates by gas chromatographic and colorimetric methods, respectively. GAE, gallic acid equivalents; trace is less than 1 g/100 g; nd, not determined. [b] Values calculated with galacturonic acid as standard and corrected with alginate values.

Table 3: Ferric reducing ability/antioxidant power values of soluble polysaccharide fractions from *Fucus vesiculosus* (Rupérez *et al.* 2002)

fraction	4 min		30 min	
	μmol Fe(II)/g	μmol Trolox/g	μmol Fe(II)/g	μmol Trolox/g
F1	78.2 ± 1.3	39.1 ± 0.6	113.9 ± 20.8	54.5 ± 9.2
F2	92.3 ± 10.4	54.9 ± 4.6	191.0 ± 17.7	97.5 ± 7.8
F3	209.8 ± 4.5	99.7 ± 2.0	263.9 ± 4.8	123.0 ± 2.1
F4	101.1 ± 1.6	51.1 ± 0.7	167.6 ± 6.7	80.0 ± 3.0

Table 4: Antioxidant capacity of *Fucus vesiculosus* and derived products (Diaz-Rubio *et al.* 2009)

	Fucus raw powder	Fucoidan 1	Fucoidan 2	*Fucus* dietary supplement	*Fucus* commercial antioxidant extract	*Fucus* raw powder extract[b]
FRAP assay (μmol Trolox/g dw)	71.6 ± 2.2	27.2 ± 2.7	9.5 ± 0.6	32.8 ± 1.3	527.2 ± 39.9	217.0 ± 6.7
ABTS assay (μmol Trolox/g dw)	119.7 ± 2.1	17.0 ± 0.6	2.2 ± 0.1	28.9 ± 0.9	81.2 ± 0.9	362.6 ± 6.5
ORAC assay (μmol Trolox/g dw)	565.9 ± 31.8	57.4 ± 8.4	41.0 ± 5.8	50.1 ± 8.2	325.9 ± 43.7	1,714.8 ± 96.5
ABTS assay						
EC_{50} (g/g ABTS)	0.59 ± 0.05	18.42 ± 1.06	14,206.13 ± 369.22	104.87 ± 13.06	1.05 ± 0.005	0.19 ± 0.02
t_{EC50} (min)	28.7 ± 2.8	24.1 ± 0.7	96.2 ± 8.0	33.0 ± 2.4	ND	28.7 ± 2.8
AE ($\times 10^{-3}$)	58.7	2.3	0.001	0.29	ND	183
Total phenolics (mg PE/100 g dw)	5,370 ± 110	960 ± 10	260 ± 10	1,600 ± 20	2450 ± 160	16272 ± 333

[a]Experimental data. Each value is the mean ± standard deviation of three replicate experiments. AE, antiradical efficiency; PE, phloroglucinol equivalents; ND, not determined (it was not possible to establish kinetic parameters in these samples). [b]Acidic methanol/water (50:50 v/v, pH 2) followed by acetone/water (70:30 v/v).

The article of Rupérez *et al.* (2002) shows that the antioxidant capacity from *Fucus vesiculosus* might be attributed to fucans and not only to the polyphenols. The article of Diaz-Rubio *et al.* (2009) suggests that polyphenols play the key role in the antioxidant capacity. Anyhow, these two articles suggest that *Fucus vesiculosus* might be used as a source of natural antioxidants.

In the study by O'Sullivan *et al.* (2011), repeated measures ANOVA combined with Dunnett's or Tukey's test is used to test if the results are statistically significant ($p < 0.05$ or $p < 0.01$). The ferric reducing antioxidant activity, radical scavenging activity and the prevention of β-carotene bleaching are all measures of protection against free radicals. This study shows that the extracts of *Fucus vesiculosus* have all three above-mentioned activities. Both catalase and superoxide dismutase are important enzymes in neutralising free radicals. In this experiment the activity of neither catalase nor superoxide dismutase increased significantly. Reduced glutathione (=GSH and consists of glutamine, cysteine and glycine) has a key role in non-enzymatic antioxidant activity. This study shows that the levels of GSH in cells treated with extracts from *Fucus vesiculosus* increases significantly ($p < 0.05$). H_2O_2 causes DNA damage in cells. This study shows that in cells treated with extract from *Fucus vesiculosus* the H_2O_2-induced DNA damage decreases significantly ($p < 0.05$). The overall conclusion of all the experiments conducted by O'Sullivan *et al.* is that extracts from *Fucus vesiculosus* show significant ($p < 0.05$) anti-oxidant activity *in vitro*. However it needs to be noted that the antioxidant activity of *Fucus vesiculosus* extracts is much lower than the one of ascorbic acid.

The study performed by Parys *et al.* (2010) does not mention if a statistical analysis has been carried out to determine whether the results are statistically significant. The study compares the radical scavenging activity of phloroglucinol, which is known to scavenge radicals, to three fucophlorethols isolated from *Fucus vesiculosus*. All analysed fucophlorethols showed radical scavenging activity comparable to that of phloroglucinol.

These experiments are typical for screening purposes. *Fucus vesiculosus* seems to have antioxidative properties *in vitro*, but the activity is lower than the one of ascorbic acid. The therapeutic consequences are not clear and should be clinically investigated.

- 17β-estradiol and progesterone levels and aromatase activity

Reference	Experimental model	Methods	Outcome
Skibola *et al.* (2005)	Dried, powdered *Fucus vesiculosus*: Maine Coast Sea Vegetables. This powder was diluted with 50% ethanol to obtain concentrations of 25, 50 and 75 µmol/L. Human granulosa cells (hLGC) from 8 women undergoing assisted reproduction treatment.	The cells were treated for 9 days with (1) 50% ethanol (=control) (2) 25 µmol/L *Fucus vesiculosus* extract (3) 50 µmol/L *Fucus vesiculosus* extract (4) 75 µmol/L *Fucus vesiculosus* extract	17β-estradiol in medium of hLGC (p: compared to before treatment) (1) 4732 ± 591 ng/L (2) 3632 ± 758 ng/L (p=0.09) (3) 3313 ± 373 ng/L (p=0.03) (4) 3060 ± 538 ng/L (p=0.03) Progesterone levels in medium of hLGC (p: compared to before treatment) (1) 6851 ± 1018 µg/L (2) 7721 ± 1415 µg/L (p=0.19) (3) 7461 ± 923 µg/L (p=0.03) (4) 7703 ± 2113 µg/L (p=0.12) The 50 and 75 µmol/L doses significantly reduced 17β-estradiol (p=0.03 for both) compard to control. The progesterone levels only increased significantly (p=0.03) for the 50 µmol/L dose.
	Estrogen and progesterone receptor binding assay Estrogen receptors α and β and the progesterone receptor.	Estrogen and progesterone receptor binding assay Estrogen receptor binding Estrogen receptors α and β, 0.5 nmol/L estradiol and addition of: (1) 0.5 µmol/L *Fucus vesiculosus* extract (2) 5 µmol/L *Fucus vesiculosus* extract (3) 50 µmol/L *Fucus vesiculosus* extract Progesterone receptor binding Progesterone receptor, 1.4 nmol/lradiolabeled progesterone and addition of: (1) 0.5 µmol/L *Fucus vesiculosus* extract (2) 5 µmol/L *Fucus vesiculosus* extract (3) 50 µmol/L *Fucus vesiculosus* extract	Estrogen receptor α (ERα)binding % inhibition (1) 7 ± 2.8% (2) 21 ± 2.1% (3) 52 ± 0.9% IC_{50} 42.2 µmol/L Inhibition constant 12.1 Estrogen receptor β (ERβ)binding % inhibition (1) 2 ± 2.7% (2) 18 ± 2.9% (3) 58 ± 5.3% IC_{50} 31.8 µmol/L Inhibition constant 5.58 Progesterone receptor (PR-B) binding % inhibition (1) -0.2 ± 5.3% (2) 12 ± 2.0% (3) 55 ± 1.4% IC_{50} 31.8 µmol/L Inhibition constant 15.8 *Fucus vesiculosus* competed with estradiol and progesterone for binding to their receptors. *Fucus vesiculosus* had a little higher affinity for ERβ compared to ERα and PR-B.
Parys *et al.* (2010)	*Fucus vesiculosus* harvested in February 2003 at Armorique was extracted with ethanol. Three fucophlorethols were isolated from the ethanol extract. Aromatase assay	Aromatase assay Following solutions were added to aromatase in concentrations ranging from 1 to 50 µg/mL: (1) Fucophlorethol consisting of 5 units of phloroglucinol (2) Fucophlorethol consisting	Aromatase assay IC_{50} values: (1) 3.3 ± 0.1 µg/mL (2) 5.6 ± 0.3 µg/mL (3) 1.2 ± 0.1 µg/mL (4) > 25 µg/mL

	Human recombinant aromatase.	of 6 units of phloroglucinol (3) Fucophlorethol consisting of 7 units of phloroglucinol (4) Phloroglucinol (=positive control)	

To test the significance (p<0.05) of the results obtained in the study by Skibola *et al.* (2005) two-way ANOVA combined with Dunnett's was used and 2-sided paired *t*-tests were performed. The experiments performed by Skibola *et al.* (2005) show that 50 and 75 µmol/L *Fucus vesiculosus* dose-significantly (p=0.03) reduces 17β-estradiol levels in human granulosa cells. The observed inhibition is concentration-dependent. These results suggest that *Fucus vesiculosus* either inhibits 17β-estradiol production or stimulates its breakdown. The inhibition of progesterone production was less prominent, and appearantly there was no concentration-response relationship. On the orher hand, there was a clearcut concentration-dependent occupancy of the oestrogen and progesterone receptors.

The results suggest that certain components of *Fucus vesiculosus* compete with estradiol and progesterone for binding to their receptors. This suggests that there are components in *Fucus vesiculosus* that are antagonists of estradiol and progesterone. In the *in vitro* study by Parys *et al.* (2010), the IC_{50} values of fucophlorethols from *Fucus vesiculosus* for the enzyme aromatase show that these fucophlorethols inhibit aromatase. Aromatase turns androgens into estrogens and estrogens play a key role in estrogen-dependent cancers. These *in vitro* results suggest that fucophlorethols from *Fucus vesiculosus* may be used in the prevention and/or treatment of estrogen-dependent cancers. However this needs further investigation, because this effect may arise from unspecific interaction of fucophlorethols with aromatase and it is unclear whether the effect would also be present *in vivo*.

The overall conclusion of both studies is that the activity of *Fucus vesiculosus* on oestrogen and progestagen receptors and production needs to be further clarified non-clinically as well as clinically. For the time being, the results cannot be translated into the monograph.

- Effects on coagulation

Reference	Experimental model	Methods	Outcome
Trento *et al.* (2001)	Polysaccharide from *Fucus vesiculosus* and *Ascophyllum nodosum*= Crinos fucansulfate Formation of thrombin assay and platelet aggregation assay 480 µl of 3 x 10^5 rabbit platelets/mm^3	Formation of thrombin assay To these platelets addition of (1) 120 µl buffer (=control) (2) 120 µl of 50 IU/mg polysaccharide (3) 25, 50, 100, 150 and 200 µg/ml thrombin 120 µl thromboplastin was added. Platelet aggregation assay To these platelets addition of (1) 10 µl of saline (= control) (2) 3 to 30 mU of 50 IU/mg Crinos fucansulfate (3) 15 to 250 mU of 188 IU/mg Opocrin sodium heparin After 2 min of incubation 5 to 10 µl of thrombin solution was added.	Formation of thrombin assay Compared to control the polysaccharide significantly (p<0.05) inhibited the formation of thrombin. The effect was dose-dependent. Decrease in thrombin production by polysaccharide compared to control: • 0.3 IU/ml: 32% (p<0.05) • 0.6 IU/ml: 53% (p<0.01) • 2.4 IU/ml: 84% (P<0.01) • 6.0 IU/ml: 98% (p<0.01) Platelet aggregation assay The thrombin-induced platelet aggregation was significantly (p<0.01) inhibited by fucansulfate. The percentage of inhibition increased with increasing concentrations of fucansulfate. IC_{50} of thrombin-induced platelet aggregation: (2) fucansulfate: 6 mU/tube (CL=1.2-7.4 mU/tube) (p=0.95) (3) heparin: 37.2 mU/tube (CL=13-61.4 mU/tube) (p=0.95) CL=confidence limit IC_{50}= half maximum inhibitory concentration

de Azevedo et al. (2009)	Fucoidan from *Fucus vesiculosus*: Sigma Aldrich	Activated partial thromboplastin time and prothrombin time 90 µl citrated normal human plasma, this was incubated for 1 min at 37°C with 10 µl of different concentrations of (1) fucoidan in saline water (2) partially desulphated fucoidan in saline water (3) desulphated fractions of fucoidan in saline water (4) three fractions of fucoidan that had been purified with 1, 2 or 3 volumes of acetone (F1, F2 and F3, respectively) in saline water (5) control: isotonic saline (= control) (6) low molecular weight heparins (LMWHs) in saline water (7) unfractionated heparin Activated partial thromboplastin time (manufacturer's instructions were followed) Prothrombin time 200 µl prothrombin time reagent was added to the mixtures. The clotting time was measured.	Activated partial thromboplastin time (aPTT) 5 µg fucoidan, F1 and F2 had significantly ($p<0.001$) higher (240 s) anticoagulant activity than control. 1 µg of LMWHs and unfractionated heparin had significantly ($p<0.001$) higher anticoagulant activity (240 s) than control. 5 µg F3 (the lowest molecular weight) showed the lowest anticoagulant activity (73.6 s) compared to other fractions, this was significant ($p<0.001$). Partially desulphated and desulphated fucoidan have 68 and 30% of the sulphate groups of fucoidan, respectively. At 5 µg their aPTT is reduced 51 and 87%, respectively, compared to fucoidan, this is significant ($p<0.001$). Prothrombin time 50 µg F1 fraction had significantly higher ($p<0.001$) anticoagulant activity (120 s) than fucoidan (81.5 s), F2 (57.1 seconds), F3 (32.5 s) and 50 µg LMWH (48.2 s). 100 µg partially desulphated and desulphated fucoidans had a significant ($p<0.001$) (82%) reduction of anticoagulant activity compared to fucoidan.
Kwak et al. (2010)	Fucoidan from *Fucus vesiculosus*: Sigma Chemical Co. Platelet aggregation assay 450 µlof human platelet-rich plasma (300000 cells/µL). Antithrombin and antifactor X_a assay A tube containing a buffer and 0.1 µg/ml antithrombin. Activated partial thromboplastin time assay Platelet-poor human	Platelet aggregation assay To the plasma 10 µl of solution was added. The solution consisted of: (1) phosphate buffered saline (PBS) (2) 0.2; 0.4; 0.6; 0.8 and 1 µg/ml fucoidan in PBS (3) 0.2; 0.4; 0.6; 0.8 and 1 µg/ml heparin in PBS 20 µM ADP was added to start the aggregation of the platelets. Antithrombin and antifactor X_a assay To this mixture addition of (1) buffer (=control) (2) 1, 5, 10, 50, 100 and 500 µg/ml fucoidan (3) 1, 5, 10, 50, 100 and 500 µg/ml heparin Thrombin or factor X_a were added, at a concentration of 0.1 and 0.005 units/ml, respectively. Depending on what was in the tube, a substrate was added. The mixture was incubated. Activated partial thromboplastin time assay The plasma was incubated with:	Platelet aggregation assay The inhibition of ADP-induced human platelet aggregation by fucoidan was concentration-dependent. The IC_{50} of fucoidan and heparin on platelet aggregation were 0.36 and 1 µg/ml, respectively. IC_{50} = half maximum inhibitory concentration Antithrombin and antifactor X_a assay Fucoidan has much weaker anti-thrombin and antifactor X_a activities than heparin. See Figure 2. Activated partial thromboplastin time assay For fucoidan the aPTT was dose-dependent.

