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SCIENCE MEDICINES HEALTH

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Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Olea europaea* L., folium

Final

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>Olea europaea</i> L. folium (olive leaf)
Herbal preparation(s)	i) Herbal substance Fresh or dried leaves ii) Herbal preparations a) Comminuted dried leaves for herbal tea b) Powdered dried leaves
Pharmaceutical form(s)	Herbal substance and comminuted herbal substance as herbal tea for oral use Powdered dried leaves in solid dosage form for oral use
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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	7
2.1. Information about products on the market	7
2.1.1. Information about products on the market in the EU/EEA Member States	7
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	10
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	13
3.1.3. Safety pharmacology	25
3.1.4. Pharmacodynamic interactions	25
3.1.5. Conclusions	25
3.2. Overview of available pharmacokinetic data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof.....	26
3.3. Overview of available toxicological data regarding the herbal substance(s)/herbal preparation(s) and constituents thereof	26
3.3.1. Single dose toxicity.....	26
3.3.2. Repeat dose toxicity.....	26
3.3.3. Genotoxicity	27
3.3.4. Carcinogenicity.....	27
3.3.5. Reproductive and developmental toxicity	27
3.3.6. Local tolerance	27
3.3.7. Other special studies.....	27
3.3.8. Conclusions	27
3.4. Overall conclusions on non-clinical data	28
4. Clinical Data	28
4.1. Clinical pharmacology	28
4.1.1. Overview of pharmacodynamic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents.....	28
4.1.2. Overview of pharmacokinetic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents.....	28
4.2. Clinical efficacy	29
4.2.1. Dose response studies.....	29
4.2.2. Clinical studies (case studies and clinical trials)	29
4.3. Clinical studies in special populations (e.g. elderly and children)	37
4.4. Overall conclusions on clinical pharmacology and efficacy.....	37
5. Clinical Safety/Pharmacovigilance	37
5.1. Overview of toxicological/safety data from clinical trials in humans.....	37

5.2. Patient exposure	42
5.3. Adverse events, serious adverse events and deaths.....	42
5.4. Laboratory findings.....	42
5.5. Safety in special populations and situations	42
5.5.1. Use in children and adolescents.....	42
5.5.2. Contraindications.....	43
5.5.3. Special warnings and precautions for use	43
5.5.4. Drug interactions and other forms of interaction.....	43
5.5.5. Fertility, pregnancy and lactation.....	43
5.5.6. Overdose.....	43
5.5.7. Effects on ability to drive or operate machinery or impairment of mental ability	43
5.5.8. Safety in other special situations	43
5.6. Overall conclusions on clinical safety.....	43
6. Overall conclusions (benefit-risk assessment).....	44
Annex	45

1. Introduction

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

Olea europaea L. belongs to the Oleaceae family. The name *O. europaea* is a synonym of *O. officinarum* Crantz; *O. pallida* Salisb. applies to both the wild *O. europaea* ssp. *sylvestris* (Mill.) Rouy (syn *O. oleaster* Hoffm. et Link, *O. sylvestris* Mill.) and domestic (cultivated) plant which is mainly known as *O. europaea* ssp. *sativa* (Hoffm. et Link) Rouy (syn = *O. europaea* L. var *europaea*, *O. europaea* ssp. *sativa* Loud., *O. europaea* L. ssp. *sativa* Arcang., *O. gallica* Mill., *O. hispanica* Mill., *O. lancifolia* Moench, *O. sativa* Gaterau). Several varieties have been recognised. More than 300 are differentiated, among which more than 150 only in Italy for oil or table olive production (Blaschek et al. 2006). The olive tree (*O. europaea*) is an evergreen that grows to approximately 6-9 metre in height. It is native to the southern European countries and throughout the entire Mediterranean region as far as Iran and beyond the Caucasus. Olive trees are also cultivated in similar climate zones in the Americas. Leaves are narrow opposite, lanceolate or linear, with entire margins and acute tips, silver-green (greyish green) on top, the underside lighter, containing fine white, scale-like hairs.

- Herbal substance(s)

According to the European Pharmacopoeia monograph "Oleae folium" 01/2009:1878, *Olea europaea* L., folium is the dried leaf of the plant containing minimum 5% of oleuropein (C₂₅H₃₂O₁₃; Mr 540.5) (European Pharmacopoeia 01/2009:1878).

The leaf is simple, thick and coriaceous, lanceolate to obovate, 30–50 mm long and 10–15 mm wide, with a mucronate apex. The upper surface is greyish-green, smooth and shiny, the lower surface paler and pubescent (European Pharmacopoeia 01/2009:1878) as well as it is referred in other National Pharmacopoeias as in Ph Belg. V, Ph Fra. IX, Ned F. 6 (Van Hellemont 1986).

The leaves are harvested throughout the year from cultivated trees and dried in the shade. The herbal substance tastes bitter. It can be identified by its microscopic characteristics, particularly the presence of many shield-shaped covering trichomes and sclerites clearly visible in the powder; these are long, have thick walls, are bent here and there, are highly refringent and end as if they were truncated. These characteristics allow verification of the identity of the herbal substance, which in addition, is characterised by the presence of triterpenes (by the red colour developed by an ether extract in the presence of acetic anhydride and sulphuric acid). The assay includes thin layer chromatography (TLC) to show the presence of oleuropein (Bruneton 1999).

Constituents of olive leaves

- Iridoid monoterpenes: including among others, oleuropein (5–9%), additionally 6-O-oleuropeine saccharose, ligstroside, oleoroside etc.
- Triterpenes: including oleanoli acid, maslinic acid etc.
- Flavonoids: luteolin, kaempferol, chrysoeriol and apigenin derivatives etc.
- Chalcones: olivine, olivine-4'-O-diglucoside etc.
- Phenolic acids: cumaric acid, caffeic acid, ferulic acid, vanillic acid etc.
- Coumarins: aesculetin, scopoletin, aesculin.

(PDR for Herbal Medicines 2007)

Additional analytical information

The main constituents of olive leaves are secoiridoids like oleuropein, ligstroside, 1 methyloleuropein, and oleoside (Gariboldi *et al.* 1986) as well as flavonoids (apigenin, kaempferol, luteolin, chrysoeriol) and phenolic compounds (caffeic acid, tyrosol, hydroxytyrosol) (Ross 2005).

Two new phenolic compounds were isolated from fruits of *O. europaea*, Hojiblanca cultivar. The first compound is the methyl acetal of the aglycone of ligstroside, while the second derivative, not yet reported in the literature, is the β -hydroxytyrosyl ester of methyl malate. These microcomponents may be responsible for hedonistic-sensorial characteristics of olive products (Bianco *et al.* 2006).

The secoiridoids is a very specific group that are abundant in Oleaceas and many other plants that are produced from the secondary metabolism of terpenes as precursors of various indole alkaloids and are usually derived from the oleoside type of glucoside oleosides, which are characterised by a combination of elenolic acid and a glucosidic residue. It can be stated that these compounds proceed from the acetate/mevalonate pathway (Gariboldi *et al.* 1986).

Oleuropein **1** (see **Figure 1**), the major constituent of the secoiridoid family in the olive trees, is a complex phenol present in large quantities in olive tree leaves, in low quantities in olive oil (Soler-Rivas *et al.* 2000) and is responsible for the bitter taste and pungent aroma of olive oil. It has been discovered in 1908 by Bourquelot and Vintilesco, and its structure was specified as being that of a heterosidic ester of elenolic acid and dihydroxyphenylethanol by Panizzi *et al.* 1960, with the empirical formula $C_{25}H_{32}O_{13}$ (Pannizi *et al.* 1960). Oleuropein can be hydrolyzed to hydroxytyrosol, elenolic acid, oleuropein aglycone and glucose (Manna *et al.* 2004). Two of its by-products are also present in the olive plant together with oleuropein **1** and the mono-demethyl-derivatives **2** and **3** (see **Figure 1**). Compound **2** is demethyl-oleuropein, which differs from oleuropein **1** in having a free carboxylic group on the pyranosic ring. Compound **3** is the oleoside methyl ester, known also as a glucoside of the elenolic acid, in which the carboxyl that esterifies the dihydroxy-phenyl-ethanol in the oleuropein **1** is here the free functionality. The two acid compounds **2** and **3** are two indicators of maturation of the olive. Their relative quantity, as regards to the oleuropein **1**, increases in fact as soon as the maturation proceeds, while the quantity of oleuropein decreases. This datum is in connection with the increase of the activity of the hydrolytic enzymes with the progress of the maturation, particularly to the activity of the esterases, responsible of the hydrolysis of the two ester bonds of the oleuropein (Amiot *et al.* 1989). The ligstroside **4** (Asaka *et al.* 1972) differs from the oleuropein **1** in the presence of a tyrosol residue instead of dihydroxy-phenyl-ethyl alcohol (see **Figure 1**). The dimethylester of the oleoside **5** (see **Figure 1**), also as glucoside of the methylester of the elenolic acid, contains the two acidic functions of the oleuropein esterified with the residue of methanol (Gariboldi *et al.* 1986). The oleuroside **6** (see **Figure 1**) is an isomer of the oleuropein, differing from **1** in the exocyclic double bond position and its structure was determined as secoxyloganin 3,4-dihydroxyphenethyl ester (Kuwajima *et al.* 1985; Khan *et al.* 2007). Triterpenes have been also isolated like maslinic acid, β -amyrin, oleanolic and maslinic acid, the occurrence of maslinic acid in fresh leaves of *O. europaea* strongly supports it is a true metabolite of the plant. Recently it has been reported that maslinic acid is produced, during the ageing of olive husks, possibly through microbial α -hydroxylation of oleanolic acid. Furthermore, to their knowledge, this appears to be the first record of isolation of β -amyrin in *O. europaea* (Mussini *et al.* 1975). Also, several alkaloids have been determined in the leaves of *Olea* like cinchonine and cinchonidine derivatives (Bezanguer-Beauquesne *et al.* 1990; Ross 2005).

Olive leaves contain around 60–90 mg/g (dry weight) oleuropein, (Le Tutour *et al.* 1992) plus significant levels of a glucosidic ester of elenolic acid and hydroxytyrosol (3,4-dihydroxyphenylethanol). However, it turns out that oleuropein and the products of its hydrolysis, oleuropein aglycone, elenolic acid, beta-3,4-dihydroxyphenethyl alcohol and methyl-o-methyl elenolate, are the major molecules of interest biologically (Fleming *et al.* 1973).

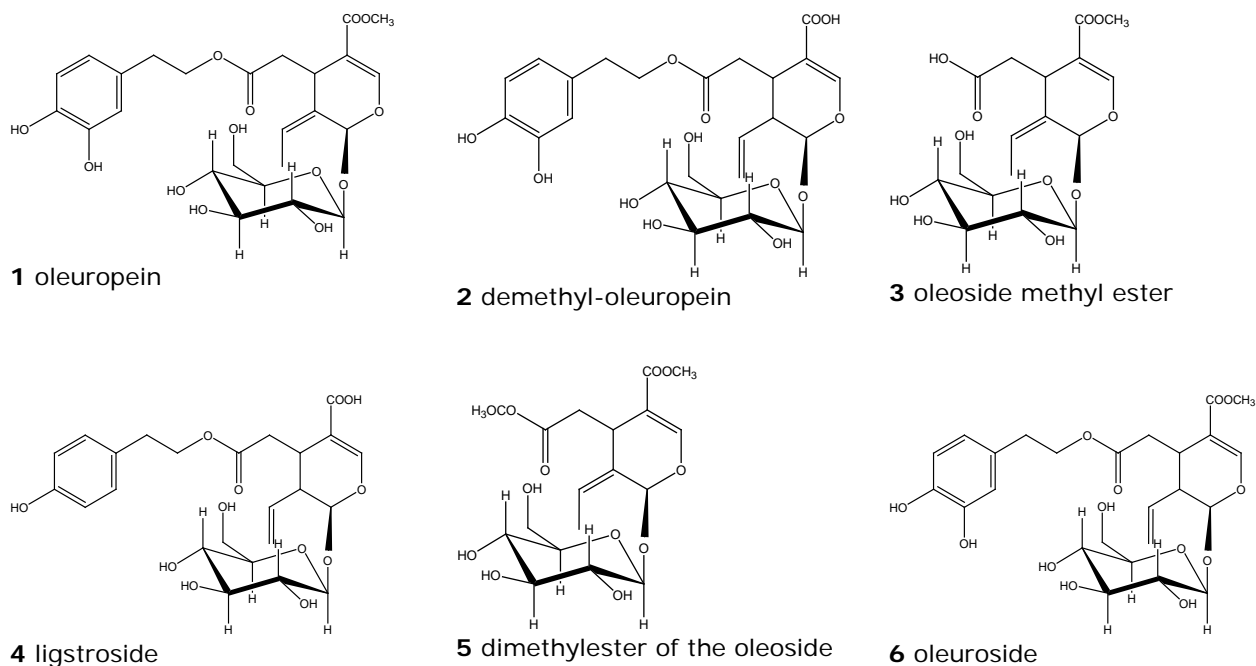


Figure 1. Relevant structures of constituents of olive leaves (Bruneton 1999).

- Herbal preparation(s)

Comminuted or powdered dried leaves.

Olive leaf extract is derived from the leaves of the olive tree. The olive leaf dry extract complies with the European Pharmacopoeia monograph "Oleae folii extractum siccum" 04/2009:2313 of European Pharmacopoeia.

- 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.

Oleae folium is reported to be used in combination with *Rauwolfia* (Holzhauer & Knobloch 1950), *Veratrum* or with *Viscum album*.

This assessment and the monograph refer exclusively to the use of *Oleae folium* as a single ingredient.

1.2. Search and assessment methodology

The assessment is based on the sources mentioned in the list of references. Publications in languages other than English (at least abstract in English or other language available) were also included in the assessment.

