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Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Salix* [various species including *S. purpurea* L., *S. daphnoides* Vill., *S. fragilis* L.], cortex

Final

Based on Article 10a of Directive 2001/83/EC (well-established use)

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)	*Salix* [various species including *S. purpurea* L., *S. daphnoides* Vill., *S. fragilis* L.], cortex
Herbal preparation(s)	a) Comminuted herbal substance. b) Powdered herbal substance. c) Dry extract (8-20:1) extraction solvent water d) Dry extract (16:23-1) extraction solvent water e) Liquid extract (1:1), extraction solvent ethanol 25% V/V. f) Tincture (1:5), extraction solvent ethanol 25% V/V. g) Dry extract (8-14:1) extraction solvent ethanol 70% V/V, 15% total salicin.
Pharmaceutical form(s)	Herbal preparation in solid or liquid dosage form or as herbal tea for oral use. Quantified herbal preparation in solid dosage form.
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1. Introduction

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

- *Herbal substance(s)*

There are about 500 species of *Salix* species called willow, mainly found in Europe and North America. The species of medical interest include *Salix alba*, *S. nigra* and *S. purpurea*, but *S. daphnoides* and *S. fragilis* along with *S. purpurea* contain the greatest yield of salicylate precursors.

According to the Ph. Eur. (01/2005:1583), the herbal substance is the whole or fragmented dried bark of young branches or whole dried pieces of current year twigs of various species of genus *Salix* including *S. purpurea* L., *S. daphnoides* Vill. and *S. fragilis* L. The drug contains not less than 1.5% of total salicylic derivatives, expressed as salicin ($C_{13}H_{18}O_7$; MW 286.3), calculated with reference to the dried drug.

The characteristic constituents are derivatives of salicin, mainly salicortin, 2'-*O*-acetylsalicortin and/or tremulacin. Other constituents are flavonoids, condensed tannins (8-20%) and catechins.

Salicylates, calculated as total salicin (and determined after hydrolysis) vary between species: 0.5% in *Salix alba*, 1-10% in *Salix fragilis*. The concentration and availability of salicylates also vary within species according to growing conditions, processing and preparation (Meier *et al*. 1985a, Meier *et al.* 1985b, Julkunen-Tiitto *et al.* 1992a, 1992b and 2001, Blashek *et al*. 1998). It should be noted that tannins may interfere with the absorption of salicylic acid.

The bark of *Salix purpurea* L. contains 4-8% of total salicin (after hydrolysis). Phenol glucosides include salicortin (up to 9%), tremulacin (rarely more than 1%) and salireposide (0.1-1.2%) with small amounts of syringin and purpurein (up to 0.4%). Other constituents include the yellow chalcone isosalipurperoside (0.15-2.2%), the flavanones eriodictyol-7-glucoside (0.18-0.4%) and (+) and (-)-naringenin-5-glucoside (0.4-1.5% each), approximatively 0.5% of (+)-catechin and 5% of polyphenols. Young twigs (bark and wood) contain the same constituents in lower concentrations than bark alone (Freischmidt *et al*. 2015).

The bark of *Salix daphnoides* Vill. contains more than 4% of total salicin. Phenol glucosides include salicortin (3-11%), tremulacin (up to 1.5%) and salicin (up to 1%) with small amounts of syringin (up to 0.2%). Other constituents include the yellow chalcone isosalipurperoside (0.2-1.5%), the flavanones (+) and (-)-naringenin-5-glucoside (0.3-1% each) and naringenin-7-glucoside (0.3-1.5%), approximatively 0.5% of (+)-catechin and 5% of polyphenols. Young twigs (bark and wood) contain the same constituents in lower concentrations than bark alone.

It is suggested that all components identified play a role in the anti-inflammatory process (Keusgen and Algauer-Lechner 2007).

There seems to be seasonal variation in the content of salicylates in willow bark. Förster *et al*. (2009) performed a qualitative analysis on samples of *Salix daphnoides*, *Salix pentandra* and *Salix purpurea* were collected over three years (2006, 2007, 2008) in Germany, Poland, Austria, Switserland and Italy. Salicylate content was analyzed with HPLC. According to their findings the authors make recommendation for optimal periods of harvesting (Förster *et al*. 2010).

Table 1: Seasonal variation of salicylate content of different *Salix* species collected over three different years. Different characters mean significant differences between different years for the same species (Föster *et al*. 2009).

	Salicylatgehalt [%]*				
	März	Juni	Juli	August	Oktober
Salix daphnoides	6,41 (a)	4,71 (b)	3,30 (bc)	2,59 (c)	4,46 (b)
Salix pentandra	2,41 (a)	1,84 (ab)	1,80 (b)	1,60 (b)	2,12 (ab)
Salix purpurea	4,02 (ab)	3,27 (bc)	2,67 (c)	2,58 (c)	4,45 (a)

* Verschiedene Buchstaben indizieren signifikante Unterschiede innerhalb einer Art und zwischen den Probezeiträumen, Tukey's HSD Test, $p < 0,05$

Esatbeyoglu *et al*. (2010) analysed 57 different samples of white willow bark (*Salix alba*) with regard to their dimeric and polymeric procyanidin composition. Polymeric procyanidins of white willow bark were found to contain the highest amount of (þ)-catechin in the extension units. The major products are procyanidins B3 and B4 which carry C4 to C8 linkages and afford purity levels of dimeric procyanidin B3 >95%. At the same time lower amounts of the C4 to C6 linked dimeric procyanidins B6 and B8 are produced (Esatbeyoglu *et al.* 2010).

Kim *et al*. (2015) isolated two new salicin derivatives, saliglandin and 6′-O-(Z)-p-coumaroylsalicin, along with fourteen known analogues were isolated from the twigs of *Salix glandulosa* Seemen. The structures of 1–16 were characterized by the use of NMR (^{1}H and ^{13}C NMR), chemical hydrolysis, and GC/MS (Kim *et al.* 2015).

Wiesneth *et al*. (2015) isolated and analysed proanthocyanidins (PAs) from an aqueous-methanolic extract of *Salix daphnoides* VILL. Procyanidin B1 (**1**), B2 (**2**), B3 (**3**), B4 (**4**), C1 (**5**), epicatechin-(4*β*→8)-epicatechin-(4*β*→8)-catechin (**6**) and epicatechin-(4*β*→8)-epicatechin-(4*β*→8)-epicatechin-(4*β*→8)-catechin (**7**) have been isolated by a combination of different chromatographic separations, mass spectrometry, 1D- and 2D-NMR, circular dichroism and polarimetry (**Figure 1**). Additionally, two fractions of very polar flavan-3-ols were compared. These "unusual" PAs were subsequently enriched and characterized by centrifugal partition chromatography (CPC). 13C-NMR, polarimetry, thiolysis, acid hydrolysis and phloroglucinol degradation. Differences in the composition of different flavan-3-ol units and the middle chain length were observed (Wiesneth *et al*. 2015).

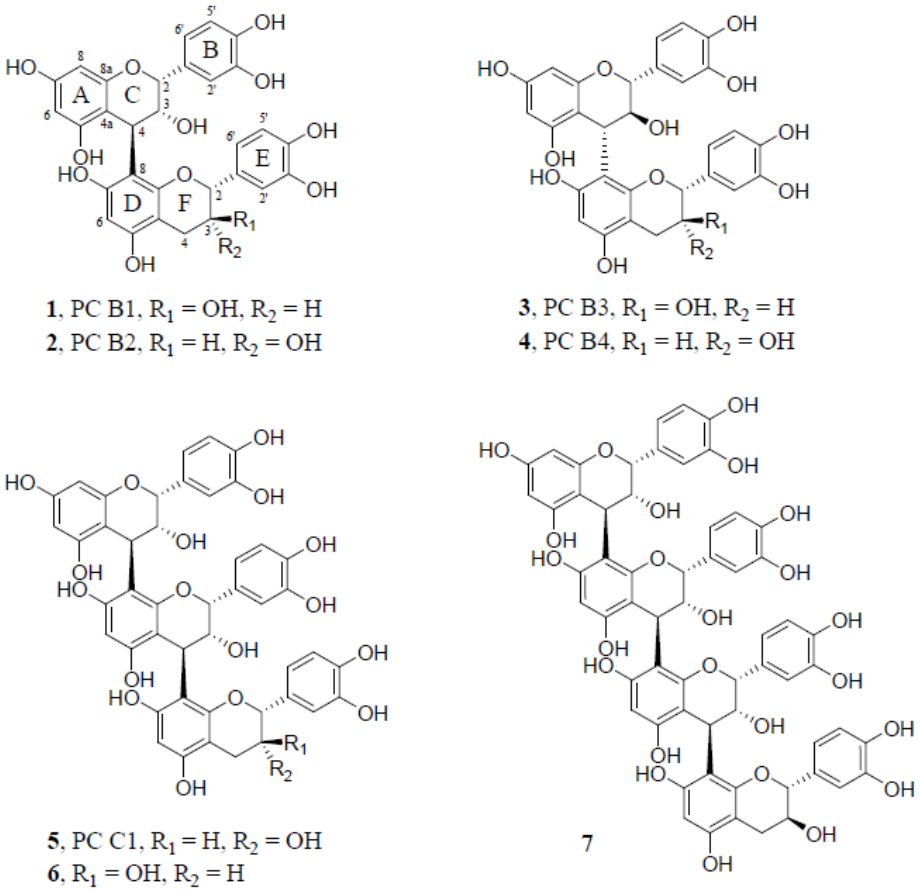

Figure 1: Structures of the isolated procyanidins (PC) 1–7 (Wiesneth *et al*. 2015).

Willow monographs are included in general reference books on herbal substances such as the American Herbal Pharmacopoeia (1999), British Herbal Pharmacopoeia (1983), British Herbal Compendium (1992), Commission E monographs (1984), ESCOP (2003), Dingerman *et al*. (2002).

DAB 10 (and DAB 10 Kommentar resp. 1991 and 1995) and Bisset (1994) recommend 2-3 g herbal substance (finely chopped or coarsely powdered) 3 to 4 times per day, with mean daily doses of 60-120 mg salicin.

Barnes *et al*. (2007) recommended 1-3 g dry bark for decoction, three times daily, corresponding to 60-120 mg total salicin daily.

The concentration of salicin in the herbal substance varies (see above). The Ph. Eur. stipulates a minimum content of 1.5% total salicin in the herbal substance. It is clear that 6-12 g powdered bark (as a decoction) will usually not deliver amounts of salicin that are comparable to the doses of salicin administered in the preparations studied in the clinical trial settings.

The herbal substance as such is however not used; only the bark reduced in size to comminuted or powdered is used (herbal preparations).

- *Herbal preparation(s)*

According to the Ph. Eur. (04/2008: 2312), willow bark dry extract contains minimum 5.0% of total salicylic derivatives, expressed as salicin ($C_{13}H_{18}O_7$; Mr 286.3) (dried extract). The extract is produced from the herbal drug by a suitable procedure using either water or a hydro-alcoholic solvent equivalent in strength to a maximum of 80% V/V ethanol.

The Ph. Eur. monograph only stipulates a minimum content of total salicylic derivatives, expressed as salicin. Each manufacturer needs to provide a range for the quantified extract used in his finished product. The 15% total salicin, as contained in the extract for which moderate clinical efficacy was demonstrated, represents an average value. The exact range needs to be established for each finished product on the basis of the manufacturer's specifications.

During the first assessment in 2008 it was discussed whether preparations in the TU part of the monograph should be quantified or not. The HMPC concluded not to use the term "quantified" in the TU part of the monograph.

ESCOP: Dried hydro-alcoholic or aqueous extracts, tinctures or liquid extracts, equivalent to 120-240 mg of total salicin per day.

The Commission E monograph (1984) on willow bark recommends liquid and solid preparations; daily dose corresponding to 60-120 mg total salicin, as antipyretic, antiphlogistic and analgesic.

In Germany, a Marketing Authorisation (MA) was granted for the following hydro-alcoholic and aqueous extracts: dry extract ethanol 70% 8-14:1 (dosage: 393.34 mg extract 1-4x per day) and dry aqueous extracts with ratio's 16-20:1; 8-16:1 (dosage: 2 x 600 mg extract per day) and dry aqueous extract (16-23:1). The maximal daily doses range from 120 mg salicin to 240 mg salicin (Wagner *et al*. 2003b, information from the Rote Liste 2002).

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

Not applicable

1.2. Search and assessment methodology

Starting from the references used for the first version additional information was searched in

- Books and book chapters: Barnes *et al*. (2007), Dingerman *et al*. (2002), Madaus (1935), McGuffin (1997), Schmid *et al*. (2002), Williamson *et al*. 2013.
- Monographs: ESCOP, Kooperation Phytotherapie

Search engines used: keywords *Salix* OR *willow* within a timeframe January 2011 to January 2016.

Scientific databases: Embase

Medical databases: PubMed

Data from EU and non-EU regulatory authorities: market overview up to November 2015.

Other resources:

- Monographs: Kooperation Phytotherapie.

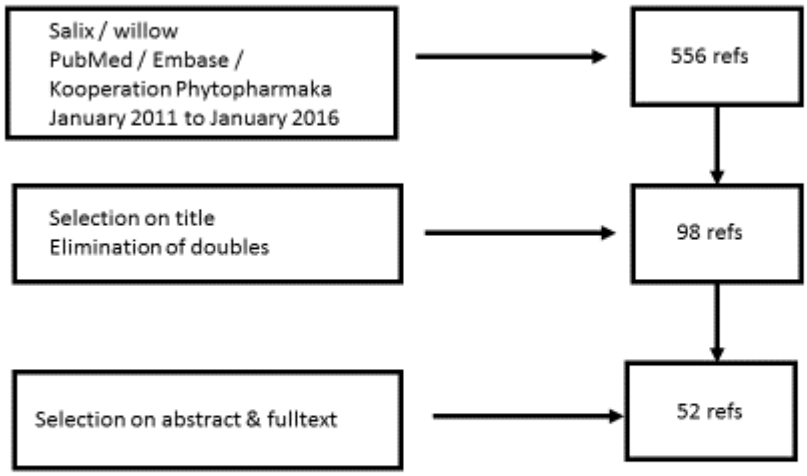

Figure 2: literature selection flowchart

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

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

Table 2: Information on medicinal products marketed in Germany

Active substance	Indication	Pharmaceutical form Posology Duration of use	Regulatory Status
1. Salicis cortex, powder	Headache, symptomatic treatment of rheumatic symptoms, symptomatic treatment of fever *(Kopfschmerzen, rheumatische Beschwerden, fieberhafte Erkrankungen)*	coated tablet 500 mg > 12 years: 2 (up to 8 coated tablets per day) Duration not limited when under medical supervision	1986, DE, WEU
2. Salicis cortex, powder	Headache, symptomatic treatment of rheumatic	coated tablet	1993, DE, WEU

Active substance	Indication	Pharmaceutical form Posology Duration of use	Regulatory Status
	symptoms, symptomatic treatment of fever *(Kopfschmerzen, rheumatische Beschwerden, fieberhafte Erkrankungen)*	500 mg > 12 years: 2 (up to 8 coated tablets per day) Duration not limited when under medical supervision	
3. Salicis cortex dry extract (8-14:1), extraction solvent: ethanol 70% (V/V)	Short term treatment of lower back pain *(Zur Kurzzeitbehandlung von Kreuzschmerzen)*	coated tablet 393.24 mg >18 years: 1-2 tablets 2 times daily Duration: maximum 4 weeks	1997, DE, WEU
4. Salicis cortex dry extract (8-14:1), extraction solvent: ethanol 70% (V/V)	Headache, symptomatic treatment of rheumatic symptoms, symptomatic treatment of fever *(Kopfschmerzen, rheumatische Beschwerden, fieberhafte Erkrankungen)*	coated tablet 393.24 mg >12 years: 1 tablet 1-2 times daily Duration not limited	1997, DE, WEU
5. Salicis cortex dry extract (16-23:1) corresponding to 120 mg salicin, extraction solvent: water	Headache, symptomatic treatment of rheumatic symptoms *(Kopfschmerzen, rheumatische Beschwerden)*	capsule, hard 480 mg >12 years: 1 capsule 2 times daily Duration not limited	2004, DE, WEU
6. Salicis cortex, cut	Symptomatic treatment of mild rheumatic symptoms (Zur Besserung von leichten rheumatischen Beschwerden)	herbal tea 2.55 g/sachet > 12 years: 1 sachet with 150 ml boiling water 3 times daily Duration maximum 2 weeks	2007, DE, WEU

Table 3: Information on medicinal products marketed in Poland

Active substance	**Indication**	**Pharmaceutical form** **Posology** **Duration of use**	**Regulatory Status**
1. Salicis cortex, powdered	Traditionally used in headache, common cold with fever and as support in arthritis	Tablets, 330 mg Posology: 1-2 tablets 3 times daily, after meals. It is advised to take with larger quantity of hot water.	Traditional use registration, 2009
2. Salicis cortex, comminuted	Used in the symptomatic treatment of fever and pain and mild rheumatic pain	Herbal tea 4 g of comminuted bark of willow pour with 1 cup (200 ml) of water and boil covered for 15 minutes. Let stand for 15 minutes, strain. Drink after meals, 3 times a day a glass of warm, freshly prepared decoction.	National registration, R/0133
Information on active or analytical marker(s) or constituents with known therapeutic activity			
1. 330 mg of powdered Salicis cortex contains not less than 20.0 mg of phenolic glycosides counted as salicin.			
2. Quality in line with Eur. Ph. Daily dose corresponds to 240 mg phenolic glycosides counted as salicin.			

