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Willow bark extract: The contribution of polyphenols to the overall effect

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Weidenrindenextrakt: Der Beitrag der Polyphenole zum Gesamteffekt

Zusammenfassung. Die Wirksamkeit von Weidenrindenextrakten in der Behandlung schmerzhafter Erkrankungen des Bewegungsapparates, wie Rückenschmerzen und Arthritis, wurde dem Gehalt an Salicinderivaten als Prodrugs für Salicylate zugeschrieben. Aus der Erfahrung klinischer Studien und pharmakologischer Testmodelle heraus kann jedoch die Gesamtsalicinfraktion nicht als zufriedenstellende Erklärung für die Wirksamkeit der Weidenrinde herangezogen werden. Darüber hinaus fehlt den Salicinen und ihren Metaboliten das Acetylierungspotenzial der ASS, so dass hier ein anderer Wirkmechanismus vorliegen muss. Ein detailliertes pharmakologisches Screening des wässrigen Weidenrindenextraktes STW 33-I zielte auf die Frage nach der Identifizierung von Fraktionen mit einem Anteil am Gesamteffekt ab. Alle angewandten *in vitro*- und *in vivo*-Modelle ließen auf einen relevanten Beitrag der Fraktion der Polyphenole und Flavonoide schließen. Die Aufklärung der für diesen Effekt verantwortlichen Einzelsubstanzen bzw. ihrer Kombinationen wird Thema zukünftiger Untersuchungen sein.

Schlüsselwörter: Weidenrinde, *Salix sp.,* Salicaceae, Polyphenole, Flavonoide, Salicin, Wirksamkeit.

Summary. The efficacy of willow bark extract in the treatment of painful mobility disorders, such as back pain and arthritis, has been attributed to the content of salicin and its derivatives as pro-drugs of salicylates. However, based on clinical experience and the evidence of experimental pharmacological studies, the fraction of total salicin cannot satisfactorily explain the clinical efficacy of willow bark. In addition, salicins and their metabolites lack the acetylating potential of ASA and must therefore possess a different mechanism of action. A detailed pharmacological screening of the aqueous willow bark extract STW 33-I addressed the question of the identification of fractions contributing to the overall effect. All *in vivo* and *in vitro* models studied pointed to relevant contributions of the fraction of polyphenols and flavonoids. The single compounds or their combinations responsible for the effect remain to be elucidated.

Key words: Willow bark, *Salix sp.*, Salicaceae, polyphenols, flavonoids, salicin, efficacy.

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Introduction

The European Pharmacopoeia defines willow bark as the whole or fragmented dried bark of young branches or dried pieces of current year twigs of various species of the genus *Salix*, including *Salix purpurea* L., *Salix daphnoides* VILL. and *Salix fragilis* L. The bark is specified to contain not less than 1.5 per cent of total salicylic alcohol derivatives, expressed as salicin [1]. Willow bark extract has a long history as a herbal remedy against fever, pain and inflammation [2]. Its use in these indications is widely accepted through positive monographs of the ESCOP and the German Commission E, and is confirmed through many clinical trials [3]. The ESCOP monograph recommends a daily dose equivalent to 120–240 mg total salicin. On the German market, there are currently two different types of willow bark preparations available: tablets based on a 70% ethanolic extract (DER 8–14:1, min. 15% total salicin), where 4 tablets contain the equivalent of 240 mg salicylic alcohol derivatives recommended by ESCOP, and more recently tablets based on the aqueous extract STW 33-I (DER 16–23:1, 23–26% total salicin), which allow the daily administration of the equivalent of 240 mg salicylic alcohol derivatives with only 2 tablets.