Scientific databases: Scifinder, Scopus; HealLink, search date January–May 2016; key words: "*Olea europaea* L., olive leaf, hydroxyl-tyrosol, oleuropein

Medical databases: Pubmed key words: *Olea europaea* L., olive leaf, hydroxyl-tyrosol, oleuropein

Clinical trials were searched in computerised general and specialised databases (MEDLINE (1966 to 2016), EMBASE, Cochrane Library, Phytodok), by checking bibliographies, and by contacting relevant manufacturers and researchers.

Other resources: Library of the National Kapodistrian University of Athens (Pharmacy and Pharmacognosy library)

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

The following information has been received on products in European Union, see table 1.

Table 1: Overview of data obtained from marketed medicinal products

Active substance	Indication	Pharmaceutical form	Regulatory Status
Olive leaf extract (1:0.71-0.86), extraction solvent: ethanol 96% V/V	Traditionally used to support cardiovascular system	Liquid and coated tablet for oral use Posology in adults: 30–50 drops, 3 times daily (or 2 times daily 45–75 drops) 100 g (= 98 ml) liquid contain 18.2 g extract, 1 g = 28 drops Daily dose: 3–5 coated tablets containing 14 mg dry extract each divided into 2–3 single doses	Germany 1976 until 2011*
Powdered or cut leaves for oral use	Traditionally used to enhance the renal excretion of water and to support the cardiovascular system.	For oral use in adults: 3 times daily (210–400 mg)	Spain since 1986
Powdered dried olive leaves	Traditionally used to promote urinary and digestive elimination functions	Hard capsules (containing 275 mg powder each) for oral use in adults: 3–5 times daily	France since 1980

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

*The marketing authorisation for the olive leaf extract (1:0.71-0.86), extraction solvent: ethanol 96% V/V was TU according to Section 109a in connection with Section 105 German Medicinal Products Act and valid until 04/2011.

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

Not applicable

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

Not applicable

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

The leaves of the olive tree *O. europaea* have been widely used in folk medicine in regions around the Mediterranean Sea and the islands therein (Bouaziz and Sayadi 2005).

Olive leaf and extracts are utilised in the complementary and alternative medicine community for its ability to act as a natural pathogens killer by inhibiting the replication process of many pathogens (Juven and Henys 1972).

Olive leaf is commonly used to fight colds and flu, yeast infections, and viral infections such as the hard-to-treat Epstein-Barr disease, shingles and herpes. Olive leaf is also used to support the heart and has been shown to reduce low-density lipoproteins (LDL), lower blood pressure and increases blood flow by relaxing the arteries (Ross 2005).

Olive leaf extract has been used as a folk remedy for combating fevers and other diseases such as malaria. In addition, several reports have shown that it has the capacity to lower blood pressure in animals, to increase blood flow in the coronary arteries, to relieve arrhythmia and to prevent intestinal muscle spasms (Samuelson 1951; Zarzuelo 1991).

Interest in the potential benefits of extracts from the olive tree originates from two main independent historical sources. The first formal medical mention of the olive leaf, an account describing its ability to cure severe cases of fever and malaria, occurred about 150 years ago. In 1854, the *Pharmaceutical Journal* published a report by Daniel Hanbury. The author wrote he discovered the effective tincture in 1843 and had used it successfully. As second source appear records that in the early 19th century, Spanish physicians sometimes prescribed olive leaves as a "febrifuge" and consequently, during the Spanish war of 1808–1813, the French Officers de Santé often used them to treat cases of "intermittent fever". This method became well known in England for treating sick Britons returning from tropical colonies. The author believed that a bitter substance in the leaves was the key healing ingredient (Cruess & Alsberg 1934; Samuelsson *et al.* 1951; Veer *et al.* 1957).

Decades later, scientists isolated a bitter substance from the leaf and named it oleuropein. It was reported that it makes the olive tree particularly robust and resistant against insect and bacterial damage. Oleuropein is an iridoid, a structural class of chemical compounds found in plants. It is present in olive oil, throughout the olive tree, and is the bitter material that is eliminated from the olives when they are cured. In 1962, an Italian researcher reported that oleuropein lowered blood pressure in animals (Panizzi L. *et al.* 1960). This triggered scientific interest in the olive leaf. Other European researchers confirmed this interesting finding. In addition, they found it could also increase blood flow in the coronary arteries, relieve arrhythmias, and prevent intestinal muscle spasms (Petkov & Manolov 1972; Juven & Henys 1972; Kubo & Matsumoto 1984). The dried or fresh leaves have been also used against malaria as antipyretic as well as diuretic (Cruess & Alsberg 1934; Samuelsson *et al.* 1951; Veer *et al.* 1957; Zarzuelo 1991).

Herbal substance: fresh or dried leaves as infusions or decoctions (Petkov & Manolov 1972; Juven & Henys 1972; Kubo & Matsumoto 1984; Duke 2002; Raynaud 2005)

Herbal preparations: Comminuted leaves for herbal tea 6–10 g/200 ml, up to three times day (daily dose of 30 g) (Petkov & Manolov 1972; Juven & Henys 1972; Kubo & Matsumoto 1984; Duke 2002; Raynaud 2005).

France: ‘Feuille d’olivier’ Olive leaf is regarded as one of the herbal substances whose efficacy and safety has been proven by thorough literature studies and long-term traditional use (Agence du Medicament, Paris 1998) stating the use of olive leaf as digestive and as mild diuretic (“pour faciliter les fonctions d’élimination urinaire et digestive, pour favoriser l’élimination renale d’eau”) (Oliviane UPSA Fr for diuresis) (Martindale 1996; Todd RG Martindale 24th ed. 1967). It has also been used in combination with a water extract of birch.

Germany: Olive leaves have been used traditionally at least since 1976, for the prevention of atherosclerosis and against hypertension (Martindale 1996). They have also been used in combination either with *Rauwolfia* (Holzhauer & Knobloch 1950), *Veratrum* or *Viscum album*.

The Commission E issued a negative monograph (Blaschek *et al.* 2006; Blumental *et al.* 1998).

Spain: Infusion of the leaf is taken orally for hypertension. Extracts of the leaf is taken orally for gastrointestinal colic. Leaves are eaten for diabetes (Khan *et al.* 2007).

Madeira: Infusion of leaves of *O. europaea* var. *maderensis* is taken orally as an antihypertensive.

Canary Islands: An infusion prepared from the fresh or dried leaf is taken orally as hypoglycemic agent. Leaves are taken orally as hypotensive and administered per rectum for haemorrhoids.

Greece: Hot water extract of the leaf is taken orally for high blood pressure (Khan *et al.* 2007).

Italy: Infusion of the fresh leaf is taken orally as a hypotensive and applied externally as a vulnerary, emollient for ingrown nails, and restorer of epithelium (Mainoli 1951).

Bulgaria: Hot water of the fresh or dried leaves is taken orally to treat hypertension (Petkov 1979).

Moreover, the following worldwide information has been received (Khan *et al.* 2007):

Argentina: Decoctions of the dried fruit and of the dried leaf are taken orally for diarrhoea and to treat respiratory and urinary tract infections (Anesini *et al.* 1993)

Brazil: Hot water of the fresh leaves is taken orally to treat hypertension and to induce diuresis.

Cuba: Hot water of the fresh leaves is taken orally to treat hypertension (Herrera Sotolongo 1952).

Kenya: Stem, fresh and dried twigs of *O. europaea* ssp. *africana* are used as a chewing stick.

Mexico: Decoction of dried leaves is taken orally for diabetes.

Morocco: Leaves are taken orally for stomach and intestinal disease and used as a mouth cleanser. Essential oil made from the leaves is taken orally for constipation, liver pain and tonic and applied externally for hair care.

Oman: Barks and leaves are applied externally for skin rash. The cataplasm prepared from leaves is applied externally for ulcers.

Reunion Island: Hot water extract of the dried *O. europaea* ssp. *africana* plant is taken orally for diabetes, diarrhoea, rheumatism, fever and gastroenteritis in infants.

Serbia (former Yugoslavia): Hot water extract of the dried leaf orally for diabetes (Ross 2005).

Tunisia: Extract of the dried leaf is taken orally for diabetes and as a hypotensive.

Ukraine: Hot water extract of dried plant leaf is taken orally for bronchial asthma.

Table 2: Overview of historical data

Herbal preparation	Documented use / Traditional use	Pharmaceutical form	Reference
Fresh or dried or comminuted dried leaves	Diuretic	6–10 g dried leaves /200 ml, up to three times day (daily dose of 30 g) Up to 2–4 weeks	Petkov & Manolov 1972; Juven & Henys 1972; Petkov 1979; Kubo & Matsumoto 1984; Duke 2002; Raynaud 2005
Fresh or dried leaves	Mild diuretic	Up to 20 g of fresh or up to 10 g of dried olive leaves in 300 ml of water, boiled until the water reach 200 ml, filter (decoction). To be consumed hot twice a day (morning and evening) Up to 2–4 weeks	Van Hellemont 1986
Dried leaves or comminuted dried as herbal tea	Mild diuretic To support cardiovascular system	7–8 g dry leaf in 150 ml water, 3–4 times daily or 2 teaspoons of leaf in hot water, steep for 30 minutes 3–4 times daily	Van Hellemont 1986; Raynaud 2005; Duke 2002; PDR 2007; Petkov & Manolov 1972; Juven & Henys 1972; Kubo & Matsumoto 1984
Comminuted or powdered dried leaves	As a mild diuretic (“pour faciliter les fonctions d’élimination urinaire et digestive, pour favoriser l’élimination renale d’eau”)	Herbal tea oral use in adults: several times daily (posology not specified)	Martindale 1996; Todd RG Martindale 24 th ed. 1967.

2.3. Overall conclusions on medicinal use

On the basis of the information on traditional and current indications and data from the overview of European market it is confirmed the existence of marketed product (see table 3).

Table 3: Overview of evidence on period of medicinal use

Herbal preparation	Indication	Posology, Strength	Period of medicinal
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Pharmaceutical form			use
Powdered dried leaves	Traditionally used to promote urinary and digestive elimination functions	Capsules of 275 mg, 3–5 times daily Daily dose: 825–1375 mg	France since 1980
Powdered or cut dried leaves for oral use	Traditionally used to enhance the renal excretion of water and to support the cardiovascular system	Capsules of 210–400 mg 3 times daily Daily dose: 630–1200 mg Posology for oral use in adults	Spain since 1986
Olive leaf extract (1:0.71-0.86), extraction solvent: ethanol 96% V/V	Traditionally used to support cardiovascular system	Liquid and coated tablet for oral use Posology in adults: 30–50 drops, 3 times daily (or 2 times daily 45–75 drops) 100 g (= 98 ml) liquid contain 18.2 g extract, 1 g = 28 drops Daily dose: 3–5 coated tablets containing 14 mg dry extract each divided into 2–3 single doses	Germany 1976 until 2011
Fresh or dried or comminuted dried leaves	Diuretic	6–10 g dried leaves /200 ml, up to three times day (daily dose of 30 g) Up to 2–4 weeks	Petkov & Manolov 1972; Juven & Henys 1972; Petkov 1979; Kubo & Matsumoto 1984; Duke 2002; Raynaud 2005
Fresh or dried leaves	Mild diuretic	Up to 20 g of fresh or up to 10 g of dried olive leaves in 300 ml of water, boiled until the water reach 200 ml, filter (decoction). To be consumed hot twice a day (morning and evening) Up to 2–4 weeks	Van Hellemont 1986
Dried leaves or comminuted dried as herbal tea	Mild diuretic To support	7–8 g dry leaf in 150 ml water, 3–4 times daily or 2 teaspoons of leaf in hot	Van Hellemont 1986; Raynaud 2005; Duke 2002; PDR 2007;

	cardiovascular system	water, steep for 30 minutes 3-4 times daily	Petkov & Manolov 1972; Juven & Henys 1972; Kubo & Matsumoto 1984
Comminuted or powdered dried leaves	As a mild diuretic ("pour faciliter les fonctions d'élimination urinaire et digestive, pour favoriser l'élimination renale d'eau")	Herbal tea oral use in adults: several times daily (posology not specified)	Martindale 1996; Todd RG Martindale 24 th ed. 1967.

Folk uses as diuretic and to support the cardiovascular system are historically well documented. However, in accordance with the requirements for traditional registration and, in particular, suitability for self-medication, HMPC considered that only the use to promote the renal elimination of water, in mild cases of water retention can be acceptable.

Therefore, the HMPC agreed (2010) that products used for more than 35 years in Germany [Liquid extract (1:0.71-0.86 solvent: ethanol 96% V/V) and Dry extract (7.9-12:1), extraction solvent: ethanol 96% V/V] should be excluded from the preparations proposed as traditional herbal medicinal products as the indication which is in use in Germany "...to support the cardiovascular system" does not comply with the indication accepted by the HMPC for self-medication products.

On the basis of the available information on traditional use, the data from the overview of the European market and bibliographic references, the existence of herbal substances and herbal preparations (marketed products) fulfilling the criteria of the safe use for a period of more than 30 years in the market is confirmed. Moreover, according to the data on clinical efficacy (see section 4.2) and the requirement to ensure a safe use in the specified conditions of use, the traditional therapeutic indication is reworded as follows: "Traditional herbal medicinal product used to promote the renal elimination of water, in mild cases of water retention after serious conditions have been excluded by a medical doctor".

The traditional use in the above mentioned indication is substantiated for the herbal substance and the following preparations for oral use:

Herbal substance:

- fresh or dried leaves (herbal tea as a decoction or infusion)

Herbal preparations:

- comminuted dried leaves (as herbal tea)
- powdered dried leaves

Based on the products on the market, the available literature references the following posologies are proposed:

Posology:

Adults and elderly

i) Herbal substance

Herbal tea: 10 g fresh leaves or 5 g of dried leaves in 150 ml of boiling water as herbal decoction 2 times daily (morning and evening).