Countries without information on products on the market (reporting September – October 2015)

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Malta, the Netherlands, Norway, Portugal, Romania, Slovakia, Slovenia, Spain, UK, Sweden.

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

Medicinal use of different parts of the willow tree has been mentioned by Pedanius Dioskorides (1st century AD) and Philippus Aureolus Theophrastus Bombastus von Hohenheim or Paracelsus (16th century) (Madaus 1935). Willow bark has had a long tradition as febrifuge dating back to the 18th century. Following the identification of salicin and the subsequent synthesis of salicylic acid and more importantly acetyl salicylic acid (end of 19th century), the interest in willow bark had decreased substantially. However, the demand for phytoanalgetica with better tolerability versus anti-inflammatory drugs has increased scientific interest in willow bark (Mayer and Mayer 1949) (McGuffin *et al*. 1997) (Kaul *et al*. 1999). Even in South-Africa willow was known by Hottentot sheperds as a remedy in case of rheumatic fever (Volmink 2008).

Willow bark has traditionally been used for muscular and arthroidal rheumatism with inflammation and pain, influenza, respiratory catarrh, gouty arthritis, ankylosing spondylitis, and specifically for rheumatoid arthritis (RA) and other systemic connective tissue disorders characterised by inflammatory changes (Barnes *et al*. 2007; Beer *et al*. 2008). The use in case of lumbar pain is less supported by scientific data (Beer & Loew 2008).

The British Herbal Pharmacopoeia (1983) and British Herbal Compendium volume 1 (1992) included a liquid extract (1:1 in 25% alcohol): 1-3 ml three times daily and a tincture (1:5) (extraction solvent ethanol 25%): 15-24 ml per day.

Wichtl (2002) / Hänsel (1991) / Hagers Handbuch (Blaschek *et al*. 1998) and ESCOP (2003) state the use as a relief of low back pain; symptomatic relief of mild osteoarthritic and rheumatic complaints.

HagerROM (2001) mentions the traditional use of powdered drug and herbal teas in case of flu-like conditions and treatment of minor pain (daily dose equivalent to 60-120 mg salicin).

The German Commission E monograph (1984) approved internal use for diseases accompanied by fever, rheumatic complaints and headaches. Apart from these indications the use in quite a lot of other conditions have been listed. The following indications can be found in standard sources: amongst others adjuvans in diabetes mellitus, anthelminticum, diaphoreticum, diureticum, dyspepsia, malaria, bronchitis, against neuralgia, sedative and as a tonicum. Traditional external use includes the use as amongst others antisepticum, adstringens, keratolyticum, rubefaciens and in case of ear infection (Benedum *et al*. 2006).

März and Kemper (2002) made an overview of preclinical and clinical investigations, confirming pharmacodynamic properties and clinical value of willow preparations.

In Germany, MAs were granted for HMPs containing:

Dry extract ethanol 70% 8-14:1 with indications: headache, fever, minor articular pain. Dosage: 1-2 x 393, 34 mg extract per day (MA dated 1997)

Dry aqueous extracts 16-20:1 and 8-16:1 with indications: headache, fever, rheumatic complaints. Dosage: 2 x 600 mg extract per day (MAs date from 1997 & 2003)

Dry aqueous extract 16-23:1 with indication of fever. Dosage: 2 x 480 mg extract per day (MA 2003)

Powdered willow bark: 500 mg per coated tablet or capsule (MAs since 1991 and 1992 respectively).

Cut herb: 1.995 g/teabag, 3-6 cups per day (MA dated 1999).

All preparations for which MA for “TU” had been granted (according to former national regulations) were included in the overview by Germany (some of them not in accordance with the actual provisions of Directive 2004/24/EC). Traditional preparations were authorised in 10-50% of WEU doses when in parallel the same preparations were authorised under WEU. In line with HMPC policy and established practice with reference to Art. 16a(3) of Directive 2001/83/EC, it is not considered appropriate that the dry extract ethanol 70% 8-14:1 appears in the TU section of the monograph, because fulfilling criteria to be eligible for WEU and included in the WEU part of the monograph.

Willow bark is also ingredient in combination products (3 WEU products and 2 TU products) and a standard MA for combination products with willow bark as a herbal tea exists.

In Spain, 400 mg powdered willow bark is administered every 8 hours.

In France, capsules containing 260 mg willow bark powder are authorised since 1988.

No single ingredient products are authorised / registered in the other Member States: willow bark is included in combination HMPs in Belgium, Malta, Czech Republic, UK, Austria, Latvia and Italy.

In Austria, a MA exists for a combination willow-bark containing HMP (120 mg aqueous extract 20-1, 15% salicin; in combination with Tilia flos and vitamin C). In addition, a combination herbal tea is on the market.

No willow bark containing products are authorised in Norway, Finland and Portugal.

For information, in Italy food supplements with the following preparations were notified:

- Capsules with a combination of 400 mg dry extract of *Salix alba* (15% salicin) and 460 mg powdered bark (notified in 2004): claim that it may favor osteo-articular well-being
- Oral solution (drops) containing an ethanol (60%) extract

In central Italy dried willow bark is applied topically to treat warts (Leporatti 1990).

Hagers Handbuch includes external use to help healing of wounds (50 g herbal substance per 0.5 l water).

The conditions associated with “fever” and “pain” in which HMPs containing willow bark are traditionally used were specified as “a) the symptomatic relief of minor articular pain” with a duration restriction to a maximum of 4 weeks, “b) the symptomatic relief of fever associated with common cold” for no longer than 3 days (in common cold, fever is experienced for 3 days), and “c) the symptomatic relief of headache”. If fever exceeds 39°C, persists or is associated with severe headache (meningitis) or if symptoms worsen during the use of the medicinal product, a doctor should be consulted. If headache persists for more than one day or is recurrent, medical advice is sought.

These TU uses are contra-indicated in children and adolescents under 18 years of age. THMPs containing willow bark are not intended to be used in case of acute osteoarthritis (OA) as this condition requires medical advice.

The posology section covers only the preparations for which posology is documented. A posology for dry bark for herbal tea preparation, dry aqueous extracts, liquid extract and powdered dry bark were specified.

Table 4: Overview of historical data

Herbal preparation	**Documented Use**	**Pharmaceutical form** **Posology** **Duration of use**	**Reference**
Salix cortex	TU as an anti-inflammatory herbal medicine in different conditions: muscular and arthrodial rheumatism with inflammation and pain, influenza, respiratory catarrh, gouty arthritis, ankylosing spondylitis, and specifically for rheumatoid arthritis (RA)	No posology specified	Kaul *et al*. (1999); Barnes (2007); Wichtl (2002); Hänsel (1991) Hagers Handbuch; Blaschek *et al*. (1998); ESCOP (2003);
Salix cortex powdered and as herbal tea	Flu-like conditions and treatment of minor pain	Daily dose equivalent to 60-120 mg salicin	HagerROM (2001)

2.3. Overall conclusions on medicinal use

Table 5: Overview of evidence on period of medicinal use

Herbal preparation **Pharmaceutical form**	**Indication**	**Strength** **Posology**	**Period of medicinal use**
Comminuted herbal substance	Indication 1) Traditional herbal medicinal product used for the relief of minor articular pain. Indication 2) Traditional herbal medicinal product used for the relief of fever associated with common cold. Indication 3) Traditional herbal medicinal product	Herbal tea: 1 to 3 g of the comminuted herbal substance in 150 ml of boiling water as an herbal infusion 3 times daily. Decoction: 4 g of comminuted bark of willow pour with 1 cup (200 ml) of water and boil covered for 15 minutes. Let stand for 15 minutes, strain. Drink after	Since 2007, DE National registration, R/0133 Poland.

Herbal preparation Pharmaceutical form	Indication	Strength Posology	Period of medicinal use
	used for headache.	meals, 3 times a day a glass of warm, freshly prepared decoction.	
Powdered herbal substance		260-500 mg 3 to 8 times daily. To be taken after meals. It is advised to drink with larger quantity of hot water.	Since 1986, DE
Dry extract (DER 8-20:1) extraction solvent water		600 mg twice daily	All preparations for which MA for "TU" had been granted (according to former national regulations) were included in the overview by Germany (some of them not in accordance with the actual provisions of Directive 2004/24/EC). Traditional preparations were authorised in 10-50% of WEU doses when in parallel the same preparations were authorised under WEU.
Dry extract (DER 16-23:1) extraction solvent water		480 mg twice daily	
Liquid extract (DER 1:1), extraction solvent ethanol 25% V/V		1 to 3 ml, three times daily	The British Herbal Pharmacopoeia (1983) and British Herbal Compendium volume 1 (1992)
Tincture (1:5), extraction solvent ethanol 25% V/V		15-24 ml per day	
Dry extract (8-14:1) extraction solvent ethanol 70% V/V, 15% total salicin	Herbal medicinal product used for the short-term treatment of low back pain.	Single dose: 393 mg to 786 mg up to 2 times daily. The daily dose is 393 to 1572 mg dry extract (8-14:1)	Since 1997, DE, WEU

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

In vitro studies

Pharmacological actions normally associated with salicylates are also applicable to willow which support most of the herbal uses, although no studies are available for willow covering most uses. Salicin is probably the most active anti-inflammatory compound in willow; it is metabolised to salicylic acid.

The hen's egg choriollantoic membrane test system has been used to study the anti- inflammatory effect of the willow bark constituents salicin and tremulacin (isolated from *Populus spp*). Onset of this anti-inflammatory effect is delayed in comparison with saligenin (salicyl alcohol), sodium salicylate and acetylsalicylic acid, indicating that the active principles may be metabolites of salicin and tremulacin (Albrecht *et al*. 1990).

Isolated tremulacin, s.c. injected at 100 mg/kg bw significantly inhibited carrageenan-induced paw oedema and peritoneal leucocyte migration in rats, and croton oil induced ear oedema and acetic acid induced writhing in mice. Inhibition of leukotriene B4 biosynthesis in pleural leucocytes also supported its anti-inflammatory activity in acute inflammatory animal models (Cheng *et al*. 1994).

A water extract of *Salix caprea* bark showed moderate inhibition of prostaglandin synthesis and PAF-induced exocytosis *in vitro* (Tunón *et al*. 1995).

A water extract of willow bark inhibited oxidation of LDL by copper ions in a number of vitro tests. Copper chelation seemed to be only partially involved in inhibition of copper-dependent oxidations and only at a certain concentration of extract (Rohnert *et al*. 1998).

Willow bark extract ethanol 70% (total salicin 15%) demonstrated a dose-dependent inhibition of COX-1 and COX-2 *in vitro* on whole-blood samples of 3 healthy volunteers, but inhibited less efficiently TNF-alpha and IL-1beta release. The concentration of extract tested did not affect cell viability (Wagner *et al*. 2003a).

It should be noted that after oral ingestion of the extract, these inhibitory effects were no longer demonstrated. Moreover salicin components may not be the only constituents responsible for the activity (Fiebig and Appel 2003).

In another *in vitro* study with primary human monocytes, the extract 70% ethanol 8-14:1 inhibited LPS-induced release of PGE2 reflecting COX-2-mediated PGE2 release. Salicin and salicylate had no effect on the parameters, while rofecoxib was included as the active control. The extract inhibited the LPS-induced release of TNF-α, IL-1β and IL-6 (Chrubasik *et al*. 2003; Fiebig & Chrubasik 2004, Wagner *et al*. 2003a).

A third study examined a water extract 33:1 in two inflammation models in rats, the 6day air pouch model and the adjuvant-induced arthritis. The extract was at least as active as acetylsalicylic acid (ASA) on a mg/kg basis in reducing inflammatory exudates and in inhibiting leukocytic infiltration as well as in preventing the rise in cytokines, was more effective than ASA in suppressing leukotrienes, but equally effective in suppressing PGs. Again, other constituents than salicin are thought to

contribute to the overall activity as the extract contains considerably lower amounts of salicylates (Khayyal *et al*. 2005).

The effects of 5 fractions of a standardized willow bark extract from *Salix daphnoides, purpurea* and *fragilis* were investigated on human monocytes. Positive controls were diclofenac and acetylsalicylic acid. The willow bark extract, as well as its 5 fractions inhibited the interferon-gamma- and lipopolysaccharide-induced inflammation in isolated human monocytes. The release of nitrites and NO was significantly reduced. Active concentrations of fractions and extract varied between 5 and 30 µg/ml. Incubation time was 90 minutes (Kelber *et al*. 2006).

A pharmacological *in vivo* and *in vitro* study on an aqueous willow bark extract (16-23:1, 23-26% total salicin) pointed to contributions of the fraction of polyphenols and flavonoids to the overall effect of willow bark on the inhibition of enzymes of arachidonic acid (AA) metabolism (COX-1, COX-2, HLE isolated enzymes, 5-LOX), inhibition of gene expression of mediators of inflammation, anti-oxidative effects whereas the contribution of salicin derivatives was found to be minor (note that no metabolic activation of the salicins took place). Dose-dependent effects of the extract (50-150 mg/kg) were found in the carrageenan-induced rat paw oedema test and the Randall-Sellitto-test (anti-nociceptive effect), comparable to 150 mg/kg ASA. The results and the mg-mg comparison with regard to salicylic derivatives again suggest that other fractions than salicins distinctly contribute to the effects of the extract (Nahrstedt *et al*. 2007).

Salicin administered orally to rats at 5 mmol/kg bw significantly reduced yeast-induced fever, producing a normal temperature, and completely prevented fever when administered simultaneously with yeast. However, salicin at this dose level did not affect the renal body temperature of afebrile rats. On the other hand, both sodium salicylate and saligenin at 5 mmol/kg lowered body temperature significantly in afebrile rats (Akao *et al*. 2002).

Other ingredients of the extract may contribute to the overall analgesic effects. These constituents may include naringenin, catechins and eriodictyol, that inhibit lipoxygenase, hyaluronidase and scavenge free radicals (Kuppsamy *et al*. 1990; Rice-Evans *et al*. 1995).

An aqueous extract of the bark of *Salix purpurea* (DER 16-23:1) at a concentration 50 µg/ml decreased ICAM-1 (Intercellular Adhesion Molecule 1) expression to 40% in human vascular epithelial cells, as compared to control cells, without any sign of toxicity. Flavonoid and chalcone glycosides were not active up to 50 µM, whereas catechol and eriodictyol at the same concentration showed significant reduction of ICAM-1 expression to 50% of controls. Other isolated flavanone aglyca like taxifolin, dihydrokaempferol and naringenin showed only weak or moderate inhibitory activity. Eriodictyol was a minor compound in the extract whereas the catechol content in the extract reached 2.3% determined by HPLC. One of the isolated cyclohexan-1,2-diol glucosides 6'-O-4-hydroxybenzoyl-grandidentin, is a new natural compound. From these *in vitro* data it can be concluded that not only flavonoids and salicin derivatives, but also catechol can probably contribute to the anti-inflammatory activity of willow bark extracts (Freischmidt *et al*. 2012).

Shakibaei *et al*. (2012) studied the anti-inflammatory property of a non-specified willow bark extract (10 µg/ml) *in vitro* with primary canine articular chondrocytes treated with Interleukine-1β. Expression of collagen type II, cartilage-specific proteoglycan (CSPG), β1-integrin, transcription factor SOX-9, COX-2, and matrix metalloproteinases MMP-9 and MMP-13 was examined by western blotting. The extracts suppressed IL-1β-induced NF-κB activation by inhibition of IκBα phosphorylation, IκBα degradation, p65 phosphorylation, and p65 nuclear translocation. These events correlated with downregulation of NF-κB targets including COX-2 and MMPs. The extracts also reversed the IL-1β-induced downregulation of collagen type II, CSPG, β1-integrin, and cartilage-specific transcription

factor SOX-9 protein expression (see **Figure 3**). In high-density cultures willow bark extracts stimulated new cartilage formation even in the presence of IL-1*β*. From the experiments it is concluded that willow bark extracts exerted anti-inflammatory and anabolic effects on chondrocytes. The observed reduction of IL-1*β*-induced NF-*κ*B activation suggests that further studies are warranted to demonstrate the effectiveness of willow bark extract (Shakibaei *et al*. 2012).