	plasma.	(1) phosphate buffered saline (PBS) (2) fucoidan in PBS (3) heparin in PBS	aPTT of fucoidan-treated plasma: • 0 µg/ml: 15 ± 0.1 s • 0.5 µg/ml: 38.6 ± 1.7 s • 1.5 µg/ml: 55.3 ± 9.4 s • 3.0 µg/ml: 258.2 ± 20.4 s For heparin the aPTTs, at all concentrations, were higher than 600 s.
Dürig et al. (1997)	Fucoidan was obtained from *Fucus vesiculosus* using the method of Bruhn *et al.* Clotting and α2-antiplasmin activity assay 450 µl human plasma Platelet aggregation assay 450 µl citrated human platelet rich plasma in a glass cuvette, that had been treated with silicone.	Clotting assay and α2-antiplasmin activity assay Plasma was incubated with 50 µl of: (1) Isotonic saline (=control) (2) Isotonic saline and fucoidan at 5, 10 and 20 µg/ml (3) Isotonic saline and different fucoidan fractions at 5, 10 and 20 µg/ml Clotting assay 100 µl Neothromtin® and 100 µl 25 mM CaCl$_2$ were added. Platelet aggregation assay To initiate the reaction the human plasma was incubated with 50 µl of: (1) Isotonic saline (=negative control) (2) Adenosine diphosphate (=positive control) (3) Isotonic saline and two different fucoidan fractions	Clotting assay Fucoidan and its fractions increased the activated partial thromboplastin time. The clotting time increased with sulphate content and molecular weight of fucoidan and its fractions. See Figure 3. α2-antiplasmin activity assay The plasminogen activator inhibitor activity increased when the sulphate content dropped. See Figure 4. Platelet aggregation assay Both fucoidan fractions tested induced potently platelet aggregation. Their action was concentration-dependent, started immediately and increased with molecular weight. See Figure 5.
Cumashi et al. (2007)	The fucoidan from *Fucus vesiculosus* used consisted of 26.1% w/w L-fucopyranose, 3.1% w/w mannose, 2.4% w/w xylose, 2.2% w/w glucose, 5% w/w galactose, 10.3% w/w uronic acids and 23.6% w/w sodiumsulphate. Coagulation assay 80 µl of human plasma Thrombin-induced platelet aggregation assay 500 µl of platelets at a concentration of 10^8/ml in a 1 mmol/L HEPES-Tyrode's solution were stirred continuously.	Coagulation assay To this plasma 20 µl of following solutions was added: (1) Masses of up to 5 µg of fucoidan in isotonic saline (2) Heparin in isotonic saline (3) Isotonic saline (=control) 100 µL of phospholipids and activator was incubated with this mixture for 2 min. 100 µL of 0.025 M CaCl$_2$ was added to induce clot formation. Thrombin-induced platelet aggregation assay To this solution (1) isotonic saline (=control) (2) 100 µg/mL fucoidan in isotonic saline (3) heparin in isotonic saline was added. Then 0.5 U/ml thrombin was added. After 5 min 30 µg/ml collagen was added.	Coagulation assay The anticoagulant activity of fucoidan from *Fucus vesiculosus* was equivalent to that of 9.4 ± 1.2 U/mg heparin. Thrombin-induced platelet aggregation Fucoidan from *Fucus vesiculosus* did not inhibit thrombin-induced platelet aggregation significantly.

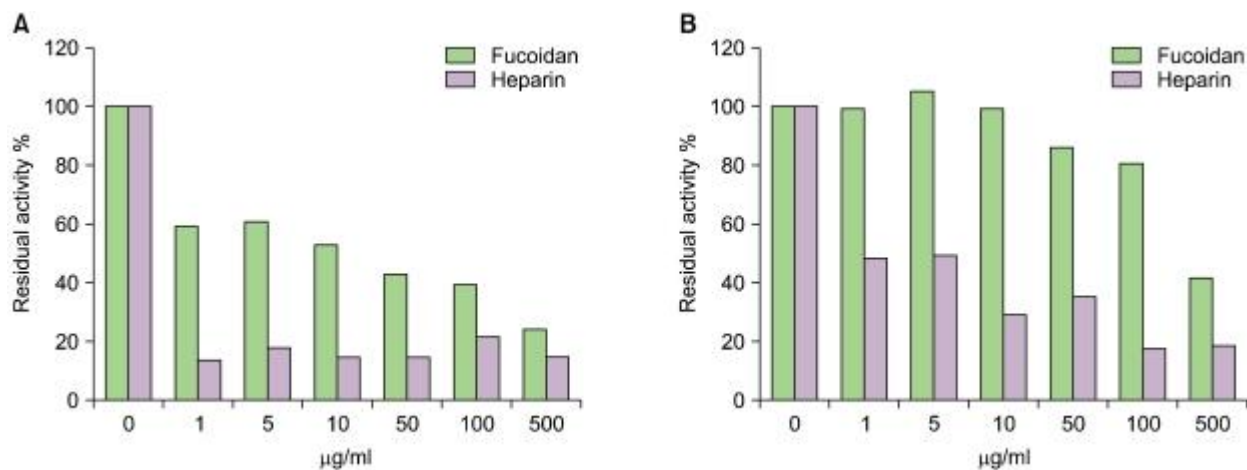**Figure 2: Inhibitory effect of fucoidan from *Fucus vesiculosus* (green) and heparin on thrombin (left) and factor X$_a$ (right) (Kwak *et al.* 2010)**
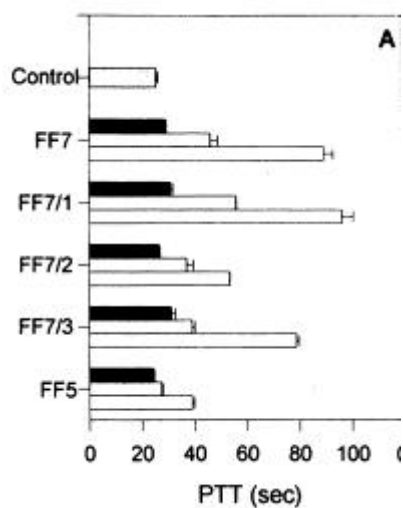

Figure 3: Activated partial thromboplastin time of fucoidan from *Fucus vesiculosus* (Dürig *et al*. 1997)
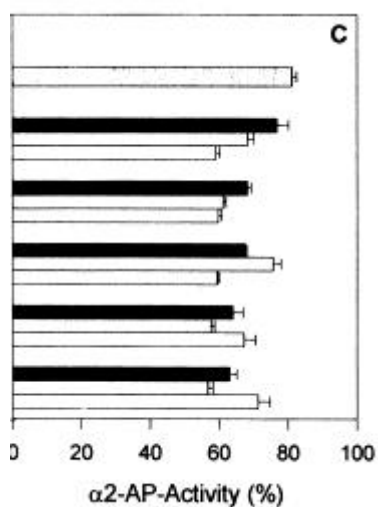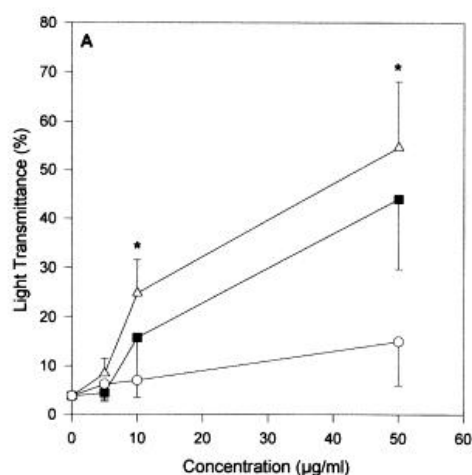

Figure 4: α$_2$-antiplasmin activity of fucoidan from *Fucus vesiculosus* (Dürig *et al*. 1997)

Figure 5: Induction of platelet aggregation by fucoidan fraction (asterisk and square) compared to control (circle) (Dürig et al. 1997)

In the study by Trento et al. (2001), the Dunnett's test was used to determine the statistical significance ($p < 0.05$) of the results. This study shows that fucansulfate in vitro significantly ($p < 0.05$) and dose-dependently inhibits the production of thrombin compared to control. Thrombin induces platelet aggregation. The study by Trento et al. also shows that in vitro fucansulfate significantly ($p < 0.01$) decreases the platelet aggregation induced by thrombin.

In the study performed by de Azevedo et al. (2009), the statistical significance ($p < 0.05$) of the results was determined using ANOVA combined with Tukey-Kramer tests. This study tests the activated partial thromboplastin time and the prothrombin time, which are both measures for coagulation, in the presence of Fucus vesiculosus extracts. The results show that these extracts significantly ($p < 0.001$) prolong both the activated partial thromboplastin and prothrombin time compared to control. However in both experiments this prolongation was much lower than that caused by low molecular weight heparins.

The study carried out by Kwak et al. (2010) does not mention if an analysis to determine the statistical significance of the results has been performed. The results show that fucoidan from Fucus vesiculosus inhibits ADP-induced aggregation of platelets. This inhibition increases with increasing concentrations of fucoidan and is approximately 2 times stronger than that of heparin. The study also shows that fucoidan from Fucus vesiculosus inhibits the activities of both thrombin and factor X_a. These inhibitions increase concentration-dependently, but are much lower than that of heparin. The experiments carried out by Kwak et al. show that fucoidan from Fucus vesiculosus prolongs the activated partial thromboplastin time, but this effect is smaller than that of heparin. The conclusion of this study is that fucoidan from Fucus vesiculosus has less potent antithrombotic activities than heparin in vitro. For instance in inhibiting thrombin and factor X_a activities and prolonging the activated partial thromboplastin time, it scores less than heparin. The only experiment in which fucoidan scored better than heparin is in the inhibition of ADP-induced platelet aggregation.

In the study carried out by Dürig et al. (1997), the Wilcoxon test for paired non parametric data is used to determine whether the results are statistically significant ($p < 0.05$). This study was designed to test the impact of the molecular weight and sulfate content of fucoidan from Fucus vesiculosus on the anticoagulant activity of fucoidan. The conclusion of the platelet aggregation assay is that if the sulfate level remains constant and the molecular weight increases, the high molecular weight fucoidan from Fucus vesiculosus increases platelet aggregation significantly ($p < 0.05$) more than low molecular weight fucoidan. The experiment in which the activated partial thromboplastin time, α_2-antiplasmin activity and plasminogen activator inhibitor activity were measured shows that if the molecular weight of the

fucoidan remains constant and the sulfate level increases, then the anticoagulant effects increase, whereas the α_2-antiplasmin inhibitor activity and plasminogen activator inhibitor activities decrease.

In contrast with the other studies on the anticoagulant activity of fucoidan from *Fucus vesiculosus in vitro* which conclude that fucoidan has anticoagulant activities, the *in vitro* study by Dürig *et al.* (1997) concludes that fucoidan can also stimulate coagulation. However Kwak *et al.* also find that fucoidan with low molecular weight stimulates coagulation very little and even has anticoagulant activities, this is consistent with the studies described above.

In the study performed by Cumashi *et al.* (2007), the statistical significance ($p < 0.05$) of the results is determind using the Student t-test. The experiments show that the anticoagulant activity of 1 mg fucoidan from *Fucus vesiculosus* is equal to that of approximately 9.4 U heparin and that fucoidan from *Fucus vesiculosus* doesn't inhibit the platelet aggregation induced by thrombin significantly.

Most of the experiments were done with human plasma or platelets (only once with rabbit platelets). The activity was compared amongst others with heparin. *Ex vivo* studies are the next step to confirm the antiplatelet activity of *Fucus* preparations or concentrates. There are no direct consequences for the therapeutic indication of the monograph. Indirectly, interference with blood coagulation could be seen as an advantage in patients with overweight and at cardiovascular risk. However, the *in vitro* activity is clearly inferior to that of heparins and there is no guarantee for an *in vivo* effect.

- Inhibition of reverse transcriptase activity of HIV by fucans

Reference	Experimental model	Methods	Outcome
Queiroz *et al.* (2008)	Fucans from *Fucus vesiculosus*: Sigma Chemical Company Avian reverse transcriptase, 2 template primers, activated DNA, poly(rA)-oligo(dT) (rA)n(dT). The template : primer ratio is 5:1.	Fucans were added at concentrations of 0.5 and 1 µg/ml.	0.5 and 1 µg/ml galactofucan inhibited reverse transcriptase powerfully. For synthetic polynucleotides the inhibitions were $87.0 \pm 1.5\%$ and $94 \pm 1.2\%$, respectively. For activated DNA the reductions dropped to 60.5 ± 1.3 and $67.0 \pm 5.2\%$, respectively. At 0.5 µg/ml fucoidan inhibited reverse transcriptase for activated DNA $84.0 \pm 4.3\%$ and for synthetic polynucleotides $98.1 \pm 4.5\%$; respectively. 1 µg/ml fucan B inhibited reverse transcriptase for synthetic polynucleotides and activated DNA $53.9 \pm 2.3\%$ and $31.0 \pm 0.9\%$, respectively. For 0.5 µg/ml these reductions were 39.4 ± 2.4 and $29.5 \pm 3.2\%$, respectively.

The study carried out by by Quieroz *et al.* (2008) does not mention if an analysis to determine the statistical significance of the results has been performed. The results show that galactofucan, fucoidan and fucan B from *Fucus vesiculosus* inhibit reverse transcriptase concentration dependently. Fucoidan has the strongest inhibiting acitivity of reverse transcriptase: 0.5 µg/ml fucoidan inhibits reverse transcriptase for activated DNA by $84.0 \pm 4.3\%$ and for synthetic polynucleotides by $98.1 \pm 4.5\%$. As no positive controls are used, it is difficult to estimate the value of the experiments.

- Effects on the immune system

Reference	Experimental model	Methods	Outcome
Oomizu *et al.* (2006)	Fucoidan from *Fucus vesiculosus* B cells were incubated with IL-4 and anti-CD40 antibodies	These cells were incubated for 7 days with 15 µg/ml fucoidan medium (=control) These solutions were added within 48 h after addition of the antibodies.	Fucoidan from *Fucus vesiculosus* significantly ($p < 0.01$) decreased the production of IgE. See Figure 6.

| Price *et al.* (2002)	Methanolic extracts of *Fucus vesiculosus*, harvested at the North Atlantic coast. Methanolic extracts are fractionated using a Sephadex column. Mast cells from the peritoneal cavity of rats.	4 groups of mast cells, the medium consisted of: Buffer Buffer + control releasing agent Buffer + control releasing agent + methanolic fractions Buffer + methanolic fractions The control releasing agent releases 50 to 60% of the histamine content of the cells.	Fucus vesiculosus inhibits histamine release from mast cells directly, as well as the histamine release induced by the releasing agent by approximately 58%. The inhibition of histamine release goes up with exposure time.
Choi *et al.* (2005)	Fucoidan from *Fucus vesiculosus*: Sigma Chemical Viability assay of immune cells 2 x 10^5 peritoneal macrophages of healthy BALB/c mice per well. 2 x 10^5 splenic lymphocytes of healthy BALB/c mice per well.	Viability assay of immune cells These macrophages and splenic lymphocytes were incubated separately for 48 h with 10, 50 and 100 µg/ml of fucoidan concanavalin A (=mitogen for macrophages and lymphocytes =positive control) lipopolysaccharide (=mitogen for macrophages and lymphocytes =positive control) vehicle (=control)	Viability assay Compared to control the mitogenic activity (=viability) of fucoidan incubated macrophages and lymphocytes significantly increased (p<0.05). Significant (p<0.05) at fucoidan concentrations of macrophages: 10, 50 and 100 µg/ml lymphocytes: 50 and 100 µg/ml For neither the increase was significant compared to the positive controls.
Kim and Joo (2008)	Fucoidan from *Fucus vesiculosus*: Sigma Bone marrow cells came from the tibia and femur of C57BL/6 mice. These bone marrow cells were used to produce dendritic cells. Fucoidan was dissolved in phosphate buffered saline (PBS), concentrations ranging from 0 to 100 µg/ml were obtained.	The different concentrations of fucoidan in PBS were added to the dendritic cells.	Fucoidan from *Fucus vesiculosus* increased the viability of dendritic cells in a dose-dependent way, but the viability dropped at a concentration of 100 µg/ml. Maximum viability was observed at 50 µg/ml fucoidan. Dendritic cells treated with fucoidan also showed a significantly higher expression of MHC class I (p<0.01) and II (p<0.001), CD54 (p<0.001) and CD86 (p< 0.001). The production of IL-12 (p<0.01) and TNF-α (p<0.001) by fucoidan treated dendritic cells was significantly higher, whereas the antigen uptake was significantly lower (p<0.05).
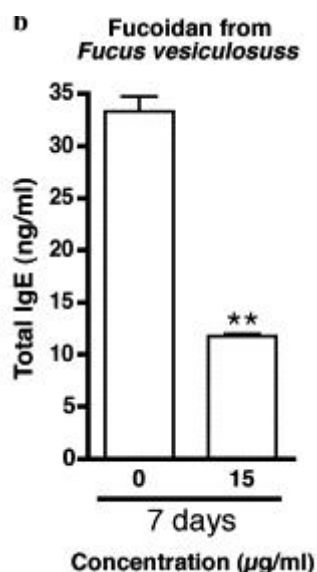
Figure 6: Significant (P<0.01) inhition of B cell IgE production by fucoidan from *Fucus vesiculosus* (Oomizu *et al.* 2006)

The study carried out by Oomizu *et al.* (2006) uses one-way and two-way ANOVA, Bonferoni's post test and the Student t-test to determine whether the results are statistically significant ($p < 0.05$). The result from the study by Oomizu *et al.* shows that fucoidan from *Fucus vesiculosus* significantly ($p < 0.01$) inhibits the release of IgE by B cells *in vitro*. However, only one, rather high concentration was used, without any concentration-effect relationship. The importance of the results is limited.