Decoction time: allow to simmer to reach 100 ml of decoction (Van Hellemont 1986).

i) Herbal substance and ii) Herbal preparations

Dried leaves or a) comminuted dried leaves

6–10 g herbal substance or comminuted herbal substance as herbal infusion up to 3 times daily. (Van Hellemont 1986; Duke 2002; Raynaud 2005).

Daily dose: 6–30 g (Van Hellemont 1986; Duke 2002; Raynaud 2005).

ii) Herbal preparation

Powdered herbal substance:

Single dose: 275 mg 3–5 times daily or 210–400 mg 3 times daily.

Daily dose: 630–1375 mg.

Duration of use

Up to 2–4 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

O. europaea and its products are well known mainly for their phenolic content (Visioli *et al.* 2002). Although tradition attributes to the olive tree leaf numerous properties (febrifuge, hypoglycaemic, hypotensive, diuretic, and more) very few of them have been studied experimentally.

Renal effects

Ethanol:water (50:50) extracts of fresh leaf from Brazil, given intragastrically (IG) to the rats in doses of 40 ml/kg, showed diuretic and anti-hypertensive activity (Ribeiro *et al.* 1986; Riberio *et al.*, 1988)

Table 4: Overview of the main non-clinical data/conclusions

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
Ethanol:water (50:50) extracts of fresh leaf from Brazil	In doses of 40 ml/kg	<i>In vivo</i> given IG to the rats	Ribeiro <i>et al.</i> 1986; 1988	Diuretic effect

3.1.2. Secondary pharmacodynamics

Recently (Vogel *et al.* 2015) reviewed the scientific literature published in the LILACS-BIREME, SciELO and MEDLINE databases for publications in English, Portuguese and Spanish with the descriptors "*Olea europaea*", "olive leaves", "olive leaf", "olive leaves extracts", "olive leaf extracts", "phenolic compounds", "polyphenols", "oleuropein", "chemical composition", and "health". Ninety-two articles

were identified, but only 38 related to the objectives of the study (benefits of the polyphenols of olive leaves to human health) and 9 articles cited in the works were included due to their relevance. The review showed that the phenolic compounds present in olive leaves, especially the oleuropein, are associated to antioxidant, antihypertensive, hypoglycemic, hypocholesterolemic and cardioprotective activity. Furthermore, studies associate the oleuropein to an anti-inflammatory effect in trauma of the bone marrow and as a support in the treatment of obesity.

The olive leaf extract is used to enhance the immune system, as an antimicrobial, antiviral, as an antioxidant, hypoglycaemic agent and in cardiovascular problems (PDR for Herbal Medicines 2007) as follows:

Smooth muscle relaxant effects

In experiments demonstrating that a dried extract of olive leaf has relaxant effects on both isolated rat ileal tissue and rat tracheal segments, the effects were not altered in the presence of calcium antagonists including verapamil and nifedipine. However, the authors discuss that it could be possible that olive leaf extract alters calcium transport through an increase in the intracellular concentration of cyclic adenosine monophosphate (Fehri *et al.* 1995).

Effects on the inflammatory response

The effects of fresh olive leaf extracts of Italian provenance were assessed *in vitro* for effects on the complementary system, both the alternative and classical pathways. Neither ethyl acetate (50 µg/ml) nor methanol (50 µg/ml) extracts inhibited the alternative pathway while both inhibited the classical pathway, at $IC_{50} > 7.7$ µ/ml (EtOAc) and > 5.8 µ/ml (MeOH) (Pieroni *et al.* 1996).

Aqueous leaf extracts from Tunisia, given intragastrically to the rat (dose unspecified), showed activity against carrageenan-induced paw oedema (Fehri *et al.* 1996).

Recent studies suggest that olive leaf extracts suppress inflammation and reduce stress oxidative injury. The authors sought to extend these observations in an *in vivo* study of rat cerebral ischemia-reperfusion injury. Four groups, each of 18 Wistar rats, were studied. One (control) group received distilled water, while three treatment groups received oral olive leaf extract (no further specification) (50, 75 and 100 mg/kg/day respectively). After 30 days, blood lipid profiles were determined, before a 60 min period of middle cerebral artery occlusion (MCAO). After 24 h reperfusion, neurological deficit scores, infarct volume, brain edema, and blood-brain barrier permeability were each assessed in subgroups of six animals drawn from each main group. Olive leaf extract reduced the LDL/HDL ratio in doses 50, 75, and 100 mg/kg/day in comparison to the control group ($P < 0.001$), and offered cerebroprotection from ischemia-reperfusion. For controls vs. doses of 50 mg/kg/day vs. 75 mg/kg/day vs. 100 mg/kg/day, attenuated corrected infarct volumes were 209.79 ± 33.05 mm³ vs. 164.36 ± 13.44 mm³ vs. 123.06 ± 28.83 mm³ vs. 94.71 ± 33.03 mm³; brain water content of the infarcted hemisphere $82.33 \pm 0.33\%$ vs. $81.33 \pm 0.66\%$ vs. $80.75 \pm 0.6\%$ vs. $80.16 \pm 0.47\%$, and blood-brain barrier permeability of the infarcted hemisphere 11.22 ± 2.19 µg/g vs. 9.56 ± 1.74 µg/g vs. 6.99 ± 1.48 µg/g vs. 5.94 ± 1.73 µg/g tissue ($P < 0.05$ and $P < 0.01$ for measures in doses 75 and 100 mg/kg/day vs. controls respectively). Oral administration of olive leaf extract reduces infarct volume, brain edema, blood-brain barrier permeability, and improves neurologic deficit scores after transient middle cerebral artery occlusion in rats (Mohagheghi *et al.* 2011).

This activity appeared to reside in several flavonoids present in the olive leaf extracts. This reported anti-complement *in vitro* activity of the Olive leaf is a proposed mechanism of its anti-inflammatory effects. From this extract of olive (*O. europaea* L.) leaves showing anti-complementary activity, the flavonoids apigenin, apigenin-4'-O-rhamnosylglucoside, apigenin-7-O-glucoside, luteolin, luteolin-4'-O-glucoside, luteolin-7-O-glucoside, chrysoeriol, chrysoeriol-7-O-glucoside and quercetin-3-O-

rhamnoside were isolated. Major isolated constituents strongly inhibited the classical pathway of the complement system (Pieroni *et al.* 1996; PDR for Herbal Medicines, 2007).

Hepatic S-transferase properties

In vivo glutathione S-transferase induction activity has been demonstrated in mice given olive leaf extracts in the diet (ethyl acetate extract: 0.4% of diet; methanol extract: 1% of diet) (Han *et al.* 2001).

Antiviral properties

Animal experiments studies suggested that olive leaf extracts possess antiviral activity against viral haemorrhagic septicaemia rhabdovirus (VHSH) (Micol *et al.* 2005). Oleuropein has been claimed in a United States patent to have potent antiviral activities against DNA and RNA viruses such as herpes mononucleosis, hepatitis virus, rotavirus, bovine rhinovirus, canine parvovirus, and feline leukaemia virus (safe and effective natural antiviral agents, the antiviral activity of a commercial extract of olive leaves *O. europaea*, and its major component, oleuropein, were tested against a model rhabdovirus such as the VHSV, which infects continental and sea farmed fish and a wide range of wild marine species in Europe, North America and Japan. The results presented showed the inhibitory action of both extract and oleuropein against VHSV when the virus was incubated with the agents before infecting the cells, suggesting a direct inactivation effect on VHSV infectivity by the compounds. Some of the cardio-vascular, antimicrobial, antioxidative, hypoglycaemic effects noted for *O. europaea* have been attributed to the secoiridoids (mainly oleuropein), phenolic compounds as well as triterpenes derived from the leaves, fruits and oil of the olive tree which have been shown through the following *in vitro* and *in vivo* studies to possess biological properties.

The virucidal activity of olive leaves is more likely to be attributed to its ability to prevent virus entry into the cells. It may be due to the interaction of olive leaf extract with Vero cell membrane and/or HSV-1 envelope. The exact mechanism of the antiviral activity is not still clear. However, it might be attributed to the prevention of attachment and absorption of virus particles to the cell and thereby blockade of their entry to the cells. In agreement with this hypothesis, olive leaves extract was shown to interact with the surface of phospholipid bilayer (Khan *et al.* 2007). Moreover it has been shown that OLE (olive leaf extract, not further specification given) is a viral inhibitor at early stages of replication, probably via blocking of viral envelope fusion (Micol *et al.* 2005).

In vitro antiviral activity of an olive leaf extract (not further defined) against HIV-1 virus (infected H9 T lymphocytes) has been demonstrated in cell culture (IC₅₀ 0.2 µg/ml). Cell-to-cell transmission of HIV was inhibited in a dose-dependent manner and HIV-1 replication was inhibited in an *in vitro* experiment (Lee-Huang *et al.* 2003). It was shown that the olive leaf has an interesting effect on *Herpes simplex virus-1* (HSV-1) *in vitro*. The *in vitro* virucidal effect of olive leaf extract on HSV-1 in concentrations > 1 mg/ml has been proven. The CC₅₀ (50% cytotoxic concentration) of olive leaf extract for Vero cells and IC₅₀ were 1.75 and 0.65 mg/ml, respectively. When applied to cell culture infected with HSV-1, one hour before challenge, olive leaf extract showed no antiviral activities. When applied to the cells followed by the virus infection one hour later, or to the media containing the virus and the combination was added to cell culture one hour later, olive leaf extract showed anti HSV-1 activities at concentrations > 1.00 mg/ml (Motamedifar *et al.* 2007).

Antioxidant activity

Experiments have been conducted to demonstrate the antioxidant activity of olive leaf extracts. In rat epithelial cells stimulated with cytokines, an olive leaf polyphenol concentrate extract (OLPC) reduced nitrite concentration and free radical production. The effects of several natural antioxidants on nitric oxide (NO) modulation and oxidative status were determined in rat epithelial lung cells (L-2).

Resveratrol and OLPC were found to be effective in reducing nitrite levels, modifying iNOS mRNA, and decreasing free radical production. OLPC affected the levels of MnSOD while resveratrol did not, indicating that they act via different pathways. In particular resveratrol and OLPC, may have therapeutic potential in the treatment of inflammatory diseases (Zaslaver *et al.* 2005).

In an *in vitro* study, kinetic measurements were performed on an 80% ethanolic extract of olive leaf as well as a pure isolated compounds of it on its possible inhibitory effects on xanthine oxidase (XO), an enzyme well known for its significant contribution to the pathological process of gout. The studied standardised extract significantly inhibited the activity of XO. Through this study the authors suggest to provide a rational basis for the potential use of olive leaves against gout in Mediterranean folk medicine (Fleming *et al.* 1973).

Antiplatelet properties

Olive leaf has been reported to inhibit platelet aggregation and production of thromboxane A2 (a stimulator of platelet aggregation with vasodilatory effects) (Petroni *et al.* 1995).

Also of interest is another study reporting that olive leaf extract inhibited both angiotensin converting enzymes (Hansen *et al.* 1996).

Antihyperglycaemic/Hypoglycemic activity

An early study, using ethanol leaf extracts (defatted with petrol ether) given by gastric intubation to the rabbit (dose not specified), showed a 17-23% decrease in blood sugar levels which reached a minimum within 6 hours and rose to normal after 48 hours (Manceau *et al.* 1942).

Aqueous decoctions of Spanish olive leaf, administered IG to the rat at a dose of 32 mg/kg, showed hypoglycemic activity against alloxan-induced hyperglycaemia. Maximum hypoglycaemic activity was obtained from samples collected in the winter months, especially in February. One of the compounds responsible for this activity was oleuropein, which showed activity at a dose of 16 mg/kg. This compound also demonstrated antidiabetic activity in animals with alloxan-induced diabetes. The hypoglycaemic activity of this compound may result from two mechanisms:

(a) potentiation of glucose-induced insulin release, and (b) increased peripheral uptake of glucose (Gonzalez *et al.* 1992).

Aqueous extracts of dried olive leaves from Italy, administered IG to male rats in a very high dosage of 500 mg/kg, reduced the blood glucose levels of normal or alloxan-induced diabetic rats (Trovato *et al.* 1993).

The hypoglycemic activity of olive leaf has been demonstrated in animals. In one study the significance of supplementation of oleuropein in reducing oxidative stress and hyperglycemia in alloxan-induced diabetic rabbits has been evaluated. In rabbits with induced diabetes, an ethanol extract (75% ethanol) of olive leaf decreased blood glucose as well. Suggested mechanisms include potentiation of glucose-induced insulin release and increased peripheral uptake of glucose. (Al-Azzawie & Alhamdani 2006; Gonzalez *et al.* 1992). Dried leaf powder extracts of Egyptian olive trees collections, when administered intragastrically to the rat, in a dose of 750 mg/kg, were found to be inactive in streptozotocin-induced hyperglycaemia (Eskander & Jun 1995).

Studies in laboratory animals have reported mainly hypoglycemic activity of olive leaves (Bennani-Kabchi *et al.* 1999; Gonzalez *et al.* 1992). The active constituent was reported to be oleuropein, with a potentiation of glucose-induced insulin release as proposed mechanism of action, as well as an increase in peripheral blood glucose uptake. Especially, in the study of Bennani-Kabchi *et al.* 1999, sand rats develop obesity, insulin resistance, hyperlipidaemia and prediabetes, when given a standard laboratory chow diet. This model has been used to demonstrate the beneficial action of *O. europaea*

var. *oleaster* leaves to regulate unbalanced metabolism. Thirtytwo sand rats fed on hypercaloric diet during 7 months, were divided into 3 groups: controls (n = 10), treated by plant (n = 13) and treated by simvastatin; hypocholesterolemic drug. The plant decoction prepared at 10% was given orally at the rate of 1.5 ml/100 g during 3 months. Results show that the plant presents a hypocholesterolemic effect (42%) related to decreases in LDL and VLDL cholesterol. In addition, hypoglycemic (16%) and antihyperglycemic (40%) effects were observed accompanied by a 27% decrease in insulin. Chronic treatment with reduced total cholesterol (32%), LDL and VLDL cholesterol. Both treatments produced no significant reduction in plasma levels of triglycerides and HDL cholesterol. No toxic effects of this plant have been observed in usual doses (Bennani-Kabchi *et al.* 1999).