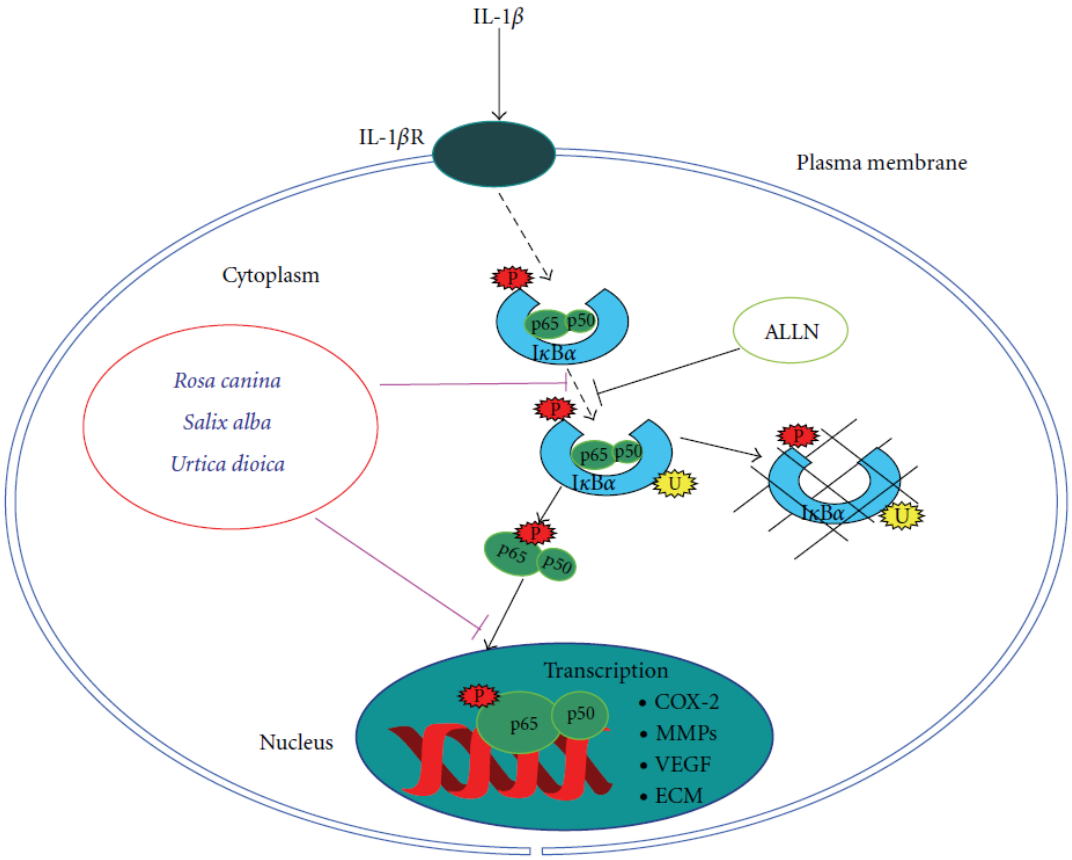

Figure 3: Inhibitory effects of willow bark extract on IL-1*β*-induced NF-*κ*B activation in chondrocytes *in vitro.* IL-1*β* stimulates the IL-1*β* receptor, initiating an intracellular signal transduction cascade, which activates the cytoplasmic I*κ*B*α* kinases (I*κκ*)-*α*, I*κκ*-*β*, and I*κκ*-*γ*. These kinases phosphorylate inactive I*κ*B*α*. Phosphorylated I*κ*B*α* is then ubiquitinated and degraded by the proteasome and active NF-*κ*B is released. NF-*κ*B translocates to the nucleus, where it activates proinflammatory and proapoptotic gene production. In chondrocytes, botanical extracts inhibit the NF-*κ*B signal transduction pathway, ubiquitination of phosphorylated I*κ*B*α* and block translocation of activated NF-*κ*B to the nucleus (Shakibaei *et al*. 2012).

Dried aerial parts of willow bark (7.5 g) were extracted in 100 ml water at 90°C for 15 min. Different concentrations of this aqueous herbal extract was incubated with THP1 macrophages, and interleukin (IL)-1b, IL-6 and tumour necrosis factor-alpha (TNF-a) were measured. At concentrations equivalent to 7 µM salicylic acid the production of cytokines was significantly reduced ($p < 0.001$) (Drummond *et al*. 2013).

Table 6: Overview of the main non-clinical data/conclusions

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
Comparable/similar preparations to preparations of the monograph	5 fractions of a willow bark extract from *Salix daphnoides, purpurea* and *fragilis* 5 and 30 µg/ml	*In vitro* Human monocytes	Kelber *et al*. 2006	Significant reduction of nitrite and NO release
	Aqueous extract of the bark of *Salix purpurea* (DER 16-23:1) at a concentration 50 µg/ml	*In vitro*: Human vascular epithelial cells	Freischmidt *et al*. 2012	Decreased ICAM-1 (Intercellular Adhesion Molecule 1) expression Inhibition of the enzymes.
	Aqueous willow bark extract (16-23:1, 23-26% total salicin) 50-150 mg/kg	*In vitro*: COX-1, COX-2, HLE isolated enzymes, 5-LOX	Nahrstedt *et al*. 2007	Dose-dependent inhibition of inflammation comparable to 150 mg/kg ASA
	Extract 70% ethanol 8-14:1	*In vivo:* Carrageenan-induced rat paw oedema		Inhibited of the LPS-induced release of TNF-α, IL-1β and IL-6
		In vitro: Primary human monocytes	Chrubasik *et al*. 2003; Fiebich *et al*. 2004, Wagner *et al*. 2003a	
Other preparations	10 µg/ml of a non-specified willow bark extract.	*In vitro*: Canine articular chondrocytes	Shakibaei *et al*. 2012	Cytokine activation and actions depressed, cartilage formation stimulated.
	Willow bark water extract equivalent with 7 µM salicylic acid.	*In vitro*: THP1 macrophages	Drummond *et al*. 2013	Significant reduction of cytokines
	Willow bark water extract 33:1	*In vivo*: the 6 day air pouch model and	Khayyal *et al*. 2005	At least as active as acetylsalicylic acid (ASA) on a mg/kg

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
		the adjuvant-induced arthritis		basis - in reducing inflammatory exudates - in inhibiting leukocytic infiltration -in preventing the rise in cytokines; -more effective than ASA in suppressing leukotrienes -but equally effective in suppressing PGs
Single substances	Salicin administered orally at 5 mmol/kg	*In vivo*: Yeast-induced fever in rats	Akao *et al*. 2002	Fever reduction
	Tremulacin, s.c. injected at 100 mg/kg	*In vivo* Rats (carrageenan-induced paw oedema) and mice (croton oil oedema and acetic acid writhing)	Cheng *et al*. 1994	In rats inhibition of: -Carrageenan-induced paw oedema Inhibition in mice of: - Peritoneal leucocyte migration - Croton oil induced ear oedema - Acetic acid induced writhing Anti-inflammatory effect
	Salicin and tremulacin	Hen's egg choriollantoic membrane test system	Albrecht *et al*. 1990	

Conclusions

Preclinical experimental data *in vitro* were obtained by using human and canine monocytes, chondrocytes, macrophages and cyclo-oxygenase activity. Carragenan-induced rat paw oedema was the most important *in vivo* model of inflammation. Willow bark extracts listed in the monograph (aqueous extract DER 16-23:1; extract 70% ethanol 8-14:1), as well as other extracts and pure substances were used. It is not clear whether the concentrations *in vitro* are clinically relevant. At least for the pure substances they are higher than what can be achieved therapeutically. The doses

administered *in vivo* must also be considered as supra-therapeutic. Sometimes equivalence with acetylsalicylic acid is obtained. Generally, the results qualitatively support the therapeutic indications listed in the monograph: anti-inflammatory, antipyretic and analgesic.

3.1.2. Secondary pharmacodynamics

Wuthold *et al*. (2004) published an analysis of 22 various extracts (aqueous and hydro-ethanolic) with HPTLC and 2 *in vitro* tests (anti-oxidative effects). The models were used to predict activity of willow bark extracts.

The potential of willow bark extracts as anticancer agents has been reported. A study demonstrated the inhibition of anchorage independent growth, motility, migration, and adhesion of colon cancer cell lines HCT-116 and HT-29 by EEB. These *in vitro* functional changes were accompanied by a restoration of Ecadherin expression, a reduction in EGFR, SNAI1, SNAI2, and Twist1 and the matrix metalloproteases MMP9 and MMP2. Many of these proteins are involved in the process of epithelial to mesenchymal transition, which is considered as a critical step in the progression of noninvasive tumor cells into malignant, metastatic carcinomas (Enayat and Banerjee 2014).

Fiebig *et al*. (2010) investigated the influence of different fractions of the STW 33-I willow bark extract on human umbilical vein endothelial cells. More particularly the content of c-GMP and the production of NO. Two fractions (A and E) increased the release of NO, whereas one fraction (C) inhibited the release. In the abstract no further details were given on the concentration of the extracts (Fiebig *et al*. 2010).

The influence of salicylalcohol, flavonoids and proanthocyanidins isolated from willow bark extract BNO 1455 on proliferation and apoptosis of human colon and different cancer cells. All compounds showed anti-proliferative activity, with 50% maximal growth inhibitory concentrations between 33.3 and 103.3 µg/ml for flavcoaonoids and proanthocyanidins fractions and 50.0 and 243.0 µg/ml for salicylalcohol derivatives and extract (Hostanska *et al*. 2007).

The willow bark STW 33-I and 4 of its fractions separated by polarity were studied on Sprague Dawly rats. The rats received different doses of the extract and its fractions. Imipramine (20 mg/kg) was the positive control. The outcomes of a forced swim were evaluated. A significant shortening of the cumulative period of immobility was seen after treatment with 15, 30, 60 mg/kg of the extract. Locomotor activity did not increase. From the neurotransmitter concentrations determined in frontal cortex, hypothalamus, hippocampus and striatum, it could be seen that serotonin seemed to be involved. According to the authors, these results confirm the hypothesis that *Salix* preparations could have a central activity that contributes to the alleviation of pain (Kelber *et al*. 2011; Ulrich-Merzenich *et al*. 2010).

Willow bark extract (WBE; not specified) prevented oxidative-stress-induced cytotoxicity of human umbilical vein endothelial cells (HUVEC) and death of *Caenorhabditis elegans*. There were concentration-dependently increased mRNA and protein expression levels of the nuclear factor erythroid2-related factor2(Nrf2) target genes hemeoxygenase-1, g-glutamylcysteineligase modifier and catalyticsubunits, p62 and intracellular glutathione (GSH) in HUVECs. Also in the nematode *C. elegans*, WBE triggered a cascade that lead to the expression of antioxidant enzymes and prevents oxidative stress through activation of Nrf2 (Ishikado *et al*. 2013).

Polyphenols including procyanidins are suggested to contribute to the overall effect of willow bark. Kaufeld *et al*. (2014) investigated the relaxant response to a highly purified and chemically defined 2,3-trans procyanidin fraction in porcine coronary arteries. The procyanidin sample produced a concentration-dependent relaxation in U46619-precontracted tissues. Relaxation was predominantly

mediated through the redox-sensitive activation of the endothelial phosphatidylinositol-3-kinase (PI3K)/Akt signaling pathway, leading to the subsequent activation of endothelial nitric oxide synthase (eNOS) by phosphorylation, as evidenced in human umbilical vein endothelial cells (HUVECs). Organ bath studies showed that *Salix* procyanidins reversed the abrogation of the relaxant response to bradykinin by oxidized low-density lipoproteins (oxLDL) in coronary arteries, suggesting a vasoprotective effect of willow bark against detrimental oxLDL in pathological conditions. The authors conclude that intracellular Ca polyphenols including procyanidins contribute to the overall effect of willow bark (Kaufeld *et al*. 2014).

Kong *et al*. (2014) investigated the antitumorigenic and antiangiogenic activity of salicin and its underlying mechanism of action. Salicin suppressed the angiogenic activity of endothelial cells, such as migration, tube formation, and sprouting from an aorta. Moreover, salicin reduced reactive oxygen species production and activation of the extracellular signal-regulated kinase pathway. The expression of vascular endothelial growth factor was also decreased by salicin in endothelial cells. When the salicin was administered to mice, salicin inhibited tumor growth and angiogenesis in a mouse tumor model. Taken together, salicin targets the signaling pathways mediated by reactive oxygen species and extracellular signal-regulated kinase (Kong *et al*. 2014).

Some compounds isolated by Kim *et al*. (2015) were evaluated for their nitric oxide (NO) inhibitory efficacy in lipopolysaccharide (LPS)-activated microglial cell (BV-2). The most active compound had a IC_{50} of 13.57 μM). Another compound increased the nerve growth factor (NGF) production with 165.24±11.1% in C6 gliomacells. Taken together, these results suggest that salicin derivatives from *Salix glandulosa* have an effect as anti-neuroinflammatory agents (Kim *et al*. 2015).

Conclusions

More recent interest raised in the antitumoral, cardiovascular and anti-oxidative properties of *Salix* preparations. Inhibition of tumor growth and angiogenesis opens new therapeutic perspectives for willow bark. Furthermore, willow bark and its extracts possess vasoprotective properties. Finally, there may be a central component in the analgesic activity of willow bark.

3.1.3. Safety pharmacology

No data available.

3.1.4. Pharmacodynamic interactions

Ulrich-Merzenich *et al*. (2013) consider the pharmacodynamic interaction between salicylates and polyphenols as an example of pharmacodynamic anti-inflammatory synergy, resulting in lower active concentrations of salicylates. This synergy may be beneficial in avoiding undesirable effects.

Ulrich-Merzenich *et al*. (2011 and 2012) studied a model for prediction of adverse events using *in vivo* gene expression profiling with phytopharmaceuticals containing salicylates and the antidepressant imipramine. Gene expression profiles (Agilent Whole Genome Array, n = 4/group) obtained from the peripheral blood of male Sprague Dawley rats treated with willow bark (WB or STW 33-I: 30 mg/kg body weight), its salicin rich ethanol fraction (EtOH-FR: 30 mg/kg body weight) or imipramine (20 mg/kg body weight) were analysed comparatively by the Ingenuity Systems Programme, which allows to conduct model calculations of thresholds for theoretical potential undesirable effects.

The number of genes regulated by the three treatments were 1673 (WB), 117 (EtOH-FR) and 1733 (imipramine). The three treatments related to 47 disease clusters. The WB extract reached the threshold for a potential AE in one disease cluster (cardiac hypertrophy), whereas the EtOH-FR

exceeded the threshold in 5 disease clusters (cardiac arteriopathy and stenosis, glomerular injury, pulmonary hypertension, alkaline phosphatase levels⇑). Imipramine treatment hit 13 disease clusters: amongst others tachycardia, palpitation, myocardial infarction, arrhythmias, heart block, precipitation of congestive heart failure; urinary retention, altered liver functions. Those correspond to known potential adverse events. Glomerular injury and altered liver functions are part of the side effect profile of salicylic acid derivatives in agreement with the findings for the salicin rich EtOH-FR (Ulrich-Merzenich *et al*. 2012).

The authors conclude that there is no linear relationship between the number of constituents of a drug (preparation) and the number of different targets hit in a biological system on the gene expression level. Therefore, the number of genetic targets in a biological system does not necessarily increase with the complexity of the treatment corresponding to the non-linear behaviour of biological systems. Regarding gene expression levels undesirable effects of single treatments are not necessarily additive in combination treatments (Ulrich-Merzenich *et al*. 2012).

Durak and Gawlik-Dziki (2014) published the results of synergy investigations between coffee and *Salix* components. They showed that both coffee and willow bark are sources of multidirectional antioxidant compounds. Synergism was observed for ability of inhibition of lipid peroxidation and reducing power, whereas in the determination of the ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) radical scavenging activity the compounds acted antagonistically. Additionally, phytochemicals from willow possess a hydrophilic character and thermostability. According to the authors this may justify their use as an ingredient in coffee beverages.

