



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

19 September 2017
EMA/HMPC/745347/2016
Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Ribes nigrum* L., folium

Final – revision 1

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

Herbal substance(s) (binomial scientific name of the plant, including plant part)	<i>Ribes nigrum</i> L., folium
Herbal preparation(s)	a) Comminuted herbal substance b) Dry extract (DER 7:1), extraction solvent water c) Powdered herbal substance
Pharmaceutical form(s)	Comminuted herbal substance as herbal tea for oral use. Herbal preparations in solid dosage forms for oral use. The pharmaceutical form should be described by the European Pharmacopoeia full standard term.
Rapporteur(s)	Revision 1: B. Jansone First version: G. Laekeman, A. Vlietinck
Peer-reviewer	B. Kroes



Table of contents

Table of contents	2
1. Introduction	4
1.1. Description of the herbal substance(s), herbal preparation(s) or combinations thereof ..	4
1.2. Search and assessment methodology	6
2. Data on medicinal use	6
2.1. Information about products on the market	6
2.1.1. Information about products on the market in the EU/EEA Member States	6
2.1.2. Information on products on the market outside the EU/EEA	8
2.2. Information on documented medicinal use and historical data from literature	8
2.3. Overall conclusions on medicinal use	11
3. Non-Clinical Data	13
3.1. Overview of available pharmacological data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof.....	13
3.1.1. Primary pharmacodynamics	13
3.1.2. Secondary pharmacodynamics	22
3.1.3. Safety pharmacology	24
3.1.4. Pharmacodynamic interactions	24
3.1.5. Conclusions	24
3.2. Overview of available pharmacokinetic data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof.....	25
3.3. Overview of available toxicological data regarding the herbal substance(s)/herbal preparation(s) and constituents thereof	25
3.3.1. Single dose toxicity.....	25
3.3.2. Repeat dose toxicity.....	25
3.3.3. Genotoxicity	25
3.3.4. Carcinogenicity.....	25
3.3.5. Reproductive and developmental toxicity	25
3.3.6. Local tolerance.....	25
3.3.7. Other special studies.....	26
3.3.8. Conclusions	26
3.4. Overall conclusions on non-clinical data	26
4. Clinical Data	27
4.1. Clinical pharmacology	27
4.1.1. Overview of pharmacodynamic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents.....	27
4.1.2. Overview of pharmacokinetic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents.....	27
4.2. Clinical efficacy	27
4.2.1. Dose response studies.....	27
4.2.2. Clinical studies (case studies and clinical trials)	27
4.3. Clinical studies in special populations (e.g. elderly and children)	27
4.4. Overall conclusions on clinical pharmacology and efficacy	27
5. Clinical Safety/Pharmacovigilance	27
5.1. Overview of toxicological/safety data from clinical trials in humans.....	27

5.2. Patient exposure	27
5.3. Adverse events, serious adverse events and deaths.....	28
5.4. Laboratory findings.....	28
5.5. Safety in special populations and situations	28
5.5.1. Use in children and adolescents.....	28
5.5.2. Contraindications.....	28
5.5.3. Special warnings and precautions for use	28
5.5.4. Drug interactions and other forms of interaction	29
5.5.5. Fertility, pregnancy and lactation.....	29
5.5.6. Overdose.....	29
5.5.7. Effects on ability to drive or operate machinery or impairment of mental ability	29
5.5.8. Safety in other special situations	29
5.6. Overall conclusions on clinical safety.....	29
6. Overall conclusions (benefit-risk assessment).....	29
Annex	30

1. Introduction

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

- Herbal substance

In accordance with the European Pharmacopoeia (8th ed., 07/2013:2528) blackcurrant leaf consists of the dried leaves of *Ribes nigrum* L. (blackcurrant leaf). The leaf is simple, lamina is up to 10 cm long and 12 cm wide, with 3 or 5 rounded triangular lobes and the median lobe is the largest (European Pharmacopoeia 8th ed., 07/2013:2528).

Description and origin of the plant

Ribes nigrum L. belongs to the family of the Grossulariaceae. The genus *Ribes* contains between 140 and 150 species. The leaves are collected during or shortly after flowering (Pharm. Française 1996; Hänsel *et al.*, 1994). A dark green upper surface and a pale greyish green lower surface is the characteristic for the slightly wrinkled leaf fragments. Furthermore, a widely spaced reticulate venation is particularly distinct on the lower surface. Glands can be seen as scattering yellowish dots. In contrast with the fresh leaves, the dried leaves have no odour or taste (Wichtl 1994; Hänsel *et al.*, 1994).

Constituents

The dried *Ribes nigrum* leaf contains not less than 1% of flavonoids, expressed as isoquercitroside. The material complies with the monograph of the European Pharmacopoeia 8th ed. (2013) and British Pharmacopoeia Vol IV (2015). According to the Pharmacopée Française the dried *Ribes nigrum* leaf contains not less than 1.5% of flavonoids, expressed as rutin (ESCOP, 2003).

The most important secondary metabolites present in the herbal substance can be subdivided into several groups of phytochemical compounds.

Polyphenolic substances, more particularly flavonoid glycosides: kaempferol, quercetin, myricetin, isorhamnetin and sakuranetin (Wyk & Wink, 2005).

Some substances can seasonally appear in the glands of leaves of *Ribes nigrum*. Sakuranetin is a methylated flavanone aglycone (4',5-diOH-7-methoxyflavanone). Its biosynthesis may be useful in the protection against parasites like *Botrytis cinerea* Pers. ex Fr. (Atkinson & Blakeman, 1982).

Hydroxycinnamic acid derivatives: chlorogenic acid and chlorogenic acid derivatives (isochlorogenic acid, neochlorogenic acid), caffeic acid, gallic acid, ferulic acid, coumaric acid, gentisinic acid (Trajkovski, 1974a; Trajkovski 1974b).

Prodelphinidins (proanthocyanidines) were identified in a methanolic extract of the leaves. They may be responsible for the anti-inflammatory properties of the herbal preparations (Tits *et al.*, 1992a, 1992b).

The presence of glycerolipids has been reported. The total fatty acid composition was unusual, because the following unsaturated fatty acids were identified: linolenic acid (alpha-18:3), together with cis-7, 10, 13-hexadecatrienoic acid (16:3) and lower amounts of stearidonic acid (18:4) and gamma-linolenic acid (gamma-18:3). This makes the lipid composition type mixed: typical of 16:3 plants but also partially typical for 18:4 plants (Dobson, 2000).

The essential oil of the leaves of *Ribes nigrum* contains mainly monoterpenic substances like alpha-pinene, myrcene, p-cymene, limonene, beta-ocimene, beta-phellandrene, linalool, terpinen-4-ol,

geraniol, citronellylacetate. Furthermore, the sesquiterpenes caryophyllene and humulene were identified, as well as methyl salicylate (Andersson *et al.*, 1963).

Ascorbic acid, carotenoids (Herbal Medicines, 2013).

Maximum content of Ca, Mg, Fe, Al, Cr and K in the black current leaves is detected in the June (Nour *et al.*, 2014).

The potassium-sodium ratios in the leaf of *Ribes nigrum* L. and decoctions of the leaves were 128:1 and 242:1 respectively. These ratios are considered as eventually contributing to the diuretic effect (Szentmihályi *et al.*, 1998).

- Herbal preparation(s)

Information about registered/authorised herbal preparations on the European market of *Ribes nigri* folium was provided by the National Competent Authorities and is presented in the overview of the market products, see section 2.1.1.