In another experimental model of diabetes, induced by streptozotocin, olive leaf failed to lower blood glucose levels or prevent glucosuria and ketonuria but it did not reduce circulating levels of liver enzymes and minimised histopathologic abnormalities in both the kidneys and liver (Onderoglu *et al.* 1999).

The oral administration of an olive leaves extract (ethanol 80%) (0.1, 0.25 and 0.5 g/kg bw) to STZ-diabetic rats and normal rats, caused a decrease in serum glucose, total cholesterol, triglycerides; moreover an increase in serum insulin in diabetic but not in normal rats (Eidi & Darzi, 2009).

In a series of animal models, normal, streptozotocin (STZ) diabetic, and sand rats were used in the inverted sac model to determine the mechanism through which olive leaf extract affected starch digestion and absorption. In the animal models, normal and STZ diabetic rats exhibited significantly reduced starch digestion and absorption after treatment with olive leaf extract (no further specification) compared with intestine without olive leaf treatment. Reduced digestion and absorption was observed in both the mucosal and serosal sides of the intestine. Though reduced, the decline in starch digestion and absorption did not reach statistical significance in the sand rats. The authors concluded that olive leaf extract may represent an effective adjunct therapy that normalizes glucose homeostasis in individuals with diabetes (Wainstein *et al.* 2012).

The inhibitory action of the olive leaf ethanol extract (OEE) on the activities of human amylases was examined *in vitro*. OEE inhibited the activities of α -amylase from human saliva and pancreas with IC₅₀ values of 4.0 and 0.02 mg/ml, respectively (Komaki *et al.* 2003). This finding is due to the inhibitory action of the flavonoids (luteolin-7-O- β -glucoside and luteolin-4'-o- β -glucoside) as well as of the triterpene oleanolic acid on α -amylase from human saliva and pancreas (Komaki *et al.* 2003).

Hypolipaedimic activity

Antihypercholesterolaemic activity has been shown in rats given a high daily dose, administered intragastrically (IG) of 500 mg/kg of a glycerine: ethanol leaf extract for 15 days. Activity was shown both in diet-induced and triton-induced hypercholesterolaemic animals (De Pasquale *et al.* 1991).

Oleuropein-rich extracts from olive leaves and their enzymatic and acid hydrolysates respectively rich in oleuropein aglycone and hydroxytyrosol, were prepared under optimal conditions. The antioxidant activities of these extracts were examined by a series of models *in vitro* (superoxide dismutase (SOD) and catalase (CAT) activities were evaluated in liver tissue). In this study the lipid-lowering and the antioxidative activities of oleuropein, oleuropein aglycone and hydroxytyrosol-rich extracts in rats fed a cholesterol-rich diet were tested. Wistar rats fed a standard laboratory diet or cholesterol-rich diets for 16 weeks were used. The serum lipid levels, the thiobarbituric acid reactive substances (TBARS) level, as indicator of lipid peroxidation, and the activities of liver antioxidant enzymes (SOD and CAT) were examined. The cholesterol-rich diet induced hyperlipidaemia resulting in the elevation of total cholesterol (TC), triglycerides (TG) and low-density lipoprotein cholesterol (LDL-C). Administration of polyphenol-rich olive leaf extracts significantly lowered the serum levels of TC, TG and LDL-C and increased the serum level of high-density lipoprotein cholesterol (HDL-C). Furthermore, the content of

TBARS in liver, heart, kidneys and aorta decreased significantly after oral administration of polyphenol-rich olive leaf extracts compared with those of rats fed a cholesterol-rich diet. In addition, these extracts increased the serum antioxidant potential and the hepatic CAT and SOD activities. The CAT and SOD activities significantly decreased in livers of rats fed a cholesterol-rich diet compared to those fed a control diet. The decrease was significantly restored ($P < 0.05$) in the presence of the olive leaves and the hydrolysate extracts. These results suggested that the hypocholesterolemic effect of oleuropein, oleuropein aglycone and hydroxytyrosol-rich extracts might be due to their abilities to lower serum TC, TG and LDL-C levels as well as slowing the lipid peroxidation process and enhancing antioxidant enzyme activity (Jemai *et al.* 2008).

Besides, the hypothesis *in vitro* by inducing LDL oxidation with copper sulphate and pre-incubating the samples with oleuropein, the bitter principle of olives, that is one of the major components of the polyphenolic fraction of olive oil. Oleuropein 10^{-5} M effectively inhibited CuSO_4 -induced LDL oxidation, as assessed by various parameters. It has been demonstrated that polyphenolic components of the Mediterranean diet interfere with biochemical events that are implicated in atherogenetic disease, thus proposing a new link between the Mediterranean diet and prevention of coronary heart diseases (Visioli & Galli 1994).

Poudyal *et al.* (2010) studied the effects of a liquid ethanolic extract from olive leaves, containing oleuropein (13.04 g/l) and its major metabolite, hydroxytyrosol (2.73 g/l), in High Carbohydrate-, High Fat-fed rats (HCHF) as experimental model of metabolic syndrome. Results were a decrease of plasma total cholesterol and triglyceride concentrations, improved oral glucose tolerance, prevented increase in abdominal fat pads and attenuated increase in abdominal circumference, reduction of plasma uric acid and malon dialdehyde concentrations, suggesting a decrease in oxidative stress. Histological evaluation of heart and liver showed reduced inflammation and fibrosis. This was accompanied by reduced left ventricle stiffness, reduced portal inflammation and fat deposition in the liver, and total restoration of liver function as well as improved aortic reactivity. (N.B. no effect on systolic blood pressure in this model was detected).

Paiva-Martins *et al.* (2014) supplemented the conventional diets of Crossbreed male pigs ($n=30$) with dried olive leaf powder at 5% (OL5) and 10% (OL10) and compared the results with pigs fed with conventional diet alone. In the pigs fed with the supplemented diet, a significant decrease in plasmatic triacylglycerols (TAGs), lower body mass and fat storage was shown, but no significant reductions for LDL cholesterol and oxLDL levels. The highest dose resulted in significant RBC membrane destabilization.

Antihypertensive effects

O. europaea extract of fresh leaves administered to rats in a single dose of 360 mg/kg daily showed spasmolytic activity against phenylephrine-induced contractions (in 5 minutes 40% and in 15 minutes 25% (Lasserre *et al.* 1983; Blaschek *et al.* 2006).

European olive leaf and shoot has been administered in the rat IG at doses of 25 mg/kg, following aconite-induced arrhythmia. In the same study, antihypertensive activity was demonstrated by glycerine:ethanol (50:50) extracts given IG to the rat at high dosages of 125-250 mg/kg, following desoxycorticosterone acetate (DCSA)-induced hypertension. Positive inotropic effects of 95% ethanol, glycerine and ethanol:glycerine (50:50) extracts were demonstrated in the rabbit at dosages of 5.0 mg/ml. Spasmolytic activity of similar extracts was demonstrated in the guinea pig at doses of 50 mg/kg against vasopressin-induced coronary spasm and hypotensive activity in the rat at doses of 100 mg/kg, given IG. Maximum hypotensive activity effect was seen 60-120 minutes after administration of each extract. Positive chronotropic effects of glycerine: ethanol (50:50) extracts were noted, when given IG to the DCSA-hypertensive rabbit at a dose of 125 mg/ml (Circosta *et al.* 1990).

The effects of a glycerioethanolic macerate of the leaves of *O. europaea* L. and of oleuropein on excitation and on the right atrial and ventricular monophasic action potential (MAP) have been studied in anaesthetised dogs using the technique of endocavitary recording. At the higher doses tested a slight increase in the sinus cycle of the sinoatrial conduction time and of the sinus node recovery time, together with a prolongation of the atrioventricular and intraventricular conduction and an increase of the atrial and ventricular MAP duration were observed. This may be due to a decrease in the repolarisation phase 3. These electrophysiological effects indicate an inhibitory action both on the swift influx of sodium and on the slow influx of calcium, as well as a decrease in potassium conductance (Occhiuto *et al.* 1990).

Leaf decoctions or lyophilised extracts of fresh olive leaves (25–50 mg/kg i.v.) administered to the rat (aorta) showed spasmolytic activity against phenylephrine-induced contractions, both in the presence of and without endothelium (IC₅₀ 1.12 mg/ml) (Zarzuelo *et al.* 1991).

Spasmolytic activity of dried leaf extracts (30% ethanol) has been demonstrated against VSK+ induced contractions, when administered (aorta) to the rabbit in doses of 1 mg/ml (Rauwald *et al.* 1994).

Some of the cardio-vascular effects noted for *O. europaea* have been attributed to the secoiridoids oleuropein increased coronary flow and oleacein (ACE inhibitory activity) (Hansen *et al.* 1996).

A special prepared proprietary named (I) olive leaf extract has been tested for its blood pressure lowering activity in rats rendered hypertensive by daily oral doses of L-NAME (N(G)-nitro-L-arginine methyl ester, 50 mg/kg) for at least 4 weeks. Oral administration of the extract at different dose levels at the same time as L-NAME for a period of 8 weeks showed a dose dependent prophylactic effect against the rise in blood pressure induced by L-NAME, best effect being induced by a dose of 100 mg/kg of the extract. In rats previously rendered hypertensive by L-NAME for 6 weeks and then treated with that dose of the extract for a further 6 weeks without discontinuation of L-NAME, normalisation of the blood pressure was observed. The findings confirm previous reports on the hypotensive effects of olive leaf. The special extract, has shown to give consistent results with little individual variability. The antihypertensive effect of the extract maybe related to a variety of factors involving reversal of vascular changes involved in the L-NAME (Khayyal *et al.* 2002).

Effects of a commercial *O. europaea* leaf extract (OLE, extract not further specified) on isolated hearts and cultured cardiomyocytes have been investigated. Isolated rabbit hearts were perfused according to the Langendorff technique and connected to a 256-channel epicardial mapping system. Voltage clamp experiments were performed in cultured neonatal rat cardiomyocytes using a perforated-patch technique. This resulted in a concentration-dependent decrease in systolic left ventricular pressure and heart rate as well as an increase in relative coronary flow and a slight, but not significant prolongation of PQ-time. There were no significant changes between the groups in the activation-recovery interval and its dispersion, total activation time, peak-to-peak amplitude, percentage of identical breakthrough-points and similar vectors of local activation. Voltage clamp experiments in cultured neonatal rat cardiomyocytes showed a significant decrease in maximum I_{Ca, L} by OLE which was reversible upon wash-out. The Authors concluded that OLE suppresses the L-type calcium channel (I_{Ca, L}) directly and reversibly. These findings might help to understand the traditional use of OLE in the treatment of cardiovascular disease (Schefflera *et al.* 2008).

Flavonoids

Antioxidant activity

A study was done to identify the major phenolic compounds present in an extract of olive leaf and estimate their antioxidant activity by their ability to scavenge the radical cation ABTS. Several structural attributes of flavonoids present in olive leaf, including 3-hydroxyl groups, influenced the

ability of these compounds to scavenge free radicals. Radical scavenging capacity increased with the number of free hydroxyl groups present in the flavonoid structure. The flavonoid rhamnoglucoside rutin was the most effective compound. The flavonoids, oleuropeosides and substituted phenols present in olive leaf extract exhibited synergism with respect to antioxidant activity (Benavente-García *et al.* 2000). Caffeic acid was also reported to have antioxidant activity through the scavenging of superoxide anion (Chimi *et al.* 1991, 1995).

Olive leaf contains flavonoids that possess antioxidant activity, and tissue antioxidant status has been proposed as a key factor in the development of diabetic complications. This may help explain why an orally administered preparation of olive leaf substantially diminished tissue damage in the kidney and liver in rats with streptozotocin induced diabetes (Onderoglu *et al.* 1999).

Flavonoids (luteolin-7-O- β -glucoside and luteolin-4'-O- β -glucoside from olive leaf extracts have showed anti- α -amylase activity from human saliva and pancreas with IC₅₀ values of 0.5 and 0.3 mg/ml, respectively (Komaki *et al.* 2003) which is in accordance with previous reported results on luteolin (Kim *et al.* 2000).

Caffeic acid, luteolin and luteolin-7-O- β -glucoside from an ethanolic extract of olive leaf, in a very recent study (Fleming *et al.* 1973) have showed a strong inhibition of xanthine oxidase (XO) an enzyme well known to contribute significantly to the pathological process of gout.

Oleuropein

Antioxidant activity

Phenolic compounds derived from the leaves, fruits and oil of the olive tree (*O. europaea* L.) have long been known to have anti-oxidative properties (Chimi *et al.* 1991; Sheabar & Neeman 1988; Petroni *et al.* 1995). More recently, LeTutour & Guedon demonstrated that oleuropein, hydroxytyrosol, and in particular, extracts of *O. europaea* leaf (containing 19% oleuropein, 1.8% flavonoid glycosides, and 3,4-dihydroxy-phenethyl esters) were more potent antioxidants than vitamin E or another established antioxidant, BHT, in a model chemical system (inhibition of oxidation of methyl linoleate in heptanol or propanol-water, initiated by 2,2'-azo-bis-isobutyronitrile (AIBN)). Another *in vitro* study (Visioli & Galli 1994) showed that oleuropein (at a concentration of 10–5 M) significantly inhibited copper sulphate-induced oxidation of LDL extracted from normal human plasma.