Assessor's comments:

There are no *in-vivo* studies nor preclinical studies specifically related to drug-drug interactions of *Salix* extracts. However, *Salix* preparations inhibit cyclo-oxygenase. Hence interactions with drugs acting on blood platelet function or blood coagulation are likely. See further considerations under chapter 5.5.4.

3.1.5. Conclusions

There seems to be no linear relationship between the number of constituents of willow bark extracts and the number of different targets hit in a biological system on the gene expression level. Interaction studies prove that the number of genetic targets in a biological system does not necessarily increase with the complexity of the treatment. A possible synergism between willow and coffee components. Synergism was observed for ability of inhibition of lipid peroxidation and reducing power.

However both findings have a speculative character, as their clinical relevance remains to be demonstrated.

Willow preparations inhibit cyclo-oxygenase. Hence interactions with drugs acting on blood platelet function or blood coagulation are likely.

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

In vitro

Salicortin was unchanged after 1 hour of incubation in artificial gastric juice (pH 1.0). After 6 hours of incubation with artificial intestinal juice ph. 7.4-7.6), salicortin was degraded to salicin with $T_{1/2}$ = 4.02 h (Meier *et al*. 1990).

Salicin is stable under acidic conditions (0.5% hydrochloric acid with or without pepsin) and produces no saligenin, even after incubation with human saliva at pH 7.2) (Steinegger *et al*. 1972, Fötsch *et al*. 1989 a and b).

ß-glucosidase extracted from almonds and ß-glucosidase from guinea pig liver converted salicin and salicortin to saligenin. However, salicin derivatives acetylated on the sugar moiety and tremulacin were not decomposed (Julkunen-Tiito *et al*. 1992a, Gopalan *et al*. 1992). Non-specific esterases from rabbit and pig liver transformed salicortin to salicin (98.1%), acetylsalicortin to acetylsalicin (75.5%) and tremulacin to tremuloidin (63.9%). Pancreatic proteases degraded salicortin to salicin and tremulacin to tremuloidin (Wutzke 1991).

Transport of salicin and saligenin into erythrocytes was rapid for saligenin (1 minute to saturation) and delayed for salicin (4 hours to saturation). The process was reversible exhibiting rapid release for saligenin and slower release for salicin. Saligenin and salicin both bind to human serum albumin but saligenin has a significantly higher affinity (Matsumoto *et al*. 1993).

Saligenin was transformed to salicylic acid by homogenised liver, lung and kidney. Gentisic acid was quantitatively detectable in homogenised liver after incubation with saligenin (Fötsch *et al*. 1989 a and b). Salicin was partially metabolised to saligenin and salicic acid after incubation with homogenised kidney from rats (Adamkiewicz *et al*. 1961).

Salicin injected into an isolated closed-off section of the male rat intestine, appendix and colon, was hydrolysed by intestinal bacteria to its main metabolite saligenin (Fötsch *et al*. 1989). Transport of salicin and saligenin through the isolated intestinal wall was confirmed using the closed-off posterior section of the male rat intestine. When salicin and saligenin were injected into the closed intestine both passed the ileal wall unchanged. Saligenin appeared to penetrate the intestinal wall faster than salicin (Adamkiewicz *et al*. 1961).

Gawlik-Dziki *et al*. (2014) investigated and compared the extractability, bio-accessibility (= activity of the bioavailable substances in the models used), and bioavailability *in vitro* (cf. dialysis) of antioxidative compounds from bark of selected *Salix* species: *S. alba (SA), S. daphnoides (SD), S. purpurea (SP),* and *S. daphnoides x purpurea (SDP)* hybrid willow clones originating from wild collection and cultivated on the sandy soil. SDP and SD contained the highest amount of phenolic glycosides. The amounts in the species investigated varied from 75 to 110 mg/g dried material. The best source of phenolics was bark of *SDP*. The highest content of flavonoids was found in *SD* bark samples, whereas SDSP bark yielded the highest concentration of bio-accessible and bioavailable phenolic acids. Bark of all tested *Salix* species showed significant antiradical activity. The activity is was dependent on extraction system and genetic factors. SDP had the highest activity with EC_{50} varying between 2 and 5 mg dried material/ml (chemical extract, buffered extract, digested, absorbed). Regardless of *Salix* genotypes, the lowest chelating power was found for chemically extractable compounds, the highest for the absorbed material of all species (< 0.2 mg dried material/ml). Bark of all *Salix* species contained ethanol-extractable compounds with reducing ability. The highest activity was found for the absorbed material of SDP with an EC_{50} < 1 mg dried material /ml. Ethanolic extracts of all four species had a lipoxygenase inhibiting activity with an EC_{50} < 0.25 mg dried material/ml (**Figure 4**). There was a variable xanthine oxidase inhibiting activity for the absorbed material of all species, with EC_{50} between 1 and 2.5 mg dried material/ml. (Gawlik-Dziki *et al*. 2014).

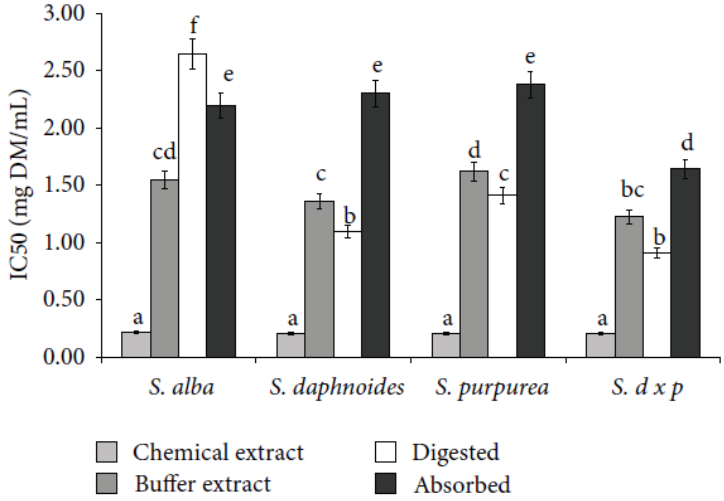

Figure 4: Effect of extraction system on LOX-inhibitory activity of extracts from bark of different *Salix* genotype. Bars (means). Bars with different characters differ significantly (Tukey-test, $P < 0.05$).

In vivo

After oral administration of salicin (400 mg/kg bw) or sodium salicylate (29 mg/kg bw) to rats, salicylic acid appeared slowly (salicin, C_{max} of 82.4 µg/ml after 5 hours) or rapidly (sodium salicylate, C_{max} of 104.2 µg/ml after 1.5 hours). Elimination was slower with sodium salicylate. The relative bio-availability of salicylic acid from salicin was only 3.25% of that from sodium salicylate (Fötsch *et al*. 1990), which was much lower than postulated after administration of 1 mmol salicin / kg bw = 268 mg/kg bw (Fötsch *et al*. 1989). Salicin appears to be a pro-drug, which is gradually transported to the lower part of the intestine, hydrolysed by intestinal bacteria to saligenin, and converted to salicylic acid after absorption. Absorption of salicin is slow compared to that of saligenin or salicylic acid (Akao *et al*. 2002).

Conclusions

The *in vitro* and *in vivo* pharmacokinetics of salicin in rats and its precursors are documented in literature. The data should be read in conjunction with the clinical pharmacology data (pharmacokinetic data).

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

3.3.1. Single dose toxicity

No data are available on single dose toxicity.

3.3.2. Repeat dose toxicity

Salicin did not induce gastric lesions in rats even at a dose of 5 mmol/kg bw. Saligenin and sodium salicylate induced severe gastric lesions in a dose-dependent manner in the range of 1-5 mmol/kg (Akao *et al*. 2002). It may be that willow bark is less prone to induce adverse reactions in the stomach than acetylsalicylic acid is. This may be due to the generation of active metabolites in the intestine after passing through the stomach as intact glycosides that do not inhibit cyclo-oxygenase in the stomach wall.

An LD 50 of 28 ml/kg is described for a hydro-alcoholic extract of willow bark (Morgan *et al*. 2005).

3.3.3. Genotoxicity

No data available.

3.3.4. Carcinogenicity

No data available.

3.3.5. Reproductive and developmental toxicity

Only indirect data on reproductive toxicity and teratogenicity are available for willow bark. No data on willow bark as a single ingredient were found. Teratogenicity of salicylates in animal models is described.

3.3.6. Local tolerance

No data available.

3.3.7. Other special studies

No data available.

3.3.8. Conclusions

Very limited data on willow bark are available.

Tests on reproductive toxicity, genotoxicity and carcinogenicity have not been performed.

3.4. Overall conclusions on non-clinical data

Generally, some pre-clinical pharmacological data support the therapeutic indications listed in the monograph: anti-inflammatory, antipyretic and analgesic.

Specific preclinical data on interactions with other substances are not available. Nevertheless, a warning in case of concomitant use with anticoagulants is recommended in the monograph.

Non-clinical information on the safety of willow bark preparations is scarce.

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

Tests on reproductive toxicity, genotoxicity and carcinogenicity have not been performed.

4. Clinical Data

4.1. Clinical pharmacology

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

It is mainly salicin and the salicyl glycosides which form salicin after hydrolysis that represents a salicylic-acid pro-drug. Salicin and salicyl glycosides have antipyretic, analgesic, anti-rheumatic and anti-phlogistic actions.

In view of the considerable variation in salicylate concentrations between different *Salix* species, the salicin content of the products should be quantified and declared.

The analgesic activity of willow bark was studied in double-blind study and open controlled studies on patients with low back pain exacerbations. Dose-dependent analgesic effects were observed. In addition, willow bark showed a moderate but significant analgesic effect in one double-blind placebo-controlled study in patients with osteoarthritis but the effect was not confirmed in a later clinical study in OA patients (Biegert *et al*. 2004).

Influence on cyclooxygenase activity and TNFα and IL-1β was studied on whole blood samples of healthy volunteers. Oral intake of a willow bark dry extract (8-14:1) ethanol 70% (total salicin 15%, equivalent to 240 mg salicin) by 3 healthy volunteers did not show significant inhibitory effects in the 4 test systems. Diclofenac was included as an active control (Wagner *et al*. 2003a).

In contrast to acetylsalicylic acid, aggregation of thrombocytes is affected to a far lesser extent by willow bark. Platelet aggregation was followed in patients receiving willow bark extract (corresponding to 240 mg salicin per day), 100 mg acetylsalicylic acid per day or placebo. Willow bark decreased AA- and ADP-induced aggregation but to a significantly lower extent than acetylsalicylic acid. Collagen-induced aggregation was not influenced by willow bark (Krivoy *et al*. 2001). Clinical relevance in patients with impaired thrombocyte function has to be further studied.

Serum salicylate concentrations during treatment suggest that a daily consumption of 240 mg of salicin as extract is bio-equivalent to 50-87 mg acetylsalicylic acid (Schmid 2001a). Other ingredients of the extract may contribute to the overall analgesic effects. These constituents may include naringenin, catechins and eriodictyol, that inhibit lipoxygenase, hyaluronidase (Kuppusamy *et al*. 1990) and scavenge free radical (Rice-Evans *et al*. 1995).

Conclusions

Willow bark is the phyto-therapeutic precursor of acetylsalicylic acid. The pharmacological actions of salicylates in humans are well-documented, and are considered to be applicable to willow. However, the serum salicylate levels that are produced by the recommended doses of willow bark are too low to explain the analgesic activity, and it has been suggested that other constituents such as flavonoids or salicin esters may contribute to the overall effect.

Dose-dependent analgesic effects of willow bark dry extract (8-14:1) ethanol 70% were observed in recent controlled clinical studies in patients with low back pain exacerbations. In OA patients, the (borderline) significant effect could not be confirmed in a later clinical study.

Orally administered willow bark dry extract (8-14:1) ethanol 70% did not significantly inhibit COX-1, COX-2 or inhibit the release of TNF alpha and IL-1beta in a small study in 3 healthy volunteers.

AA and adenosine diphosphate (ADP)-induced platelet aggregation was decreased in patients receiving willow bark extract. This information should be included in both WEU and TU sections of the monograph for safety reasons.

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

In a 24 hours pharmacokinetic study in 10 healthy volunteers (Schmid *et al*. 2001a), intake of standardized willow bark extract (1360 mg, equivalent to 240 mg salicin, dose divided into 2 tablets at T0h and another 2 tablets at T3h), resulted in salicylic acid as the major metabolite of salicin detected in the serum (86% of total salicylates), besides salicyluric acid (10%) and gentisic acid (4%). Peak levels were reached within 2 hours after oral administration. Peak serum levels of salicylic acid were on average 1.2 mg/L and the AUC was equivalent to that expected form an intake of 87 mg acetylsalicylic acid. Considerably higher peak levels of salicylic acid are observed after analgesic doses of acetylsalicylic acid.

Renal elimination occurred predominantly in the form of salicyluric acid (71% of total salicylates), followed by salicylic acid (15%) and gentisic acid (14%). No saligenin or salicin could be detected in serum or urine. After 24 hours, on average 15.8% of the orally ingested dose of salicin was detected in the urine as salicylates. Since approximately 5% of the salicylates had not yet been excreted by the kidneys after 24 hours, it could be estimated that at least 16.6% of the ingested salicin had been absorbed and metabolized to salicylates.

Based on the *in vivo* findings in rats, it was repeatedly suggested that in humans salicin is also hydrolysed by the flora of the lower intestine prior to absorption of the aglycone (salicyl alcohol). This is contradicted by the studies of Schmid *et al*. (2001a), Steinegger *et al*. (1972, 4 g pure salicin) and Pentz *et al*. (1989) combination product of caffeine and willow bark) that found salicylic acid in the serum as early as 1 hour after ingestion, and peak levels recorded after 1-3 hours. This suggests that absorption takes place in the upper intestine, and possibly in the stomach. After oral administration, salicin is obviously hydrolysed before or during absorption. The resulting salicyl alcohol is oxidized to salicylic acid, which is the first detectable metabolite in the serum. After parenteral or rectal administration in humans, salicin is excreted unchanged in the urine (Steinegger *et al*. 1972).

Schmid *et al*. (2002) reviewed the metabolism of willow compounds after administering an extract of *Salix purpurea* and *daphnoides* to 10 human volunteers (6 men; 4 women; mean 34.6 years of age). They ingested 2 tablets with 340 mg willow extract, corresponding to 120 mg salicin derivatives before taking a standard breakfast (8:30 am) and two other tablets at 11:30 am, corresponding to a total of 240 mg salicin derivatives. Blood collection started one hour after the first intake. The results are given in **Figures 5** to **7**.

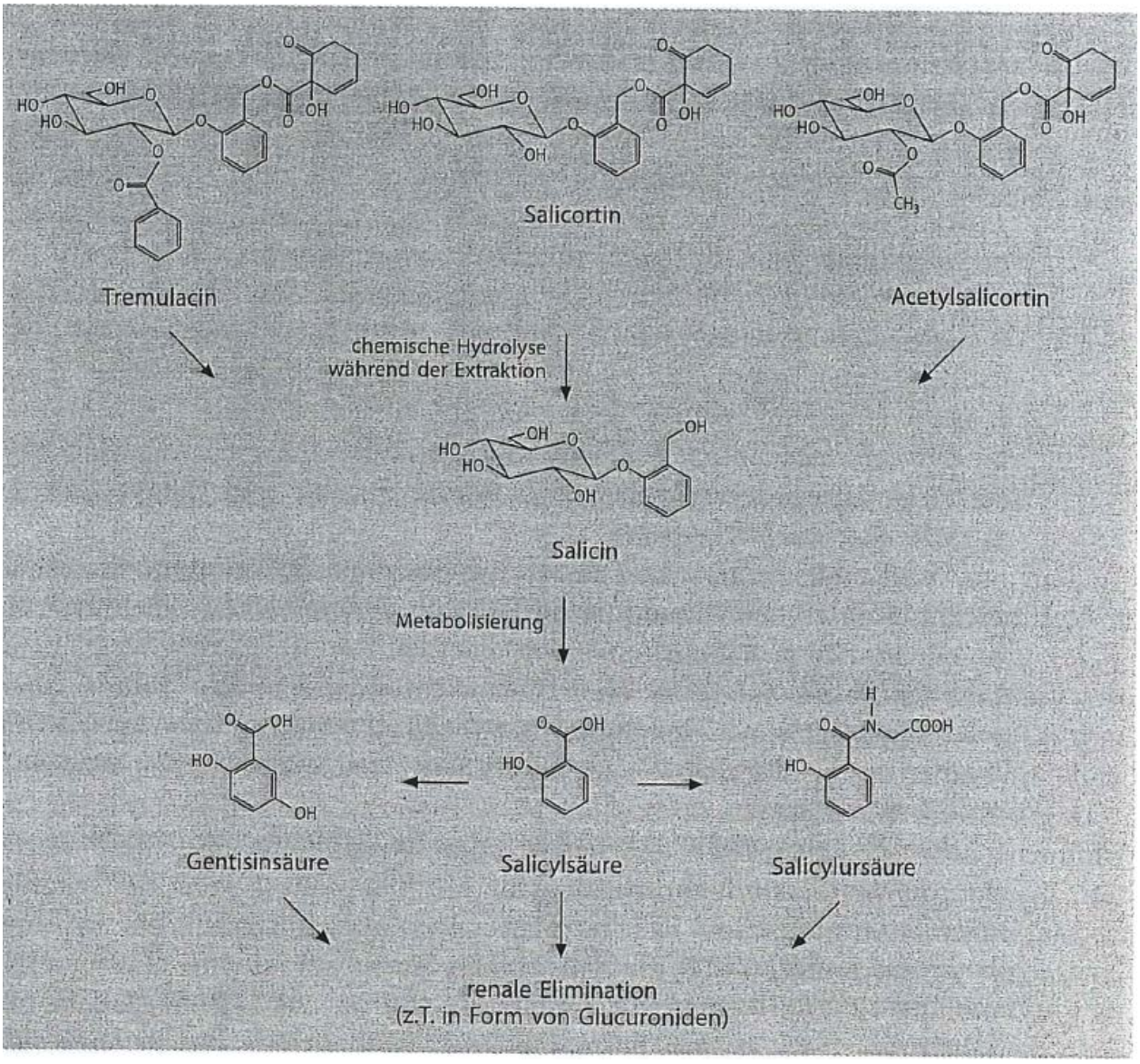

Figure 5: Main metabolites of salicin derivatives in humans (Schmid *et al*. 2002).