In France, Spain and Poland the following herbal preparation of *Ribes nigrum* L., folium is present as monocomponent medicinal products:

- comminuted herbal substance for tea preparation
- dry extract (DER: 7:1; extraction solvent water) in solid dosage form as hard capsule
- powdered herbal substance in solid dosage form as hard capsule

Table 1: Information obtained from pharmacopoeias and handbooks

Reference, year	Herbal preparation
Herbal Medicines (Fourth edition), 2013	As tea of finely cut dried leaves for infusion, 2-4 g per cup (150 mL) three to four times daily; Dry extract (7:1, water), 169 mg per capsule, 1 -3 capsules daily; Powdered herbal substance, 340 mg per capsule, three times daily.
Hänsel <i>et al.</i> , 1994; Delfosse, 1998; Wichtl, 1994; Wyk & Wink, Gruenwald <i>et al.</i> , 2000	As tea of finely chopped leaves, 2-4 g taken several times a day.

- 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.

This assessment report and the Community herbal monograph refers exclusively to *Ribes nigri* folium as a single ingredient. The Community herbal monograph describes the use of the comminuted herbal substance for tea preparations, powdered herbal substance in solid dosage form as hard capsule and dry extract in solid dosage form as hard capsule.

1.2. Search and assessment methodology

Electronic databases and other sources used to assess information available on traditional use, pharmaceutical, non-clinical, clinical data and current indications on *Ribes nigrum* L., folium using search keywords: *Ribes nigrum* L., Ribis nigri folium, blackcurrant leaf, cassis. No restrictions to language were set. The search of information was performed from June till September 2016.

Articles and references were retrieved from:

- Scientific databases: PubMed, ScienceDirect, Scopus, Scifinder, Web of Science, EMBASE, EBSCO.
- Medical databases: UpToDate.
- Toxicological databases: TOXLINE.
- The Cochrane Library: 1 reference was found using *Ribes nigrum* L., folium or blackcurrant leaf as search terms.
- Libraries: hand searches in handbooks, textbooks and Pharmacopoeias on *Ribes nigrum* L. folium and blackcurrant leaf at the various libraries: EMA, University of Latvia, Rigas Stradinu University, The State Agency of Medicines of Latvia, National library of Latvia.
- Databases of electronic books: Dawsonera.
- Search engines used: Google, Google Scholar.

Other databases: The World Health Organization, National Center for Complementary and Alternative Medicine (NCCAM).

2. Data on medicinal use

2.1. Information about products on the market

2.1.1. Information about products on the market in the EU/EEA Member States

Information on medicinal products marketed in the EU/EEA

Table 2: Overview of data obtained from marketed medicinal products

Active substance	Indication	Pharmaceutical form, Posology, Duration of use	Regulatory Status
<i>Ribes nigrum</i> L., folium Dry extract (extraction solvent water; DER: 7:1)	a) Traditionally used to treat minor articular pain. b) Traditionally used to support renal and digestive elimination systems.	Herbal preparations in solid dosage form: hard capsule Method of administration: oral use Posology: adults only Single dose: 169 mg of extract/hard capsule Daily dose: 169 mg of extract/hard	France TUR From 1990 until 2011 it was authorised/registered in France as a medicinal product. Currently there are no monocomponent medicinal products containing <i>Ribes</i>

Active substance	Indication	Pharmaceutical form, Posology, Duration of use	Regulatory Status
		capsule 1 to 3 times daily Duration of use: a) 4 weeks b) 2-3 weeks	<i>nigrum</i> L., folium as dry extract authorised/registered in France
<i>Ribes nigrum</i> L., folium Powdered herbal substance	a) Traditionally used in the symptomatic treatment of minor painful joint conditions. b) Traditionally used to promote urinary and digestive elimination functions.	Herbal preparations in solid dosage form: hard capsule Method of administration: oral use Posology: adults only Single dose: 1 hard capsule contains 340 mg of powder Daily dose: 1 hard capsule 3 times daily Max dose: till 5 hard capsules if necessary Duration of use: 4 weeks 2-3 weeks	TUR use in France since 1987
<i>Ribes nigrum</i> L., folium Powdered herbal substance	a) Traditional herbal medicinal product for relief of minor articular pain. b) Traditional herbal medicinal product to increase the amount of urine to achieve flushing of the urinary tract as an adjuvant in minor urinary complaints.	Herbal preparations in solid dosage form Method of administration: oral use Posology: adults only Single dose: 1 hard capsule contains 340 mg of powder Daily dose: 3 capsules per day, 1020 mg Duration of use: 2 to 4 weeks 2 to 4 weeks	Spain It was registered by former registration scheme in January 1992. TUR since May 2009, according to article 16 of Directive 2001/83 was granted.
<i>Ribes nigrum</i> L., folium Comminuted herbal substance	Traditionally used as an aid in mild rheumatic complaints.	Herbal preparations for infusion as herbal tea Method of administration: oral use Posology: adults only Daily dose: 2 to 4 g pour with 200mL of boiling water, infuse under cover for 10-15	TUR use in Poland since 1978

Active substance	Indication	Pharmaceutical form, Posology, Duration of use	Regulatory Status
		min. Drink freshly prepared infusion 3 times a day. Duration of use: 4 weeks	

This overview is not exhaustive. It is provided for information only and reflects the situation at the time when it was established.

Information on relevant combination medicinal products marketed in the EU/EEA

Hungary

Combination product

230 g of the preparation contains:

- Crataegi extractum (extraction solvent red vine + alcohol): 178.71
- *Crataegus spp.*, folium cum flore
- *Ribes nigrum* L., folium
- *Crataegus spp.*, fructus
- Melissae herba
- Kalium asparticum: 0.46 g
- Magnesium asparticum: 0.23 g

Indication: prevention of cardiac complaints in elderly, relief of symptoms of temporary nervous cardiac complaints (e.g. palpitations, perceived extra heart beat due to mild anxiety).

All together there are six combination products containing *Ribes nigrum* L., folium in the old 'healing products' category.

Information on other products marketed in the EU/EEA (where relevant)

Latvia

The herbal preparations that contain *Ribes nigrum* L., folium are available on the market as food supplements (combination products).

2.1.2. Information on products on the market outside the EU/EEA

Not applicable

2.2. Information on documented medicinal use and historical data from literature

Indications exclusively in folk medicine for the *Ribes nigrum* leaf are: arthritis, rheumatic complaints, diarrhoea, spasmodic cough as well as it has been traditionally used as an infusion to cure joint

complaints (traditionally classified as 'rheumatism') (Wichtl, 1994; Leclerc 1983; Rombi, 1991). The only indication mentioned in the ESCOP monograph is 'adjuvant in the treatment of rheumatic conditions' (ESCOP, 2003).

Anti-inflammatory posology according to ESCOP (2003), Van Hellemont (1985) and Delfosse (1998):

- Dried leaves as an infusion: from 1.5 to 4 g per cup (= 150 mL; 3-4 cups daily) to 20-50 g per litre (250 to 500 mL daily), infused during 15 minutes.
- Fluid extract (1:1): 5 mL 2x daily. The extraction solvent is not specified.

Ribes nigri folium is also described in folk medicine as a diuretic, eliminating uric acid. The preparations mentioned are aqueous decoctions (Decaux, 1930; Hänsel *et al.*, 1994).

Some authors mention the use of tea preparations containing *Ribes nigrum*, *Fraxinus excelsior* and *Ulmaria officinalis*.

According to some authors, the essential oil of *Ribes nigrum* stimulates the renal epithelium and enhance diuresis (Garnier *et al.*, 1961; Rombi, 1991). Rarely preparations of *Ribes nigri folium* were locally applied on wounds (Wichtl, 1994), as well as the leaves were applied on the head against migraine (Decaux, 1930).