The study carried out by Price *et al.* (2002) shows that *Fucus vesiculosus* extracts inhibit histamine release from mast cells *in vitro* by approximately 58%. Again, no concentration dependence was investigated, which reduces the importance of the obesrvations.

In the study by Choi *et al.* (2005), the statistical significance ($p < 0.05$) of the results is determined using analysis of variance combined with Dunnett's test and Duncan's multiple range test. When macrophages and lymphocytes are exposed to fucoidan from *Fucus vesiculosus*, the viability of macrophages and lymphocytes increases significantly ($p < 0.05$) *in vitro*. The meaning of thes results remains rather experimental, i.e. the use in pro-inflammatory conditions

In the study by Kim and Joo (2008), the statistical significance ($p < 0.05$) is determined using the Tukey-Kramer multiple comparisons test. This study shows that fucoidan from *Fucus vesiculosus* increases the viability of dendritic cells significantly ($p < 0.01$). The production of IL-12 ($p < 0.01$) and TNF-α ($p < 0.001$) by fucoidan treated dendritic cells also was significantly higher, whereas the antigen uptake was significantly lower ($p < 0.05$). TNF-α and IL-12 play important roles in the protection against pathogens. The significantly ($p < 0.001$) increased expression of MHC class I and II, CD54 and CD86 on leukocytes suggests that fucoidan has immunostimulatory effects and stimulates the maturation of dendritic cells. However the concentrations to obtain the effect were too high to create persepctives *in vivo*.

As an overall conclusion on the effect of fucoidan and other extracts from *Fucus vesiculosus* on the immune system, it can be stated that the consequences are limited to experimental conditions. There is no direct translation to the monograph.

- Fucoidan in atherosclerosis and restenosis prevention

Reference	Experimental model	Methods	Outcome
Kwak *et al.* (2010)	Fucoidan from *Fucus vesiculosus*: Sigma Chemical Co. Cytokine production assay Human umbilical vascular endothelial cells (HUVECs) were plated in 96-well plates. Proliferation, migration and adhesion assay Rat aortic smooth muscle cells (RAoSMCs)	Cytokine production assay Addition to the medium of: (1) fucoidan (2) heparin To the media 10 ng/ml tumor necrosis factor-alpha was added. After 16 h the concentration of 16 inflammatory cytokines and chemokines was determined. Proliferation assay 5×10^4 RAoSMCs per well. The medium was changed to: (1) medium (2) 50, 100, 500 and 1000 µg/ml fucoidan in medium (3) 50, 100, 500 and 1000 µg/ml heparin in medium Migration assay 5×10^5 RAoSMCs/ml. Different concentrations of heparin or fucoidan were added. The RAoSMCs were fixed and stained, following that the migrated cells were counted.	Cytokine production assay The levels of GM-CSF, IL-6, MCP-1 and RANTES of the HUVECs treated with fucoidan dropped. Fucoidan from *Fucus vesiculosus* inhibited the pro-inflammatory cytokines stronger than heparin. Proliferation, migration and adhesion assay Fucoidan inhibited the proliferation, migration and adhesion of RAoSMCs more than heparin. The inhibition of adhesion of RAoSMCs by fucoidan is dose-dependent.

		Adhesion assay Fucoidan-preincubated RAoSMCs were incubated in wells that had been precoated with vitronectin.	

Unfortunately the study performed by Kwak *et al.* (2010) does not mention any p-values. As a consequence it is unclear whether the results are statistically significant. The results from the study by Kwak *et al.* show that fucoidan from *Fucus vesiculosus* decreases the production of several inflammatory cytokines by human umbilical endothelial cells *in vitro*. This suggests that fucoidan from *Fucus vesiculosus* has a qualitative potential in the prevention of atherosclerosis and restenosis, because in both processes inflammation plays a role. This study also shows that fucoidan from *Fucus vesiculosus* inhibits the proliferation, migration and adhesion of rat aortic smooth muscle cells *in vitro* more than heparin. However it should be noted that high concentrations were used which may be difficult to obtain *in vivo*.

- Inhibitory effect of fucoidan on polymorphonuclear leukocyte recruitment

Reference	Experimental model	Methods	Outcome
Cumashi *et al.* (2007)	The fucoidan from *Fucus vesiculosus* used consisted of 26.1 % w/w L-fucopyranose, 3.1 % w/w mannose, 2.4 % w/w xylose, 2.2 % w/w glucose, 5 % w/w galactose, 10.3 % w/w uronic acids and 23.6 % w/w sodiumsulphate.	On slides that were coated with platelets, addition of (1) 5 ml of a polymorphonuclear leucocyte suspension (=positive control). (2) monoclonal antibodies that block P-selectin, which is present on activated platelets. After 10 minutes a polymorphonuclear leucocyte suspension was added (=negative control). (3) 100 µg/ml fucoidan from *Fucus vesiculosus*, after 15 min of incubation a polymorphonuclear leucocyte suspension was added (=test). All solutions were washed after 2 min.	The monoclonal antibodies inhibited the adhesion of polymorphonuclear leucocytes nearly entirely. Fucoidan from *Fucus vesiculosus* decreased this interaction, but this was not significant.
Ritter *et al.* (1998)	Fucoidan (0.36 mg/ml blood) derived from *Fucus vesiculosus*. *(no further information on origin)* Isolated rat hearts from adult male Sprague-Dawley rats (400 to 600 g) with 30 min ischemia following deposition of leukocytes in coronary capillaries and venules.	(1) Nonischemic control hearts (2) Untreated postischemic hearts reperfused at low flow (3) Postischemic hearts reperfused at low flow treated with fucoidin (0.36 mg/ml blood)	Leukocyte accumulation in both capillaries and venules reduced ($p<0.05$) in 3 compared to 2, but was still greater than the control ($p<0.05$). Persistence of leukostasis in both capillaries and venules decreased ($p<0.05$) in 3 compared to 2. This was the same in 1 and 3.

In the study carried out by Cumashi *et al.* (2007), the statistical significance ($p<0.05$) of the results is determined using the Student t-test. The results show that fucoidan from *Fucus vesiculosus* does not significantly inhibit the adhesion of polymorphonuclear leukocytes *in vitro*. This suggests that possible anti-inflammatory properties of fucoidan cannot be explained by an anti-adhesive action on the polymorphonuclear leukocytes.

The significant decrease in persistence and accumulation of leukocytes in capillaries and venules, shown in the article by Ritter *et al.* (1998), indicate that fucoidan affected the adhesion process in a rat heart model after ischemia. However the observed effect was obtained with a high concentration.

- Effects on metastasis

Reference	Experimental model	Methods	Outcome
Cumashi *et al.* (2007)	The fucoidan from *Fucus vesiculosus* used consisted of 26.1 % w/w L-fucopyranose, 3.1% w/w mannose, 2.4% w/w xylose, 2.2% w/w glucose, 5% w/w galactose, 10.3% w/w uronic acids and 23.6% w/w sodiumsulphate. Adhesion of breast cancer cells to platelets assay A surface was coated with platelets.	Adhesion of breast cancer cells to platelets assay MDA-MB-231 breast cancer cells that had been preincubated for 10 min with (1) isotonic saline (2) fucoidan in isotonic saline (final concentration is 100 µg/ml) were added to the surface. After 1 h the adherent cells were fixed using hematoxylin and eosin.	Adhesion of breast cancer cells to platelets assay Fucoidan from *Fucus vesiculosus* inhibited the adhesion of breast cancer cells to the platelets by circa 80%. This was significant (p<0.01).

In the study carried out by Cumashi *et al.* (2007), the statistical significance (p<0.05) of the results is determined using the Student t-test. The study shows that fucoidan from *Fucus vesiculosus* significantly (p<0.01) inhibits tumour-platelet interactions *in vitro*. The concentration needed for the effect was high and maybe not realistic when considered *in vivo*.

- Protection against irradiation-induced apoptosis

Reference	Experimental model	Methods	Outcome
Byun *et al.* (2008)	Fucoidan from *Fucus vesiculosus*: Sigma Bone marrow cells obtained from the tibias and femurs of C57BL/6 mice had a concentration of 1×10^6 cells/ml. The growth medium didn't contain any cytokines. For irradiation a ^{60}Co γ-source was used.	The bone marrow cells were treated with (1) medium (control) (2) 50 µg/ml fucoidan (3) 50 µg/ml fucoidan + 1 Gy radiation (4) 1 Gy radiation	Addition of 2 to 50 µg/ml fucoidan to the medium significantly (p<0.05) decreased the number of bone marrow cells that died in the absence of cytokines. The cell viability of the bone marrow cells after irradiation increased significantly (p<0.01) when fucoidan was added to the medium in concentrations from 10 to 50 µg/ml. Fucoidan selectively (p<0.05) protects granulocytes in bone marrow cells from death, it also significantly (p<0.01) increases the antigen presenting cell function of bone marrow cells and significantly increases the bone marrow cell production of TNF-α (p<0.001) and IL-12 (p<0.001), but for the latter not when the cells were irradiated.
Rhee and Lee (2011)	Fucoidan from *Fucus vesiculosus*, consisting of 27.5% w/w fucose, 26.3% w/w sulphate and 14.7% w/w ash, was supplied by Heawon Biotech Inc. Cell viability assay Human monoblastic leukemia cells (U937)	Cell viability assay To 2×10^5 human monoblastic leukemia cells/ml (1) fucoidan in final concentrations of 1, 10 and 100 µg/ml in phosphate buffered saline (2) phosphate buffered saline(=control) was added. After 48 h of incubation these cells were γ-irradiated at 8 Gray using ^{137}Cs, the absorbed dose rate was circa 1 Gray/min. The cells were allowed to recover during 8 days, after that cell viability was measured.	Cell viability assay The cell viability of the irradiated cells treated with fucoidan increased dose-dependently. The increase was between 53 to 80% higher when compared to control. This is significant (p<0.05).

In the study by Byun *et al.* (2008), the significance ($p<0.05$) of the results is determined using the Turkey-Kramer multiple comparisons test. This study shows that in bone marrow fucoidan from *Fucus vesiculosus* selectively protects granulocytes from cell death due to irradiation ($p<0.01$) and deprevation of cytokines ($p<0.05$) *in vitro*. It also has significant ($p<0.05$) immunostimulatory effects by stimulating the production of IL-12 and TNF-α and increasing the number of granulocytes *in vitro*.

In the study performed by Rhee and Lee (2011), the statistical significance ($p<0.05$) is determined using life table methods with Mantel-Peto-Cox summary of Chi square. The results show that the viability of cells that are treated with fucoidan from *Fucus vesiculosus* and then-irradiated increases significantly ($p<0.05$) compared to not fucoidan-treated cells.

The results of the study by Rhee and Lee (2011) are similar to those of Byun *et al.* (2008). They show that the viability of γ-irradiated cells increases significantly ($p<0.05$) after treatment with high concentrations of fucoidan from *Fucus vesiculosus.*

- Effects of fucoidan on myotoxicity

| Reference	Experimental model	Methods	Outcome
Angulo and Lomonte (2003)	135 kDa fucoidan from *Fucus vesiculosus*: Sigma Cytotoxic assay 9 types of crotaline snake venom from *Bothrops asper (4 types)*, *Cerrophidion godmani* (2 types), *Atopoides nummifer* (2 types) and *Bothriechis schlegelii* (1 type). Murine C2C12 skeletal muscle myoblasts (ATCC CRL-1772). (1) venom control (9 types) (2) venom (9 types) + fucoidan (different molar ratios) (3) control for 0 % toxicity (4) control for 100 % toxicity (5) fucoidan control (different concentrations- see (2))	Cytotoxic assay Growth medium of the Murine C2C12 skeletal muscle cells was replaced by: (1) 30 µg of venom (9 types) (2) 30 µg of venom (9 types) + fucoidan (different molar ratios) that had preincubated (3) medium (4) medium + 0.1% Triton X-100 (5) fucoidan (concentration-see (2)) Lactate dehydrogenase in the medium is indicative of cytotoxicity.	Cytotoxic assay Fucoidan from *Fucus vesiculosus* reduces cytotoxicity of the 9 types of venom. The reductions of cytotoxicity range from 50 to 100%. See figure 7.
	Cytotoxic C-terminal region assay Amino acids 115 to 129 of the C-terminal end of 3 crotaline venoms were used. These amino acids induce the same cytotoxic effects as their parent venoms. Murine C2C12 skeletal muscle myoblasts (ATCC CRL-1772). (1) peptide control (3 groups of peptides) (2) peptide + fucoidan	Cytotoxic C-terminal region assay Added to the muscle myoblasts: (1) 100 µg peptides (3 groups) (2) 100 µg peptides + fucoidan (3 groups; fucoidan:peptide; 1:4); 30 min preincubation	Cytotoxic C-terminal region assay The cytotoxic effect of the peptides preincubated with fucoidan was completely inhibited when compared to the cytotoxic effect of the peptides. See figure 8.
	Fucoidan-myotoxin complex formation assay *Bothrops asper* myotoxin I, II, III and IV. The formation of complexes between fucoidan and the myotoxins was assessed using turbidimetry.	Fucoidan-myotoxin complex formation assay To 200 µg of each myotoxin 1 µl of 20 mg/ml fucoidan was added successively.	Fucoidan-myotoxin complex formation assay Insoluble complexes were formed that consisted of fucoidan and myotoxin. These complexes were at their maximum at the molar of fucoidan:myotoxin; 0.1:1. The complexes dissolved again when there was a fucoidan excess.
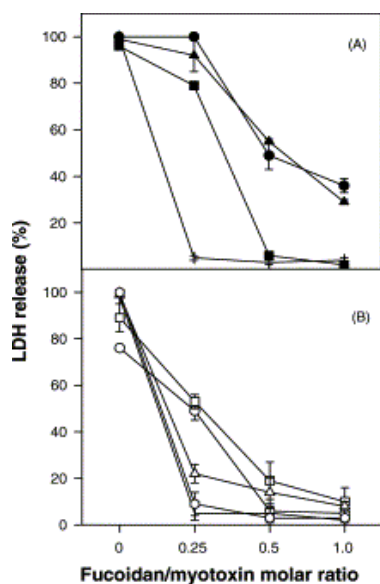
Figure 7: Inhibition of lactate dehydrogenase, which is released in case of myotoxic effect, by fucoidan from *Fucus vesiculosus* (A: *B. Asper* myotoxins, B: *C. Godmani* myotoxins; symbols represent different myotoxins) (Angulo and Lomonte 2003)
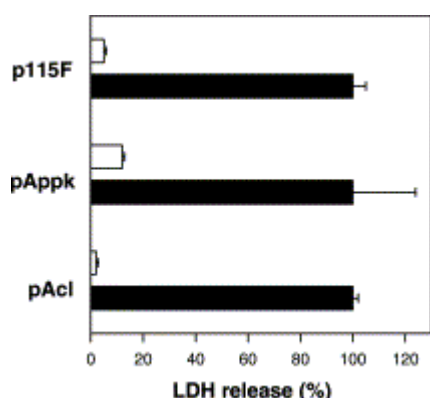
Figure 8: Inhibition of lactate dehydrogenase, which is released in case of myotoxic effect, by fucoidan (black) from *Fucus vesiculosus* compared to control (white) (Angulo and Lomonte 2003

Unfortunately the study performed by Angulo and Lomonte (2003) does not mention if a statistical analysis has been carried out to determine the statistical significance of the results. Therefore it is unknown whether the results are statistically significant. On the other hand, the effect is concentration-dependent. The results of this study show that *in vitro* the toxicity of snake venom to skeletal muscle cells decreases in the presence of fucoidan from *Fucus vesiculosus*. The toxicity of snake venom to skeletal muscle cells is due to the phospholipases A_2 in the venom. The tests also show that fucoidan causes this effect by forming insoluble complexes with the phospholipases A_2.