Oleuropein and hydroxytyrosol, two phenolic compounds contained in olives and olive oil, are known to possess several biological properties, many of which may be related, partially at least, to their antioxidant and free radical-scavenger ability. Hence, together with their scavenging activity against the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH test), the antioxidative effect of oleuropein and hydroxytyrosol were investigated in a model system consisting of dipalmitoylphosphatidylcholine / linoleic acid unilamellar vesicles (DPPC/LA LUVs) and a water-soluble azo compound as a free radical generator (LP-LUV test). The results obtained were also interpreted in the light of biophenol interactions, studied by differential scanning calorimetry, with dimyristoylphosphatidylcholine (DMPC) vesicles as a biological membrane model. The results obtained in the DPPH and LP-LUV tests confirm the good scavenger activity and antioxidant effect of oleuropein and hydroxytyrosol. However, while both compounds exhibit comparable effectiveness in the DPPH test (hydroxytyrosol being slightly more active than oleuropein), oleuropein seems, in the LP-LUV test, a better antioxidant than hydroxytyrosol. In addition, oleuropein shows a better antioxidant activity in the membranous system than in homogenous solution. Furthermore, oleuropein, but not hydroxytyrosol, interacts with DMPC vesicles, causing shifts, toward lower values, of the calorimetric peak temperature, associated to the gel to liquid crystal phase transition, typical for DMPC multilayers. The authors hypothesized that hydroxytyrosol can serve as scavenger of aqueous peroxy radicals near the membrane surface, while

oleuropein acts also as a scavenger of chain-propagating lipid peroxy radicals within the membranes (Saija *et al.* 1998).

Rabbits with induced diabetes showed a decrease in oxidative stress markers when treated with oleuropein (Al-Azzawie & Alhamdani 2006). Other experiments support the antioxidant activity of the phenols oleuropein and hydroxytyrosol (Benavente-Garcia *et al.* 2000; Briante *et al.* 2001, 2002; Visioli *et al.* 2002; Caturla *et al.* 2005).

The antioxidative effect of oleuropein and hydroxytyrosol were investigated in a model system consisting of dipalmitoylphosphatidylcholine/linoleic acid unilamellar vesicles (DPPC/LA LUVs) and a water-soluble azo compound as a free radical generator (LP-LUV test). The results obtained were also interpreted in the light of biophenol interactions, studied by differential scanning calorimetry, with dimyristoylphosphatidylcholine (DMPC) vesicles as a biological membrane model. The results obtained in the DPPH and LP-LUV tests confirm the good scavenger activity and antioxidant effect of oleuropein and hydroxytyrosol. However, while both compounds exhibit comparable effectiveness in the DPPH test (hydroxytyrosol being slightly more active than oleuropein), oleuropein seems, in the LP-LUV test, a better antioxidant than hydroxytyrosol. Besides oleuropein shows a better antioxidant activity in the membranous system than in homogenous solution. Furthermore, oleuropein, but not hydroxytyrosol, interacts with DMPC vesicles, causing shifts, toward lower values, of the calorimetric peak temperature, associated to the gel to liquid-crystal phase transition, typical for DMPC multilayers. The authors hypothesized that hydroxytyrosol can serve as scavenger of aqueous peroxy radicals near the membrane surface, while oleuropein acts also as a scavenger of chain-propagating lipid peroxy radicals within the membranes (Saija *et al.* 1998).

Cardiovascular effects

Studies have shown that oleuropein possesses a wide range of pharmacologic and health promoting properties including antiarrhythmic, spasmolytic, immune-stimulant, cardio protective, hypotensive, and anti-inflammatory effects (Petkov & Manolov 1978; Visioli *et al.* 1995, 1998; Circosta *et al.* 1990; Diaz *et al.* 2000; Somova *et al.* 2004). Many of these properties have been suggested as a result from the antioxidant character of oleuropein (Visioli *et al.* 2002). Furthermore, most of the reported antioxidant characteristics of oleuropein are drawn from *in vitro* investigations (Amro *et al.* 2002; Stupans *et al.* 2002; Ferroni *et al.* 2004), and even those who involved animals or human subjects the antioxidant activity of oleuropein was demonstrated in a condition at which there is no established oxidative challenge (Visioli *et al.* 2000). The results obtained from this study may provide further information on the antioxidative effect of oleuropein in an animal model of oxidative stress.

It is well known that the olive leaf has antioxidant properties associated with phenolic constituents and oleuropein (Turner *et al.* 2005). Oleuropein, has been reported to decrease the oxidation of LDL cholesterol (Visioli *et al.* 1994). Oxidized LDL is the most damaging form of cholesterol and can initiate damage to arterial tissues, thereby promoting atherosclerosis.

The effects of oleuropein were studied on the electromechanical properties of isolated guinea-pig atria. In spontaneously beating right atria, oleuropein decreased the amplitude of contractions ($IC_{50} = 1.3 \pm 0.2 \times 10^{-4}$ M), slightly decreased atrial rate, and lengthened sinus node recovery time and also inhibited peak contractile force in electrically driven left atria incubated in normal ($IC_{50} = 1.5 \pm 0.5 \times 10^{-4}$ M) and in 27 mM K^+ Tyrode solution ($IC_{50} = 1.7 \pm 0.4 \times 10^{-4}$ M). These negative inotropic effects were not accompanied by significant changes in the characteristics of action potentials recorded in atria incubated in either normal or depolarising solutions. These results indicate that oleuropein produced an electromechanical uncoupling that cannot be attributed to an inhibitory effect on Ca^{2+} entry through L-type channels (Duarte *et al.* 1993).

Petkov and Manolov (1972) observed in their investigations of the cardiovascular effects of oleuropein in animals that 3–50 mg/kg oleuropein given i.p. caused a slight stimulation of the respiratory rate in anaesthetised cats. In dogs with experimentally induced hypertension, 10–30 mg/kg oleuropein caused a sharp, long-lasting drop in both systolic and diastolic blood pressure in three out of four animals, and a lesser, shorter-lived decrease in blood pressure in the fourth dog. The same investigators found that oleuropein caused an increase in blood flow through the coronary vessels of isolated rabbit heart preparations, but no change in coronary flow in anaesthetised cats at doses of 10–30 mg/kg. However, in a model of experimentally disturbed coronary circulation, oleuropein (30 mg/kg intravenously) largely abolished the characteristic ECG (electrocardiogram) changes caused by Pituitrin (which diminishes coronary blood flow) in conscious rabbits, when given 1 minute after the Pituitrin injection. The authors found that oleuropein eliminated cardiac arrhythmia in dogs with induced hypertension for 1.5–2 hours, normalised cardiac rhythm in rabbits with barium chloride-induced arrhythmia for about 1 hour, and prevented or reduced the duration of disturbed cardiac rhythm in rats with calcium chloride-induced arrhythmia. The pharmacological mechanisms underlying any of these effects on the heart and vasculature are unknown. Also, in doses of 10–30 mg/kg, it caused a brief depressed state with decreased motor activity in two out of four conscious dogs with induced hypertension, and was badly tolerated in a third dog, causing excitation, scratching, and vigorous jolting movements, red, watery eyes, and hyperaemic (warm, reddened) abdominal skin. Some of the cardio-vascular effects noted for *O. europaea* have been attributed to the secoiridoids oleuropein increased coronary flow and oleacein (ACE inhibitory activity) (Hansen *et al.* 1996).

Ruiz-Gutierrez *et al.* (2000) investigating the effects of oleuropein on lipids and fatty acids in heart tissue, did not report any adverse behavioural or other effects (for example, on food consumption, body weight, heart weight or heart total lipid content) in rats given intraperitoneal injections of 25 or 50 mg/kg daily for 3 weeks. Oleuropein did significantly reduce the linoleic acid content and the ratio of unsaturated to saturated fatty acids in heart polar lipids, depleted heart levels of vitamin E, and itself became incorporated in heart tissue, but the significance of these findings is unclear. However, heart tissue that had been pre-treated with oleuropein *in vitro* was not susceptible to peroxidation.

In vivo, studies in rats indicate that oleuropein prevents oxidative myocardial injury (Manna *et al.* 2004).

Herbal preparations in animal experiments in rabbit and rats found a hypotensive effect of oleuropein, possibly via direct action on smooth muscle. Oleuropeoside also may exert a vasodilatory activity.

Finally, the anti-ischemic, anti-oxidative and hypolipidemic effects of oleuropein in anaesthetised rabbits were recently evaluated. It has been seen that the plasma lipid peroxidation products and carbonyl concentrations compared with the control groups in which these factors increased relative to baseline due to ischaemia and reperfusion. Treatment for 6 weeks with both doses of oleuropein (10 and 20 mg/ml) reduced total cholesterol and triglycerides concentrations. This is the first experimental study *in vivo* that suggests the possibility of using oleuropein in the treatment of ischemia (Andreadou *et al.* 2006).

Anti-atherosclerosis

In the study of Huang *et al.* (2010) using apoE knockout mouse model fed with high fat diet (rodent model of hypercholesterolemia) oleuropein significantly reduced the extent of atherosclerosis found in the aorta of apoE knockout mice.

Anti-hyperglycaemic activity

Oleuropein is reported to have an anti-hyperglycaemic effect in diabetic rats (Gonzalez *et al.* 1992). However, regarding the antioxidant feature of oleuropein, it is still unknown if oleuropein may exert other beneficial effects in diabetes as in attenuating oxidative stress.

Patients with diabetes mellitus are likely to develop certain complication such as retinopathy, nephropathy and neuropathy as a result of oxidative stress and overwhelming free radicals. Treatment of diabetic patients with antioxidant may be of advantage in attenuating these complications. Oleuropein has been endowed with many beneficial and health promoting properties mostly linked to its antioxidant activity. Al-Azzawie & Alhamdani aimed to evaluate the significance of supplementation of oleuropein in reducing oxidative stress and hyperglycaemia in alloxan-induced diabetic rabbits. After induction of diabetes, a significant rise in plasma and erythrocyte malondialdehyde (MDA) and blood glucose as well as alteration in enzymatic and non-enzymatic antioxidants was observed in all diabetic animals. During 16 weeks of treatment of diabetic rabbits with 20 mg/kg body weight of oleuropein the levels of MDA along with blood glucose and most of the enzymatic and non-enzymatic antioxidants were significantly restored to establish values that were not different from normal control rabbits. Untreated diabetic rabbits on the other hand demonstrated persistent alterations in the oxidative stress marker MDA, blood glucose and the antioxidant parameters. The Authors concluded that oleuropein may be of advantage in inhibiting hyperglycemia and oxidative stress induced by diabetes and suggest that administration of oleuropein may be helpful in the prevention of diabetic complications associated with oxidative stress (Al-Azzawie & Alhamdani 2006).

Antimicrobial activities

A variety of antibacterial actions of oleuropein and its associated compounds have been demonstrated *in vitro*. The component usually associated with olive leaf's antimicrobial properties is oleuropein (Petkov & Manolov 1972; Juven & Henys 1972).

Fleming *et al.* (1973) isolated six major phenolic compounds from green olives; one particular compound, possibly a hydrolysis product of oleuropein, was much more inhibitory than oleuropein itself to the lactic acid bacterium *Leuconostoc mesenteroides* FBB 42. Later on, the oleuropein aglycone and elenolic acid were found to strongly inhibit the growth of three further species of lactic acid bacteria – *Lactobacillus plantarum*, *Pediococcus cerevisiae* and *Lactobacillus brevis* (Fleming *et al.* 1973). Since the aglycone is composed of elenolic acid bound to β -3,4-dihydroxyphenylethyl alcohol and the latter compound was not inhibitory, the investigators concluded that elenolic acid was the inhibitory part of the aglycone molecule. Oleuropein itself was not inhibitory to these bacteria, but did inhibit three species of non-lactic acid bacteria – *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas solanacearum*. In addition, an acid hydrolysate of an extract of oleuropein (containing hydrolysis products of oleuropein not specifically identified) inhibited the growth of a further eight species of bacteria. Some more recent *in vitro* studies have shown that oleuropein and/or its hydrolysis products also inhibit the germination and sporulation of *Bacillus megaterium* (Rodriguez *et al.* 1988) and inhibit outgrowth of germinating spores of *Bacillus cereus* T. (Tassou *et al.* 1991).

Oleuropein has also been reported to directly stimulate macrophage activation in laboratory studies (Visioli *et al.* 1998i). Besides, oleuropein has shown anti-microbial activity against yeasts, fungi, molds and other parasites (Aziz *et al.* 1998; Koutsoumanis *et al.* 1998). The activity of oleuropein, was investigated *in vitro* against *Mycoplasma hominis*, *Mycoplasma fermentans*, *Mycoplasma pneumoniae* and *Mycoplasma pirum*. Oleuropein inhibited mycoplasmas at concentrations from 20 to 320 mg/l. The MICs of oleuropein to *Mycoplasma pneumoniae*, *Mycoplasma pirum*, *Mycoplasma hominis* and *Mycoplasma fermentans* were 160, 320, 20 and 20 mg/l, respectively (Furneri *et al.* 2002). Hydroxytyrosol demonstrated broader antimicrobial activity than oleuropein and is comparable to ampicillin and erythromycin in spectrum and potency (Bisignano *et al.* 1999; Khan *et al.* 2007).

Other activities

Other clinical effects of oleuropein are the potentiation of cellular and organismal protection through the macrophage-mediated response (Visioli *et al.* 1998i) and the inhibition of platelet aggregation and eicosanoid production (Petroni *et al.* 1995). Olive oil and its main phenolic constituent (oleuropein) prevent inflammation-induced bone loss in the ovariectomised rat (Puel *et al.* 2004).

Natalini and co-workers have postulated that oleuropein might be a modulator of metabolism via pepsin activation and inhibition of others enzymes such as trypsin (Polzonetti *et al.* 2004).