Table 3: Overview of historical data

Herbal preparation	Documented use / Traditional use	Pharmaceutical form	Reference
Comminuted herbal substance or equivalent preparations	Adjuvant in the treatment of rheumatic conditions	20-50 g of dried leaf per 1 L, infused for 15 minutes. Daily dosage: 250-500 mL; Fluid extract (1:1) 5 mL Daily dosage: twice daily. Taken before meals.	ESCOP Monographs, 2003
Comminuted herbal substance or equivalent preparations	Traditional diuretic. Leaves are taken in cases of rheumatism and urinary problems	As a tea, 2-4 g of finely chopped leaves taken several times a day	Wyk and Wink, 2005
Comminuted herbal substance or equivalent preparations	Usage based on tradition in folk medicine: as diuretic, uses for gout, rheumatic complaints, diarrhoea, spasmodic cough.	Single dose: 2-4 g of finely cut dried leaf, pour boiling water over and steep for 5-10 minutes and then strain. 1 cup several times daily.	Wichtl, 1994
Comminuted herbal	Usage based on	Single dose: 2-4 g of	Gruenwald et al., 2000

Herbal preparation	Documented use / Traditional use	Pharmaceutical form	Reference
substance or equivalent preparations	tradition in folk medicine: arthritis, gout and rheumatism, diarrhoea, colic, jaundice and liver ailments, painful micturition, urinary stones, convulsive coughs and whooping cough.	black current leaves in boiling water (150 mL) for the tea, strain after 15 min. preparation. Daily dosage: 1 cup to be drunk several times a day.	
Comminuted herbal substance or equivalent preparations	Usage medicine: arthritis, gout and rheumatism, diarrhoea, colic, jaundice and liver ailments, painful micturition, urinary stones, convulsive coughs and whooping cough, joint pain, diuretic	Single dose: 2-4 g /150 mL in boiling water over and steep for 10 min and then strain. Daily dosage: 3-4 cups to be taken several times a day.	Hänsel et al., 1994
Comminuted herbal substance or equivalent preparations Powdered herbal substance Dry extract (7:1, water)	For minor articular pain and minor urinary complaints (as a diuretic)	Dry extract (7:1, water), Single dose: 169 mg per capsule. Daily dosage: 3 capsules daily. Powdered herbal substance Single dose: 340 mg per capsule. Daily dosage: up to 5 capsules daily. Infusion as herbal tea of dried leaves: 2-4 g per cup (150 mL). Daily dosage: 3-4 times daily. Fluid leaf extract (1:1) Single dose: 5 mL Daily dosage: 2 daily	Herbal Medicines, 2013

Herbal preparation	Documented use / Traditional use	Pharmaceutical form	Reference
		before meals.	

2.3. Overall conclusions on medicinal use

According to the information provided by the National Competent Authorities in the overview of the marketed products (see section 2.1.1.), medicinal products, containing *Ribes nigrum* L., folium, have been available for 30 years in the markets of France, Poland and Spain therefore fulfilling the criteria of traditional use in EU in accordance with Directive 2004/24/EC.

Herbal tea (2-4 g up to 3 times daily) has been used since 1978 in Poland for minor rheumatic complaints and can be accepted according to the 30 years of use stipulated in Directive 2004/24/EC.

Powdered herbal substance in capsules has been marketed in France (since 1987) and in Spain (since 1992) in different posologies (France: 340 mg up to 5 capsules per day; Spain: 340 mg 3 times daily). At time of monograph systematic review (in 2016) the period of use fulfils the 30 years of traditional use as stipulated in Directive 2004/24/EC, therefore, this preparation can now be included in the monograph.

Dry extract (7:1; extraction solvent water) is marketed in France as capsules (169 mg per capsule; posology: up to 3 capsules daily) meets the 15-year tradition in Europe, but does not comply with the 30 years of traditional use as stipulated in Directive 2004/24/EC. Nevertheless, this preparation is included in the monograph based upon the following justification:

- The extract is made with water, a procedure comparable to herbal tea preparations.
- The drug-extract ratio is 7 to 1 and 169 mg is taken as a single dose up to 3 times a day. One capsule corresponds to 1183 mg of herbal substance. For herbal tea, 2 to 4 g herbal substance is infused; virtually, more material can be extracted by preparing the herbal tea. The number of daily doses is similar for the extract and the infusion.

Table 4: Overview of evidence on period of medicinal use

Herbal preparation	Indication	Posology, Strength	Period of medicinal use
Comminuted herbal substance	Traditionally used as an aid in mild rheumatic complaints	Herbal preparations for infusion as herbal tea Posology: adults only Dose: 2 to 4 g in 200 mL of boiling water, infuse for 10-15 min. Drink freshly prepared infusion 3 times a day. Method of administration: oral use Duration of use: 4 weeks	TUR use in Poland since 1978
Powdered herbal substance	a) Traditionally used in the symptomatic	<i>Adults</i> Herbal preparations in solid dosage form: hard capsule	TUR use in France since 1987 (and TUR use in Spain

Herbal preparation	Indication	Posology, Strength	Period of medicinal use
	<p>treatment of minor painful joint conditions.</p> <p>b) Traditionally used to promote urinary and digestive elimination functions.</p>	<p>Posology:</p> <p>Single dose: 1 hard capsule contains 340 mg of powder</p> <p>Daily dose: 1 hard capsule 3 times daily Max dose: till 5 hard capsules if necessary (in France)</p> <p>Method of administration: oral use</p> <p>Duration of use: a) 4 weeks b) 2-3 weeks</p>	<p>since 2009)</p>
<p>Dry extract (extraction solvent water; DER: 7:1)</p>	<p>a) Traditionally used to treat minor articular pain.</p> <p>b) Traditionally used to support renal and digestive elimination systems</p>	<p>Herbal preparations in solid dosage form: hard capsule</p> <p>Posology: adults only</p> <p>Single dose: 169 mg¹) of extract/hard capsule</p> <p>Daily dose: 169 mg¹ of extract/hard capsule 1 to 3 times daily</p> <p>Method of administration: oral use</p> <p>Duration of use: a) 4 weeks b) 2-3 weeks</p>	<p>Currently there are no mono-component medicinal products containing <i>Ribes nigrum</i> L., folium as dry extract authorised/registered in France as TUR</p> <p>It was registered in France as a medicinal product from 1990 until 2011</p>

The use of leaves of *Ribes nigrum* in children and adolescents under 18 years of age is not recommended due to lack of data.

Because the HMPC could not find a suitable indication for the traditional use "to support digestive elimination", this use was not included in the monograph.

The following indications for *Ribes nigrum* L., folium are proposed for the EU herbal monograph:

Traditional herbal medicinal product based upon long-standing use

Indication a): Traditional herbal medicinal product for relief of minor articular pain.

¹ In the monograph this value is rounded to 170 mg

Adults and elderly:

- Comminuted herbal substance for infusion as herbal tea preparation
Single dose: 2 to 4 g per cup, 3 times daily.
Daily dose: 6–12 g.
- Dry extract (DER 7:1, water)
Single dose: 170 mg, 1–3 times daily.
Daily dose: 170–510 g.
- Powder in hard capsule
Single dose: 340 mg, 3–5 times daily.
Daily dose: 1020–1700 mg (3–5 hard capsules per day)

Duration of use: 4 weeks

Indication b): Traditional herbal medicinal product to increase the amount of urine to achieve flushing of the urinary tract as an adjuvant in minor urinary complaints.