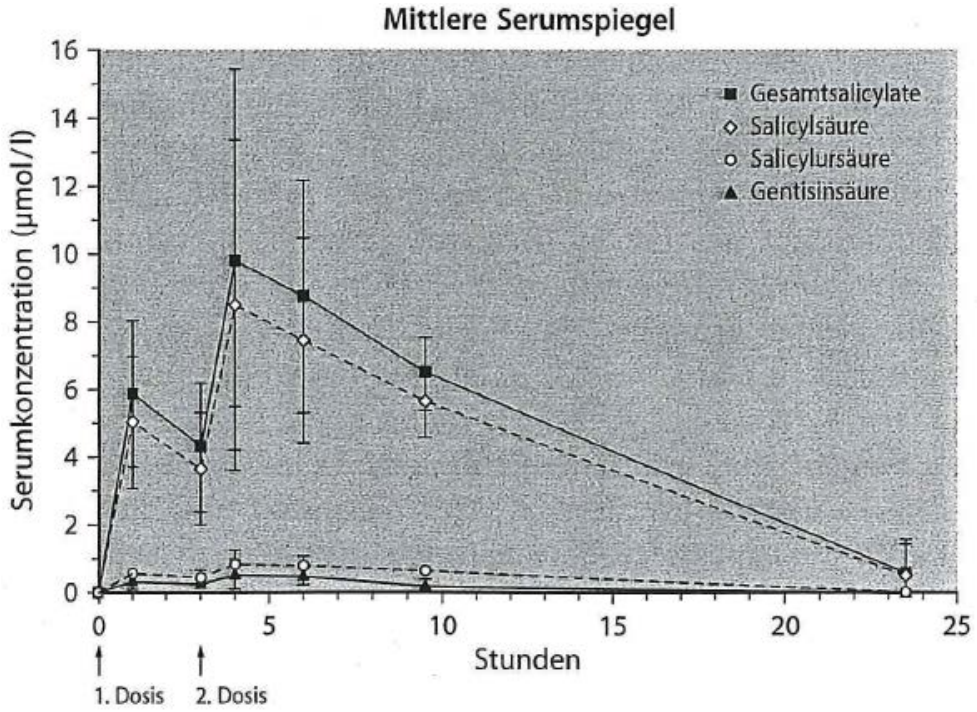

Figure 6: Mean serum levels of salicylic acid, gentisinic acid and salicyluric acid. Each point represents the mean values for 10 human volunteers, with the 95% confidence intervals. 'Gesamtsalicylate' is the result of the sum of the three salicylates (Schmid *et al*. 2002).

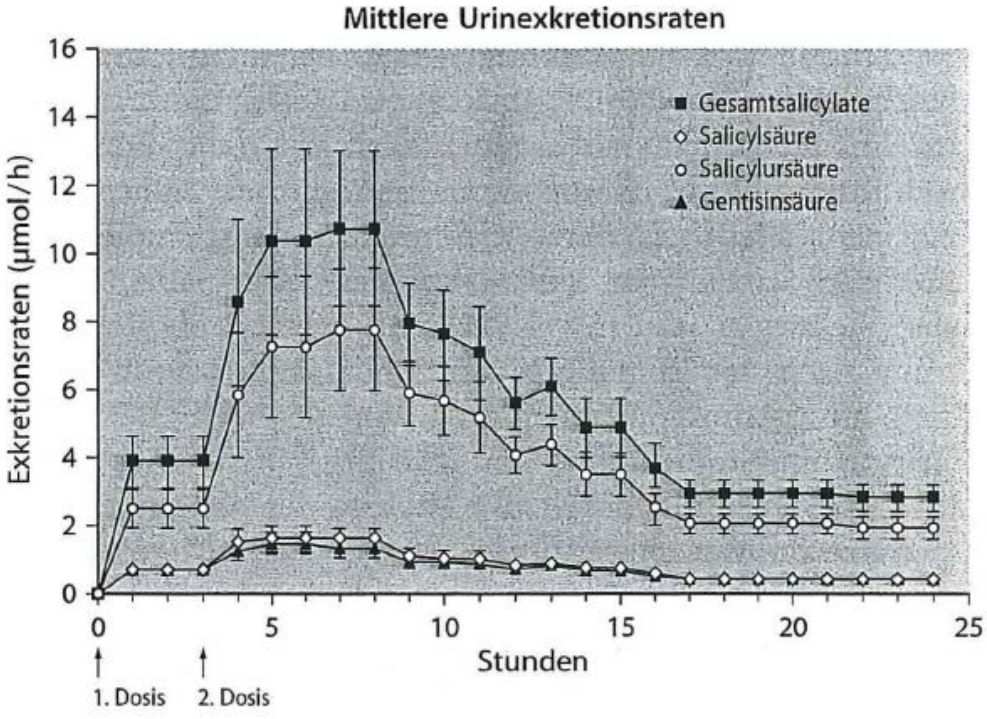

Figure 7: Excretion of salicylic acid derivatives and their sum (= Gesamtsalicylate) for 10 human volunteers. Each point represents the mean with the 95% confidence interval (Schmid *et al*. 2002).

Knuth *et al*. (2013) performed kinetic comparative studies in wistar rats and in man. After oral administration of 100 mg/kg b.w. (235.8 µmol/kg) salicortin to Wistar rats, they detected peak serum concentrations of 1.43 mg/L (13.0 µM) catechol after 0.5 hours in addition to salicylic acid by HPLC-

DAD after serum processing with β-glucuronidase and sulphatase. Both metabolites could also be detected in the serum of healthy volunteers following oral administration of a willow bark extract (Salicis cortex, *Salix* spec., Salicaceae; extraction solvent not specified) corresponding to 240 mg of salicin after processing with both enzymes. In humans, the C_{max} (1.46 mg/L, 13.3 µM) of catechol was reached after 1.2 hours. The predominant phase-II metabolite in humans and rats was catechol sulphate. Without serum processing with glucuronidase and sulphatase, no unconjugated catechol could be detected in human and animal serum samples. As catechol is described as an anti-inflammatory compound, these results may contribute to the elucidation of the mechanism of the action of willow bark extract (Knuth *et al*. 2013).

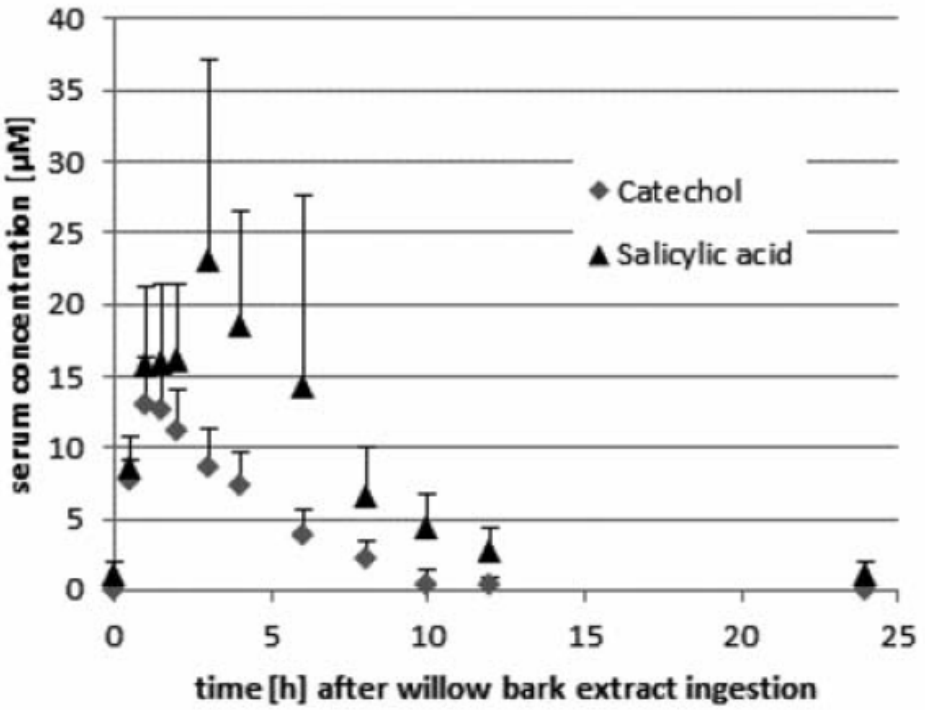

Figure 8: Catechol and salicylic acid concentration in glucuronidase- and sulphatase-processed serum samples of healthy volunteers after ingestion of WBE corresponding to 240 mg of salicin; n = 8; mean ± SD (Knuth *et al*. 2013).

4.2. Clinical efficacy

In spite of its long (traditional) use, only a few controlled trials have been conducted with willow bark to support its analgesic and/or antipyretic action. Wegener (2009) published an overview of 10 clinical studies with more than 1400 patients suffering from low back pain. The clinical studies (all located in the therapeutic area of (minor) articular disorders) are summarized below (cut-off January 2016).

4.2.1. Dose response studies

Low back pain (LBP)

Chrubasik *et al*. (2000). Treatment of low back pain exacerbations with willow bark extract: a randomized double-blind study. Am J Med 109:9-14

Methods:

Randomized double blind clinical trial, 3 arms, no report of randomization method, 4 weeks

Participants:

210 patients, N=70 in each group, 191 completed the trial. Inclusion criteria: >18 years, at least 6 months of intermittent low back pain that could not be attributed to identifiable causes, a current exacerbation of their pain at rest and with movement that caused pain of at least 5 of 10 on a VAS and that was expected to require at least 4 weeks of treatment. The characteristics of the participants were similar in the 3 groups (e.g. radiation into leg(s), neurological signs), except that the high-dose salicin group had a greater invalidity, physical impairment index and overall Arhus LBP score and Beck depression inventory. Exclusion criteria are presented.

Intervention:

placebo versus daily dose ~ 120 mg salicin (786 mg dry standardized willow bark extract 8-14:1; extraction solvent 70% ethanol V/V; 15% salicin; Plantina manufacturer, Assalix) versus daily dose ~ 240 mg salicin (1572 mg dry standardized willow bark extract 8-14:1; extraction solvent 70% ethanol V/V; 15% salicin; Plantina manufacturer, Assalix); daily dose divided into 2. Tramadol was the sole rescue medication

Primary outcome parameter: % of patients pain-free without tramadol for at least 5 days during the final week of the study

Secondary outcome: Change from baseline in modified Arhus score; % of patients requiring tramadol.

Results: dose-dependent analgesic effects were observed:

Primary outcome: 6% responders in the placebo group, 21% in the low dose group and 39% in the high dose group (P< 0.001). Similar results were obtained when drop-outs were excluded.

A significant increase in proportion of patients without rescue medication in the high dose group was apparent after 1 week of treatment and became progressively greater during the 4 weeks of treatment. The smaller effect seen in the 120 mg group was significantly different from placebo by the second week of treatment.

Significantly more patients in the placebo group required tramadol during each week of the study

Declines in the modified Arhus score (overall and its individual components) were significant. Change in overall Arhus score and its pain component was significantly greater in the 240 mg than in the 120 mg group.

Adverse effects

Willow bark groups: N=140 patients): 1 patient suffered a severe allergic reaction (exanthema, pruritis, swollen eyes; 120 mg group, could be attributed); other adverse effects (N=2) attributed to tramadol).

Placebo group: N=70: 3 cases of mild abdominal pain in placebo group (with or without diarrhoea)

Assessor's comment: The study is of good quality. The results indicate a dose-dependent analgesic effect of willow bark dry extract.

4.2.2. Clinical studies (case studies and clinical trials)

4.2.2.1. Low Back Pain

(See also **Table 7**)

Chrubasik *et al*. (2000). Treatment of low back pain exacerbations with willow bark extract: a randomized double-blind study. Am J Med 109:9-14

Details see 4.2.1.

Chrubasik S, Kunzel O et al. (2001a). Treatment of low back pain with a herbal or synthetic anti-rheumatic: a randomized controlled study. Willow bark extract for low back pain. Rheumatology 40:1388-93.

Methods:

Open randomized active-controlled clinical trial, 2 arms, 4 weeks.

Participants:

228 patients, N=114 with per group. 183 patients completed the trial. Inclusion criteria: >18 years, at least 6 months of non-specific LBP that could not be attributed to identifiable causes. Pain was recorded on VAS, the modified Arhus index and its pain component, and the Total Pain Index. Groups at baseline differed slightly in duration of LBP, and the NSAID group included more patients with pain radiating into legs and was in slightly more pain. Exclusion criteria are presented.

Intervention:

Daily dose ~ 240 mg salicin (4 capsules of Assalix = 1572 mg standardized willow bark extract 8-14:1 DER extraction solvent ethanol 70% V/V; 15% salicin) versus 12.5 mg rofecoxib (1 single tablet). Patients had free access to conventional treatments (including whatever medication they usually used in the event of severe pain, but also NSAIDs, acupuncture, physical therapy).

Outcome parameters:

Pain on a VAS, modified Arhus index, its pain component and the total pain index (TPI), physician and patient-rated success and the acceptability of the treatment on a verbal scale.

Results

- After 4 weeks of treatment, the Arhus index had improved by 20% (both groups) and its pain component by 30%, and the TPI by 35%.
- Number of pain-free patients (VAS <2) was about 20 in both groups
- Patients that resorted to NSAIDs and/or tramadol: 9 in willow bark group (average of 120 mg diclofenac equivalents and 5 mg tramadol) versus 12 in rofecoxib group (average of 42 mg diclofenac equivalents and 17 mg tramadol).
- Patients that resorted to other treatments: 13 in willow bark group versus 17 in rofecoxib group.
- Patients' and physicians' judgments of effectiveness were largely concordant.
- The multivariate analyses of changes in Arhus score and TPI did not identify significant differences related to willow bark versus rofecoxib.

Adverse effects

Willow bark group (N=114):

- Allergy: 1 possible, 3 likely, 1 clear connection.
- GI (dyspepsia, vomiting, heartburn, diarrhoea): 7 possible, 3 likely, 1 clear connection.

- Dizziness: 1 possible.
- Headache: 1 possible.
- Blood pressure instability: 1 possible.

Rofecoxib group (N=114):

27 adverse effects in total; asthma, dyspepsia, nausea, diarrhoea, heartburn, ulcer, GI bleeding, dizziness, headache, oedema.

Assessor's comment:

The open study design may induce bias and jeopardizes results/conclusions regarding equivalence or non-equivalence of both interventions. Furthermore, (slight) differences in baseline characteristics of the groups are noted (willow bark group slightly favoured). It is also noted that patients with lower disease activity were enrolled in this study (compared with Chrubasik et al. 2000). Free access to other treatments, even though resorted to by a fairly small and comparable number of patients in both groups, does not facilitate conclusions on the efficacy of willow bark versus rofecoxib.