- Effect of fucoidan on tumour viability

Reference	Experimental model	Methods	Outcome
Aisa *et al*. (2005)	Fucoidan from *Fucus vesiculosus*: Sigma 1 x 10^5 human lymphoma HS-Sultan cells	Growth inhibition assay These cells were incubated for 48 hours with (1) medium (= control) (2) 10 and 100 µg/ml fucoidan	Growth inhibition assay The number of cells treated with 100 µg/ml fucoidan that were in the sub-G1 phase increased significantly (p<0.01) compared to control. This effect increased time-dependently, the percentage of cells in sub-G1 phase compared to control:

	Growth inhibition assay and apoptosis assay 1 x 10^5 human lymphoma HS-Sultan cells		• 0 h: 1.1% • 24 h: 4% • 36 h: 28.7% • 48 h: 89% The number of 100 µg/ml fucoidan treated cells in cell cycle arrest in G1 or G2/M did not change compared to control.
		Apoptosis assay These cells were incubated for 48 h with (1) medium (= control) (2) 100 µg/ml fucoidan (3) pan-caspase inhibitor + 100 µg/ml fucoidan (added after preincubation)	Apoptosis assay Percentage of HS-Sultan cells in apoptosis: (1) 6.3% (2) 79.9% (3) 54.9% Percentage of HS-Sultan cells expressing the active form of caspase-3 after treatment with 100 µg/ml fucoidan • After 24 h: 9.2% • After 48 h: 37.7% Percentage of fucoidan treated cells that expressed less Rh123 compared to before treatment • After 0 h: 4.5% • After 24 h: 45.6% • After 48 h: 97.5% After 24 h the percentage of phosphorylation of • p38 did not differ • GSK decreased • ERK decreased
	CD62L assay 1 x 10^5 IM9 and MOLT cell lines	CD62L assay These cells were incubated for 48 h with: (1) 50 µg/ml anti-L-selectin antibody, after 1 hour 100 µg/ml fucoidan was added (2) 100 µg/ml fucoidan	CD62L assay The percentage of apoptotic cells did not differ significantly between the two groups.
Hyun et al. (2009)	Fucoidan from Fucus vesiculosus: Sigma HCT-15 human colon carcinoma cells. The concentration of these cells was 1.5 x 10^5 cells/ml.	0, 1, 10, 30, 50 and 100 µg/ml fucoidan was added to the medium. DNA fragmentation, which is a characteristic of apoptosis, was spotted with gel electrophoresis.	1, 10, 30, 50 and 100 µg/ml fucoidan inhibited the proliferation of the cells by 1.8, 24.3, 49.8, 54 and 62%, respectively. All the reductions, except for the reduction by 1 µg/ml fucoidan, were significant (p<0.001) when compared to the control. The IC_{50} of fucoidan on cell proliferation inhibition is 34 µg/ml. These experiments also show that Bcl-2 was downregulated, the expression of Bax increased and the activity of ERK, p38 kinase, caspase-3 and caspase-9 increased in the presence of fucoidan.
Ale et al. (2011, Marine Drugs)	Fucus vesiculosus extract consisting of (in mg/g dry weight): 139 ± 5 mg/g fucose, 2 ±0.6 mg/g rhamnose, 2.8 ± 0.2 mg/g arabinose, 28 ± 1 mg/g galactose, 2.5 ± 1.8 mg/g glucose, 13 ± 2 mg/g xylose, 0.2 ±0.4 mg/g mannose, 19 ± 2 mg/g uronic acid and 342 ± 45 mg/g sulphate.	Viability assay These cells were incubated with (1) medium (=control) (2) Fucus vesiculosus extract at concentrations of 0.1, 0.2, 0.4, 0.6, 0.8 and 1 mg/ml.	Viability assay Compared to control the viability of melanoma B16 cells decreased significantly (p≤0.05). The decrease of viability was dose-dependent. See Figure 9. 1 mg/ml Fucus vesiculosus decreased the viability by 94%.

	Viability, apoptosis and Caspase-3 activity assay 6 x 10^4 melanoma B16 cells per well	Apoptosis assay These cells were incubated with (1) medium (=control) (2) 0.2 mg/ml *Fucus vesiculosus* extract The translocation of phospholipid phosphatidylserine, which is a constituent of the cell membrane, from the inner to the outer plasma membrane leaflet was measured. This translocation is indicative of apoptosis.	Apoptosis assay Compared to control the melanoma B16 cells incubated with 0.2 mg/ml *Fucus vesiculosus* extract showed 30 ± 5 % (n=2) higher apoptosis level. This is significant (p≤0.05).
		Caspase-3 activity assay These cells were incubated with (1) medium (=control) (2) *Fucus vesiculosus* extract at concentrations of 0.2, 0.4 and 0.8 mg/ml.	Caspase-3 activity assay In most cases caspase-3 starts apoptosis in mammalian cells. Compared to control the melanoma B16 cells incubated with *Fucus vesiculosus* extract showed a significantly (p≤0.05) higher caspase-3 activity at all concentrations. See Figure 10.
Choi *et al.* (2005)	Fucoidan from *Fucus vesiculosus*: Sigma Chemical Cytotoxicity assay of immune cells 2 x 10^5 peritoneal macrophages of healthy BALB/c mice per well. 2 x 10^5 splenic lymphocytes of healthy BALB/c mice per well.	Cytotoxicity assay of immune cells These macrophages and splenic lymphocytes were incubated separately for 48 h with (1) 10, 50 and 100 µg/ml of fucoidan (2) concanavalin A (=mitogen for macrophages and lymphocytes =positive control) (3) lipopolysaccharide (=mitogen for macrophages and lymphocytes =positive control) (4) vehicle (=control) Splenic lymphocytes were added to the YAC-1 tumour cell line. Macrophages were added to the B16 tumour cell line.	Cytotoxicity assay of immune cells Compared to control the viability of both the YAC-1 tumour cell line, mixed with fucoidan incubated lymphocytes, and the B16 tumour cell line, mixed with fucoidan incubated macrophages, decreased significantly (p<0.05). For both this effect was present at the 10, 50 and 100 µg/ml dose. These data suggest that fucoidan stimulates the immune cell secretion of molecules that play a role in toxicity towards tumours.
	Phagocytosis and secretion assay of immune cells 2 x 10^5 peritoneal macrophages of healthy BALB/c mice per well.	Phagocytosis and secretion assay of immune cells These macrophages were incubated for 48 h with (1) 10, 50 and 100 µg/ml fucoidan (2) vehicle (=control) After that the macrophages were added to YAC-1 tumour and B16 tumour cell line. Lysosomal phosphatase and myeloperoxidase activity were measured. Nitrite and H$_2$O$_2$ production were also measured. Tumour necrosis factor α (TNF-α), interleukine-1β (IL-1β) and interleukine-6 (IL-6) concentrations were measured in the supernatant.	Phagocytosis and secretion assay of immune cells Compared to control lysosomal phosphatase (p<0.001) and myeloperoxidase activity significantly (p<0.05) increased at all concentrations of fucoidan. Both enzymes play a role in phagocytosis. Compared to control macrophage nitrite and H$_2$O$_2$ production increased significantly (p<0.05) at all concentrations of fucoidan. Production of TNF-α and IL-6 were significantly (p<0.05) increased in all fucoidan treated macrophages compared to control, but IL-1β did not.
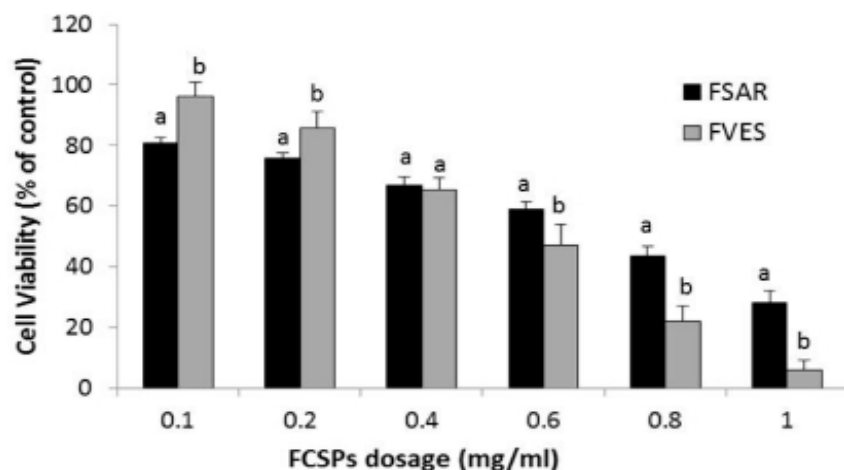
Figure 9: Significant (P≤0.05) inhibition of viability of melanoma B16 cells by fucoidans from *Fucus vesiculosus* (FVES), compared to control (Ale *et al*. 2011, *Marine Drugs*)
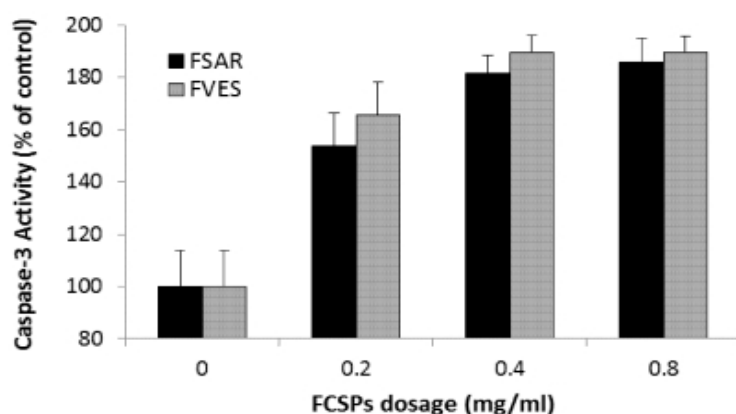
Figure 10: Inhibition of caspase-3 activity by fucoidan from *Fucus vesiculosus* (FVES), compared to control (Ale *et al*. 2011, *Marine Drugs*)

Several studies about the induction of apoptosis in tumour cell lines have been performed. Most of them concern fucoidan from *Fucus vesiculosus* and one extract of *Fucus vesiculosus*.

The study performed by Aisa *et al*. (2005) does not mention if an analysis has been done to determine the statistical significance of the results. In spite of this, the article does mention that there is a significant (p<0.01) difference in growth between the control group and the group treated with 100 µg/ml fucoidan from *Fucus vesiculosus*. This suggests that one needs to be careful drawing conclusions from this article. The *in vitro* study found that human lymphoma cells treated with fucoidan from *Fucus vesiculosus* proliferate less compared to the control group. This study also found that the cells treated with fucoidan from *Fucus vesiculosus* expressed more caspase-3 and less Rh123, GSK and ERK compared to control *in vitro*. This article assumes this changed expression is correlated with the increased apoptosis level of the cells.

In the study carried out by Hyun *et al*. (2009), every experiment was conducted at least three times and the statistical significance (p<0.001) of the results was determined using one-way ANOVA and Dunnett's test. The experiments show that concentrations of 10, 30, 50 and 100 µg/ml fucoidan from *Fucus vesiculosus* inhibit human colon carcinoma cell proliferation significantly (p<0.001). The experiments also suggest that there is a correlation between downregulation of Bcl-2, increasing concentrations of Bax and active ERK, p38 kinase, caspase-3, caspase-9 and the inhibition of the proliferation of the cancer cells all caused by fucoidan from *Fucus vesiculosus*. The conclusion of this

study is that fucoidan from *Fucus vesiculosus* significantly (p<0.001) induces apoptosis in colon carcinoma cells *in vitro*.

In the study by Ale *et al.* (2011, *Marine Drugs*), the statistical significance (p<0.05) of the results is determined using analysis of variance. This study is designed to examine the effects of fucoidan from *Fucus vesiculosus* on melanoma B16 cells. The results show that fucoidan significantly (p≤0.05) reduces the viability of melanoma B16 cells and according to the results this effect is, at least partly, achieved by a significant (p≤0.05) increase of apoptosis and caspase-3 activity. The conclusion of this study is that fucoidan from *Fucus vesiculosus* significantly (p<0.05) inhibits growth and induces apoptosis in melanoma B16 cells *in vitro*.

In the study carried out by Choi *et al.* (2005), the statistical significance (p<0.05) is determined using ANOVA combined with Dunnett's test and Duncan's multiple range test. This study shows that addition of a combination of fucoidan from *Fucus vesiculosus* and macrophages or lymphocytes to YAC1 or B16 tumour cells causes significant (p<0.05) higher tumour cell death compared to control. The study also shows that compared to control the activity of myeloperoxidase and lysosomal phosphatase increases significantly (p<0.05) in macrophages treated with fucoidan. Both enzymes are produced by macrophages and play a role in phagocytosis. Furthermore fucoidan-treated macrophages produce significantly (p<0.05) higher amounts of TNF-α, IL-6, nitrite and H_2O_2. Nitrite is an indicator of NO production and NO and H_2O_2 are molecules which have cytotoxic effects. Both TNF-α and IL-6 play important roles in immunomodulation. The conclusion of this study is that addition of fucoidan from *Fucus vesiculosus* to macrophages and lymphocytes significantly (p<0.05) increases their ability to kill melanoma and lymphoma cells *in vitro*. The overall conclusion of these studies concerning the effect of fucoidan from *Fucus vesiculosus* on tumour viability is that fucoidan significantly (p<0.05) increases apoptosis in different tumour cell lines. The clinical significance is not clear.

- Effect on angiogenesis

Reference	Experimental model	Methods	Outcome
Cumashi *et al.* (2007)	The fucoidan from *Fucus vesiculosus* consisted of 26.1% w/w L-fucopyranose, 3.1% w/w mannose, 2.4% w/w xylose, 2.2% w/w glucose, 5% w/w galactose, 10.3% w/w uronic acids and 23.6% w/w sodiumsulphate. Human umbilical vein endothelial cells (HUVECs) on matrigel form structures that look like capillaries. To test the effect of fucoidan on this process, matrigel in the absence of growth factor was coated on wells. A suspension of HUVECs was put in the wells.	To this suspension following solutions were added (1) isotonic saline (2) fucoidan in isotonic saline (final concentra-tion is 100 µg/ml) After 18 to 20 h of incubation the formation of capillary-like structures was evaluated.	At 100 µg/ml fucoidan from *Fucus vesiculosus* did not significantly inhibit the formation of capillary-like structures.

In the study performed by Cumashi *et al.* (2007), the statistical significance (p<0.05) of the results is determined using the Student t-test. The results of this study suggest that fucoidan from *Fucus vesiculosus* does not inhibit angiogenesis.