Adults and elderly:

Herbal preparations in solid dosage form: hard capsule

- Dry extract (DER 7:1, water)
Single dose: 170 mg, 1–3 times daily.
Daily dose: 170–510 g.
- Powder in hard capsule,
Single dose: 340 mg, 3–5 times daily
Daily dose: 1020–1700 mg (3–5 hard capsules per day)

Duration of use: 2 weeks

3. Non-Clinical Data

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

3.1.1. Primary pharmacodynamics

Analgesic effects

Ethanollic extracts

In vivo studies:

Mongold *et al.*, (1993) reported that a *Ribes nigrum* L., folium extract (500 g dried leaves immersed in 15% ethanol/water in 5 L) for 10 days, then filtered and lyophilised) induced an analgesic effect in the acetic-induced writhing test in mice as well as in the Hot-plate response test in mice.

Anti-inflammatory activity

Ethanolic extracts

In vivo studies:

Anti-inflammatory activities were observed by Mongold *et al.*, (1993) in several *in vivo* models:

- in the carrageenan-induced acute inflammation model *Ribes nigrum* L., folium extract (extraction solvent: 15% ethanol), demonstrated dose-dependent (75 and 150 mg/kg, i.p.) inhibition of acute inflammatory oedema, the effect of 150mg/kg dose was comparable to indomethacin (5 mg/kg, i.p.);
- cotton pellet granuloma: *Ribes nigrum* L., folium extract (150 mg/kg, i.p.) inhibited granuloma formation – the relative wet granuloma weights for the control (465±19 mg), *Ribes nigrum* L., folium extract (351±15 mg) and indomethacin (378±8 mg);
- Freund adjuvant induced arthritis: the *Ribes nigrum* L., folium extract (150 mg/kg – 18.71%; 300 mg/kg – 34.65%) expressed dose-dependent reduction of hind-paw oedema.

An extract of *Ribes nigrum* L., folium (maceration of 60 g black currant leaves with 1000 mL of 14% ethanol; 1 mL/kg and 10 mL/kg, p.o.) demonstrated anti-inflammatory activity by the inhibition of carrageenan-induced inflammation compared to reference compounds indomethacin and niflumic acid in acute and chronic studies in Sprague-Dawley rats (Declume *et al.*, 1989).

In vitro studies:

Ribes nigrum L., folium extract (containing 60% of proanthocyanidins of the total polyphenol content) increased the CD39-positive endothelial cell fraction in a concentration-dependent manner (for the 2.5 µg/mL it increases up to 10%; for 15 µg/mL up to 33%). It also enhanced endothelial nitric oxide synthase (eNOS) activation. T495 phosphorylation was decreased by 31±6% for the dose of 2.5 µg/mL and 48±6% for 15 µg/mL, whereas S1177 phosphorylation increased by 13±3% for the dose of 2.5 µg/mL and 18±7% for 15 µg/mL compared to untreated cells (Luzak *et al.*, 2014).

Anti-inflammatory activity of fresh *Ribes nigrum* L., folium extract (extraction solution: acetone/water/acetic acid (70:28:2)) was tested on the total myeloperoxidase (MPO, dose 50 ng/mL) released by activated neutrophils measured by an ELISA assay. The higher concentration (1 mg/mL) of *Ribes nigrum* L., folium gave about 15-20% of inhibition. MPO inhibition was also observed in a Specific Immunological Extraction Followed by Enzymatic Detection assay, *Ribes nigrum* L., folium extract showed a significant dose-dependent inhibition: the lowest a concentration tested, 0.5 µg/mL for leaf extract, showed 60% inhibition (Tabart *et al.*, 2012).

Isolated constituents

In vitro studies:

Shiba *et al.*, (2008) observed that flavonoids (quercetin, and its metabolites) inhibited the formation of dityrosine catalysed by the myeloperoxidase (MPO) enzyme in a dose-dependent manner (1.25 – 100 µM) *in vitro* (HL-60 cells).

The experimental model consisted of an LT2 cell line originating from human umbilical vein endothelium cells. A proanthocyanidin-enriched fraction was obtained from leaves from *Ribes nigrum* with acetone extraction (70% V/V in water). Purification was done on reversed phase chromatography. A significant inhibition of TNF-α (Tumor Necrosis Factor) stimulated ICAM-1 (Intercellular Adhesion Molecule 1) expression but not IL-8 and VEGF155 mRNA expression was observed with proanthocyanidins in concentrations from 10 µg/mL to 60 mg/kg (Garbacki *et al.*, 2005).

An *in vitro* decrease of PGE2 production in human Chondrocytes was also observed with prodelphinidines, obtained from the *Ribes nigrum* L., folium, A concentration of 10^{-4} M of gallic catechin and its dimer inhibited the formation of prostaglandins comparable with indomethacin 10^{-5} M: 53%, 57% and 67% respectively. Further, the selectivity on COX-2 inhibition was confirmed (Garbacki *et al.*, 2002).

Ex vivo studies:

An anti-inflammatory activity on isolated rabbit hearts was reported for rutin and isoquercitrin, obtained from the *Ribes nigrum* L., folium (Chanh *et al.*, 1986).

In vivo studies:

Anti-inflammatory effects of proanthocyanidins (PACs) were observed in rat's models of carrageenan induced paw oedema and carrageenin-induced pleurisy. Pre-treatment with PACs (10, 30, 60 and 100 mg/kg, i.p.) reduced in a dose time dependent manner paw oedema induced by carrageenin and also inhibited carrageenin-induced pleurisy in rats: particularly reducing lung injury, pleural exudate formation, polymorphonuclear cell infiltration, pleural exudate levels of TNF- α , IL-1 β and CINC-1, pleural exudate levels of nitrite/nitrate (NO $_x$). The mechanism of action of the PACs differs from that of indomethacin. Indomethacin treated rats showed that a low the volume of pleural exudate, and a reduced content in leukocytes and in TNF- α , IL-1 β , IL-6 and IL-10 but not in NO $_x$ (Garbacki *et al.*, 2004).

Garbacki *et al.*, (2005) observed anti-inflammatory activities of proanthocyanidin-enriched fraction (PACS) of *Ribes nigrum* L., folium (the extraction solvent was acetone 70% V/V in water purification by reversed phase chromatography) in Wistar rats with a dose of 10, 30 and 60 mg/kg/per animal. The following effects were observed:

- a dose-dependent inhibition of the carrageenin-induced pleurisy by reducing pleural exudate formation and PMNs infiltration;
- leukocyte cell adhesion molecules mobilization was not down-regulated on granulocytes;
- a decrease in the production of endothelial cell adhesion molecules on the lung sections.

Rodelphinidins (5, 10, 40 and 60 mg/kg) isolated from of dried *Ribes nigrum* L., folium extract demonstrated anti-inflammatory activities in a rat carrageenan paw oedema model by reducing paw oedema 18% for 5 mg/kg; 40% -10 mg/kg and 55% - 40 mg/kg, whereas reference compounds indomethacin 4 mg/kg reduced it by 44% and aspirin 200 mg/kg by 47% (Tits *et al.*, 1991).

Diuretic activity

Ethanollic extracts

In vivo studies:

The model used was a salidiuretic action. The intervention consisted of oral administration of a fluidextract (extraction solvent was ethanol; 1:1) of blackcurrant leaf in rats. The diuretic action of an equivalent of 1500 mg dried leaf/kg was comparable to the effect of furosemide at 50 mg/kg (Rácz-Kotilla & Rácz 1977).