Chrubasik *et al*. (2001b). Potential economic impact using a proprietary willow bark extract in outpatient treatment of low back pain: an open non-randomized study. Phytomedicine 8(4):24 1-251

Methods:

Open, non-randomised study (post-marketing surveillance) with 3 arms; 4 weeks.

Patients:

451 patients > 18 years (N=115 in 120 mg salicin group, N=112 in 240 mg salicin group, N=224 in "placebo" group) with acute exacerbations of chronic (at least 6 months) nonspecific LBP. The baseline characteristics of the 3 groups were slightly different: the "placebo" group had a shorter duration of exacerbation but their pain tended to be more severe as judged by the Arhus index and TPI.

Interventions

Daily dose of 120 mg salicin + conventional treatment, versus 240 mg salicin + conventional treatment versus conventional treatment alone. Salicin groups received standardized willow bark extract, (Assalix = standardized willow bark extract 8-14:1 DER extraction solvent ethanol 70% V/V; 15% salicin), respectively 2 capsules per day (120 mg salicin) or 4 capsules per day (240 mg salicin). Conventional treatment, prescribed by GPs or orthopaedists, included analgesics, NSAIDS, acupuncture.

Objective:

Study of safety and economic impact of including a regular intake of willow bark extract in the conventional treatment scheme. Outcome parameters: pain-free patients with or without additional treatment, modified Arhus index and total pain index.

Results

The study design does not allow conclusions on efficacy of willow bark because conventional treatment that was resorted to was variable between groups.

- When limiting to the patients included in this study that only used willow bark (no conventional treatment), pain relief of 240 mg dose seems to be superior to 120 mg and control group:

41% pain-free after 4 weeks in 240 mg group versus 8% pain-free in the 120 mg group (results for the 240 mg group are fairly consistent with Chrubasik, *et al*. (2000)).

- 18% of the "placebo" group (with conventional treatment) were pain-free after 4 weeks versus 5.7% in the placebo group of Chrubasik *et al*. 2000.

Adverse effects

Willow bark groups, N=112+115 patients: GI (6), allergic skin reaction (3)

Assessor's comment:

Important flaws in the study design make conclusions on the efficacy of willow bark based on the results impossible. The open study design may induce bias and jeopardizes results/conclusions regarding equivalence or non-equivalence of interventions. Furthermore, (slight) differences in baseline characteristics of both groups are noted (willow bark group slightly favoured). Patients had access to other conventional treatments (via GP/orthopaedist), and these treatments were not comparable between the groups.

The adverse effects are taken into account for evaluation of clinical safety.

Gagnier *et al*. (2007) published a systematic Cochrane review of the randomized clinical trials to determine the effectiveness of herbal medicine compared with placebo, no intervention or standard/accepted/conventional treatments for nonspecific LBP. A total of 10 studies met the criteria, among those the above-discussed studies of Chrubasik (2000), Chrubasik (2001a) plus Krivoy *et al*. (2001, on effects on human platelet aggregation). Methodological quality of the trials was assessed. A trial was considered high quality if more than 50% of internal validity items scored positively (quality criteria and definitions are given; Chrubasik (2000), Chrubasik (2001a) are classified as "high"). The clinical relevance of each study was assessed independently by 2 reviewers (all criteria fulfilled by Chrubasik *et al*. 2001a). Because of insufficient data and clinical heterogeneity, a qualitative analysis was conducted using a rating system (Strong/moderate/limited/conflicting/no evidence). The trial of Chrubasik *et al*. 2000 suggests there is moderate evidence that 240 mg salicin dose of a willow bark extract reduces pain more than placebo and 120 mg of salicin. The trial of Chrubasik *et al*. 2001a suggests that there is moderate evidence that there are no differences in effectiveness between a 240 mg salicin dose of a willow bark extract and 12.5 mg rofecoxib per day in treatment of acute episodes of chronic nonspecific LBP in the short term. The authors conclude that a daily 240 mg salicin dosage of willow bark is effective in the short-term treatment of acute episodes of chronic non-specific LBP. Additional trials testing against standard treatments are needed to confirm efficacy/equivalency/the relative safety of these herbals to standard medications such as NSAIDs, paracetamol.). The same authors reanalysed the available clinical data in a more recent Cochrane review. No new studies with *Salix* were included and the conclusions of the authors remained the same: willow bark, in a standardized daily dose of 120 and 240mg of salicin, reduces pain more than placebo, a standardized daily dose of 240mg is as effective as of 12.5 mg of the nonsteroidal anti-inflammatory drug rofecoxib (Gagnier *et al*. 2016; Oltean *et al*. 2014). In another Cochrane analysis it was recommended that *Salix* preparations should be compared with paracetamol as a standard therapy, i.o. rofecoxib, which was taken from the market in 2004 (by Oltean *et al*. 2014).

Shara and Stohs (2015) grant *Salix* preparations an efficacy in case of joint pain and osteoarthritis. They state that, although willow bark extracts are generally standardized to salicin, other ingredients in the extracts including other salicylates as well as polyphenols, and flavonoids may also play prominent roles in the therapeutic actions. Furthermore adverse effects appear to be minimal as compared to non-steroidal anti-inflammatory drugs including aspirin (Shara and Stohs 2015).

Vlachojannis *et al*. (2009) reviewed 6 clinical trials with ethanolic extracts of *Salix*. The authors expressed the need for studies with a higher dose equivalents than 240 mg salicin (Vlachojannis *et al*. 2009).

4.2.2.2. Osteoarthritis and Rheumatoid Arthritis

(See also **Table 8**)

Schmid, Ludtke *et al*. (2001b). Efficacy and tolerability of a standardized willow bark extract in patients with osteoarthritis: randomized placebo-controlled double blind clinical trial. Phytother Res 15:344-50.

Methods

Randomized, placebo-controlled double-blind clinical trial with 2 arms; 4-6 days wash-out, then 2 weeks trial.

Patients

78 patients, N=39 per group. Inclusion criteria: >18 years, OA of hip or knee, verified according to the clinical, laboratory and radiographic criteria of the American College of Rheumatology (ACR). Baseline characteristics are similar between both groups except that the baseline WOMAC pain score was lower for the willow bark group. Exclusion criteria are described. 5 patients withdrew during the study and 10 were excluded from the per-protocol analysis.

Intervention

Placebo versus daily dose ~ 240 mg salicin (340 mg standardized willow bark extract *Salix daphnoides* and *S. purpurea*, 17.6% total salicin, ~ 60 mg salicin per coated tablet). Daily dose was divided into 2 tablets twice daily. No additional analgesics, NSAIDs or systemic corticoids were allowed during wash-out and study phases.

Primary endpoint:

Difference in pain dimension WOMAC OA Index between day 0 and day 14.

Secondary endpoints:

Differences in the stiffness and physical function dimensions of the WOMAC, daily VAS on pain and physical function and final overall assessments by patients and investigators.

Results

- A (borderline) significant superiority of willow bark over placebo with regard to WOMAC pain dimension after 2 weeks (intent-to-treat : p= 0.047; per-protocol analysis: p= 0.0196)
- No significant differences between the 2 groups with regard to the secondary parameters, except for patients' and investigators' assessment (willow bark significantly superior).

Adverse effects

Willow bark group, N=38 patients: allergic skin reactions (6), GI (3). No evaluation presented on causality.1 patient in the willow bark group withdrew due to allergic symptoms.

Assessor's comment:

A moderate analgesic effect was observed in the willow bark group; a difference in pain dimension in the treated group compared to the placebo group just reached statistical significance. There are deficiencies in the quality of the methodology of the study that may affect the outcome/conclusions:

namely the relatively low number of patients, the shortness of the duration (the maximum treatment effect was probably not yet reached after 2 weeks) and the differences in baseline WOMAC pain dimension scores between the 2 groups. The extraction solvent and DER of the used willow bark extract is not given. Additional studies, with NSAID (diclofenac) control group were stated to be in preparation.

Biegert C *et al*. (2004). Efficacy and safety of willow bark extract in the treatment of OA and RA: results of 2 randomised double blind controlled trials. J Rheumatol 31, 2121-30. Originally described in Biegert 2003.

Methods

Randomised, double-blind controlled clinical trial; 3 arms (2 groups in RA trial); 7 days wash-out, then 6 weeks trial

Patients

OA trial: 127 patients; N=43 in willow bark group, N=43 in control group, N=41 in placebo group. Inclusion criteria: > 18 years, OA of hip or knee, verified according to the clinical, laboratory and radiographic criteria of the American College of Rheumatology (ACR) with WOMAC pain score of at least 30mm. Baseline characteristics are similar between the groups. Exclusion criteria are described. 106 patients completed the study and were included in the efficacy and safety analysis. The willow bark group received significantly less physical therapy.

RA trial: 26 patients, N=13 in each group. Inclusion criteria: diagnosis of RA according to ACR: RA functional class I, II or III, evidence of at least moderate disease activity (criteria given). The willow bark group showed a more active disease in all baseline arthritis assessments. Exclusion criteria are described.

Intervention

OA trial: Placebo versus salicin 240 mg per day (393 mg extract *Salix daphnoides* ethanol 70% 8-14:1 ~ 60 mg salicin per coated tablet) versus diclofenac 100 mg per day. Daily dose was divided into two tablets twice daily. No additional analgesics/NSAIDs/systemic or intra-articular corticoids were allowed. Aspirin was allowed up to 100 mg daily. Physical therapy could be continued but had to remain unchanged.

RA trial: Placebo versus salicin 240 mg per day (393 mg extract 8-14:1 ~ 60 mg salicin per coated tablet). Daily dose was divided into two tablets twice daily. Disease modifying anti-rheumatic drugs (except TNF-inhibitors) were allowed as concomitant therapy if taken since at least 6 months before (and dosage stable). NSAIDS and analgesics had to be discontinued; up to 100 mg aspirin per day was allowed.

Primary endpoint:

OA trial: pain sub-score of the WOMAC OA index

RA trial: patient's assessment of pain rated on a 100 mm VAS.

Secondary endpoints:

OA trial: WOMAC stiffness and function sub-scores and WOMAC total index, and patients' and investigators' assessment of overall efficacy.

RA trial: included number of tender/swollen joints, physical function (HAQ), disability index, patients' assessment of severity of morning stiffness (100 mm VAS), patients' and assessors' assessment of overall efficacy, Quality of life (SF-36), ESR, CRP, number of patients who met the ACR criteria for improvement.

Results:

OA trial

- Primary: WOMAC scores decreased for willow bark (but not significantly) and diclofenac (P=0.0002)
- Secondary: willow bark only significantly improved the physical function sub-score of the SF-36, while diclofenac was (highly) significantly superior over placebo with regard to all endpoints except the mental component of Quality of life and the investigators' assessment of overall efficacy (P=0.05).

RA trial

- Primary: pain on the VAS decreased for willow bark but not significantly. A power estimate of the study showed that that a true difference in pain reduction between willow bark and placebo of 15 mm (suggested as the minimum clinically relevant difference) or more can be excluded with a probability of 93%.
- Secondary: no significant changes between willow bark and placebo.

Adverse events:

- Willow bark group in OA trial (N=43): GI (7), plus allergy (exanthema, 1). Significantly lower adverse events in willow bark versus diclofenac.
- Willow bark group in RA trial (N=13): allergy (mild itching, 1).

Assessors comment:

The studies are in general of high quality but numbers of patients are small.

With regard to the OA trial, the study did not confirm efficacy of willow bark in OA as willow bark only significantly improved the physical function sub-score of the SF-36 while the WOMAC OA index (primary endpoint) was not significantly decreased. OA is the most common form of degenerative joint disease. The sensitivity of the study was demonstrated by the (highly) significant superiority of the control-group (diclofenac) over placebo.

With regard to the RA trial, again no efficacy was demonstrated for willow bark in RA, the most common inflammatory rheumatic disease. The number of patients included in the RA trial is very small and is therefore considered as a pilot study.

Beer and Wegener 2008. Willow bark extract (Salicis cortex) for gonarthrosis and coxarthrosis –Results of a cohort study with a control group. Phytomedicine (2008) 15: 907–913.

Methods

Open, multicentric observational study with reference treatment, 90 patients treated with a standardised willow bark extract preparation, 41 patients with a standard therapy prescribed by a doctor and 8 patients with a combination of the two.

Patients

Adults aged between 50 and 75 with degenerative complaints of the major and minor joints. The inclusion criteria were coxarthrosis with hip pain diagnosed by a specialist or gonarthrosis with knee pain according to the statement of a specialist.

Intervention

The study medication used was a standardised dry extract of willow bark (DER 8-14:1 solvent ethanol 70% V/V). Film tablet containing 393.24 mg dry extract containing 60 mg salicin were administered 1–2 tablets twice a day (equivalent to 120–240 mg salicin or 786.48–1572.96 mg dry extract). Evaluation was performed after 3 and 6 weeks.

Primary endpoint(s)

Effectiveness and tolerance were determined by the doctor (clinical findings, recording of adverse events, global tolerance) and by the patients (WOMAC questions concerning pain and stiffness, questions on general state of health).

Secondary endpoints(s)

Undesirable effects of treatment.

Results

A total of 88 patients in the willow bark group and 40 patients in the reference group completed the study. The doctors' and patients' judged effectiveness in both groups to be comparable. After 6 weeks the effectiveness of the willow bark extract was better than conventional therapy. Also in de subgroup of chronically ill sick patients (43 months), after 6 weeks the effectiveness of both forms of treatment was comparable. However, the effect was slower to set in the willow bark group than in de reference group.

Adverse events

There were no side effects in the willow bark group, whereas one case of reflux occurred in the reference group and in the combination group.

Assessor's comments:

The results of the study suggest that willow bark extract is as effective as conventional reference therapy in the treatment of degenerative complaints of the major and minor joints. However, the result of this study cannot be seen as conclusive because the trail was an open study with a limited number of patients.

Lardos *et al*. (2004) carried out a randomised double blind clinical trial with 60 patients (intention to treat) with hip or knee arthrosis. The study included 3 arms (N=17 diclofenac 150 mg per day; N=22 aqueous extract equivalent to 90 mg salicin per day; N=21 aqueous extract equivalent to 180 mg salicin per day). Inclusion and exclusion criteria are described. A 3 week wash-out period was followed by 3 weeks study period. No additional analgesics/NSAIDs were allowed during the study. Primary endpoint: pain on a 100 mm VAS and evaluation of physical function according to Steinbrocker. All 3 interventions statistically improved both endpoints after 3 weeks' treatment (diclofenac > salicin 90 mg ~ salicin 180 mg). Dose-dependency in analgesic activity (willow bark arms) was not observed.

Assessor's comment:

The study indicates analgesic effects of an aqueous extract of willow bark in patients with arthrosis. The sample size is however small; the study is considered as a pilot study. The herbal preparation is not fully characterised (DER). Comparability of the 3 arms at baseline is difficult to interpret. No dose-dependency in effect could be observed.

An unpublished trial was provided by Poland. Samochowiec (2001) studied the efficacy of willow bark extract (in patients with arthrosis (knee or hip) in a double-blind, randomized controlled clinical trial during 3 weeks. Stage II and III (according to Kellgren) patients received either sodium diclofenac (3 x 50 mg daily, N= 17), *Salix* tablet (quantity extract, and equivalent salicin not known) + 2 placebo tablets per day (N=22), or 2 *Salix* tablets + 2 placebo tablets per day (N=20). The exact administration scheme is unclear. Analgesics and anti-inflammatory drugs were not allowed during the study. Baseline characteristics of the 3 groups (functional capacity according to Steinbrocker, subjective pain evaluation on VAS, stiffness etc. were fairly comparable. Primary and secondary endpoints are not clearly defined. All treatments significantly improved pain on VAS, pain during walking and walking downstairs on even surface, pain during passive and active motion, functional capacity and decreased impairment of daily activity. No significant differences between the 3 groups were observed. 1 patient withdrew (*Salix*) due to malaise. Gastroscopy and laboratory findings were not affected by any of the treatments.

Assessor's comment:

The willow bark preparation is insufficiently characterized. It is not possible to evaluate the results in relation to the other clinical trials with willow bark. Patient numbers are rather small, and end points should be more clearly defined.

Werner, 2004. In a post-authorisation surveillance study on willow bark dry extract (8-14:1, ethanol 70%; daily doses equivalent to 120 or 240 mg total salicin), 922 physicians observed 4731 patients with chronic back pain or arthralgia after 3-4 weeks and after 6-8 weeks. Pain intensity was assessed (scale) and was decreased.

Assessor's comment:

Full details of the post-authorisation study of Werner are missing, only an abstract was available.