- Effects of fucophlorethols from *Fucus vesiculosus* on cyclo-oxygenase-1

Reference	Experimental model	Methods	Outcome
Parys *et al.* (2010)	*Fucus vesiculosus* harvested in February 2003 at	Following solutions were added to Cyclo-oxygenase-1 in concentrations ranging	Percentage of cyclo-oxygenase-1 inhibition:

		from 1 to 50 µg/ml:	(1) 39%
	Armorique was extracted with ethanol. Three fucophlorethols were isolated from the ethanol extract.	(1) Fucophlorethol consisting of 5 units of phloroglucinol	(2) 39%
		(2) Fucophlorethol consisting of 6 units of phloroglucinol	(3) 44%
		(3) Fucophlorethol consisting of 7 units of phloroglucinol	(4) 90%
		(4) Phloroglucinol (=positive control)	

The study performed by Parys *et al.* (2010) does not mention if a statistical analysis has been carried out. Therefore it is unknown if the results are statistically significant. This study shows that fucophlorethols from *Fucus vesiculosus* inhibit cyclo-oxygenase-1. The concentration needed for a relevant clinical effect may be too high to be realistic.

- Effects on inflammation

Reference	Experimental model	Methods	Outcome
Trento *et al.* (2001)	Polymorphonuclear leukocytes sticking assay Fucansulfate from *Fucus vesiculosus* and *Ascophyllum nodosum* = Crinos fucansulfate Male New Zealand rabbits	Polymorphonuclear leukocytes sticking assay Addition of polymorphonuclear leukocytes (=PMN) to 200 mg of thoracicic rabbit aorta. Thrombin was added. The number of PMNs that sticked to the aorta was measured. To these cells addition of (1) 3.125 IU/ml fucansulfate (2) 12.50 IU/ml fucansulfate (3) 50.00 IU/ml fucansulfate (4) 1.35 IU/ml heparin (5) 5.40 IU/ml heparin (6) 21.60 IU/ml heparin (7) 86.40 IU/ml heparin	Polymorphonuclear leukocytes sticking assay Before treatment 5.3% of PMNs sticked to aorta. Percentage of PMNs sticking to aorta compared to control (1) -11% (2) -61% (3) -85% (4) -63% (5) -68% (6) -76% (7) -82% The decrease is significant (p<0.01) for all concentrations of both fucansulfate and heparin.

In the study carried out by Trento *et al.* (2001), the statistical significance ($p<0.05$) of the results is determined using the Dunnett's test. The study shows that fucansulfate from *Fucus vesiculosus* significantly ($p<0.01$) decreases the number of polymorphonuclear lymphocytes that adhere to the rabbit aorta. This decrease suggests that fucansulfate from *Fucus vesiculosus* has an anti-inflammatory effect by interacting with thrombin. It is not clear what may be the *in vivo* consequences of this finding *in vivo*.

In vivo experiments related to the therapeutic indication of the monograph

- Effects on glucose levels

Reference	Experimental model	Methods	Outcome
Roy *et al.* (2011)	3 groups of male Wistar rats, fed by gavage with 50:50 corn:starch and safflower oil solution followed by test solution. (1) control (2) positive control (3) rats treated with phlorotannins from *Fv* Phlorotannin extract from *Fucus vesiculosus*, tradename InSea², supplied by InnoVactiv inc. The extract did not contain any alginates and	By gavage: composition of test solution (1) vehicle (2) acarbose 15 mg/kg bw (3) phlorotannin from *Fv* 7.5 mg/kg bw Glucose and insulin levels were measured 30, 60, 120 and 360 min after feeding.	Reduction of raise in blood glucose level 30 min after gavage compared to control (2) -100% (=no raise) (3) -90% (p<0.05 versus control) Reduction of peak increase in insulin compared to control (3) -40% (p=0.12 versus control) Reduction of area under the curve of insulin compared to control (3) -22% (not statistically significant) Prolongation of absorption

	was partially demineralised. The extract contained minimum 10% polyphenols and the majoritiy of the other constituents are polysaccharides and minerals.		(1) <120 min (3) 360 min
Lamela *et al.* (1989)	Normal male New Zealand rabbits. *Fucus vesiculosus*, harvested on Porto Nadelas beach, was boiled with 95% ethanol for 1 h (= extract 1), algae were dried, turned into powder and extracted again with 95% ethanol (= extract 2). Extract 1 and 2 are mixed and dried in vacuo. Blood samples were taken before and 2, 4 and 6 h after administration to monitor serum glucose levels.	After 20 h of fasting the ethanol extract of *Fucus vesiculosus* was administered intragastrically to the rabbits in following concentrations: (1) 5 g/kg (2) 10 g/kg (3) 20 g/kg	Serum glucose levels: (1) Dose 5 g/kg • 0 h: 120.5 ± 6.8 mg% • 2 h: 127.4 ± 8.7 mg% • 4 h: 134.6 ± 7.8 mg% • 6 h: 125.3 ± 7.8 mg% (2) Dose 10 g/kg • 0 h: 124.3 ± 2.1 mg% • 2 h: 118.5 ± 1.6 mg%* • 4 h: 114.7 ± 1.2 mg%* • 6 h: 127.3 ± 2.3 mg% (3) Dose 20 g/kg • 0 h: 127.5 ± 3.1 mg% • 2 h: 143.3 ± 6.2 mg% • 4 h: 133.6 ± 6.9 mg% • 6 h: 133.6 ± 2.2 mg% *significantly different from control ($p < 0.01$)

In the study performed by Roy *et al.* (2011), the statistical significance ($p<0.05$) is determined using the Student t-test. This study shows that the administration of phlorotannins from *Fucus vesiculosus* by gavage significantly ($p<0.05$) decreases the raise in the blood glucose after a meal compared to control. This could, at least partly, be due to the extended absorption caused by phlorotannins, which is observed in this study. Furthermore phlorotannins from *Fucus vesiculosus* also decrease the peak increase in insulin and the area under the curve for insulin, but these effects were not significant. In order to evaluate the therapeutic consequences, more clinical experience is needed. In the study carried out by Lamela *et al.* (1989) the statistical significance ($p<0.01$) of the results is determined using the Student t-test. The results of this study show that extracts from *Fucus vesiculosus* don't have any significant effect on blood glucose levels.

The results of the study by Roy *et al.* (2011) are promising, but they are not concordant with the results of the study by Lamela *et al.* (1989), so the effect of *Fucus vesiculosus* on serum glucose levels needs further investigation.

***In vivo* experiments not directly related to the therapeutic indication of the monograph**

- Effect on coagulation

Reference	Experimental model	Methods	Outcome
Trento *et al.* (2001)	Polysaccharide from *Fucus vesiculosus* and *Ascophyllum nodosum* = Crinos fucansulfate <u>Thrombin-induced hypotension assay</u> Male Sprague-Dawley rats	250 IU/mg heparin IV. Injection of 375 IU/kg thrombin IV, 25 min later: (1) 2 ml/kg saline IV (=control) (2) 250, 500 and 1,000 IU/kg fucansulfate IV (3) 250, 500 and 1,000 IU/kg heparin IV 5 min later: 375 IU/kg thrombin IV.	Thrombin has hypotensive effects. Compared to the first injection of thrombin, the second injection (after 250 and 500 IU/kg administration of fucansulfate or heparin) caused significantly ($p<0.05$) less hypotension.
	<u>Thrombus formation assay</u> Male New Zealand rabbits were anaesthetised. Two loose knots (2 centimeters beween them) were made	<u>Thrombus formation assay</u> Injection in the ear marginal vein of (1) vehicle (=control) (2) 125 IU/kg fucansulfate	<u>Thrombus formation assay</u> Percentage decrease of thrombus dry weight compared to control (2) -46% (3) -64%

|			
	in the right jugular vein, this vein was stabilised with saline.	(3) 187.5 IU/kg fucansulfate (4) 250 IU/kg fucansulfate (5) 11.38 IU/kg heparin (6) 22.75 IU/kg heparin (7) 45.50 IU/kg heparin After 5 min thrombin injection in carotid artery. 15 s later, the knots were tightened. 15 min after that, the knots were opened and the thrombus was collected.	(4) -97% (5) -58% (6) -90% (7) -99% The decrease is significant ($p < 0.01$) for all concentrations of both fucansulfate and heparin.
de Azevedo *et al.* (2009)	Fucoidan from *Fucus vesiculosus*: Sigma Aldrich Hemorrhagic activity was tested in a rat tail model: using a razor blade a wound was made in a rat tail. Wistar rats: 5 groups of 5 rats: (1) control (2) sulphated fucoidan (3) desulphated fucoidan (4) heparin (5) low molecular weight heparin	(1) Wounded tail was put in isotonic saline and rubbed with gauze. Wounded tails of non-control rats were dipped in a 'solution' for 2 min and then rinsed elaborately with saline. Composition of 'solution': (2) fresh saline + sulphated fucoidan from *Fv* (3) fresh saline + desulphated fucoidan from *Fv* (4) fresh saline + heparin (5) fresh saline + low molecular weight heparin After that all tails were dipped in isotonic saline to observe bleeding time.	Sulphated and desulphated fucoidans displayed significantly lower ($p < 0.001$) hemorrhagic activity when compared to heparin and low molecular weight heparin. See Figure 11.
Kwak *et al.* (2010)	Slc:ICR male mice, supplied by Jungan Animal Co. The carotid artery injury model was used: a filter paper with 25% $FeCl_3$ was used to initiate occlusion of the left common carotid artery. Intravenous injection of fucoidan from *Fucus vesiculosus* or heparin (both supplied by Sigma Chemical Co.) at different concentrations. In each group are three mice.	Intravenous injection of (1) vehicle (= control) (2) 0.05 mg/kg bw fucoidan (3) 0.07 mg/kg bw fucoidan (4) 0.1 mg/kg bw fucoidan (5) 0.05 mg/kg bw heparin (6) 0.07 mg/kg bw heparin (7) 0.1 mg/kg bw heparin (8) 0.13 mg/kg bw heparin (9) 0.16 mg/kg bw heparin	The ED_{50} is the concentration that doubles the total occlusion time of the left common carotid artery. The average total occlusion time is 12.5 min with vehicle. ED_{50} Fucoidan: 0.54 mg/kg bw Heparin: 1.24 mg/kg bw
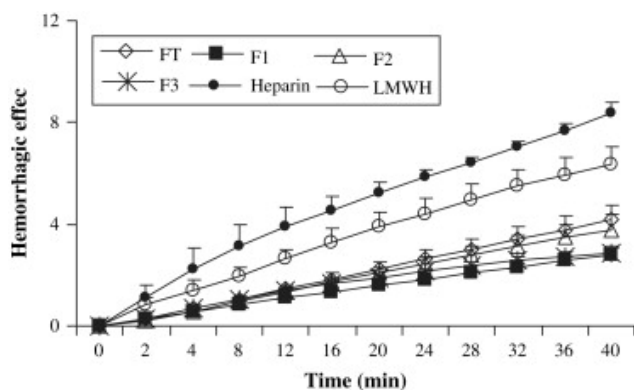
Figure 11: Hemorrhagic effect of different fractions of *Fucus vesiculosus* (de Azevedo *et al*. 2009)

In the study carried out by Trento *et al.* (2001), the statistical significance ($p < 0.05$) of the results is determined using the Dunnett's test. The results of this study show that the decrease in blood pressure that is caused by thrombin is significantly ($p < 0.05$) counteracted if fucansulfate from *Fucus vesiculosus* is administered intravenously before thrombin exposure. Furthermore the results of this study show that if fucansulfate from *Fucus vesiculosus* is administered before an exposure to thrombin, the mass of the thrombus was significantly ($p < 0.05$) lower compared to that of control. However this effect was lower than that of heparin.

In the study performed by de Azevedo *et al.* (2009), the statistical significance ($p < 0.05$) of the results is determined using ANOVA and Tukey-Kramer test. This study shows that fucoidan from *Fucus vesiculosus* has significantly ($p < 0.05$) lower hemorrhagic activity than heparin and low molecular weight heparin. This suggests that fucoidan from *Fucus vesiculosus* would have lower risk of haemorrhages compared to heparin and low molecular weight heparin.

The study carried out by Kwak *et al.* (2010) does not mention if an analysis to determine the statistical significance of the results has been performed. The experiment shows that the total time to occlude the left carotid artery in mice increases after the administration of fucoidan from *Fucus vesiculosus.* The concentration of fucoidan from *Fucus vesiculosus* that was needed to double the occlusion time compared to control was lower (0.54 mg/kg bw) than that of heparin.

Conclusion: the antithrombotic activity of fucoidan from *Fucus vesiculosus* seems to be comparable with heparin (high and low molecular weight) in the experimental models used. The question remains whether in clinical conditions improvements of hard endpoints will be obtained.

- Effects on oxalate deposition in kidneys

Reference	Experimental model	Methods	Outcome
Veena *et al.* (2007)	Fucoidan from *Fucus vesiculosus*, supplied by Sigma Chemicals. Male Wistar albino rats, 4 groups of 6 rats. (1) Control (2) Hyperoxaluria and oxalate deposition in kidneys (3) Drug control: fucoidan subcutaneous (4) Hyperoxaluria and fucoidan subcutaneous	Rats received (1) Vehicle (2) Ethylene glycol (0.75% in drinking water) (3) Fucoidan from *Fv* 5 mg/kg bw dissolved in saline subcutaneous (4) Ethylene glycol (0.75% in drinking water) and from the 8th day on fucoidan from *Fv* 5 mg/kg bw dissolved in saline subcutaneous Liver and kidney excised at the end of the experiment (after 28 days) and homogenated.	Group 2 compared to group 1 **Kidney:** ALP, β-GLU, γ-GT, GST, GR, G6PD activities decreased, SOD, CAT, GPX activities decreased by 23, 35%, 31.80% and 34.02% respectively GSH, ascorbic acid, alfa-tocoferol levels decreased significantly, LDH, XO, LPO activities increased significantly ($p < 0.01$ for all of the above) **Liver:** LDH, XO and GAO activity increased significantly ($p < 0.001$) Group 4 compared to group 2 **Kidney:** Activities of ALP, β-GLU, γ-GT, LDH, XO, SOD, CAT, GPX, GST, GR, G6PD and levels of GSH, ascorbic acid, alfa-tocoferol normalise nearly completely. **Liver:** LDH, XO, GAO activities normalise nearly completely. Group 4 values differ significantly from group 2 values ($p < 0.05$ for all of the above) **Group 3 compared to group 1:** no significant differences.

ALP = alkaline phosphatise (unit is μmol of phenol liberated/min/mg protein)
γ-GT = γ-Glutamyl transferase (unit is nmol x 10 *p*-nitroaniline liberated/min/mg protein)
β-Glu = β-glucuronidase (unit is nmol of *p*-nitrophenol liberated/min/mg protein)
LDH = lactate dehydrogenase
GAO = Glycolic acid oxidase
XO = xanthine oxidase

LPO = lipid peroxidation
SOD = superoxide dismutase
CAT = catalase
GPX = glutathione peroxidase
GST = Glutathione S-transferase
GSH = glutathione
G6PD = glucose-6-sulphate dehydrogenase
APTT = activated partial thromboplastin time

In the study performed by Veena *et al.* (2007), the statistical significance ($p<0.05$) of the results has been determined using one-way ANOVA. The administration of ethylene glycol to rats induces oxidative stress, which damages the epithelium of the kidney which then may induce the formation of stones. The results from this study show that fucoidan from *Fucus vesiculosus* reduces the oxidative stress in the kidney by significantly ($p<0.05$) increasing the levels of antioxidant enzymes and non-enzymic anti-oxidants and significantly ($p<0.05$) reducing the levels of lactate dehydrogenase, glycolic acid oxidase and xanthine oxidase. The three latter enzymes are responsible for metabolising oxalate. Oxalate and his metabolites are free radicals which cause oxidative stress. Furthermore the results show that fucoidan from *Fucus vesiculosus* significantly ($p<0.05$) reduces the levels of alkaline phosphatase, γ-glutamyl transferase and β-glucuronidase, which are all indicators of cellular damage. Whether fucoidan from *Fucus vesiculosus* could be used to prevent stone formation that is due to oxidative stress in the kidneys, remains to be clinically investigated, before any conclusion can be drawn.