Table 5: Overview of the main non-clinical data/conclusions

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
<i>Ribes nigrum</i> L., folium (dried) extract Extraction solvent: 70% (V/V) aqueous acetone	0.25-15 µg/mL	<i>In vitro:</i> Human umbilical vein endothelial cells	Luzak <i>et al.</i> , 2014	The anti-inflammatory activity demonstrated by increasing the CD39-positive endothelial cell fraction and enhanced endothelial nitric oxide synthase activation
<i>Ribes nigrum</i> L., folium extract, extraction solvent: 1 g of fresh leaves was ground with 1 g of quartz and 10 mL of extraction solution: acetone/water/acetic acid (70:28:2)	Extracts concentrations: 50, 25, 10, 7.5, 5, 2.5, 1, 0.5 µg/mL	<i>In vitro:</i> neutrophils The oxidant response of neutrophils and on myeloperoxidase (MPO) activity; effects on the release of MPO by stimulated neutrophils; effects on the specific activity of MPO measured by The Specific Immunological Extraction Followed by Enzymatic Detection (SIEFED)	Tabart <i>et al.</i> , 2012	The anti-inflammatory activity demonstrated by inhibition of myeloperoxidase activity and ROS production on activated neutrophils
<i>Ribes nigrum</i> L., folium extract, extraction solvent: 15% ethanol, lyophilised	a) Writhing induced by acetic acid; b) Hot-plate response in mice: Ribes extract 200 mg/kg, i.p. and morphine 4 mg/kg, i.p.	<i>In vivo:</i> <u>Swiss mice</u> a) Writhing induced by i.p. injected 0.75% acetic acid solution (10 mL/kg), extract given i.p. 30 min before acetic acid injection. ED ₅₀ estimated: the dose of the drug that reduced by 50% the number of mice exhibiting writhing compared to the control animals;	Mongold <i>et al.</i> , 1993	The analgesic effects demonstrated in Writhing induced by acetic acid test, Hot-plate response test in mice

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
		b) Hot-plate response in mice: Ribes extract has shown a peripheral analgesic effect		
<i>Ribes nigrum</i> L., folium extract, extraction solvent: 14% ethanol, lyophilised	Extract 1 mL/kg and 10 mL/kg, p.o. In chronic studies (21 and 28 days oral treatment) <i>Ribes nigrum</i> L., folium extract at the dose of 0.33, 1 and 10 mL/kg.	<i>In vivo</i> : Plethysmometric measurements in Sprague-Dawley rats – extract was given p.o. 30 minutes before carrageenan injection.	Declume <i>et al.</i> , 1989	<i>Ribes nigrum</i> L., folium extract (1 and 10 mL/kg) dose-dependently (30% and -54%, respectively) reduced rat paw oedema after 4h, compared to indomethacin (for 2.5 mg/kg was 63% and 5 mg/kg - 66%) and niflumic acid (25 mg/kg was 19% reduction and 50 mg/kg was 70%). In chronic studies at the dose of 0.33, 1 and 10 mL/kg the reduction of oedema was 30%, 42.5% and 46%, respectively, for indomethacin (1.66 mg/kg) it was 49% and niflumic acid (12.5 mg/kg) reduction was 53%.
<i>Ribes nigrum</i> L., folium	a) The carrageenan-	<i>In vivo</i> :	Mongold <i>et</i>	The anti-inflammatory

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
extract, extraction solvent: 15% ethanol, lyophilised	<p>induced paw oedema: Ribes extract 100 and 200 mg/kg i.p. or indomethacine (5 and 10 mg/kg).</p> <p>b) Cotton pellet granuloma: <i>Ribes</i> extract 100 mg/kg i.p. or indomethacine (3 mg/kg), once daily during 7 days, i.p..</p> <p>c) Freund adjuvant induced arthritis: <i>Ribes</i> extract 100 and 200 mg/kg i.p. or indomethacine (3 mg/kg), once daily during 14 days, i.p.</p>	<p><u>Wistar rats</u>;</p> <p>a) carrageenan-induced acute inflammation: <i>Ribes</i> extract and indomethacine was injected 1h prior to the subplantar injection of 1 % of carrageenan suspension into the left paws of the rat and the volume of the paw up to the ankle joint was measured by plethysmography;</p> <p>b) cotton pellet granuloma: granulomatous lesions were made by inplanting two sterilized cotton pellet (30±1 mg) subcutaneously into dorsal region of the rat;</p> <p>c) Freund adjuvant induced arthritis: was induced by single injection of <i>Mycobacterium butyricum</i> (0.05 mL) in the top of the third part of the tail</p>	<i>al.</i> , 1993	<p>activity demonstrated by the inhibition of carrageenan-induced acute inflammation, (paw oedema after 3 hours was reduced 70% by the extract 100 mg/kg and 77% by indomethacin);</p> <p>cotton pellet granuloma (weight of granuloma on the day 8 was reduced 18.6% by the extract 150 mg/kg and 24% by indomethacin 3 mg/kg);</p> <p>Freund adjuvant induced arthritis (paw volume was reduced by 18.7% for the extract 100 mg/kg, 34.6% by the extract 200 mg/kg and 37.7% by indomethacin 3 mg/kg)</p>
<i>Ribes nigrum</i> L., folium liquid extract (Extraction solvent: ethanol; DER 1:1)	Diuretic quotient = 1.56 when 50 mL fluid extract are given in dilution of 3%/kg body weight.	<i>In vivo</i> Rats	Rácz-Kotilla & Rácz, 1977	The diuretic effect of an equivalent of 1500 mg/kg dried <i>Ribes nigrum</i> L., folium was comparable to the effect of furosemide at the

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
				dose of 50 mg/kg.
Isolated constituents				
Quercetin and its metabolite quercetin-3-glucuronide	Concentrations 1.25–100 µM	<i>In vitro</i> : HL60 cells; <i>In vivo</i> : human atherosclerotic aorta	Shiba <i>et al.</i> , 2008	The anti-inflammatory activity of quercetin dehydrate and sulfatase H-1 in both models
Isolated prodelphinidines, extracted from the <i>Ribes nigrum</i> L., folium, extraction solvent: acetone (70% V/V in water), purified using reversed phase and Sephadex LH20 column chromatography	Concentrations (1 to 100 µg/mL) of different prodelphinidines	<i>In vitro</i> : purified fractions of prodelphinidines were evaluated on the cultivated human chondrocytes from cartilage: a positive effect on the production of proteoglycans with concentrations from 1 to 100 µg/mL; a positive effect on type II collagen production with concentration from 1 to 100 µg/mL; an inhibitory effect on the prostaglandin E2 (PGE2) production mainly with concentrations from 10 to 100 µg/mL.	Garbacki <i>et al.</i> , 2002	The anti-inflammatory activity of prodelphinidines was demonstrated in human chondrocytes showing the selectivity on COX-2 inhibition.
Total flavonoids, rutin and isoquercitrin, extracted from the <i>Ribes nigrum</i> L., folium, extraction solvent: ethyl acetate	No further data about the exact composition are available	<i>Ex vivo</i> : isolated rabbit hearts; arachidonic acid (100 µg) was used as a substrate.	Chanh <i>et al.</i> , 1986	Total flavonoids extracted from the leaves of <i>Ribes nigrum</i> , inhibited both biosynthesis and release of PG-like substances. They were more active than rutin and isoquercitin. The IC ₃₀ were respectively 1.03 + 0.24 mg/mL, 3.76 + 0.24 mg/mL and 2.31 + 0.40 mg/mL.