Saller *et al*. 2008. In an observational study with duration 6-8 weeks, 204 physicians treated 877 patients with different types of rheumatic pain (OA, RA, LBP, soft tissue disorders) with willow bark dry extract (8-14:1, ethanol 70%, 15% total salicin). The scope is to get a better estimate of the frequency of ADR and a broader picture of efficacy. Additional anti-inflammatory drugs were co-prescribed in 39.3% of the cases. Pain intensity was assessed (scale). Final data were compared with the corresponding values at baseline. No blood chemistry, coagulation nor haematology data were recorded. Pain scores tended to decrease. 38 patients (4.3%) reported a total of 46 ADRs relating predominantly to GI (3.1%) and skin (1.6). There were no serious ADRs.

Assessor's comment:

This concerns an observational study, no control group is included. Records of dose administered (1572 mg or 786 mg dry extract) are not presented. Baseline characteristics are not given (per grouped diagnosis). No conclusions can be drawn with regard to efficacy.

Mills *et al*. 1996. A 2-month randomized non-cross over study in 82 patients with chronic arthritis pain showed a small but statistically significant improvement in symptoms with a low dosage combination

willow bark formulation (containing 100 mg *Salix alba* extract, guaiacum, black cohosh, sarsaparilla and poplar bark) compared to placebo.

Müller *et al*. 2010. 350 patients suffering from low back pain and pain due to osteoarthritis were observed during 6 months. They were using a water extract of *Salix* (DER 16-23:1) (daily dose and compliance not mentioned). The progression of their pain intensity was evaluated using a 100-point visual analogue scale (VAS). Mean improvement for the *Salix* extract alone was 23.5 on the VAS. Patients combining *Salix* and NSAIDs reported a mean improvement of 18.8 whereas combination of *Salix*, NSAIDs and opioids resulted in an improvement of 21.2 (no standard deviation given). The authors conclude that the *Salix* extract reduces back pain and pain due to osteoarthritis both as monotherapy and in combination with other medicines.

The same authors reported about an observational study of 333 patients with osteoarthritis, rheumatoid arthritis or low back pain. The patients were treated with a water extract of *Salix* (DER 16-23:1) during a mean period of 3.3 ($\pm$ 0.9) weeks, 85% of the patients receiving a daily dose of 480 mg. Satisfaction rate (good/very good) was reached in 80% of the patients. According to the physicians the results were comparable to NSAIDs and paracetamol. More than 90% of the patients reported a good to very good tolerability (Müller-Fassbender *et al*. 2007).

Stange *et al*. (2014) followed 436 patients with musculoskeletal pain of different ethology during 24 weeks. The patients were taking a water extract of *Salix* (16-21:1). The mean visual analogue score (VAS 100) dropped from 58.4 $\pm$ 22.6 to 31.8 $\pm$ 22.5 ($P<0.05$ Wilcoxon). NSAIDs were used by 28.9% of the patients (mostly ibuprofen). Adverse effects were reported more often after 3 weeks (4.8%) than after 24 weeks (0.3%) (Uehleke *et al*. 2013a; Uehleke *et al*. 2013b, abstract; Stange *et al*. 2014, abstract).

An overview of 15 systematic reviews of herbal medicines used in the treatment of osteoarthritic complaints and chronic low back pain was published by Chrubasik *et al*. (2007). The evidence was found as conflicting for willow bark due to the confirmatory study of Biegert *et al*. (2004) in OA and RA with negative result (no statistically significant results).

Setty *et al*. (2005) reviewed herbal preparations commonly used in the treatment of rheumatic indications. The resurgent interest in willow bark as a treatment for chronic pain syndromes was illustrated by summary of the clinical trials. The authors concluded that trials longer than 4 weeks must be performed before declaring salicin's safety and efficacy as the conditions are chronic (OA).

The clinical studies are re-iterated by a number of articles, including März *et al*. (2002), Chrubasik and Pollak (2002), Wagner *et al*. (2003b and c), Kaul *et al*. (1999), Bruneton (2002), Bogduk (2004).

4.2.2.3. Migraine prophylaxis

Tanacetum parthenium and *Salix alba* either alone or in combination were shown to strongly inhibit binding to 5-$HT_{2A/2C}$ receptors (targets of prophylacting agents such as methysergide, pizotifen, oxetorone, cyproheptadine) while only *Salix alba* (and the combination) recognized 5-HT_{1D} receptors (targets of triptans), leading to the hypothesis that the combination would provide superior migraine prophylactic activity compared with Tanacetum alone (randomized double-blind placebo-controlled clinical trials with Tanacetum alone show mixed results).

Shrivastava *et al*. (2006) performed a prospective open-label study in 12 patients diagnosed migraine without aura (IHS criteria), aged > 18 years. After a 6 weeks' baseline-period (3-15 attacks / 6 weeks observed), twelve weeks' treatment with a combination product of Tanacetum parthenium 300 mg and *Salix alba* 300 mg (salicin content ≥ 1.5%) twice daily was administered to determine the effects on

migraine attack frequency (primary outcome parameter), intensity and duration (secondary outcome parameters). Attack frequency was reduced by 57.2% after 6 weeks (P=0.029) and 62.6% at 12 weeks (P=0.025) in 9 out of 10 patients (no significant improvement between 6 and 12 weeks) with 70% of patients having a reduction of at least 50%. Attack intensity was reduced by 38.7% after 6 weeks and 62.6% after 12 weeks in 10 out of 10 patients (both significant), with 70% having a reduction of at least 50%. Attack duration decreased by 67.2% after 6 weeks and 76.2% after 12 weeks in 10 out of 10 patients (both significant). Two patients were excluded for reasons unrelated to treatment. No adverse events occurred. In patients with more than 2 migraine attacks per month, current prophylaxis reduces the number of attacks by up to 50% but in only half the patients. A placebo-effect of approximately 30% is generally observed in migraine prophylaxis studies. The results of this open pilot trial demand a randomized double-blind placebo-controlled trial with a larger patient population (including those with aura).

Table 7: Clinical studies in humans, indication low back pain

Type	Study	Test Product(s)	Number of Subjects	Type of Subjects	Outcomes	Statistical analysis	Clinical relevance
Analgesic action Chrubasik *et al*. 2000	R / DB 3 arms 4 weeks	Willow bark extract DER 8-14:1 extraction solvent 70% ethanol *placebo *120 mg salicin eq. *240 mg salicin eq. Per day Rescue medication = tramadol	N = 210 (70 per group) ≥18 years Drop out = 19	>6 months of intermittent low back pain	% of patients pain-free during 5 days without tramadol. P = 6% 120 mg = 21% 240 mg = 39% Change from baseline (modified Arhus score) P < 120 mg < 240 mg	No information available	Severe allergic reaction N=1
Chrubasik *et al*. (2001a)	O / R	Willow bark extract DER 8-14:1 extraction solvent 70% ethanol 240 mg salicin eq. Rofecoxib 12,5 mg	N=228 Drop out = 45	Low back pain without clear origin	VAS modified Arhus index Improved by 20% in both groups TPI Improved by 30% Pain free N=20 in both groups Rescue = NSAID/tra madol Willow: N=9 Rofecoxib: N=12	Multivariate analysis	Concordance between patients and physicians Undesirable effects: Willow: N=19 Rofecoxib: N=27

Type	Study	Test Product(s)	Number of Subjects	Type of Subjects	Outcomes	Statistical analysis	Clinical relevance
Safety and economic impact Chrubasik *et al*. (2001b)	O / NR / PMS	Willow bark extract 8-14:1 extraction Placebo 120 mg salicin eq. 240 mg salicin eq. 4 weeks	N = 451 >18 years	Acute exacerbation of chronic (>6 months) nonspecific LBP	% of patients pain free after 4 weeks: 240 mg: 41% 120 mg: 8% Patients on non-conventional treatment Pl: 18% Patients on conventional treatment Pl: 5.7% Without conventional treatment	No information available	Slight differences between the groups at start. Conventional treatment, prescribed by GPs or orthopaedists, included analgesics, NSAIDS, acupuncture Willow UE: N=9

Table 8: Clinical studies in humans, indication osteoarthritis and rheumatoid arthritis

Type	Study	Test Product(s)	Number of Subjects	Type of Subjects	Outcomes	Statistical	Clinical relevance
Schmid *et al*. (2001b)	R/Pl/DB	Pl 240 mg salicin eq. No rescue medication	N=78 Drop-out = 15	OA of hip or knee	Primary: WOMAC: difference Secondary stiffness / physical function	Primary ITT Willow>Pl p=0.047 PP Willow>Pl p=0.0196	Willow UE = 9

Type	Study	Test Product(s)	Number of Subjects	Type of Subjects	Outcomes	Statistical	Clinical relevance
Biegert *et al*. (2004)	R/DB/Pl	OA-trial Pl Diclofenac 100 mg 240 mg salicin eq. 6 weeks RA-trial Pl / 240 mg salicin eq. DMARDs allowed / no NSAIDs	OA group N=127 Drop-out = 21 RA group N=26	OA hip / knee RA: at least moderate disease activity	Primary OA: WOMAC RA: 100mm VAS Secondary OA: stiffness/function RA: composite	OA primary Willow < diclofenac (p=0.0002) OA secondary Only physical function subs core better with willow Diclofenac better on all criteria (p=0.05) RA trial Willow: VAS NS	Willow: UE N=9
Beer & Wegener (2008)	O/MC 6 weeks	DER 8-14:1 solvent ethanol 70% V/V eq. to 240 mg salicin	N=138 Willow: 90 (drop-out:2) Standard Therapy: 41 (drop-out: 1) Combination: 8	50-75 y Diagnosed coxarthrosis or gonarthrosis	Primary: WOMAC Secondary: UE	No information available	Effect of willow and conventional therapy comparable
Lardos *et al*.	R/DB	Willow extract (water)	N=60	Hip or knee	Primary: VAS and physical	ITT	No dose-activity

Type	Study	Test Product(s)	Number of Subjects	Type of Subjects	Outcomes	Statistical	Clinical relevance
(2004)	3 weeks	= 90 and 180 mg salicin per day Diclofenac = 150 mg per day No analgesics/NSAIDs allowed		arthrosis	function (Steinbrocker)	All interventions active as compared with the start	relation
Samochowiec (2001)	DB/R/C	Na-diclofenac 150 mg/d Willow extract (?) Pl No rescue medication allowed	N=59 Drop-out: 1	Hip or knee arthrosis stage II and III	Pain (VAS) stiffness	No information available	No clear-cut description of endpoints
Saller *et al*. 2008 ADRs	O/MC		N=877	OA, RA, LBP, soft tissue disorders	Number a type of ADRs	No information available	GI and skin
Müller *et al*. 2010	O 6 months	water extract of *Salix* (DER 16-23:1) doses ? NSAIDs Opioids	N=350	low back pain and pain due to osteoarthritis	VAS *Salix* > *Salix* + NSAIDs + opioids > *Salix* + NSAIDs >	No information available	Non inferiority
Müller-Fassbender *et*	O 3.3 (±	water extract of *Salix* (DER 16-23:1): 480	N=333	osteoarthritis, rheumatoid	Satisfaction level	No information available	>90% very good

Type	Study	Test Product(s)	Number of Subjects	Type of Subjects	Outcomes	Statistical	Clinical relevance
al. (2007)	0.9 weeks	mg		arthritis or low back pain.	In 80% of patients		
Stange *et al*. (2014)	O 24 weeks	water extract of *Salix* (16-21:1): dose?	N=436	musculoskeletal pain	VAS 100 Dropped: 58.4 ± 22.6 to 31.8 ± 22.5	Wilcoxon $p<0.05$	UE: 4.8% (3 weeks); 0.3% (24 weeks)

ADRs = Adverse Drug Reactions

C = Controlled

DB = Double Blind

GI = Gastro-Intestinal

GPs = General Practitioners

ITT = Intention To Treat

LBP = Low Back Pain

MC = Multicenter

NR = Non Randomised

O = open study design

OA = Osteoarthritis

Pl = Placebo

PMS = Post Marketing Surveillance

PP = Per Protocol

R = Randomised

RA = Rheumatoid Arthritis

UE = Undesirable Effects

VAS = Visual Analogue Scale

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

No studies performed.

4.4. Overall conclusions on clinical pharmacology and efficacy

Under WEU: General conclusion on the clinical studies on analgesic effects of willow bark:

The disease studied, the design and quality of the published trials was variable (see assessors comments per study, see also Gagnier *et al*. 2007). Shortcomings in some of the controlled clinical trials are: small numbers of patients and/or short duration of the study, slightly different baseline characteristics which hamper conclusions on changes towards baseline, open study design, and access to rescue analgesics/NSAIDs/corticoids again hampering conclusions on efficacy of willow bark. The willow bark preparations are not always carefully characterized and described (extraction solvent, DER). The quantity of salicin should be stated although other constituents may contribute to the activity. The composition with regard to salicylates and other constituents varies among extracts (Kammerer *et al*. 2005). Results for a particular extract cannot be extrapolated to other extracts.

Taking into account the body of available published trials and their respective trial quality and outcomes, the controlled clinical trials published so far provide moderate evidence for the analgesic activity of a daily dose of willow bark extract ethanol 70% 8-14:1 corresponding to 240 mg salicin (single ingredient preparation). Based on the double-blind, placebo/active-controlled randomized clinical trials (Chrubrasik *et al*. 2000, Schmid *et al*. 2001b, Biegert *et al*. 2004), it can be concluded that willow bark is superior over placebo in a dose-dependent manner in the clinical setting of low back pain. An additional 3-arm trial including placebo and active comparator is recommended. With regard to the analgesic effects in OA and RA, willow bark exerts none to a moderate analgesic activity. It should be taken into account that responders in the placebo group are in general relatively high in pain trials. Additional studies should have sufficient power.

Based on the available clinical studies, daily intake of willow bark dry extract ethanol 70% (total salicin content 15%), equivalent to 240 mg total salicin is advised. The daily dose should be divided into 2 doses. The patient is referred to the physician in case of worsening or no improvement after the first week of use. This limitation of duration of use is based/in accordance with the clinical studies, where improvement is observed after 1 week of treatment with willow bark (Chrubasik *et al*. 2000). The use is not recommended under 18 years of age.

When revising the assessment report in 2016, no new clinical trials could be found. However meta-analysis of the existing studies seem to confirm the daily dose equivalent with 240 mg salicin as an effective dose in reducing pain and equivalent to 12.5 mg rofecoxib (Oltean *et al.* 2014).

5. Clinical Safety/Pharmacovigilance

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

See chapter 5.3

5.2. Patient exposure

Minor adverse effects have been reported in a relatively small number of patients. Based on the published clinical data, from a total of 2734 patients and healthy volunteers treated with various single-ingredient preparations containing willow bark, adverse events, predominantly mild, were

reported in 95 cases (3.5%), predominantly GI and allergic reactions (including 2 severe). Data obtained with combination products are not included in this overview of adverse events.

In a post-authorisation surveillance study on willow bark dry extract (8-14:1, ethanol 70%; daily doses equivalent to 120 or 240 mg total salicin), 922 physicians observed 4731 patients with chronic back pain or arthralgia after 3-4 weeks and after 6-8 weeks. “63 patients reported ADR, no serious ADR occurred. GI side effects were notified with an incidence of 0.93%, in most cases as abdominal pain (incidence = 0.59%). No GI bleeding or ulceration was mentioned. Skin reactions or potential allergic reactions were notified with an incidence of 0.30%. Frequency of ADR notification was independent of dosage and did not increase with treatment duration” (Werner 2004, abstract).

Assessor’s comment:

Full details of the post-authorisation study of Werner are missing, only an abstract was made available which makes assessment of the study impossible.

5.3. Adverse events, serious adverse events and deaths

The following undesirable effects were reported in randomized clinical trials.

Chrubasik *et al.* (2000): Willow bark groups: N= 70+70 patients

- 1 patient suffered a severe allergic reaction (exanthema, pruritis, swollen eyes; 120mg group, could be attributed).
- Note that the other adverse effects (N=2) were attributed to tramadol (rescue medication).

Chrubasik *et al*. (2001a): Willow bark group, N= 114 patients

- Allergy : (1 possible, 3 likely, 1 clear connection)
- GI (dyspepsia, vomiting, heartburn, diarrhoea) (7 possible, 3 likely, 1 clear connection)
- Dizziness: (1 possible)
- Headache: (1 possible)
- Blood pressure instability (1 possible)

Chrubasik *et al*. (2001b) Willow bark groups, N=112+115 patients

- GI (9)
- Allergic skin reaction (3)
- No evaluation of causality presented
- Schmid *et al*. (2001b) Willow bark group, N=38 patients:
- Allergic skin reactions (6)

No evaluation of causality presented.