- Effects on hormones

Reference	Experimental model	Methods	Outcome
Skibola *et al.* (2005)	Dried powdered *Fucus vesiculosus* from Maine Coast Sea Vegetables. 24 female adult rats with a normal estrous cycle are divided into 3 groups of 8 and are given (1) Vehicle control (2) Low dose *Fv* (3) High dose *Fv*	Per os daily in the morning (1) 2 g of apple (2) 2 g of apple + 175 mg/kg bw *Fv* extract (3) 2 g of apple + 350 mg/kg bw *Fv* extract	Mean number of days of estrous cycle (p: compared to control) (1) 4.3 ± 0.96 (2) 5.4 ± 1.7 (p=0.05) (3) 5.9 ± 1.9 (p=0.02) Mean number of days of diestrus phase within estrous cycle (p: compared to control) (1) 0.97 ± 0.22 (2) 1.4 ± 0.54 (p=0.02) (3) 2.1 ± 0.88 (p=0.02) The number of days of estrus, proestrus and metestrus phase did not change significantly.
	Mean serum 17β-estradiol and progesterone levels (1) Vehicle control (2) Low dose	Mean serum 17β-estradiol and progesterone levels Per os daily in the morning (1) 2 g of apple (2) 2 g of apple + 175 mg/kg bw *Fv*	Mean serum 17β-estradiol levels (p: compared to control) (1) 48.9 ± 4.5 ng/L (2) After 2 weeks: 40.2 ± 3.2 ng/L (p=0.13) After 4 weeks: 36.7 ± 2.2 ng/L (p=0.02) Serum progesterone levels did not change significantly.
	Mean serum 17β-estradiol levels for high level rats 8 rats with high levels of serum 17β-estradiol	Mean serum 17β-estradiol levels for high level rats Per os daily in the morning (1) 2 g of apple + 350 mg/kg bw *Fv*	Mean serum 17β-estradiol levels for high level rats (p: compared to control) • Before treatment: 68.6 ng/L • After 1 week of treatment: 42.8 ng/L (p=0.02) 2 rats did not respond to the treatment. Progesterone levels did not change significantly for high 17β-estradiol rats.

In the study performed by Skibola *et al.* (2005), the statistical significance ($p<0.05$) of the results is determined using two-way ANOVA, Dunnett's pairwise comparison and paired t-tests. This study shows that in rats dried, powdered *Fucus vesiculosus* significantly ($p\leq0.05$) increases the number of days of estrous cycle as well as the number of days of diestrus phase within the estrous cycle ($p=0.02$). Furthermore *Fucus vesiculosus* also significantly (after 4 weeks $p=0.02$) reduces the serum 17β-estradiol levels. These results are consistent with the results of a study in 3 women (see Clinical data), but in that study dried, powdered *Fucus vesiculosus* also significantly ($p=0.002$) increases the plasma progesterone level. This could be due to the fact that in the studies with rats the blood was taken in the morning and in the morning the 17β-estradiol levels are at their peak, but the progesterone levels are not. This was a weakness of the study.

- Effects on the immune system

Reference	Experimental model	Methods	Outcome
Ale *et al.* (2011)	Fucoidan from *Fucus vesiculosus*: Sigma-Aldrich Co. C57BL/6J mice: Clea Japan Inc. 4 groups of 3 mice: (1) control (2) positive control (3) fucoidan from *Fucus vesiculosus* (4) fucoidan from *Sargassum sp.*	During 4 days the mice received intraperitoneal injections: (1) no administration (2) administration of Poly 100:1 (3) fucoidan from *Fv* 50 mg/kg bw (4) fucoidan from *Sargassum sp.* 50 mg/kg bw After treatment the spleens of the mice were resected, spleen cells were cultured and the natural killer cell activity was measured.	Increase of natural killer cell activity: (1) 5.1 ± 2.1 % (2) 26.2 ± 8.9 % (3) 11 ± 1.7 % (4) 14 ± 3.8 % Both fucoidans significantly ($p\leq0.05$) increased the natural killer cell activity compared to control. Fucoidan from *Sargassum* sp. induced a significantly higher ($p\leq0.05$) natural killer cell activity than fucoidan from *Fucus vesiculosus*.

In the study by Ale *et al.* (2011), the statistical significance ($p<0.05$) of the results is determined using analysis of variance. The results of this study show that fucoidan from *Fucus vesiculosus* significantly ($p\leq0.05$) increases the natural killer cell activity compared to control. It can be questioned whether the modest increase with high doses of fucoidan creates perspectives for specific research.

- Inhibitory effect on muscle necrosis caused by snake venom

Reference	Experimental model	Methods	Outcome
Angulo and Lomonte (2003)	135 kDa fucoidan from *Fucus vesiculosus*: Sigma. Phospholipase A2 from crotaline snake venom causes muscle necrosis, which is reflected in higher plasma levels of creatine kinase. There are 9 types of crotaline snake venom. Preincubation test: 20 groups of 4 CD-1 mice: (1) Placebo control (2) Fucoidan control (3) Crotaline snake venom (9 types) (4) Crotaline snake venom (9 types) + fucoidan Independent administration test 5 groups of 4 CD-1 mice: (1) *Bothrops asper* venom control (2) *Bothrops asper* venom + low dose fucoidan	<u>Preincubation test</u> Intramuscular injection of (1) 100 µlof phosphate buffered saline (2) fucoidan from *Fv* (same concentration as in 4) (3) 75 µg crotaline snake venom (9 types) (4) 75 µg crotaline snake venom (9 types) + fucoidan in 1:1 molar ratio (30 minutes pre-incubation) <u>Independent administration test</u> Intramuscular injection of 50 µg *Bothrops asper* venom+ immediately an injection at the same spot of (1) phosphate buffered saline (2) 90 µg fucoidan from *Fv*	<u>Preincubation test</u> 70 to 95 % reduction of plasma creatine kinase activity of (4) compared to (3). *See figure 12* No increase of plasma creatine kinase levels of fucoidan control (2) compared to placebo control (1). <u>Independent administration test</u> The low (2) and high (3) doses of fucoidan from *Fucus vesiculosus* significantly ($p<0.05$) reduced the plasma creatine kinase activity when compared to venom control (1). The reduction was circa 50%. See figure 13. Intramuscular injection of only fucoidan (4) and (5) in the

	(3) *Bothrops asper* venom + high dose fucoidan (4) fucoidan low dose control (5) fucoidan high dose control	(3) 270 µg fucoidan from *Fv* No *Bothrops asper* venom, only intramuscular injection of: (4) 90 µg fucoidan from *Fv* (5) 270 µg fucoidan from *Fv*	independent administration test did not cause a significant increase of plasma creatine kinase levels compared to placebo control (1) of the preincubation test.

Figure 12: Inhibition of plasma creatine kinase activity, its activity increases if there are myotoxic effects, after intramuscular injection of snake venom that had preincubated with fucoidan from *Fucus vesiculosus*, compared to control (Angulo and Lomonte 2003)
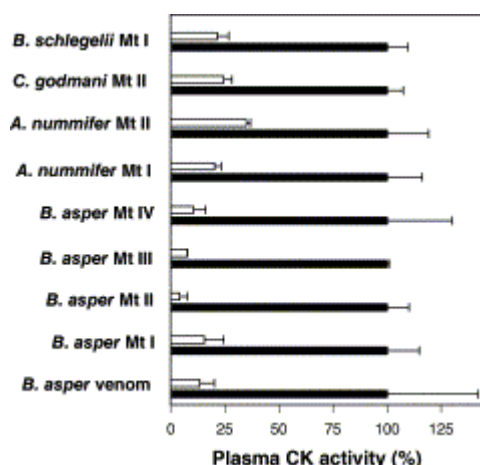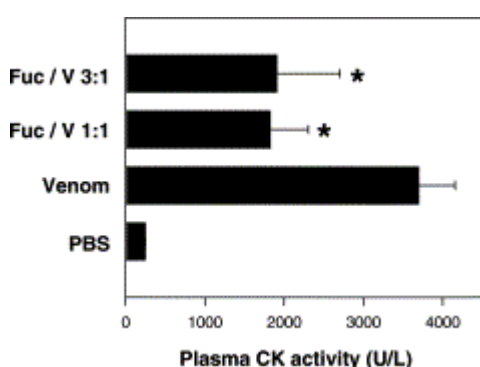

Figure 13: Inhibition of plasma creatine kinase activity, its activity increases if there are myotoxic effects, after intramuscular injection of snake venom immediately followed by injection of fucoidan from *Fucus vesiculosus*, compared to control (Angulo and Lomonte 2003)

Unfortunately the study performed by Angulo and Lomonte (2003) does not mention if a statistical analysis has been carried out to determine the statistical significance of the results. Therefore it is unknown whether the results are statistically significant. This study shows that 135 kDa fucoidan from *Fucus vesiculosus* reduces the muscle necrosis in mice by 70 to 95% when fucoidan is preincubated with the snake venom. Furthermore the study also shows that when 90 and 270 µg of fucoidan is injected intramuscularly in mice immediately after intramuscular injection of snake venom, the muscle necrosis is reduced by approximately 50%. The latter experiment resembles reality more than the first experiment. This suggests that even when fucoidan from *Fucus vesiculosus* is injected immediately after a snake bite, there still will be a lot of muscle necrosis. The relatively high molecular weight of this fucoidan may reduce its diffusion and distribution in the tissue. It may be useful to do experiments using lower molecular weight fucoidans to monitor their effect on muscle necrosis after a snake bite.

- Effects on serum triglyceride levels

Reference	Experimental model	Methods	Outcome
Lamela *et al.* (1989)	Normal male New Zealand rabbits. *Fucus vesiculosus*, harvested on Porto Nadelas beach, was boiled with 95 % ethanol for 1 h (= extract 1), algae were dried, turned into powder and extracted again with 95% ethanol (= extract 2). Extract 1 and 2 are mixed and dried in vacuo. Blood samples were taken before and 4 and 6 h after administration to monitor serum triglyceride levels.	After 20 h of fasting the ethanol extract of *Fucus vesiculosus* was administered intragastrically to the rabbits in following concentrations: (1) 5 g/kg (2) 10 g/kg (3) 20 g/kg	Serum triglyceride levels: 1) Dose 5 g/kg • 0 h: 93.8 ± 12 mg% • 4 h: 93.8 ± 8.1 mg% • 6 h: 90.8 ± 2.4 mg% (2) Dose 10 g/kg • 0 h: 59.2 ± 7.1 mg% • 4 h: 65.7 ± 2.6 mg% • 6 h: 65.5 ± 4.1 mg% (3) Dose 20 g/kg • 0 h: 57.2 ± 7.1 mg% • 4 h: 56.3 ± 8.9 mg% • 6 h: 49.5 ± 4.7 mg% *significantly different from control ($p < 0.01$)

In the study carried out by Lamela *et al.* (1989), the statistical significance ($p<0.01$) of the results is determined using the Student t-test. The results of this study show that *Fucus vesiculosus* has no significant effects on serum triglyceride levels in New Zealand rabbits.

- Inhibitory effects on damage caused by irradiation

Reference	Experimental model	Methods	Outcome
Rhee and Lee (2011)	Fucoidan from *Fucus vesiculosus*, consisting of 27.5% w/w fucose, 26.3% w/w sulphate and 14.7% w/w ash, was supplied by Heawon Biotech Inc. Healthy Balb/c mice were γ-irradiated at 8 Gray using ^{137}Cs.	Before irradiation following solutions were injected intraperitoneally: (1) phosphate buffered saline (=control) (2) 1, 10 and 100 mg/kg fucoidan in phosphate buffered saline After irradiation blood was drawn from the retro-orbital plexus every week during 4 weeks.	Thrombocyte counts on day 21 and 28: (1) 32 and 45%, respectively (2) 49 and 60% ($p<0.05$), respectively Hematocrit ratio on day 21 and 28: (1) 60 and 68%, respectively (2) 75% on day 28 Erythrocyte counts from day 14 to day 28: (1) 64 to 67% (2) 75 to 80% Leucocyte protection ($p<0.05$ on day 28 compared to control) by fucoidan was similar to thrombocyte protection. Mean number of mice survival days after irradiation: (1) 9 days (2) 16, 21 and 29 days for the 1, 10 and 100 mg/kg fucoidan, respectively (not significant) 50-day actuarial mice survival rate (1) 0% (2) 12, 20 and 30% for the 1, 10 and 100 mg/kg fucoidan, respectively (not significant) Fucoidan treated irradiated mice had significantly ($p<0.05$) less hypoplasia when compared to irradiated controls, the protective effect against hypoplasia increases with dose. All mice showed abnormalities in the lungs, liver and spleen after irradiation.

In the study performed by Rhee and Lee (2011), the statistical significance ($p<0.05$) is determined using ANOVA or the Bonferroni multiple comparison method. The results of the study by Rhee and Lee show that fucoidan from *Fucus vesiculosus* significantly ($p<0.05$) increases the leukocyte and thrombocyte count 28 days after irradiation compared to control. Fucoidan also increases the erythrocyte levels and hematocrite levels and the survival time of the mice after irradiation compared to control, but these effects are not significant. The relevance of the statistical differences can be questioned, because of high doses needed to obtain a reasonable survival.

3.2. Overview of available toxicological data regarding the herbal substance(s)/herbal preparation(s) and constituents thereof

Single and repeat dose toxicity

Reference	Experimental model	Methods	Outcome
Zaragoza *et al.* (2008)	*Fucus vesiculosus* was harvested at 2 different places at the North coast of France. The algae were worked up into 2 powder extracts, these powder extracts were processed in 1% carboxymethylcellulose. Acute toxicity Swiss mice and Sprague – Dawley rats were used to find the LD_{50}. Subacute toxicity 5 groups of 14 Sprague-Dawley rats (7 males and 7 females) (1) control (2) extract 1, low dose (3) extract 1, high dose (4) extract 2, low dose (5) extract 2, high dose LD_{50}= median lethal dose of 50% of subjects	Acute toxicity (1) rats: extract 1 intragastrically (2) rats: extract 1 intraperitoneally (3) rats: extract 2 intragastrically (4) rats: extract 2 intraperitoneally (5) mice: extract 1 intragastrically (6) mice: extract 1 intraperitoneally (7) mice: extract 2 intragastrically (8) mice: extract 2 intraperitoneally Subacute toxicity During 4 weeks daily via an intragastric canula (1) vehicle = 1 % carboxymethylcellulose (=control) (2) 200 mg/kg bw extract 1 (3) 750 mg/kg bw extract 1 (4) 200 mg/kg bw extract 2 (5) 750 mg/kg bw extract 2 Bw= body weight	Acute toxicity: LD_{50} (1) 1,000-2,000 mg/kg bw (2) 250-2,000 mg/kg bw (3) >2,000 mg/kg bw (4) ≥500 mg/kg bw (5) 1,000-2,000 mg/kg bw (6) 150-200 mg/kg bw (7) ≥500 mg/kg bw (8) 250-500 mg/kg bw Subacute toxicity Treated female rats ate 13% less than their controls, this is not significant. The study doesn't mention if the following results are significant. The number of white cells shrinked in groups that received extract 2 (up to 50%) or high dose of extract 1 (up to 70%) compared to control. Coagulation parameters did not change. α-amylase increased for the high dose of extract 1 (44% rise) and for both doses of extract 2 (36% rise). Increase in Na^+-excretion for extract 1 and 2 (68 and 90%, respectively), but the plasma [Na^+] did not change. There was no fecal blood, this means there were no lesions in the gastrointestinal tract. The relative weight of kidneys raised in male rats that received high doses of extract 2 (25% rise), but the functional parameters of these organs were normal.