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
Prodelphinidins of dried <i>Ribes nigrum</i> L., folium, extract, extraction solvent: aqueous acetone, obtained by medium pressure liquid chromatography (MPLC)	Prodelphinidins at the dose of 5, 10, 40 and 60 mg/kg; indomethacin 4 mg/kg; aspirin 200 mg/kg; i.p. injected	<i>In vivo</i> : In carrageenan paw oedema model in rats	Tits <i>et al.</i> , 1991	Reduction paw oedema by prodelphinidins of dried <i>Ribes nigrum</i> L., folium was 18% for 5 mg/kg; 40% -10 mg/kg and 55% - 40 mg/kg; indomethacin 4 mg/kg reduced by 44% and aspirin 200 mg/kg by 47%
Proanthocyanidin-enriched fraction (PACS) of <i>Ribes nigrum</i> L., folium, extraction solvent: acetone (70% V/V in water purification by reversed phase chromatography)	Model 1 Proanthocyanidins (PACs) at the doses of 10, 30, 60, 100 mg/kg or saline; i.p. injection. Model 2 PACs at the doses of 10, 30, 60 or 100 mg/kg or indomethacin 10 mg/kg, i.p. injection. Carrageenan at the dose of 0.1 mL and 10 mg/mL	<i>In vivo</i> : Wistar rats Model 1 Carrageenin induced paw oedema: Pre-treatment of PACs was done before and 1 h, 2 h and 4 h after the injection of carrageenan. The carrageenin (0.1 mL, 10 mg/mL into the plantar region of the right hind paw) was injected 30 minutes after the PACs. Model 2 Carrageenin-induced pleurisy: pretreated with saline, PACs or indomethacin 30 min before the intrapleural injection of the carrageenin in the, pleural cavity opened after 4 hours	Garbacki <i>et al.</i> , (2004)	The anti-inflammatory effect of proanthocyanidins was demonstrated in rat's models of carrageenin-induced paw oedema and carrageenin-induced pleurisy. The main mechanism of this effect of PACs lies in an interference with the migration of the leukocytes and inhibition of <i>in vivo</i> nitric oxide release.
Proanthocyanidin-enriched fraction (PACS) of <i>Ribes nigrum</i> L., folium,	Pre-treatment with proanthocyanidins (PACs) at the doses of 10, 30 or 60 mg/kg or	<i>In vivo</i> : Male Wistar rats Carrageenin-induced pleurisy: injection of carrageenin	Garbacki <i>et al.</i> , (2005)	Anti-inflammatory activity demonstrated in rats by PACS at the doses of 10, 30 and 60

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
extraction solvent: acetone (70% V/V in water purification by reversed phase chromatography)	saline, injected i.p.	in the right pleural cavity 30 minutes after the test substances, Pleural cavity opened after 4 hours. Measurements: accumulation of exudate volume and PMNs. Flow cytometry: analysis of leukocyte cell adhesion molecules (LFA-1, Mac-1 and VLA-4) mobilization in circulating granulocytes. Immunohistochemistry on lung sections: detection of endothelial cell adhesion molecules (ICAM-I and VCAM-I).		mg/kg/rat
Proanthocyanidin-enriched fraction (PACS) of <i>Ribes nigrum</i> L., folium, extraction solvent: acetone (70% V/V in water purification by reversed phase chromatography)	0.4, 4, 40, 400, mg/g	<i>In vitro</i> : endothelial LT2 cells stimulated with TNF- α and upon PACs treatment were evaluated for ICAM-1, IL-8 and VEGF mRNA expression	Garbacki <i>et al.</i> , (2005)	Anti-inflammatory activity demonstrated in endothelial LT2 cells

3.1.2. Secondary pharmacodynamics

Antioxidative effects

In vitro studies:

The polyphenolic fraction of the water extract of *Ribes nigrum* L., folium (extraction solvent: water containing 200 ppm of SO₂, the ratio of solvent to leaves 3:1) showed antioxidant activity, protecting the pig erythrocyte membrane against free radicals induced by UV radiation (Bonarska-Kujawa *et al.*, 2014). In the study the effect of the extract on osmotic resistance, shape of erythrocytes was determined with spectrophotometric methods. The result suggest that the extract of *Ribes nigrum* L., folium protected erythrocytes against the UVC radiation, by the strengthening the membrane and inducing echinocytes (Bonarska-Kujawa *et al.*, 2014).

The antioxidant activity of the polyphenolic (mainly flavonols) extracts of *Ribes nigrum* L., folium in relation to the membrane of erythrocytes and lipids extracted from red blood cell membranes (RBCL) exposed to chemical oxidizing agent 2,2'-azobis-2-methyl-propanimidamide, dihydrochloride (AAPH) was studied fluorometrically, while effects of the extracts on the properties of membranes were examined using calorimetric, IR spectroscopy and fluorimetric methods. According to the authors, the results indicate that the compounds contained in the extracts protect erythrocyte and lipid membranes against oxidation (Cyboran *et al.*, 2014).

A spectrophotometric method was used to investigate the influence of 0.1-0.5 mg/mL of a water extract of *Ribes nigrum* L., folium on osmotic resistance of erythrocytes. On basis of the result the authors conclude that the extract of *Ribes nigrum* L., folium makes the erythrocytes less susceptible to changes in the medium tonicity and may prevent the membrane from stiffening in some pathological states (Cyboran *et al.*, 2012). Fourteen compounds, including four 7,70-epoxyignans, three tetrahydrofuran-type sesquilignans, and a spirocyclic dilignan, isolated from the leaves of *Ribes nigrum* extract (dried leaves of *Ribes nigrum* (3.0 kg) extracted with ethanol/water (7 L x 3 kg, 70:30 V/V)) were evaluated for their antioxidant activities using superoxide anion scavenging assay and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. Of all the compounds tested, ribesin D and ribesin G showed the most potent superoxide anion scavenging activity with EC₅₀ values of 1.24 and 1.12 µM, respectively (Sasaki *et al.*, 2013).

The protective effect of two synthetic antioxidants (quercetin and caffeic acid) and 70% hydroalcoholic extract of blackcurrant leaves was tested in preventing and/or reducing the membrane lipid oxidation due to free radical attack performed by using fluorescence spectroscopy techniques. According to the authors, these results show that natural antioxidants have a much higher antioxidant activity against free radicals than synthetic compounds but they degrade after two hours of oxidation (Golea *et al.*, 2012).

The antioxidant capacity of the blackcurrant leaf extract (1g of fresh leaves ground with 1 g of quartz in 10 mL of extraction solution: acetone/water/acetic acid (70:28:2)) was tested using several assays. Significant antioxidant activity was seen in the Trolox Equivalent Antioxidant Capacity (TEAC) assay (scavenging of the radical 2,2-azino-bis(3-ethylbenzothiazoline)-6 sulphonic acid, ABTS) - 53.7 ± 6.2 mg TE/g FW. The Cellular Antioxidant Activity (CAA) of blackcurrant leaves extracts were measured using the CAA assay on EAHy926 cells - CAA value was 12.89 ± 0.77 µmol QE/g of LE. The influence of *Ribes nigrum* L., folium extract on the (reactive oxygen species) ROS production of PMA-activated neutrophils was determined through a lucigenin dependent chemiluminescence assay indicating a significant dose-dependent inhibition, inducing 50% of inhibition with the concentration of 5 ± 0.5 µg of leaf extract/mL (Tabart *et al.*, 2012).

The extract (extraction solvent: water containing 200 ppm of SO₂, the ratio of solvent to leaves was 3:1) of *Ribes nigrum* L., folium (0.005-0.05 mg/mL) exhibited an antioxidant activity. It protected the membrane lipids of pig erythrocyte against oxidation, significantly reducing the level of free radicals in erythrocyte suspension detected by the Fluorimetric and Spectrophotometric method. The antioxidant activity of the extract of *Ribes nigrum* L., folium is connected mainly with activity of quercetin glucosides, the extract contains about 77% of these compounds (Cyboran *et al.*, 2011).