Note that 1 patient in the willow bark group withdrew due to allergic symptoms.

Biegert *et al*. (2004): Willow bark group in OA trial (N=43) and in RA trial (N=13)

- GI (7)
- Allergy (exanthema, 1) (mild itching, 1)

No evaluation of causality presented.

Schmid *et al*. (2001a): willow bark group N=10 volunteers

Adverse events not recorded / reported

Krivoy *et al*. (2001): willow bark group N=35 patients

Adverse events not recorded / reported.

Literature reports a case of anaphylaxis resulting from the use of a willow-bark containing dietary supplement in a patient with a history of aspirin allergy (Boullata *et al*. 2003).

Plants that contain more than 10% tannins (willow bark: 8-20%) have potential adverse effects including stomach upset, nausea, vomiting (Rotblatt 2002).

Vlachojannis *et al*. (2011) reported that *Salix* extracts are not interfering with platelet aggregation, which proves a mechanism of action different from the NSAIDs and acetylsalicylic acid.

Undesirable effects are reflected in section 4.8 of the monograph.

5.4. Laboratory findings

No data available.

5.5. Safety in special populations and situations

Intrinsic as well as extrinsic factors are considered.

5.5.1. Use in children and adolescents

Adverse effects and signs of toxicity normally associated with salicylates (such as gastric and renal irritation, hypersensitivity, blood in stools, tinnitus, nausea and vomiting) may occur. Salicin is documented to cause skin rashes.

Moro *et al*. (2011) reported about a hypovolemic shock due to severe gastrointestinal bleeding in a 4-year old boy taking a herbal syrup with *Filipendula ulmaria* and *Salix spp*. The preparation was marketed as food and prescribed by his paediatrician to treat a mild cold accompanied by fever. Quali-quantitative analysis confirmed the presence of salicylates in the syrup. Naranjo algorithm showed a probable correlation between the onset of symptoms and the consumption of the herbal remedy. The child recovered after receiving intensive care. This adds to the justification of restricting *Salix*-containing products to adults and elderly (Moroa *et al*. 2011).

In view of the lack of more toxicity data on willow bark, the usual precautions associated with salicylate therapy are also applicable to willow bark. Therefore, in individuals with known hypersensitivity to aspirin, asthma, active peptic ulceration, haemophilia and other bleeding disorders, gout should be aware of the possible risks associated with the intake of willow bark (Clauson *et al*. 2005; Aronson 2006).

Concurrent administration of willow bark with other salicylate-containing products should be avoided as such combination may increase the risk of gastric irritation.

Hypersensitivity to salicylates or other NSAIDS

There is a considerable cross-reactivity of acetylsalicylic acid with other NSAIDS and the now widely banned tartrazine. For willow bark preparations, the risk for an idiosyncratic response (skin reactions, bronchospasm) in sensitive individuals cannot be excluded; the use of willow bark is therefore contra-indicated. Mechanism of (aspirin) hypersensitivity: the current theory relates to inhibition of COXs and interference with PEG2 synthesis allowing PGF2 to predominate in susceptible individuals. Avoidance of aspirin and substances to which there is a cross-sensitivity is the only satisfactory solution.

Asthma Patients with existing asthma and nasal polyps or chronic urticaria have a greater frequency of hypersensitivity. Because of the relatively high incidence of aspirin-induced broncho-constriction, urticaria or anaphylaxis, aspirin should not be used in patients with asthma or those already believed to be hypersensitive to salicylates, NSAIDS or tartrazine (Rotblatt 2002). The use of willow bark in asthma patients is contra-indicated as severe reactions could be induced.

Children Reye's syndrome was previously regarded as a side-effect of aspirin, but it has become clear that the syndrome cannot be assigned to a specific cause. Reye's syndrome presents itself a few days after the prodrome of a viral illness, including influenza A and B, adenovirus, varicella virus and rheovirus. Various other factors have been incriminated such as pesticides. Only in case of aspirin, some epidemiological studies have been performed but the clarity of the link between Reye's syndrome and aspirin has been questioned (Pugliese *et al*. 2008).

Despite the lack of understanding of the syndrome and the fact that a clear, conclusive link between the syndrome and aspirin (salicylates) is not yet established, the decision has been taken in many countries to advice against the use of salicylates in children (Rotblatt 2002). Because of the clinical importance of the syndrome and the avoidable risk, use of salicylates in patients below 16 years is questioned.

Assessors's comment:

The approach to the risk-benefit balance differs between European countries. In Belgium there are low-dose preparations on the market with 100 mg of acetylsalicylic acid, with posology's for children starting from an age of 6 months. The SPC contains a warning (no contraindication) stating that acetylsalicylic acid should only be used in children below 12 years of age when other medicines failed. It states also that in case of loss of consciousness or persistent vomiting during treatment, Reye's syndrome may be suspected. Finally it is mentioned that although a direct link with acetylsalicylic acid has not been proven with certainty, the treatment must be immediately interrupted (SPC Aspirin Junior 2016).

In the *Salix* monograph of 2009 a special warning on Reye's syndrome for patients under 18 years was included: "*In children and adolescents under 18 [product name] should only be used on medical advice and only in cases when other therapies failed to succeed. In a child or adolescent who has become very unwell with severe vomiting, drowsiness or loss of consciousness following a viral infection, a serious disease may be suspected. Reye's syndrome is an extremely rare but life threatening condition which requires immediate medical attention*". Although, – up to now - no occurrence of Reye's syndrome after intake of *Salix* preparations has been reported, the HMPC decided, as a precautionary measure, to include in the revised monograph a contraindication for children and adolescents because of the lethal outcome of this syndrome. There are no pharmacovigilance reports on serious undesirable effects in children and adolescents.

5.5.2. Contraindications

As a precautionary measure, the HMPC decided to include in the revised monograph a contraindication for children and adolescents (see above). Furthermore, considering the SmPC of authorized acetylsalicylic acid containing products, the following contraindications are also included:

- Hypersensitivity to the active substance.

When willow bark preparations are taken according to the normal dosage recommendations, they will produce relatively low salicylate serum levels. Still, reactions in sensitive individuals cannot be ruled out.

-
- Hypersensitivity to salicylates or to other NSAIDs (e.g. history of angioedema, bronchial spasm, or chronic urticaria in response to salicylates or to other NSAIDs).
- Asthma due to sensitivity to salicylates.
- Active peptic ulcer disease.
- Third trimester of pregnancy.
- Glucose-6-phosphate dehydrogenase deficiency.

There is a case report of a woman with G6PD who developed massive haemolysis after taking an herbal preparation containing salicin (Baker *et al*. 1987). In Sardinia there is a high incidence of G6PdH deficiency. Salicylates have been contraindicated by all the experts for these patients. Therefore, the use of willow bark in case of G6PD deficiency is contra-indicated.

- Children and adolescents under 18 years of age due to the risk of Reye's syndrome.
- Severe liver or renal dysfunction.
- Coagulation disorders.

Precautions associated with salicylate therapy are also applicable to willow bark. In case of severe liver or renal dysfunction, coagulation disorders (risk of haemorrhagia), gastric/duodenal ulcer, willow bark should be contra-indicated in these patients.

5.5.3. Special Warnings and precautions for use

Regulatory action for salicylates:

The Belgian authorities issued an advice against use in children below 12 years in case of suspicion of viral infection. A class labelling in the section 4.4 (not 4.3) was imposed for acetylsalicylic acid containing medicinal products: only to be used for these patients in case other products lack efficacy; information on symptoms of Reye's syndrome is given; a statement is included that relationship between syndrome and acetylsalicylic acid is not yet established with certainty.

Other precautions with regard to intake of salicylates

As a matter of precaution special warnings and precautions for use are included in the monograph.

'Traditional use':

- The product is not intended to be used in case of acute arthritis as this condition requires medical advice.

- If fever exceeds 39°C, persists or is associated with severe headache or if symptoms worsen during the use of the medicinal product, a doctor should be consulted.
- For tinctures, extracts, containing ethanol, the appropriate labelling for ethanol, taken from the 'Guideline on excipients in the label and package leaflet of medicinal products for human use', must be included.

'Well-established use' and 'Traditional use':

- Concomitant use with salicylates and other NSAIDs is not recommended without medical advice.
- If the symptoms worsen during the use of the medicinal product, a doctor or a pharmacist should be consulted.

5.5.4. Drug interactions and other forms of interaction

Only the interactions documented with willow bark are included. A number of theoretical interactions listed for acetylsalicylic acid (anti-hypertensive agents, uricosurica and others) were not included.

Interaction (pharmacokinetic and pharmacodynamic) with oral anticoagulants (heparin, coumarine derivatives) is plausible and of therapeutic importance and therefore included: Krivoy *et al*. (2001) investigated whether treatment with willow bark during treatment of LBP affected platelet aggregation. 35 patients having acute exacerbations of LBP were enrolled in a double-blind placebo-controlled study to receive for 28 days *Salix daphnoides* and *Salix purpurea* extract with 240 mg salicin per day, "Assalix"(N=19) versus placebo (coated tablets, N=16). A further 16 patients with stable chronic ischemic heart disease were given 100 mg acetylsalicylate per day during the study period. After 28 days of treatment, platelet aggregation was measured. Willow bark significantly decreased AA- and ADP-induced aggregation but to a significantly lesser extent than acetylsalicylate did. The mean percentages of maximal AA-induced platelet aggregation were 61% (willow bark), 78% (blank) and 13% (acetylsalicylate). Collagen-induced aggregation was not influenced by willow bark (or acetylsalicylate). Further investigation is needed to clarify the clinical relevance of these findings in patients with impaired thrombocyte function or with vitamin K antagonistic treatment (structural similarity of salicylate and warfarin).

Salicylates are extensively bound to plasma proteins. A recent study on the pharmacokinetics of salicin after oral administration of a standardized willow bark extract (Schmid *et al*. 2001a) demonstrates that the AUC of salicylate after ingestion of a dose corresponding to 240 mg salicin was equivalent to that expected from an intake of 87 mg acetylsalicylic acid; bio-availability was 43.3%; peak serum levels were 1.2 mg/L and were reached within 2 hours after ingestion. Pharmacokinetic interactions due to plasma protein binding cannot be ruled out. Salicylic acid does not irreversibly acetylate COX-1. Taking into account the study of Krivoy *et al*. (2001) and the fact that salicylates are highly bound to plasma proteins, the potential for interaction cannot be ruled out.

Shalansky *et al*. (2007) carried out a prospective longitudinal study (171 adults) to determine the risk of bleeding and supratherapeutic international normalised ratios (INR) associated with use of complementary and alternative medicine (CAM) in patients receiving warfarin. Statistically significant associations between the use of willow bark and bleeding events were identified. The risk of a supratherapeutic INR was not increased. After adjustment for the identified non-CAM risk factors, association was not statistically significant.

The combined use of willow bark with acetylsalicylic acid/other NSAIDS is not recommended even though an increased risk of gastric irritation has never been described (Rotblatt 2002). The MLWP

decided in September 2007 to add a warning that concomitant use with salicylates and other NSAIDs is not recommended without medical advice.

The very high concentration of tannins present may interfere with absorption of other products.

According to Williamson *et al*. (2013) no drug-drug interactions with willow are reported. Pharmacokinetic studies suggest that doses of willow bark extract can achieve levels of salicylic acid that are equivalent to an 87 mg dose of aspirin. Interaction of willow bark with medicines acting upon platelets or blood coagulation should be taken into account. Williamson *et al*. (2013) also recommend to avoid combinations of willow bark with antiplatelet drugs. However if concurrent use is felt desirable it would seem sensible to warn patients to be alert for any signs of bruising or bleeding, and report these immediately, should they occur. Therefore relevant warnings are included in the monograph.

5.5.5. Fertility, pregnancy and lactation

Salicylates cross the placenta. Acetylsalicylic acid is teratogenic in rodents, but till now there is no clear evidence of teratogenesis when used in human pregnancy. Increased PG production during pregnancy and/or placental metabolism may have protective roles.

Due to increased bleeding risk, delay of parturition and induction of early closure of the ductus arteriosis, use of acetylsalicyl acid/NSAIDs is contra-indicated in the third trimester of pregnancy (Aronson 2006; Barnes *et al*. 2007).

Conflicting reports have been documented concerning the safety of acetylsalicylic acid taken during the first and second trimester of pregnancy. The safety of willow bark has not been established. Occasional ingestion of salicylates does not seem to be a problem (no contra-indication in Belgium for first and second trimester), but due to lack of conclusive data on the use during the first and second trimester of the pregnancy are not available, the use is not recommended as a general precaution

Salicylates appear in breast milk and have been reported to cause macular rashes in babies. The two major pathways of salicylate degradation (formation of salicyluric acid and salicyl phenol glucuronide) become saturated at relatively low body levels of the drug. The drug is slowly eliminated by the newborn infant.

Because data on the use during lactation are not available, the use is not recommended as a general precaution.

5.5.6. Overdose

No toxic effects reported.

Taking into account the relatively low serum levels after oral intake of willow bark and the high content of tannins in willow bark (GI disturbances) which makes intake of large amounts less likely, it was agreed not to include the symptoms of overdose with acetylsalicylic acid.

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

No data available.

5.5.8. Safety in other special situations

Chrubasik (2002) stated that willow bark does not enhance menstrual bleeding. The author supported this statement referring to observations with a commercial product equivalent to a daily dose of 240 mg.

5.6. Overall conclusions on clinical safety

Clinical data, from a total of nearly 600 patients and healthy volunteers treated with various single-ingredient preparations containing willow bark, adverse events were predominantly mild.

Adverse effects and signs of toxicity normally associated with salicylates may occur. In view of the lack of more toxicity data on willow, the usual precautions associated with salicylate therapy are also applicable to willow. Therefore individuals with known hypersensitivity to aspirin, asthma, active peptic ulceration, haemophilia and other bleeding disorders, gout should be aware of the possible risks associated with the intake of willow bark. Appropriate contra-indications and special warnings and precautions for use are introduced in the monograph (WEU and TU).

6. Overall conclusions (benefit-risk assessment)

In spite of its long (traditional) use, only a few controlled trials have been conducted with willow bark to support its analgesic and/or antipyretic action. Recent renewed interest in willow bark resulted in a number of clinical trials studying the efficacy in acute exacerbations of low back pain, osteoarthritis and rheumatoid arthritis. The design and quality of the published trials was variable.

Taking into account the body of available published trials and their respective trial quality and outcomes, the controlled clinical trials published so far provide moderate evidence for the analgesic activity of a daily dose of willow bark extract 8-14:1 (solvent 70% ethanol) corresponding to 240 mg salicin (single ingredient preparation) in low back pain (WEU). Proposed ATC code: N02BG (other analgesics and antipyretics).

For the symptomatic treatment of fever and pain, only general evidence is available (TU). Based on data in text books and information provided by interested parties, the following are included in the monograph: powdered and comminuted herbal substance, dry extract (DER 8-20:1) extraction solvent water, dry extract (DER 16-23:1) extraction solvent water, liquid extract (DER 1:1) extraction solvent ethanol 25% V/V, tincture (1:5), extraction solvent ethanol 25% V/V.

In view of the lack of adequate toxicity data on willow bark, the usual precautions for use associated with salicylate therapy are also applicable to willow. Appropriate contra-indications and special warnings and precautions for use are introduced in the monograph. More particularly willow preparations are contraindicated for children and adolescents under 18 years of age (WEU and TU).

Allergic reactions (rash, pruritis, urticaria, asthma, exanthema) and gastrointestinal symptoms (nausea, vomiting, abdominal pain, diarrhoea, dyspepsia, heartburn), may occur. The frequency cannot be estimated accurately due to the limited number of patients in controlled clinical trials. Nevertheless, atopic patients should be warned against the use of willow bark preparations. Due to the mechanism of action warnings should be included with regard of gastro-intestinal irritation and damage. Furthermore, there is a theoretical risk for patients using anticoagulants. No herbal drug interactions have been reported in humans.

The use during the first and second trimester of pregnancy and during lactation is not recommended as salicylates cross the placenta. Willow bark preparations are also contraindicated in the third trimester

of pregnancy. They should not be used during breast feeding as small amounts of salicylated also appear in breast milk. No fertility data available.

On the basis of the available information salicin and its metabolites are considered by the HMPC as contributing to the activity of the herbal substance and herbal preparation(s) and therefore are classified as active markers.

Tests on reproductive toxicity, genotoxicity and carcinogenicity have not been performed. Therefore, a list entry is not proposed.

Annex

List of references