The molluscicidal (anti-schistosomal) activity of oleuropein against the mollusc *Biomphalaria glabrata* has been reported showing an LD₅₀ 250 ppm within 24 hours (Kubo & Matsumoto 1984; Blaschek *et al.* 2006).

Oleuropein has been patented in the United States for antiviral activity against viral diseases, including herpes, mononucleosis, and hepatitis (Fredrickson 2000).

In vitro studies have demonstrated that oleuropein acts as an anti-tumour compound (Saenz *et al.* 1998), inhibits platelet-activating factor activity (Andrikopoulos *et al.* 2002), enhances nitric oxide production by mouse macrophages (Visioli *et al.* 1998i) and decreases inflammatory mediator production (Miles *et al.* 2005).

The antioxidant / anticancer potential of phenolic compounds isolated from olive tree (Owen *et al.* 2000), as well as the *in vitro* cytotoxicity to human cells in culture of some phenolics from olive oil, has been reported by Babich & Visioli 2003 as well as by Hamdi & Castellon 2005; who have shown the activities of oleuropein, as an anti-tumor agent and cytoskeleton disruptor.

Besides oleuropein exhibits proteasome stimulatory properties *in vitro* and confers life span extension of human embryonic fibroblasts (Katsiki *et al.* 2007).

Elenolic acid

Antiviral properties

In addition to its antibacterial actions, elenolic acid has been shown to be a potent inhibitor of a wide spectrum of viruses. Olive leaf extract has reported antiviral activity, caused by the constituent calcium elenolate, a derivative of elenolic acid (Renis 1970; Heinze *et al.* 1975). The isolated calcium salt of elenolic acid was tested as a broad-spectrum antiviral agent active against all viruses tested (Soret 1969). Some viruses inhibited by calcium elenolate *in vitro* include rhinovirus, myxoviruses, *Herpes simplex* type I, *Herpes simplex* type II, Herpes zoster, *Encephalomyocarditis*, Polio 1, 2, and 3, two strains of leukemia virus, many strains of influenza and para-influenza viruses vaccinia, pseudorabies, influenza A (PR8), Newcastle disease, parainfluenza 3, Coxsackie A21, encephalomyocarditis, polio 1, 2 and 3, vesicular stomatitis, Sindbis and reovirus 3 (Deering) viruses (Renis 1975; Soret 1969; Hirschman 1972). Calcium elenolate also inhibits the RNA-dependent DNA polymerase I enzymes (reverse transcriptases) of murine leukaemia viruses (MuLV(M) and Rauscher), (Hirschman 1972) and the DNA polymerase II and III enzymes of *Escherichia coli* (Heinze *et al.* 1975) *in vitro*.

The mechanism of action of the antiviral activity is reported to include:

- i) ability to interfere with critical amino acid production essential for viruses
- ii) ability to contain viral infection and/or spread by inactivating viruses or by preventing virus shedding, budding, or assembly at the cell membrane
- iii) ability to directly penetrate infected cells and stop viral replication

- iv) in the case of retroviruses, it is able to neutralise the production of reverse transcriptase and protease
- v) stimulation of phagocytosis.

Antiviral properties

Soret (1969) showed that calcium elenolate effectively reduced viral titres *in vivo* when given before and/or after inoculation of hamsters with myxovirus parainfluenza type 3 (HA-1 virus, strain C-243). Treatment with calcium elenolate, but not placebo, prevented spread of viral infection to the lungs.

Triterpenes

Hypoglycemic effect of α -amylase

The triterpene oleanolic acid on the activities of human amylases was examined *in vitro*. It has inhibited the activities of α -amylase from human saliva and pancreas with IC₅₀ value of 0.1 mg/ml (Komaki *et al.* 2003).

Cardiovascular effects

A bioassay directed study of triterpenoids isolated from the leaves of *O. europaea* from Greece, from wild African olive and from cultivar of *O. europaea* grown in Cape Town was reported. The experiment was undertaken since the preliminary analyses showed that the African wild olive leaf is rich in triterpenoids and contain only traces of oleuropein which is typical for European olive leaves. The anti-hypertensive, diuretic, anti-atherosclerotic, antioxidant and hypoglycaemic effects of authentic oleanolic and ursolic acid and the three isolates were studied on Dahm-salt-sensitive insulin resistant rat genetic model of hypertension. All the three isolates in a dose 60 mg/kg body weight for 6 weeks treatment prevented the development of severe hypertension and atherosclerosis and improved the insulin resistance of the experimental animals (Somova *et al.* 2002; 2003). The same derivatives have been acted as beta-adrenergic antagonists, blocking the effect of adrenaline and isoprenaline. The three triterpenes could provide an effective and cheap and accessible source of additive to conventional treatment of hypertension, complicated by stenocardia and cardiac failure in the African population (Somova *et al.* 2004).

3.1.3. Safety pharmacology

Laboratory experiments evaluating safety pharmacology were not fully performed. Therefore, safety parameters and the benefit-risk ratio must be derived from general toxicological properties of the components and the traditional use of olive leaf extracts.

3.1.4. Pharmacodynamic interactions

Pharmacodynamic drug interactions of whole extracts or isolated constituents have not been reported.

3.1.5. Conclusions

The published data on pharmacological activities with respect to the proposed indications (Traditional herbal medicinal product used to promote the renal elimination of water, in mild cases of water retention) and preparations are limited. On the basis of existing pharmacological data mainly on olive leaf constituents, antihypertensive, hypolipidemic and diuretic, antioxidant effects, are reported. Furthermore, hypoglycaemic (in high doses), antimicrobial, antiviral, smooth muscle relaxant as well as effects on the inflammatory response were described.

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

Herbal substance/Herbal preparations

No data on olive leaf has been found or reported.

Oleuropein

There are insufficient data in the literature to fully understand the bioavailability of polyphenols such as oleuropein, hydroxytyrosol and tyrosol. It is known that oleuropein is poorly absorbed due to its large size and planar configuration (Edgecombe *et al.* 2000). It is however hypothesised that since oleuropein is a glucoside, it could probably access a glucose transporter (SGLT1) found on the epithelial cells of the small intestine, permitting its entry into the cells. Conversely, Holmann *et al.* (1995) postulated that the absorption of the quercetin glycoside (a similar polyphenolic) involved active sugar transporters (Singh *et al.* 2008).

Other non-clinical studies have shown that oleuropein is rapidly absorbed after oral administration in rats, with maximum plasma concentration occurring 2 h after administration. Hydroxytyrosol was its most important metabolite. Both compounds are rapidly distributed and excreted in urine as glucuronides or in very low concentrations as free forms. (Tan *et al.* 2003; Boccio *et al.* 2003).

Oleuropein is among the herbal constituents that act as mechanism-based inhibitors of various Cytochrome P450 enzymes (CYPs) (Zhou *et al.* 2007).

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

3.3.1. Single dose toxicity

The LD₅₀ of an extract of olive leaf (not specified) (*O. europaea*) was 1300 mg/kg, i.p. in mouse; and > 3000 mg/kg orally in mouse (Duke 2002; Blascheck *et al.* 2006). Besides, an extract of olive leaf at doses of 1 mg/ml, was not toxic to human cells (Lee-Huang *et al.* 2003).

Leaf methanol extract (no further specification) from *O. europaea* subsp. *africana* (Mill.) P.S. was studied in *in vivo* mice antidiarrheal models. LD₅₀ in mice of 3475 mg/kg p.o determined during investigation of the extract testing it for antidiarrheal activity (Amabeoku and Bamuamba 2010).

Oleuropein

Petkov and Manolov (1972) gave single daily intraperitoneal doses of oleuropein to albino mice ranging from 100 to 1000 mg/kg (in solutions of 1, 5 and 10%). No toxic effects or deaths during the 7-days post-treatment period were observed, and consequently oleuropein's LD₅₀ could not be determined in this study (Petkov & Manolov 1972; Blascheck *et al.* 2006).

Calcium elenolate

Elliott *et al.* (1969 cited in Khayyal *et al.* 2002) determined the LD₅₀ for calcium elenolate to be 120 mg/kg in mice when given intraperitoneally, and 160 mg/kg in rats via the intraperitoneal route and 1700 mg/kg via the oral route.

3.3.2. Repeat dose toxicity

No data on olive leaf are available. Toxicological studies was conducted to investigate the repeated dose oral toxicity of a proprietary named (II) water-soluble extract of the leaves of the olive tree (*O.*

europaea L.), in accordance with internationally accepted protocols. The extract tested did not cause mortality or toxic effects in CrI: (WI)BR Wistar rats in a 90-day repeated-dose oral toxicity study at doses of 360, 600, and 1000 mg/kg bw/d. The no observed adverse effect level in the 90-day study was 1000 mg/kg bw/d for both male and female rats, the highest dose tested. This study was made with a specifically prepared extract (oleuropein > 40% (Netherlands)) (Clewell *et al.* 2015).

Calcium elenolate

Elliott *et al.* (1969) found calcium elenolate to be well tolerated in rats given daily oral doses of 0, 30, 100 or 300 mg/kg for 1 month. The only treatment-related change observed was a yellowing of the nonglandular fore-stomach in 40% of the rats receiving the highest dose (300 mg/kg). In 7-month old beagle dogs given daily oral doses of 0, 3, 10 or 30 mg/kg calcium elenolate for 1 month, all but the highest dose were well tolerated – three out of the four dogs receiving 30 mg/kg showed a mild gastric irritation with sporadic vomiting. Tissue analysis revealed a few small gastric erosions in these animals.

3.3.3. Genotoxicity

A battery of toxicological studies was conducted to investigate the genotoxicity of a proprietary named (II) water-soluble extract of the leaves of the olive tree (*O. europaea* L.), in accordance with internationally accepted protocols. There was no evidence of mutagenicity in a bacterial reverse mutation test and in an *in vitro* mammalian chromosomal aberration test nor was any genotoxic activity observed in an *in vivo* mouse micronucleus test at concentrations up to the limit dose of 2000 mg/kg bw/d (Clewell *et al.* 2015).

Assessor's comment:

According to available scientific literature genotoxicity studies have been carried out only on the above mentioned specifically prepared proprietary name (II) extract and the outcome cannot be extrapolated to the herbal preparations proposed in the monograph (comminuted and powdered leaves as well as infusion/decoction).

3.3.4. Carcinogenicity

No studies on carcinogenicity were found in the literature.

3.3.5. Reproductive and developmental toxicity

No teratogenicity studies have been carried out according to available scientific literature.

3.3.6. Local tolerance

No data available.

3.3.7. Other special studies

No data available.

3.3.8. Conclusions

There are only limited non-clinical safety data for olive leaf extracts and some limited toxicological data on the toxicity of oleuropein and calcium elenolate, mainly published in the 70's.

Data on genotoxicity, carcinogenicity and reproductive and developmental toxicity are missing.

3.4. Overall conclusions on non-clinical data

The published data on pharmacological activities with respect to the proposed indications (Traditional herbal medicinal product used to promote the renal elimination of water, in mild cases of water retention) and preparations are limited. On the basis of existing pharmacological data mainly on olive leaf constituents, antihypertensive, hypolipidemic and diuretic, antioxidant effects, are reported. Furthermore, hypoglycaemic (in high doses), antimicrobial, antiviral, smooth muscle relaxant as well as effects on the inflammatory response were described.

Some of these data support the traditional use of *O. europaea* and preparations thereof in the proposed indication.

Limited data are available on pharmacokinetics. For the herbal substance or the herbal preparation no data are available and therefore no conclusion can be drawn. There are some pharmacokinetic data available for oleuropein and its metabolites. Oleuropein is also among the herbal constituents which behave as mechanism-based inhibitors of various CYPs.

Data on genotoxicity, carcinogenicity as well as reproductive and developmental toxicity studies are missing.

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

In a double-blind placebo-controlled crossover study in healthy adults (n=18), a single consumption of an olive leaf extract with 51 mg oleuropein and 10 mg hydroxytyrosol (HT) showed improved vascular function (Digital volume pulse-stiffened index) and reduced production of inflammatory cytokine IL-8 (Lockyer *et al.*, 2015).

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

The only pharmacokinetic data reported in literature concern specific proprietary named extracts from olive leaves.

The absorption of OLE phenolics (a specific olive leaf extract containing 51 mg oleuropein; 10 mg hydroxytyrosol), was studied, indicating the bioavailability of HT (hydroxytyrosol), tyrosol, HValc (homovanillic alcohol) and oleuropein, as by the measured presence of HT, tyrosol, HValc, EDA (oleuropein aglycone dialdehyde), oleuropein and EA (oleuropein aglycone) in urine following the intake of the special olive leaf extract. In this randomised, double-blind, placebo-controlled, cross-over, intervention trial was conducted with eighteen healthy volunteers (nine male, nine female), who consumed either OLE, or a matched control (separated by a 4-week wash out) on a single occasion. Urine was analysed for phenolic metabolites by HPLC and HT and oleuropein derivatives peaked in urine after 8–24 h (Lockyer *et al.* 2015).

Assessor's overall conclusions on pharmacokinetics:

Data on pharmacokinetics of *Oleae folium*, extracts or relevant components are limited in human beings.

4.2. Clinical efficacy

4.2.1. Dose response studies

No pharmacodynamic studies were performed to support the posology and daily dose proposed.

4.2.2. Clinical studies (case studies and clinical trials)

Renal effects

Diuretic activity was observed in human adult patients given an olive leaf infusion (5 ml) or decoction (3 ml) by mouth once daily for 20–25 days. A daily increase in urinary output of 100–145 ml was noted for both dosage forms, with no effect on blood Na, K or chloride levels (Capretti & Bonaconza 1949).