Anthelmintic activity

In vivo studies:

The development of free-living larvae (*Oesophagostomum dentatum*) was significantly inhibited by *Ribes nigrum* L., folium extract (acetone/water, 3:7) at different concentrations (125, 250, 500 and 1000 µg/mL) (Williams *et al.*, 2014).

Antibacterial activity

In vitro studies:

The essential oil (obtained by hydro-distillation) from the *Ribes nigrum* L., folium containing Δ³-carene (18.7%), β-caryophyllene (17.7%), sabinene (11.6%), *cis*-β-ocimene (10.6%) and α-terpinolene (10.6%) showed the antimicrobial activity against 14 micro-organisms (including *Escherichia coli*, *Streptococcus faecalis*, *Staphylococcus aureus*, *Candida albicans* and *Trichophyton mentagrophytes* isolates) detected by the broth microdilution method (Stevic *et al.*, 2010).

Antiviral effect

In vitro and *in vivo* studies:

Antiviral activity of a water extract (DER not specified) of *Ribes nigrum* L., folium (0–1 mg/mL) against influenza A virus *in vitro* was observed when the virus was pre-incubated prior to infection or when added directly after infection, however with no antiviral effect when infected cells were treated 2, 4, or 8 h after infection, indicating that the extract blocks a very early step in the virus infection cycle. *In vivo* (the C57BL/6 mouse infection model) study showed that intranasal application of the extract (500 µg) inhibits progeny virus titers in the lung up to 85% after 24 h (Haasbach *et al.*, 2014).

Ehrhardt *et al.* (2013) tested a water extract (DER not specified) of *Ribes nigrum* L., folium against influenza A viruses infections *in vitro* and *in vivo*. The extract inhibited Influenza A virus replication in a concentration dependent (50, 100 or 200 µg/mL) manner in human alveolar type II epithelial cell line A549 model. The extract did not exert any significant negative effects on cell proliferation or survival, and did not alter transcription or translation processes (Ehrhardt *et al.*, 2013). *Ribes nigrum* L., folium extract (50 and 100 µg/mL) was tested on the A549 cells using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide] - Cell Proliferation Assay that is based on an enzymatic reaction of the mitochondrial succinic dehydrogenase. The extract did not affect the cell morphology and viability and did not interfere with cellular proliferation and metabolism (Ehrhardt *et al.*, 2013). In *in vivo* study, BALB/c mice were infected with a sub-lethal dose of influenza virus A/FPV/Bratislava/79 (H7N7). Further animals were exposed to the same extract as *in vitro* studies: 2 bar of aerosolized extract of *Ribes nigrum* L., folium (prepared from the 10 and 15 mg/mL of stock solutions) at the dose of 1.5 mL/per mouse for 10 min twice a day for three (lung titer) or five (body weight) consecutive days twice a day by using the COAALA Mouse Aerosol Application System. The results showed a reduction of virus titers in the lung of infected animals already at the day three of infection (Ehrhardt *et al.*, 2013).

Antihypertensive effect

In vivo studies:

The model used was the antihypertensive effect on cats. Doses liquid extract (extraction solvent was ethanol; 1:1) equivalent to of 400 mg dried blackcurrant leaf/kg were compared to tolazoline 0.75 mg/kg and 1.0 mg/kg. The antihypertensive effects of both were comparable, but the effect of the leaf extract lasted for 20 minutes as compared to 5 minutes for tolazoline (Rácz-Kotilla & Rácz 1977).

In another study normotensive rats were used. An infusion of blackcurrant leaf (20 g/L) was administered intravenously at a dose equivalent to 360 mg dried leaf per kg. There was a 45% fall in blood pressure, which after 30 minutes was still 30% (Laserre *et al.*, 1983).

3.1.3. Safety pharmacology

No data available.

3.1.4. Pharmacodynamic interactions

No data available.

3.1.5. Conclusions

Anti-inflammatory, analgesic, diuretic and antioxidative effects of *Ribes nigrum* L., folium extracts and isolated constituents (flavonoids, proanthocyanidins including prodelphinidines) have been demonstrated in the experimental models *in vitro*, *ex vivo* and *in vivo*. The preparations tested consisted mainly of alcoholic liquid extracts, which were mostly lyophilised. They were administered per orally as well as intraperitoneally.

Several inflammatory parameters have been reported reversed, especially the formation of oedema and the cellular components as illustrated by reduced exudate, infiltration of polymorph nuclear leukocytes, release of interleukins and cytokines and the formation NO-components.

Apart from the anti-inflammatory activity also an analgesic activity has been demonstrated in the acetic acid induced writhing test and the hot-plate response with mice.

The medicinal use of *Ribes nigrum* L., folium described in several monographs and well-known handbooks as well as indications of the products available on the EU market are supported by the effects observed in the non-clinical studies:

- for the relief of minor articular pain;
- to increase the amount of urine to achieve flushing of the urinary tract as an adjuvant in minor urinary complaints.

Effects reported but not related to the indications proposed for the *Ribes nigrum* L. folium in the monograph are antioxidative effects, antimicrobial activity, antiviral activity and antihypertensive effect.

None of the reported non-clinical pharmacological studies described indicate a cause for safety concern for the *Ribes nigrum* L., folium.

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

No data with regard to absorption, distribution, metabolism, elimination and pharmacokinetic interactions with other medicinal products are available.

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

3.3.1. Single dose toxicity

Ribes nigrum L., folium liquid extract (1:1) was administered intraperitoneally to mice. The intraperitoneal LD₀ and LD₅₀ were 22 and 49 g/kg respectively. The LD₁₀₀ was estimated at 90 g/kg (ESCOP, 2003).

In another study with mice a lyophilisate obtained by maceration of 100 g leaf per litre 15% ethanol was administered. Intraperitoneal LD₅₀ was 1.09 g/kg. Oral doses up to 3 g/kg did not show overt toxicity (ESCOP, 2003; Mongold 1993).

3.3.2. Repeat dose toxicity

Subacute toxicity

Ribes nigrum L., folium was administered to rats as a lyophilised 15% ethanolic extract (1 g of extract was equivalent to 1.8 g of leaf). The extract was administered orally in daily doses of 2 g/kg/day (21 days) and 1.34 g/kg/day (28 days) respectively. No signs of toxicity or gastric ulceration was observed (ESCOP, 2003).

Ribes nigrum L., folium was administered to rats as a lyophilisate obtained by maceration of 100 g leaf per litre 15% ethanol. The extract was administered orally during 10 days without specification of the dose. No change in feeding pattern, fluid consumption or body weight was seen. Blood analysis and histopathological evaluation of 14 organs did not reveal any abnormalities (ESCOP, 2003, Mongold, 1993).

Chronic toxicity

Feeding mice with a daily dose of 3 g/kg of dried leaves during 6 months did not reveal any toxicity (Hänsel *et al.*, 1994).

3.3.3. Genotoxicity

No data available.

3.3.4. Carcinogenicity

No data available.

3.3.5. Reproductive and developmental toxicity

No data available.

3.3.6. Local tolerance

No data available.

3.3.7. Other special studies

The haemolytic activity of the water extract (extraction solvent water containing 200 ppm of SO₂) of *Ribes nigrum* L., folium (at the concentrations from the 0,01 up to 0.1 mg/mL) was conducted on fresh, heparinized blood and haemoglobin concentration in the supernatant (expressed as percentage of haemoglobin concentration of totally haemolysed cells) was assumed as the measure of the extent of haemolysis. At between 0.01 and 0.1 mg/mL, the extract did not induce haemolysis but protected erythrocytes against the UVC radiation (Bonarska-Kujawa *et al.*, 2014).