In the study by Zaragoza *et al.* (2008), the significance of the results is determined using the unpaired Student t-test and the one-factor analysis of variance. Although these statistical analyses were performed, the study doesn't mention the statistical significance of all of the results. The results show that *Fucus vesiculosus* treated female rats ate 13% less than their controls. Furthermore the number of white cells shrinked and α-amylase increased in groups that received extract 2 (to 50%) or high

dose of extract 1 (to 70%) compared to control. However the coagulation parameters did not change and there was no fecal blood, the latter means there were no lesions in the gastrointestinal tract.

Although the Na^+-excretion of the rats treated with extract 1 and 2 (68 and 90%, respectively) increased, the plasma $[Na^+]$ did not change. The study also shows that the relative weight of liver and kidneys increased in male rats that received high doses of extract 2 (25%), but the functional parameters of these organs were normal.

3.3. Overall conclusions on non-clinical data

In vitro a lot of studies have been performed on *Fucus vesiculosus* and its different components, such as fucoidan, phlorotannins and fucophlorethols. The subjects of these studies were very diverse, namely the effects on skin, cholesterol, oxidants, estrogen, progesterone, glucose levels, coagulation, viruses , immune system, atherosclerosis, restenosis, leukocyte recruitment, metastasis, irradiation of cells, snake venom, CYP1A, tumour viability, accumulation of advanced glycation end products, angiogenesis and cyclo-oxygenase-1. Most of these activities are not relevant for the therapeutic indication maintained in the monograph. They were nevertheless kept into the assessment report in order to give a large view on the herbal substance and its preparations.

Fucus vesiculosus extracts reduce trans-sialidase activity (which would be correlated to intracellular cholesterol accumulation), reduces 17β-estradiol levels and competed with estradiol and progesterone for binding to their receptors, reduces coagulation (inhibits the formation of thrombin and inhibits thrombin-induced platelet aggregation and plasminogen activator activity, increases activated partial thromboplastin time and prothrombin time) and inhibits the myotoxic effects on skeletal muscle cells after addition of snake venom.

Phlorotannins from *Fucus vesiculosus* inhibit α-amylase and α-glucosidase and inhibit the accumulation of advanced glycation products by scavenging reactive carbonyls.

Fucophlorethols from *Fucus vesiculosus* inhibits CYP1A and aromatase.

Fucoidan from *Fucus vesiculosus* inhibits cyclo-oxygenase-1 and tumour-platelet interactions (the latter effect reduces metastasis risk), inhibits leukocyte recruitment (this is an anti-inflammatory effect), IgE release from B cells and histamine release from mast cells, reverse transcriptase and reduces inflammation and inhibits proliferation, migration and adhesion of smooth muscle cells. Fucoidan from *Fucus vesiculosus* induces apoptosis in several tumour cell lines by increasing caspase-3 activity, increases cell viability after irradiation, increases production of several pro-inflammatory cytokines and increases the viability of macrophages and lymphocytes and increases fibroblast-populated gel culture contraction. Fucoidin from *Fucus vesiculosus* lead decreases the accumulation and persistence of leukocytes in capillaries and venules (anticoagulant properties).

However the question remains whether the extract can reach the action site at a sufficient dose *in vivo* and if the *in vitro* models are representative for *in vivo* effects.

In vivo, phlorotannin from *Fucus vesiculosus* decreases the glucose level after gavage. *Fucus vesiculosus* increases the number of days in the oestrous cycle and reduces 17β-estradiol levels. Fucansulfate decreases thrombus formation and it also decreases the adherence of polymorphonuclear lymphocytes to the rabbit aorta (this is an anti-inflammatory effect), it inhibits kidney stone formation that is caused by oxidative stress by increasing the levels of several anti-oxidative enzymes, it reduces snake venom induced muscle necrosis if it is injected soon enough and it inhibits death of leukocytes and thrombocytes after irradiation and it increases survival time after irradiation. Fucoidan from *Fucus vesiculosus* increases natural killer cell activity. The results obtained with high doses are modest.

A difference has to be made between the effects obtained with *Fucus vesiculosus* preparations and the results from experiments with concentrated compounds from Fucus when considering traditional use.

There were no relevant signs of toxicity during the examination period for the used doses in the study conducted by Zaragoza *et al.* (2008). It would be useful to have more toxicity studies at our disposal. For example studies on genotoxicity, carcinogenicity, reproductive and developmental toxicity, immunotoxicity or other special toxicities were not performed. Despite the lack of these data, *Fucus vesiculosus* is used since centuries without severe documented toxicological responses.

4. Clinical Data

4.1. Clinical Pharmacology

4.1.1. Overview of pharmacodynamic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

No data available.

4.1.2. Overview of pharmacokinetic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

- Effects of fucoidan on CYP enzymes

Reference	Experimental model	Methods	Outcome
Parys *et al.* (2009)	*Fucus vesiculosus* harvested in February 2003 at Armorique, St. Efflam, France was extracted with ethanol. Three fucophlorethols were isolated from the ethanol extract. CYP1A inhibition assay CYP1A from β-naphtoflavone-induced rat hepatoma cells.	CYP1A inhibition assay Following solutions were added in concentrations ranging from 1 to 50 µg/ml: (1) fucophlorethol consisting of 5 units of phloroglucinol (2) fucophlorethol consisting of 6 units of phloroglucinol (3) fucophlorethol consisting of 7 units of phloroglucinol (4) phloroglucinol (=positive control)	CYP1A inhibition assay IC_{50} values: (1) 20 ± 0.4 µg/ml (2) 17.9 ± 1 µg/ml (3) 33.7 ± 3 µg/ml (4) > 50 µg/ml

CYP1A is a phase I enzyme, which is used in this experiment as a phase I marker enzyme. The study by Parys *et al.* (2009) shows that fucophlorethols from *Fucus vesiculosus* inhibit CYP1A activity. The inhibition of phase I enzymes by fucophlorethols may be useful, because phase I enzymes may play a role in the activation of carcinogens.

4.2. Clinical Efficacy

No data available.

4.2.1. Dose response studies

No data available.

4.2.2. Clinical studies (case studies and clinical trials)

- Effects on skin

Reference	Experimental model	Methods	Outcome
Fujimura *et*	*Fucus vesiculosus* was supplied by Ichimaru Pharcos. The aqueous	Topically twice daily (morning and evening) during 5 weeks: (1) Placebo gel	*Fucus vesiculosus* extract (2) compared to control (1):

	extract of *Fucus vesiculosus* was 1.5% w/v. 10 females, 23 to 36 years old (mean 28.4 years). (1) cheek 1: control (2) cheek 2: *Fucus vesiculosus* extract	(2) Gel with 1% of aqueous extract of *Fucus vesiculosus* A specialist measured thickness and mechanical properties of the skin in the morning and evening before and after treatment in a half-face double blind way.	Skin thickness • Morning: -0.096 mm ($p<0.005$) • Evening: -0.109 mm ($p<0.005$) Skin final distension • Morning: 0.007 mm ($p<0.05$) • Evening: 0.010 mm ($p<0.05$) Skin thickness increases and skin distension decreases with age. The *Fucus vesiculosus* extract significantly ($p<0.05$) decreases skin thickness and significantly increases ($p<0.05$) skin distension.
al. (2002)			

In the study by Fujimura *et al.* (2002), the statistical significance of the results has been determined using the paired Student t-test. Skin thickness increases and skin distension decreases with age. This study shows that the *Fucus vesiculosus* extract significantly ($p<0.005$) decreases skin thickness and significantly ($p<0.005$) increases skin distension. Whether *Fucus vesiculosus* may be a topical anti-aging agent remains to be demonstrated.

- Effects on weight management

There are no clinical studies with monopreparations of *Fucus vesiculosus*.

A clinical study with a commercial preparation containing pomegranate seed oil and brown seaweed extract was conducted in obese postmenopausal women with a non alcoholic fatty liver disease, and women with normal liver fat. The preparation was able to reduce body weight, body and liver fat content, and plasma C-reactive protein (Abidov *et al.* 2010). No further details are given as the study does not fit with the objectives of the monograph. There was a conflict of interest in the article of Abidov *et al.* (2010); furthermore it was not specified which brown seaweed the product contains. This was not found on the manufacturer's website. Statistically, there was no correction for multiple testing and no exact p values were provided.

- Effects on osteoarthritis of the knee

There are no clinical studies with monoprearations containing *Fucus vesiculosus*.

A study was conducted with a commercial preparation containing 3 species of brown algae among which *Fucus vesiculosus* in a limited number of patients with osteoarthritis of the knee. The preparation reduced the comprehensive osteoarthritis test score (Myers *et al.* 2010). No further details on this study are given as the herbal preparation does not fit with the objectives of the monograph. It was an open-label pilot study. It was not clear whether the visual analogue scale was validated. A one-tailed test was used to calculate significance. The authors did not correct for multiple testing.

- Effects on oestrogen, progesterone and menstrual cycle

Reference	Experimental model	Methods	Outcome
Skibola (2004)	Case report of 3 pre-menopausal women: (1) 43 years, hypermenorrhea, polymenorrhea, dysmenorrhea, luteal phase	All 3 women participated in the low dose (= 700 mg *Fucus vesiculosus* daily) treatment.	Menstrual cycle length (p: compared to before treatment) Before treatment: (1) 16.3 ± 0.6 days (2) 23.0 ± 1.7 days

	deficiency and endometriosis (2) 42 years, hypermenorrhea, polymenorrhea and dysmenorrhea (3) Hypermenorrhea, dysmenorrhea and endometriosis Dried, powdered *Fucus vesiculosus* was supplied by Maine Coast Sea Vegetables and made into 350 mg capsules. • 3 menstrual cycles: before treatment • 2 menstrual cycles: low dose *Fucus vesiculosus* treatment • 2 menstrual cycles high dose *Fucus vesiculosus* treatment	Patient (1) participated in the high dose (=1,400 mg *Fucus vesiculosus* daily) treatment.	(3) 27.3 ± 0.6 days After low dose treatment: (1) 26.0 ± 1.4 days (p<0.002) (2) 28.5 ± 0.7 days (p=0.03) (3) 31.5 ± 0.7 days (p=0.005) After high dose treatment: (1) 31.2 ± 1.1 days (p<0.001) (3) 36.0 ± 2.8 days (p=0.01) <u>Total days of menstruation</u> (p: compared to before treatment) Before treatment: (1) 9.3 ± 0.6 days (2) 8.0 ± 1.0 days (3) 6.3 ± 1.5 days After low dose treatment: (1) 6.3 ± 1.8 days (p=0.06) (2) 5.3 ± 2.5 days (p=0.06) (3) 5.8 ± 0.4 days (p=0.65) After high dose treatment: (1) 4.5 ± 0.7 days (p<0.003) (3) 3.5 ± 0.7 days (p=0.10) <u>Plasma estradiol and progesterone levels tested in patient 1</u> (p: compared to before treatment) <u>17β-estradiol:</u> • <u>Before: 626 ± 91 pg/ml</u> • <u>Low dose: 164 ± 30 pg/ml (p=0.04)</u> • <u>High dose: 92.5 ± 3.5 pg/ml (p=0.03)</u> <u>Progesterone:</u> • <u>Before: 0.58 ± 0.14 ng/ml</u> • <u>Low dose: 8.4 ± 2.6 ng/ml (p=0.1)</u> • <u>High dose: 16.8 ± 0.7 ng/ml (p=0.002)</u>

In the study performed by Skibola (2004), the statistical significance ($p \leq 0.05$) of the results is determined using unpaired t tests. This study shows that in three women 700 and 1400 mg dried, powdered *Fucus vesiculosus* significantly ($p \leq 0.05$) increases the menstrual cycle length and significantly ($p \leq 0.05$) reduces the days of menstruation. For patient 1, the plasma estradiol levels are significantly ($p \leq 0.05$) lower and the progesterone levels are significantly higher (p=0.002 after high dose treatment) when compared to the levels before treatment. The higher dose induced higher effects. However the doses applied can be discussed, when considering the possible amount of iodine ingested (see chapter 5.3). In general, these results are of limited value.

4.2.3. Clinical studies in special populations (e.g. elderly and children)

No data available.

4.3. Overall conclusions on clinical pharmacology and efficacy

Fucus vesiculosus decreases skin thickness and increases skin distension, it also decreases plasma estradiol levels and increases menstrual cycle length and reduces the number of days of menstruation. A commercial preparation of *Fucus vesiculosus* showed beneficial effects in weight management of postmenopausal women. Another commercial extract reduced the complaints of osteoarthritis of the knee.

The value of these clinical studies is limited. Variable inclusion criteria were used to include patients and the patients investigated are not directly representative for the target population in relation to the indication found in the monograph. For the intervention, mixed preparations and sometimes high doses

were used, which should be reconsidered when taking into account the amounts of iodine possibly ingested. *Most of the outcomes are not related to the therapeutic indication of the monograph.*

5. Clinical Safety/Pharmacovigilance

5.1. Overview of toxicological/safety data from clinical trials in humans

- Effects on thyroid gland

Reference	Experimental model	Methods	Outcome
Clark *et al.* (2003)	Double-bind prospective clinical trial on 36 healthy euthyroid subjects for 4 weeks. Randomly assigned into three groups: (1) Placebo (2) Low-dose kelp (3) High-dose kelp Kelp capsules: Nature's Way, containing a total of 660 µg of iodine Alfalfa capsules: Nature's Way, Springville, UT	Daily intake of (1) 4 alfalfa capsules (2) 2 kelp capsules and 2 alfalfa capsules (Nature's Way) (3) 4 kelp capsules (Nature's Way)	Compared to before treatment: (1) No change (p>0.05) in TSH, total T_3 and urinary iodine concentrations, TRH stimulation test results and basal metabolic rate. FT_4 concentrations significantly reduced (p=0.01). (2) TSH increased (p=0.04) FT_4: not significant (p=0.73) Total T_3: not significant (p=0.85) Urinary iodine: increase (p=0.0008) TRH stimulation test results: not significant (p=0.06) Basal metabolic rate: not significant (p=0.87) (3) TSH increased (p=0.002) FT_4: not significant (p=0.93) Total T_3: reduced (p=0.04) Urinary iodine: increase (p=0.003) TRH stimulation test results: increase (p=0.0002) Basal metabolic rate: not significant (p=0.10) The increase in iodine excretion appeared to be dose-dependent.

The findings of the study by Clark *et al.* (2003) on the influence on thyroid hormones are similar to trials with iodine supplementation. This indicates that the iodine present in bladderwrack products is bioavailable. They were not corrected for multiple testing (some results can become not significant if corrected for multiple testing). The increase of TSH and reduction of T_3 by the Fucus preparation points to a possible inhibition of the thyroid function due to the content of iodine in the capsules (660 µg up to 4 times daily).

5.2. Patient exposure

No data available.

During the assessment, reference was made to the specified conditions of use oforlistat as an OTC medicine, according to which treatment is limited to 6 months.

5.3. Adverse events and serious adverse events and deaths

The chapter on adverse events and serious adverse events and deaths is subdivided into a general part on iodine in the environment and recommendations for daily intake. A second part considers possible side effects of Fucus containing preparations.

5.3.1. Iodine in the environment and recommendandations for intake

General considerations

According to the information in the SPC of iodine containing medicines in some European countries (e.g. Germany) patients should not use *Fucus vesiculosus* in case of the following conditions: (1) pathologies of the thyroid gland; (2) cardiovascular pathologies e.g. recent myocardial infarction, coronary disease, angina pectoris, hypertension; (3) adrenal insufficiency; (4) women suffering from postmenopausal osteoporosis.

Fucus vesiculosus ingestion could create collecting duct or inner medullary defects which is the onset of diabetes insipidus. Fanconi's syndrome has been reported after ingestion of *Fucus vesiculosus*. Consumption of *Fucus vesiculosus* grown in a contaminated environment, for example with arsenic or heavy metals, can lead to ingestion of these toxic entities. This can cause glomerular injury which is manifested in proteinuria and hematuria (Luyckx and Naicker 2008, Barnes *et al*. 2007).