Antihypertensive effects

Olive leaf extract (400 mg of aqueous olive leaf extract with no further details) had an antihypertensive effect in patients with essential arterial hypertension. Patients were separated into two groups: first timers who had never been previously treated with hypotensive medication (n=12) and a second group who had previously benefited from some sort of anti-hypotensive therapy such as diuretic or beta-blocker medication (n=18). For the second group, all therapeutic medications were removed 15 days prior to the beginning of the study. Both groups then received placebo gel capsules for 2 weeks. For the 3 months that followed, the placebo was replaced with similar gel capsules, each containing 400 mg of aqueous olive leaf extract. Patients took 4 capsules daily for total dose of approximately 1.6 g olive leaf extract daily. A decrease in blood pressure occurred in all patients. As a side note, the authors also reported a small decrease of glycaemia and calcium in the groups (Cherif *et al.* 1996).

A double-blind, randomised, parallel and active-controlled clinical study was conducted to evaluate the anti-hypertensive effect as well as the tolerability of a proprietary name (I) olive leaf extract in comparison with captopril in patients with stage-1 hypertension (Susalit *et al.* 2011).

The proprietary name (I) olive leaf extract, manufactured from the dried leaves of *O. europaea* L., is an ethanol (80% m/m) extract. After a patented filtration process, the crude extract was dried. The drug to extract ratio (DER) was 3–7:1. Characteristic components in the extract were 18–26% (m/m) oleuropein, 30–40% (m/m) polyphenols as well as verbascoside and luteolin-7-glucoside (Khayyal *et al.* 2002; Perrinjaquet-Mocchetti *et al.* 2008; Susalit *et al.* 2011).

In the study by Susalit *et al.*, two-hundred thirty-two patients, were enrolled. Of them, 162 (69.8%) subjects completed the study, 16 (6.9%) dropped out from the study due to various reasons and 54 (23.3%) had no available post-treatment data. Among those subjects that completed the study, 14 (6%) were incompliant with respect to study medication (consumption of study medication <80%), resulting in 148 (63.8%) patients evaluable for per-protocol efficacy analysis. Additionally, this study also investigated the hypolipidemic effects of olive leaf extract in such patients. It consisted of a run-in period of 4 weeks continued subsequently by an 8-week treatment period. Olive leaf extract (I) was given orally at the dose of 500 mg twice daily in a flat-dose manner throughout the 8 weeks. Captopril was given at the dosage regimen of 12.5 mg twice daily at start. After 2 weeks, if necessary, the dose of captopril would be titrated to 25 mg twice daily, based on subject's response to treatment.

The primary efficacy endpoint was reduction in systolic blood pressure (SBP) from baseline to week-8 of treatment. The secondary efficacy endpoints were SBP as well as diastolic blood pressure (DBP) changes at every time-point evaluation and lipid profile improvement. Evaluation of BP was performed

every week for 8 weeks of treatment; while of lipid profile at a 4-week interval. Mean SBP at baseline was 149.3 ± 5.58 mmHg in olive group and 148.4 ± 5.56 mmHg in captopril group; and mean DBPs were 93.9 ± 4.51 and 93.8 ± 4.88 mmHg, respectively.

The authors report that after 8 weeks of treatment, both groups experienced a reduction of SBP as well as DBP from baseline; while such reductions were not significantly different between groups. Means of SBP reduction from baseline to the end of study were -11.5 ± 8.5 and -13.7 ± 7.6 mmHg in olive and captopril groups, respectively; and those of DBP were -4.8 ± 5.5 and -6.4 ± 5.2 mmHg, respectively. Also a reduction of triglyceride level was observed in the olive group, but not in the captopril group. In conclusion, olive leaf extract, at the dosage regimen of 500 mg twice daily, was similarly effective in lowering systolic and diastolic blood pressures in subjects with stage-1 hypertension as captopril, given at its effective dose of 12.5–25 mg twice daily.

The same olive leaf extract (I), was tested as a food supplement in an open study including 40 borderline hypertensive monozygotic twins. Twins of each pair were assigned to different groups receiving 500 or 1000 mg-for 8 weeks, or advice on a favourable lifestyle. Body weight, heart rate, blood pressure, glucose and lipids were measured fortnightly. The authors reported that blood pressure changed interestingly within pairs, depending on the dose, with mean systolic differences of ≤ 6 mmHg (500 mg vs control) and ≤ 13 mmHg (1000 vs 500 mg), and diastolic differences of ≤ 5 mmHg. After 8 weeks, mean blood pressure remained unchanged from baseline in controls (systolic/diastolic: $133 \pm 5/77 \pm 6$ vs $135 \pm 11/80 \pm 7$ mmHg) and the low-dose group ($136 \pm 7/77 \pm 7$ vs $133 \pm 10/76 \pm 7$), but had decreased for the high dose group ($137 \pm 10/80 \pm 10$ vs $126 \pm 9/76 \pm 6$). Cholesterol levels decreased for all treatments with a dose dependent within-pair differences for LDL-cholesterol. None of the other parameters showed significant changes or consistent trends. The authors conclude that the study confirmed the antihypertensive and cholesterol-lowering action of extract (I) in humans (Perrinjaquet-Moccetti *et al.* 2008).

Hypoglycemic effects

The efficacy of 500 mg oral olive leaf extract taken once daily in tablet form was examined versus matching placebo in improving glucose homeostasis in adults with type 2 diabetes (T2DM). In this controlled clinical trial, 79 adults with T2DM were randomised to treatment with 500 mg olive leaf extract tablet taken orally once daily or matching placebo. The study duration was 14 weeks. Measures of glucose homeostasis including Hba1c and plasma insulin were measured and compared by treatment assignment. In the randomised clinical trial, the subjects treated with olive leaf extract exhibited significantly lower HbA1c and fasting plasma insulin levels; however, postprandial plasma insulin levels did not differ significantly by treatment group. The authors concluded that olive leaf extract is associated with improved glucose homeostasis in humans. Olive leaf extract was prepared from olive leaves as described by Zaslaver *et al.* 2005. Briefly, the leaves were randomly picked from the Barnea cultivar in the Jezreel Valley region of Israel and immediately freeze dried on dry ice. After being thoroughly rinsed with sterile distilled water to remove dust, insecticides, and contaminating material, the olive leaves were ground and Soxhlet extracted with hexane for 3 h followed by 80% aqueous ethanol for 6 h. The alcoholic extract was concentrated under reduced pressure at 25C, and the powder was encapsulated (Wainstein *et al.* 2012).

In a randomised, double-blinded, placebo controlled, crossover trial with 46 overweight men, participants received for 12 weeks either capsules with placebo or olive leaf extract (III) (New Zealand) equating to a daily dose of 51.1 mg oleuropein and 9.7 mg hydroxytyrosol, to study the effects of olive polyphenols on glucose homeostasis in humans. The authors reported that supplementation with olive leaf polyphenols for 12 weeks significantly improved insulin sensitivity and pancreatic β -cell secretory capacity in overweight middle-aged men at risk of developing the metabolic syndrome (de Bock *et al.* 2013ii).

Hypocholesterolaemic effects

In a double blind, 12 months controlled study, 250 mg of olive leaf phenolics (II) (oleuropein > 40 %, Netherlands) and 1000 mg Ca (treatment) or 1000 mg Ca alone (placebo) daily were administered orally in postmenopausal women. Levels of pro-osteoblastic marker osteocalcin increased in the treatment group as compared to placebo. Lumbar spine bone mineral density (BMD) remained stable, while it decreased in the placebo group. Improved lipid profiles, with significant decrease in total- and LDL-cholesterol in the treatment group was observed (Filip *et al.* 2014)

Table 5: Clinical study on renal effects.

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
Objective of the study Efficacy study Capretti & Bonaconza 1949	Controlled clinical trial	5 ml olive leaf infusion or 3 ml leaf decoction by mouth once daily for 20–25 days	Not reported	Adult patients (no further specification)	A daily increase in urinary output of 100–145 ml was noted for both dosage forms, with no effect on blood Na, K or chloride levels.	Not reported Old trial	Diuretic activity was reported. Details of the study as it is an old one are insufficient.

Table 6: Clinical studies in hypertension and on hypoglycaemic and hypocholesteroleamic effects

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
Antihypertensive effects Efficacy study Cherif <i>et al.</i> 1996	Double blind placebo controlled Duration: 3 months	400 mg of aqueous olive leaf extract (no further info given) 4 caps daily (approx 1.6 g olive leaf extract)	30 subjects	Patients with essential arterial hypertension separated into two groups: not previously treated with hypotensive drugs (n=12) previously used hypotensive therapy	A decrease in blood pressure in all patients were reported	Not reported	Small number of participants.

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
				(diuretics or beta-blocker) (n=18). Medication removed. Both groups received placebo gel capsules for 2 weeks before starting the treatment			
Anti-hypertensive effect as well as the tolerability in patients with stage-1 hypertension + hypolipidemic effects Comparative efficacy study versus captopril Susalit <i>et al.</i> 2011	Double-blind, randomised parallel and active-controlled clinical trial Duration: run-in period of 4 weeks continued subsequently by an 8-week treatment period. Evaluation of BP performed every week; lipid profile at a 4-week interval Primary endpoint: from baseline to week-8	Ethanollic (80% m/m) extract (DER 3–7:1) (18–26% m/m oleuropein, 30–40% m/m polyphenols) 500 mg twice daily, given orally for 8 weeks Control: Captopril 12.5 mg twice daily at start. After 2 weeks, if necessary, captopril 25 mg	232 patients enrolled 16 (6.9%) dropped out 54 (23.3%) no available post-treatment data 14 (6%) incompliant medication (<80%)	Patients with stage-1 hypertension Mean SBP at baseline was 149.3 ± 5.58 mmHg in olive group and 148.4 ± 5.56 mmHg in captopril group; and mean DBPs were 93.9 ± 4.51 and 93.8 ± 4.88 mmHg, respectively.	Primary endpoint (reduction in systolic blood pressure (SBP)): significant reduction of SBP in both groups (–11.5 ± 8.5 in olive and –13.7 ± 7.6 mmHg in captopril group; DBP: –4.8 ± 5.5 in olive and –6.4 ± 5.2 mmHg captopril group). Secondary endpoints: significant reduction of triglyceride level in the olive group, but not in the captopril group.	Not reported	63.8% of the patients were evaluable at the end of the study

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
	of treatment. Secondary endpoints: SBP and diastolic blood pressure (DBP) changes at every time-point evaluation and lipid profile improvement.	twice daily.	consumption) 148 (63.8%) patients evaluable for per-protocol efficacy analysis				
Anti-hypertensive effect + hypolipidemic effects Efficacy study as a food supplement Perrinjaquet-Moccetti <i>et al.</i> 2008	Open clinical study Duration: 8 weeks Heart rate, blood pressure, glucose and lipids measured fortnightly	Ethanollic (80% m/m) extract (DER 3–7:1). (18–26% m/m oleuropein, 30–40% m/m polyphenols) Treatment I: 500 mg/daily Treatment II: 1000 mg/daily Control: advice on a favourable lifestyle Oral	40 twins	Borderline hypertensive monozygotic twins	Body weight, blood pressure changed significantly within pairs, depending on the dose. After 8 weeks, mean blood pressure unchanged from baseline in controls (systolic/diastolic: 133 ± 5/77 ± 6 vs 135 ± 11/80 ± 7 mmHg) and the low-dose group (136 ± 7/77 ± 7 vs 133 ± 10/76 ± 7), but significantly decreased for the high dose group (137 ± 10/80 ± 10 vs	Not reported	Open study with small number of participants.

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
		administration			126 ± 9/76 ± 6). Cholesterol levels decreased for all treatments with significant dose dependent within-pair differences for LDL-cholesterol.		
Efficacy study Wainstein <i>et al.</i> 2012	Randomised placebo controlled clinical trial Duration of treatment: 14 weeks	Treatment: 500 mg of special olive leaf extract taken once daily in oral tablets Control: matching placebo	79 adults with T2DM randomised to treatment or matching placebo	Adults with type 2 diabetes (T2DM) Study comparing / improving glucose homeostasis in adults with type 2 diabetes (T2DM)	Subjects treated with olive leaf extract exhibited lower HbA1c and fasting plasma insulin levels	Not reported	Improving glucose homeostasis in adults with type 2 diabetes (T2DM) Interestingly lowering HbA1c and fasting plasma insulin levels; however, postprandial plasma insulin levels did not differ significantly by treatment group.
Effects of olive polyphenols on glucose homeostasis	Randomised, double-blinded, placebo controlled, crossover trial	Treatment: capsules with olive leaf extract (III)	46 overweight men (No	Overweight participants at risk of developing the metabolic syndrome	Insulin sensitivity and pancreatic β-cell secretory capacity improved in the	Not reported	Small number of participants and no randomisation given

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
Efficacy study de Bock <i>et al.</i> 2013ii	Duration: 12 weeks	(New Zealand) corresponding to a daily dose of 51.1 mg oleuropein and 9.7 mg hydroxytyrosol Control: placebo	randomisation given)		treatment group		No clinically relevant treatment.
Efficacy study Filip <i>et al.</i> 2014	Double blind, placebo controlled study Duration: 12 months	Treatment: 250 mg phenolics from olive leaf (oleuropein > 40% - Netherlands) + 1000 mg Ca daily (treatment) Control (placebo): 1000 mg Ca alone daily Oral administration	Number of subjects not specified	Postmenopausal women (No further specification)	Levels of pro-osteoblastic marker osteocalcin increased in the treatment group as compared to placebo. Lumbar spine BMD remained stable, while it decreased in the placebo group. Improved lipid profiles, with significant decrease in total- and LDL-cholesterol in the treatment group.	Not reported	Insufficient information for the clinical study design

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

No information available.

4.4. Overall conclusions on clinical pharmacology and efficacy

At present, the mechanism of action of olive leaf or olive leaf extracts cannot be considered clarified. Phenolic compounds such as oleuropein, phenolic acids and flavonoids are quantitatively important constituents of the whole olive leaf extract. The systemic bioavailability of these constituents is probably relatively low and variable.