Cyboran *et al.*, (2012) also observed that the polyphenols contained a water extract of *Ribes nigrum* L., folium, do not induce haemolysis in concentrations of 0.1-0.5 mg/mL. The Cellular Antioxidant Activity of *Ribes nigrum* L., folium (1 g of fresh leaves ground with 1 g of quartz in 10 mL of extraction solution: acetone/water/acetic acid (70:28:2)) was measured using the CAA assay on EAHy926 cells. Leaf extracts had the highest CAA value (12.89 ± 0.77 µmol QE/g of LE).

Tabart *et al.*, (2012) performed a viability assay using MTT (3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide; Cell Growth Determination Kit MTT) and found that the *Ribes nigrum* L., folium (1 g of fresh leaves ground with 1 g of quartz in 10 mL of extraction solution: acetone/water/acetic acid (70:28:2)) extracts (0.1 to 1 mg extract/mL) is not cytotoxic on endothelial cells.

In the range of 0.00001–1 mg/mL the water-soluble extract of *Ribes nigrum* L., folium showed no cytotoxic effect on three cell lines (MDCK, A549 and HeLa cells). Cytotoxicity was only observed with peripheral blood mononuclear cells (CC50 of 0.5 ± 0.3 mg/mL) the extract did not affect the proliferative status of human lymphocytes (Haasbach *et al.*, 2014).

3.3.8. Conclusions

The data on toxicology of *Ribes nigrum* L., folium and relevant preparations are limited.

However, the medicinal use of *Ribes nigrum* L., folium is considered safe because no adverse effects have been reported during the long-standing use as a medicinal product in France, Poland and Spain.

Due to the lack of adequate data on genotoxicity a list entry cannot be proposed.

3.4. Overall conclusions on non-clinical data

The medicinal use of *Ribes nigrum* L., folium described in several monographs and well-known handbooks as well as of the products available on the EU market for the traditional herbal medicine is supported by the effects observed in the non-clinical (*in vitro*, *ex vivo* and *in vivo* data) studies:

- for the relief of minor articular pain;
- to increase the amount of urine to achieve flushing of the urinary tract as an adjuvant in minor urinary complaints.

Pharmacological effects not related to the indications proposed for the *Ribes nigrum* L., folium in the monograph are antioxidative effects, antimicrobial, antiviral and antihypertensive effect.

None of the reported non-clinical pharmacological studies described indicate a cause for safety concern for the *Ribes nigrum* L., folium.

The use in the European Union and available data indicate no toxicological concern and potential risks associated with *Ribes nigrum* L., folium use.

As there is no information on reproductive and developmental toxicity, the use during pregnancy and lactation cannot be recommended.

Tests on genotoxicity and carcinogenicity have not been performed.

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 clinical data available.

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

No data available.

4.2. Clinical efficacy

4.2.1. Dose response studies

No dose response studies available.

4.2.2. Clinical studies (case studies and clinical trials)

No clinical studies reported.

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

No clinical studies in special populations reported.

4.4. Overall conclusions on clinical pharmacology and efficacy

For the *Ribes nigrum* L., folium no data from clinical studies are available therefore, in accordance with Directive 2001/83/EC the well-established use cannot be supported.

The traditional herbal medicinal use of *Ribes nigrum* L., folium for the indications which are proposed in the monograph is supported by products in the EU market and information available in several monographs, pharmacopoeias and well-known handbooks.

5. Clinical Safety/Pharmacovigilance

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

No data collected.

5.2. Patient exposure

No data available.

5.3. Adverse events, serious adverse events and deaths

No data available.

5.4. Laboratory findings

None reported

5.5. Safety in special populations and situations

5.5.1. Use in children and adolescents

The use in children and adolescents has not been investigated and is not supported by the traditional use. Therefore, the use in children and adolescents under 18 years of age is not recommended.

5.5.2. Contraindications

Oedema due to heart failure or renal insufficiency is mentioned as a possible contra-indication without any further specification (Hänsel *et al.*, 1994; Gruenwald *et al.*, 2000).

The monograph includes a contraindication for persons with hypersensitivity to the active substance and in conditions where a reduced fluid intake is recommended (e.g. severe cardiac or renal disease).

5.5.3. Special warnings and precautions for use

No data available. However, to exclude serious diseases and worsening of the complaints the following warnings are proposed for the monograph:

For the indication 'Traditional herbal medicinal product for relief of minor articular pain':

- 'articular pain accompanied by swelling of joints, redness or fever, should also be examined by a doctor'.

For the indication 'Traditional herbal medicinal product to increase the amount of urine to achieve flushing of the urinary tract as an adjuvant in minor urinary complaints':

- 'if complaints or symptoms such as fever, dysuria, spasms or blood in the urine occur during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted';
- 'To ensure an increase of the amount of urine, adequate fluid intake is required during treatment'. In the revised monograph this warning/recommendation text is moved from the section '4.2. Posology and method of administration' part 'method of administration' to the section '4.4. Special warnings and precautions for use' which is considered to be more relevant section for this warning. Furthermore, this modification is also in accordance with other recently adopted EU herbal monographs for traditional herbal medicinal products'.

For both indications:

- The use in children and adolescents under 18 years of age has not been established due to lack of adequate data.
- If the symptoms worsen during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.

5.5.4. Drug interactions and other forms of interaction

No data available.

5.5.5. Fertility, pregnancy and lactation

No data is available, therefore, use of *Ribes nigrum* L., folium cannot be recommended during pregnancy and lactation.

No fertility data available.

5.5.6. Overdose

No data available.

5.5.7. Effects on ability to drive or operate machinery or impairment of mental ability

No data available.

5.5.8. Safety in other special situations

No data available.

5.6. Overall conclusions on clinical safety

For *Ribes nigrum* L., folium there is no clinical safety data available. Also, the use in children or adolescents is not documented in literature.

The available information in the literature and pharmacovigilance data of marketed products do not indicate safety concerns for *Ribes nigrum* L., folium. As a precautionary measure a contraindication and warnings are included in the monograph.

6. Overall conclusions (benefit-risk assessment)

Due to the lack of data from clinical studies, well-established use for *Ribes nigrum* L., folium in accordance with Article 10a of Directive 2001/83/EC, is considered not fulfilled.

The traditional medicinal use of *Ribes nigrum* L., folium according to Directive 2004/24/EC is considered fulfilled based on the information available in several pharmacopoeias, relevant medicinal handbooks, and the information provided by the National Competent Authorities. The available information substantiates the presence of medicinal products on the EU market throughout a period more than 30 years, including at least 15 years within the EU. In the 2017 revision of the monograph, powdered herbal preparation was added because this preparation has been on the market in France for more than 30 years.

The traditional herbal medicinal use of *Ribes nigrum* L., folium for the indications and posologies which are proposed in the monograph is supported by products on the EU market, *in vitro* and *in vivo* pharmacological results and information available in several monographs, pharmacopoeias and well-known handbooks:

For oral use:

- 1) 'Traditional herbal medicinal product for relief of minor articular pain'

2) 'Traditional herbal medicinal product to increase the amount of urine to achieve flushing of the urinary tract as an adjuvant in minor urinary complaints'

The indications are considered suitable for self-medication.

There are no published reports on serious side effects with the herbal substance or herbal preparations thereof.

There are no concerns of possible drug interactions.

No data on fertility, reproductive and developmental toxicity and the usage in children and adolescents is available. Therefore, the use is not recommended in children, adolescents and during pregnancy and lactation.

A European Union list entry is not supported due to lack of adequate data on genotoxicity.

The therapeutic areas for browse search on the EMA website are 'Pain and inflammation' and 'Urinary tract and genital disorders'.

No constituents with known therapeutic activity or active markers could be identified by the HMPC.

Annex

List of references