There are serveral case reports on *Fucus vesiculosus*. One on arsenic toxicosis in a 54-year-old woman (Amster *et al.* 2007); this case report however is objected by an employee of a trade association that represents the natural products industry (Fabricant 2007). Moreover there were two cases of elevated urinary excretion of arsenic caused by the intake of food supplements containing bladderwrack (Walkiw and Douglas 1975). Finally one on therapy failure of iodine-131 due to high iodine intake from selenium supplements. These supplements contained bladderwrack as an additive (Arum *et al*. 2009).

With regard to therapeutic use of *Fucus vesiculosus*, the discussion is mainly related to the ingestion of iodine, added to the daily amount provided by food and environment.

Iodine in food and the environment

The iodide content of foods and total diets differs depending on geochemical, soil, and cultural conditions. The major natural food sources are marine fish (mean 1,220 µg/kg, up to 2.5 mg/kg), shellfish (mean 798 µg/kg, up to 1.6 mg/kg), marine algae, seaweed (1,000-2,000 µg/kg) and sea salt (up to 1.4 mg/kg). In industrialised countries, the most important sources of iodides are dairy products, e.g. whole cow's milk (mean: 27-47 µg/kg), UK winter milk (mean: 210 µg/kg), UK summer milk (90 µg/kg), eggs (mean: 93 µg/kg), and grain and cereal products (mean: 47 µg/kg depending on the soil). Other food sources are freshwater fish (mean: 30 µg/kg), poultry and meat (mean: 50 µg/kg), fruits (mean: 18 µg/kg), legumes (mean: 30 µg/kg) and vegetables (mean: 29 µg/kg) (EFSA 2006).

Non-food sources are iodine-containing medication, topical medicines, antiseptics (povidone-iodine), X-ray contrast media (~5,000 mg/dose yielding 1-4 g in cholecystography, >10 g in urography), iodised oil for oral Iodine or i.m. use, mineral dietary supplements (up to 190 mg iodide/dose), tablets or capsules of seaweed-based dietary supplements (0.045-5 mg iodide/dose) and kelp tablets as dietary supplement (up to 57 mg iodide/dose). Marine macroalgae produced in China, Japan, the Philippines, North and South Korea are products grown in aquaculture from brown, red and green algae and can have an extremely high iodine content, particularly in marketed products derived from dried material (up to 6,500 mg iodine/kg dry product). A product known as Kombu-powder contains about 0.5% iodine (EFSA 2006). Hence the need for well-qualitatively and quantitatively defined herbal preparations.

Daily intake of iodine

The daily intake of iodine from diet sources may vary from country to country.
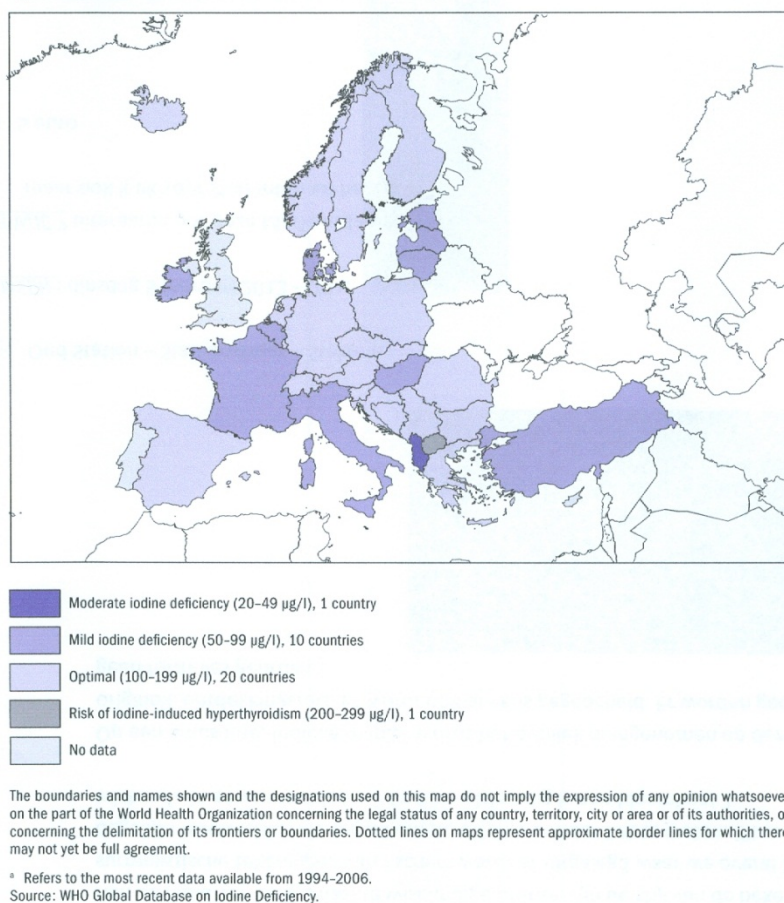
In Germany, the median daily iodine intake varies from about 64-118 (mean: 45.3) µg I/day for males aged 4-75 years and from 59-114 (mean: 44.2) µg I/day for females aged 4-75 years. For infants aged 6 months, children and young adults up to the age 18 years the mean iodine intakes varied from 31-64 µg/day for males and from 28-56 µg/day for females. In those taking iodine supplements once/week the corresponding mean levels were 124 µg I/day for males and 109 µg I/day for females compared to 107 µg I/day for males and 102 µg I/day for females not taking any supplements.

In Denmark, the median intake was about 119 µg I/day for males and 92 µg I/day for females.

In The Netherlands, the median intake was about 145 µg/day for males and 133 µg/day for females.

In Great Britain, the median dietaryintake from all sources was 226 µg/day for males and 163 µg/day for females, the 97.5th percentile reaching 434 µg/day in males and 359 µg/day in females. Survey data in young children aged 1½-4½ years show for high milk consumers in winter 247 µg/day to 309 µg/day (EFSA 2006).

Figure 14: Daily iodine intake in European countries (Andersson *et al.* 2007)

■	Moderate iodine deficiency (20–49 µg/l), 1 country
■	Mild iodine deficiency (50–99 µg/l), 10 countries
□	Optimal (100–199 µg/l), 20 countries
■	Risk of iodine-induced hyperthyroidism (200–299 µg/l), 1 country
□	No data

The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

a Refers to the most recent data available from 1994–2006.
Source: WHO Global Database on Iodine Deficiency.

According to Andersson *et al.* (2007), iodine intake is optimal in 20 European countries (between 100 and 199 µg/day), whereas 11 countries have a mild to moderate iodine deficiency. Only Macedonia has a risk of iodine-induced hyperthyroidism with a mean intake of more than 200 µg/day (Figure 14).

Bladderwrack (kelp) contains, according to the European Pharmacopoeia 7.0, between 0.03 and 0.2% iodine on dried substance. This implies that *Fucus vesiculosus* preparations may contribute to the dietary iodine intake.

Recommendations

The World Health Organization advises a daily iodine intake of 100 to 150 µg. Some countries or organisations advise daily iodine intake levels up to 200-300 µg. During pregnancy and lactation there is an increased urinary loss of iodine caused by a higher renal blood flow. Therefore a higher daily iodine intake is advised (up to 230 µg) (SCF 2002).

According to some sources doses exceeding 150 µg iodine per day may cause the side effects of increased thyroid gland function such as heart palpitation, increased heart rate, trembling, changes in blood pressure and increased basal metabolism, though rarely reported. High iodine intake can also cause aggravation of acne. Therefore long-term use in subjects with normal thyroid function should be avoided. A generalised allergic reaction can occur (Yarnell and Abascal 2006, Verhelst 2010, Barnes *et al.* 2007). However this information is not in line with reports about about intake of high doses.

The parameters altered in dose-response studies included an elevation of serum TSH levels in response to iodine intake and the enhanced response in TSH levels to TRH stimulation. They were all of a biochemical nature and not associated with any clinical adverse effects. However, elevated serum levels of TSH are not necessarily clinically adverse, but could be regarded as indicators of an existing risk of induced hypothyroidism. There is uncertainty whether the subtle changes observed, such as an enhanced response to TRH, would have significant adverse biological consequences even if sustained over longer periods, because all observed values remained within the normal ranges for the parameters determined. It remains uncertain whether chronic exposure to these small doses would have any relevant clinical consequences in normal euthyroid individuals (EFSA 2006).

An upper limit (UL) can be established on the basis that the noted biochemical changes in TSH levels and the TSH response to TRH administration were marginal and unassociated with any clinical adverse effects at estimated intakes of 1,700 and 1,800 µg/day. Although the studies on which these UL estimates are based were all only of short duration, involved only a small number of individuals, and lacked precision of the actual total dietary intakes, their results were supported by the study covering a 5-year exposure at approximately similar iodide intake levels of 30 µg/kg bw/day (equivalent to approximately 1,800 µg iodide/day) in which no clinical thyroid pathology occurred. A safety factor of 3 is thus considered adequate and provides an UL for adults of 600 µg/day. The UL of 600 µg is also considered to be acceptable for pregnant and lactating women based on evidence of lack of adverse effects at exposures significantly in excess of this level. Since there is no evidence of increased susceptibility in children, the ULs for children were derived by adjustment of the adult UL (Table 5) on the basis of body surface area (body weight $^{0.75}$) (EFSA 2006).

Table 5: Tolerable upper daily intake level for children derived from body surface area (body weight $^{0.75}$) (EFSA 2006)

Age in years	Tolerable upper intake level (UL) for iodine (µg per day)
1-3	200
4-6	250
7-10	300
11-14	450
15-17	500

In the US the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes of the Food and Nutrition Board together with Health Canada are pursuing a joint project which proposes a tolerable upper level of intake for iodine for adults of 1,100 µg/day. WHO has suggested a provisional maximum tolerable daily intake of 1 mg/day from all sources, equivalent to 17 µg/kg bw. In countries with long-standing IDD the intake should not exceed 500 µg/day to avoid the occurrence of

hyperthyroidism. In France, the Expert Committee on Human Nutrition has suggested an UL of 500 µg I/day in countries with long-standing Iodine Deficiency Disease to avoid the occurrence of hyperthyroidism (EFSA 2006).

Upper levels of tolerance and toxicity of iodine

Very high doses are required to reach the toxic levels of iodine. Chronic iodine intake can cause iodism, the doses at which this occurs differ and were not determined. Sensitivity to iodine-induced hyperthyroidism is race-dependent, for example the Japanese and African populations appear to be less sensitive. Studies conducted on subchronic exposure of iodine deal with the same problem. Supplements up to 3 mg appeared to have no effect on serum TSH levels, whereas 50 to 250 mg significantly increased these levels, however the effect was small after 10 to 14 days intake. These studies did not evaluate the dietary iodine intake. Therefore total iodine intake remains unclear. One single dose of 2,000 to 3,000 mg of iodine is probably lethal to humans (SCF 2002).

5.3.2. Possible side effects with *Fucus* containing preparations

Standard sources refer to kelp for adverse effects and precautions (Sweetman 2009).

Bladderwrack (kelp) can concentrate various heavy metals. Auto-immune thrombocytopenic purpurea and disorderd erythropoiesis in a patient who had been taking kelp for 6 weeks was attributed to the arsenic content of the preparation (Pye *et al.* 1992).

Clinical hyperthyroidism has also been reported in patients taking kelp-containing preparations as part of a slimming regimen or dietary supplement (Eliason 1998).

The FDA has advised that preparations containing compounds such as kelp, which may be taken orally in bulk laxatives or weight-control preparations should be taken with a full glass of water or, if the patient has difficulties in swallowing, they should be avoided. Such compounds will swell into masses that may obstruct the oesophagus if not taken with sufficient water (Sweetman 2009). This information has been only partially implemented into the monograph, as the selected posology is restricted and the pharmaceutical form facilitates swallowing.

5.4. Laboratory findings

No data available.

5.5. Safety in special populations and situations

Precautions should be taken: hypertension, kidney diseases (Verhelst 2010) and anemia (fucoidan may lead to reduced gastrointestinal absorption of iron) (Barnes *et al.* 2007).

Fucus vesiculosus is contra-indicated in following cases: hyperthyroidism, Graves or Basedow disease, Hashimoto thyroiditis, after partial resection of the thyroid gland, excess of iodine, pregnancy or lactation, children under five years, hypersensitivity to halogens, malicious diseases and tuberculosis (Zimmermann and Delange 2004, Barnes *et al.* 2007, De Smet *et al.* 1993, De Smet *et al.* 1997).

Interactions are possible in following circumstances: lithium carbonate, thyroid medication, antihypertensive drugs, blood-diluting-agents and iodine containing drugs (De Smet *et al.* 1997, Verhelst 2010).

One case report of hyperthyroidism was published about a 60-year-old male patient diagnosed with bipolar disorder and under treatment with lithium. Since his myocardial infarction in 2001, the patient was treated with ramipril (1.25 mg 2 times daily), bisoprolol (2.5 mg/d), simvastatine (10 mg/d) and

acetylsalicylic acid (100 mg/d). He was operated several times for an anal fistula, which is why he started taking a herbal product that contained besides 0.125 g of *Fucus vesiculosus* also *Rhamnus purshiana* 0.170 g and *Frangula* 0.222 g per tablet. The patient took this preparation regularly (once a day). The thyroid hormones were controlled every 4 months. In 2008, he developed hyperthyroidism: T4: 2.13 ng/dl (normal levels 0.9-1.7) and TSH 0.01 mIU/l (normal levels 0.27-4.2). The condition was treatable with metamizole and thyroxine (Arbaizar and Llorca 2011).

Although other literature considers the interaction with vitamin K-antagonists unlikely (Williamson 2009).

5.6. *Overall conclusions on clinical safety*

The quality of Fucus preparations should be carefully controlled, especially as heavy metals is concerned. It is not excluded that by traditional use of Fucus preparations, the recommended daily intake of iodine can be more than the acceptable one of 100 to 150 μg. Although there is some case reporting (among others on interaction between lithium and *Fucus*) and data about the endocrinological consequences of the intake of iodine are available, there are no alarming signals from pharmacovigilance or intake of iodine from food. Although a maximum iodine content should be considered for the extracts, it is not clear what may be the threshold. Apart from an upper limit, it is highly recommendable to set limits to the duration of treatments and to recommend regular monitoring (e.g. every 4 months) of thyroid function.

6. Overall conclusions

Quality

The quality of the herbal substance and its extracts must be guaranteed, especially as contaminations with heavy metals are concerned. Furthermore the total iodine content should be known and the daily intake should not be exceeded.

Safety

It has been demonstrated that the intake of iodine-containing Fucus preparations can influence thyroid function. The maximum content of iodine is 0.2%, according to the monograph in the European Pharmacopoeia. The amount of iodine by intake of Fucus vesiculosus adds to the daily intake from food. Therefore a maximum intake is defined as the regular intake enhanced with the iodine from Fucus. The resulting intake of total iodine should not exceed the upper limit defined by EFSA of 600 μg (see also benefit-risk).

Efficacy

Fucus and its components have extensively studied preclinically. More particularly cardiovascular, immunological, endocrinological, anti-oxidative and antitumoral activities have been investigated.

Based upon market information provided by the members, a traditional use of Fucus preparations as an adjuvant in slimming diets can be granted, and a corresponding monograph can be conceived. However some conditions should be considered with regard to the maximum daily intake of total iodine and the duration of treatment. There are a few clinical data on the use of combined preparations containing Fucus. The outcomes of these studies cannot be translated to the monograph. As there is no evidence for a well established use, traditional use can be granted for the monopreparation.

Benefit-risk

The benefit-risk analysis remains positive for Fucus vesiculosus, provided that the total intake of iodine does not exceed 600 μg/day. For this reason, it is specified in the monograph that the upper daily limit of 400 μg total iodine per day following intake of *Fucus vesiculosus* containing medicinal products should not be exceeded.

Therapeutic area for browse search

Overweight, weight loss, adjuvant.

Community list entry

Due to the lack of genotoxicity testing, no Community list entry can be established.

Annex

List of references