Four existing clinical studies could support the traditional use with a mild diuretic activity as well as antihypertensive activity. However, there is a lack of rigorous clinical research assessing the effects of preparations of olive leaf.

A double-blind, randomised, parallel and active-controlled clinical study was conducted to evaluate the anti-hypertensive effect as well as the tolerability of olive leaf extract in comparison with captopril in 148 patients with stage-1 hypertension (Susalit *et al.* 2011). In another study, the same extract was tested as a supplement in an open study including 40 borderline hypertensive monozygotic twins and the antihypertensive and cholesterol-lowering action of it in the tested humans were reported (Perrinjaquet-Moccetti *et al.* 2008).

Recently other clinical trials were performed using (not well specified) olive leaf extracts (de Bock *et al.* 2013i; Filip *et al.* 2014; Wong *et al.* 2014) for several different purposes (effects on glucose homeostasis, in weight control, etc.) with generally good safety in humans. These results cannot be further evaluated as they are not confirmed due to shortcomings of the above mentioned clinical trials.

According to the published *in vitro* and *in vivo* studies as well as the existing old and not well documented clinical study (Caparetti & Bonaconza 1949), but also the two more recent clinical trials (Susalit *et al.* 2011; Perrinjaquet-Moccetti *et al.* 2008), a mild diuretic effect as well as a supportive effect to the cardiac system (through decrease of blood pressure) is plausible.

5. Clinical Safety/Pharmacovigilance

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

The safety profile of olive leaf and olive leaf extracts can be described as acceptable from the limited existing clinical studies and from its use from products on the market. The safety results obtained from the clinical studies conducted so far (see table 7) show that the oral use of olive leaf extracts are well tolerated by most patients. No drug-related serious or moderate adverse events were reported during the existing clinical trial.

The antihypertensive effect of an olive leaf extract (400 mg of aqueous olive leaf extract with no further details) was studied in patients with essential arterial hypertension. Patients were separated into two groups: first timers who had never been previously treated with hypotensive medication (n=12) and a second group who had previously benefited from some sort of anti-hypotensive therapy such as diuretic or beta-blocker medication (n=18). Patients took 4 capsules daily for total dose of approximately 1.6 g olive leaf extract daily. No adverse effects were reported during treatment with olive leaf extract and patients especially noted a disappearance of gastric disturbances that they had previously experienced on beta-blockers medications (Cherif *et al.* 1996).

Information on adverse reactions comes mainly from the comparative efficacy study of a proprietary name (I) olive leaf extract (DER 3–7:1, extraction solvent ethanol 80% m/m containing 18–26% m/m oleuropein, 30–40% m/m polyphenols) versus captopril (Susalit *et al.* 2011). A total of 1057 adverse events were reported by 168 (94.4%) study subjects, 83 subjects (49.4%) belonged to the olive group and 85 (50.6%) to the captopril group. The majority of adverse events were tolerably mild (99.8%) and comparable between groups. The most common adverse events which contributed to more than 5% of the total events observed during the study were coughing (4.6% in olive and 7% in captopril group) and vertigo (5.9% in olive and 6.3% in captopril group). Less frequently, muscle discomfort, headache, fatigue, malaise, myalgia and muscle cramp were reported and comparable between groups, constituting less than 5% of the total events. Vertigo, muscle discomfort and headache were judged to be possibly related to both olive leaf extract and captopril. All these adverse events had resolved at the end of the study. Based on the laboratory safety evaluation, it was observed that administration of olive leaf extract to stage-1 hypertensive subjects did not affect liver and renal functions. Neither did it affect haematological parameters and electrolyte balance of study participants. Even though some of the laboratory safety parameters were statistically different from baseline values, all of them remained within the normal range at the end of the study period, and thus such changes were not clinically relevant. The evaluation of all safety parameters and occurrence of adverse events showed that olive leaf extract was safe and tolerable in patients with stage-1 hypertension.

Table 7: Clinical safety data from clinical trials

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Adverse reactions	Comments
Antihypertensive effects Efficacy study Cherif <i>et al.</i> 1996	Double blind placebo controlled Duration: 3 months	400 mg of aqueous olive leaf extract (no further info given) 4 caps daily (approx 1.6 g olive leaf extract)	30 subjects	Patients with essential arterial hypertension separated into two groups: not previously treated with hypotensive drugs (n=12) previously used hypotensive therapy (diuretics or beta-blocker) (n=18). Medication removed. Both groups received placebo gel capsules for 2 weeks before starting the treatment.	No adverse effects were reported during treatment and patients noted a disappearance of gastric disturbances (reported previously on beta-blockers medication).	No adverse effects were reported
Anti-hypertensive effect as well as the tolerability in patients with stage-1 hypertension + hypolipidemic	Double-blind, randomised parallel and active-controlled clinical trial Duration: run-in period	Ethanollic (80% m/m) extract (DER 3–7:1). (18–26% m/m oleuropein, 30–40% m/m polyphenols) 500 mg twice daily, given orally for 8 weeks	232 patients enrolled 16 (6.9%) dropped out, for various reasons 54 (23.3%) no available post-	Patients with stage-1 hypertension.	The most common adverse events which contributed to more than 5% of the total events observed were coughing (4.6% in olive and 7% in captopril group) and vertigo (5.9% in olive	Adverse events were comparable in the two groups and the evaluation of all safety parameters and occurrence of showed that

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Adverse reactions	Comments
effects Comparative efficacy study versus captopril Susalit <i>et al.</i> 2011	of 4 weeks continued subsequently by an 8-week treatment period.	Captopril 12.5 mg twice daily at start. After 2 weeks, if necessary, captopril 25 mg twice daily.	treatment data 14 (6%) in-compliant medication (<80% consumption). 148 (63.8%) patients evaluable for per-protocol efficacy analysis.		and 6.3% in captopril group).	olive leaf extract was safe and tolerable in patients with stage-1 hypertension.
Effects of olive polyphenols on glucose homeostasis Efficacy study de Bock <i>et al.</i> 2013	Randomised, double-blinded, placebo controlled, crossover trial Duration: 12 weeks	Treatment: capsules with olive leaf extract (III) (New Zealand) equating to a daily dose of 51.1 mg oleuropein and 9.7 mg hydroxytyrosol Control: placebo	46 overweight men (no randomisation given)	Overweight participants at risk of developing the metabolic syndrome	Only 1 mild adverse event reported in placebo group. Liver function tests showed no differences in AST, ALP, ALT, or GGT among participants in treatment arm.	Efficacy study with well accepted safety
Efficacy study Of the lipid profiles (total- and LDL-cholesterol) group. Filip <i>et al.</i> 2014	Double blind, placebo controlled study Duration: 12 months	Treatment: 250 mg phenolics from olive leaf (oleuropein > 40% - Netherlands) + 1000 mg Ca daily (treatment) Control (placebo): 1000 mg Ca alone	Subjects not specified	Postmenopausal women (no further specification)	Overall incidence of adverse events was similar across the two study groups. No serious adverse events were related to the treatment. No clinically relevant treatment	No clinically relevant treatment related adverse events

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Adverse reactions	Comments
		daily Oral administration			related events were recorded during the study	
Objective of the study Efficacy study Wong <i>et al.</i> , 2014	Double-blind, placebo controlled, crossover Duration of treatment: 6-weeks	Treatment: Blend of Olive leaf extract (no further specification?) + green coffee bean extr extract+ beetroot extr extract(proprietary name Switzerland)	37 subjects (out of 52 who started the trial)	Adults with mildly elevated, but untreated hypertension (130–160 mmHg systolic and 85–100 mmHg diastolic)	One serious adverse event was reported, that resolved without incident; 3 out of 4 mild adverse events occurred during the active treatment phase	Combination of olive leaf extract with green coffee bean and beetroot extracts were studied

5.2. Patient exposure

No data available on the exposure of patients for the diuretic activities of the olive leaf.

5.3. Adverse events, serious adverse events and deaths

Intra-ocular use of olive leaf may irritate the surface of the eye (Brinker 1998). If olive leaf extract preparations are administrated to patients with biliary tract stones, these may be a risk of causing biliary colic through promoting the secretion of bile (Lockyer *et al.*, 2015).

Pollinosis, in the form of rhinitis or bronchial asthma has been reported (PDR for Herbal Medicines 2007).

In the clinical trial (Susalit *et al.* 2011) with olive leaf extract a total of several adverse events were reported by 83 subjects (49.4%) belonged to the olive group. The majority of adverse events were tolerably mild (99.8%) The most common adverse events which contributed to more than 5% of the total events observed during the study were coughing (4.6% in olive group) and vertigo (5.9% in olive group). Less frequently, muscle discomfort and headache, were reported in a less than 5% of the total events.

Serious adverse events and deaths

None reported.

Assessor's comment:

The safety profile of olive leaf extracts can be described as acceptable from the existing clinical studies (Susalit *et al.* 2011; Perrinjaquet-Moccetti *et al.* 2008) and from its use of products on the market. The safety results obtained from the clinical studies conducted so far show that the oral use of olive leaf extracts are well tolerated by most patients. The majority of adverse events were tolerably mild while the most common ones (5% of the total events observed during the studies) were coughing and vertigo (4.6% and 5.9% respectively in Olive group). Less frequently, muscle discomfort and headache, were reported.

There are no reported drug-related serious or moderate adverse events. It is proposed in the literature that olive leaf extract preparations administrated to patients with biliary tract stones, could cause a risk of biliary colic through promoting the secretion of bile. (PDR for Herbal Medicines 2007)

5.4. Laboratory findings

No data available.

5.5. Safety in special populations and situations

5.5.1. Use in children and adolescents

Olive leaf is not intended for use in children.

5.5.2. Contraindications

Hypersensitivity to the active substance. The herbal substance and comminuted herbal substance as herbal tea for oral use, is contraindicated 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

To ensure a safe use the following statement should be labelled:

Patients with cardiac disease or renal impairment should seek medical advice before taking Olea medication.

The use in children and adolescents under 18 years of age has not been established due to lack of adequate data.

If symptoms worsen during the use of the medicinal product, a medical doctor or a qualified health care practitioner should be consulted.

5.5.4. Drug interactions and other forms of interaction

Drug interactions from clinical trials or case studies have not been reported so far.

5.5.5. Fertility, pregnancy and lactation

No fertility data available.

The use of medicinal products from olive leaf is not recommended during pregnancy and lactation as no data are 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 studies on the effect on the ability to drive and use machines have been performed.

5.5.8. Safety in other special situations

Not applicable

5.6. Overall conclusions on clinical safety

No side effects have been reported during the use of olive leaf preparations.

In the absence of data in special patient populations, olive leaf is intended only for adults and, in accordance with general medical practice, it is recommended not to use the herbal medicinal products containing olive leaf during pregnancy and lactation.

The safety profile of olive leaf and olive extracts can be judged as good from the existing clinical data and from their long term use, more than 30 years, in the European market. The available literature, on pharmacological and toxicological studies, does not give reason for safety concerns. No serious adverse

events or deaths as well as no drug interactions from clinical trials or case studies have been reported so far.

6. Overall conclusions (benefit-risk assessment)

The positive effects of olive leaf to enhance the excretion of urine and to support somehow the cardiovascular function (through its hypotensive activity) have long been recognised empirically. The use is made plausible especially by *in vitro* and *in vivo* pharmacological data. There are not many available clinical studies using herbal preparations containing the herbal substance of olive leaf. A recent double-blind, randomised, parallel and active-controlled clinical study was conducted to evaluate the anti-hypertensive effect as well as the tolerability of a proprietary name olive leaf extract in comparison with captopril in 148 patients, with stage-1 hypertension for 8 weeks (Susalit *et al.* 2011). The evaluation of all safety parameters and occurrence of adverse events showed that olive leaf extract was safe and tolerable in patients with stage-1 hypertension. In another study the same extract, having antihypertensive actions in rats (Khayyal *et al.* 2002), was tested as a supplement in an open study in 40 borderline hypertensive monozygotic twins for 8 weeks, confirming signals of the antihypertensive and cholesterol-lowering action in the tested humans (Perrinjaquet-Moccetti *et al.* 2008). These data cannot be extrapolated to other olive leaf preparations.

There are not sufficient data from well-designed clinical trials to support well-established use in this indication.

Moreover, safety concerns remain with respect to a cardiovascular indication, i.e. the demarcation between mild functional complaints and organic symptoms. More serious conditions may not be easily distinguished by patients. Even after exclusion of such conditions, it should be avoided that patients may be encouraged for self-treatment, where clearly medical supervision and medically supervised medication is required.

Therefore the HMPC endorsed only an indication of olive leaf preparations as traditional herbal medicinal products in the following indication:

Traditional herbal medicinal product used to promote the renal elimination of water, in mild cases of water retention, after serious conditions have been excluded by a medical doctor.

The following herbal substances/preparations have been traditionally used for more than 30 years and their safe use can be stated on the basis of the well-known, long-lasting and traditional use in the folk medicine and as registered medicinal products:

- fresh or dried leaves
- comminuted or powdered dried leaves for herbal tea
- Powdered dried leaves.

Following the acceptance of the above mentioned indication, two herbal preparations [Liquid extract (1:0.71-0.86 solvent: ethanol 96% V/V). and Dry extract (7.9-12:1), extraction solvent: ethanol 96% V/V.], which have been on the German market for more than 40 years were excluded from the monograph as the indication in Germany (“...to support the cardiovascular system”) does not comply with the indication accepted by the HMPC as well as for self-medication.

In the absence of data in special patient populations, olive leaf is intended only for adults and elderly and, in accordance with general medical practice, the use of the herbal medicinal products containing olive leaf during pregnancy and lactation is not recommended.

Due to the lack of adequate data on genotoxicity, a European Union list entry is not supported.

Typical analytical marker is oleuropein.

Annex

List of references