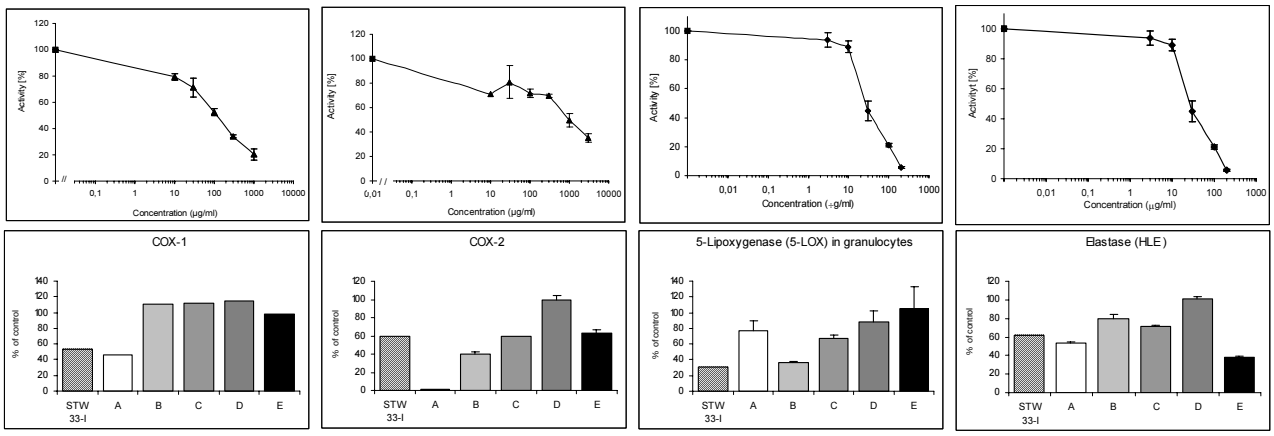

Fig. 1. Inhibitory effects of STW 33-I and its fractions on COX-1, COX-2, HLE (isolated enzymes) and 5-LOX (human granulocytes). Upper graphs: Estimation of EC_{50} of total extract. Lower graphs: Testing of fractions with concentrations corresponding to the EC_{50} of the total extract (100 µg/ml for COX-1, 800 µg/ml for COX-2, 30 µg/ml for 5-LOX and 60 µg/ml for HLE) [8]

While salicin has always been traditionally used as a biological marker for the activity of willow bark, and was even considered the major active constituent in the past, little attention has been given to the role of polyphenols and flavonoids in willow bark preparations. Willow bark contains up to 20% flavonoids and condensed tannins. Typical representatives of the flavonoids are glucosides of naringenin, isosalipurpuroside or eriodictyol [4]. Other polyphenols also form a major fraction in aqueous extracts from willow bark: recently the flavan-3-ols catechin, epicatechin, gallocatechin, catechin-3-O-(1-hydroxy-6-oxo-2-cyclohexene-1-carboxylic acid)-ester, the dimeric procyanidins B1, B3 and the trimeric procyanidins epicatechin-(4β→8)-catechin-(4α→8)-catechin and epicatechin-(4β→8)-epicatechin-(4β→8)-catechin were isolated from the bark of *S. purpurea* L. A fraction containing higher oligomeric procyanidins was investigated by ^{13}C-NMR; data indicate an average degree of oligomerization of 4 to 5 flavan-3-ol units with dihydroxylated B-rings and predominance of 2,3-*cis*-stereochemistry [5].

Salicin was already discovered in 1831. The isolated compound was used for the treatment of rheumatic pain. Salicin is a prodrug and thus a precursor of salicylic acid. Since salicylic acid derivatives, mainly in the form of acetyl salicylic acid (ASA), still count among the most important non-steroidal anti-inflammatory drugs, willow bark was considered a natural form of ASA. However, besides the fact that salicylic acid is not ASA, this oversimplified view was already questioned in early clinical research: Mayer and Mayer [6] concluded from their results that other fractions from willow bark extract must have significantly contributed to the overall efficacy.

In a more recent clinical trial, Lardos et al. [7] tested aqueous willow bark extracts with two different dosage regimens for total salicin (90 versus 180 mg/day) in patients with arthrosis. They used diclofenac as an active reference drug. There was no correlation between the dose of total salicin and efficacy of the extracts [7], thus supporting the view that another fraction, other than salicin, strongly contributes to their efficacy.

The aim of a multidisciplinary screening program, from which some of the relevant results are presented here (the individual results have been presented in scientific symposia and publications of the different working groups) was to systematically study the pharmacological properties of an aqueous willow bark extract, STW 33-I, and to examine the relative contribution of salicin vs. those of the other components in typical models of inflammation *in vitro* and *in vivo*.

Fractions of willow bark extract

The aqueous willow bark extract STW 33-I was sequentially separated into five fractions of different polarity, using sequentially toluene, ethyl acetate, n-butanol and ethanol in addition to the remaining aqueous extract. The toluene fraction A provided the smallest yield (0.4%). It contained mainly unidentified polyphenols. Fraction B, prepared with ethyl acetate, had a yield of 9.0%, and contained unidentified polyphenols and flavonoids. The butanol-fraction C (23.6%) was shown to contain flavonoids. The fraction with the highest yield (25.2%) was the ethanolic fraction D, which contained salicin. Finally, the remaining aqueous fraction E (10.3%) was shown to contain proanthocyanidins.

Inhibition of enzymes of arachidonic acid metabolism

The inhibitory effect of the aqueous extract and the different fractions on cyclooxygenases, COX-1 and COX-2, as well as on lipoxygenase, 5-LOX, was studied in human granulocytes *in vitro*. Initially, a concentration-effect curve was plotted for the total extract. In order to compare the effect of the different fractions, they were tested in concentrations corresponding to the EC_{50} of the total extract. The most marked inhibition was found in fractions A (effect mainly on COX-1) and B (effect mainly on COX-2 and 5-LOX), both of which contain essentially polyphenols [8]. In all assays the contribution of the salicin fraction was negligible (Fig. 1); however, it

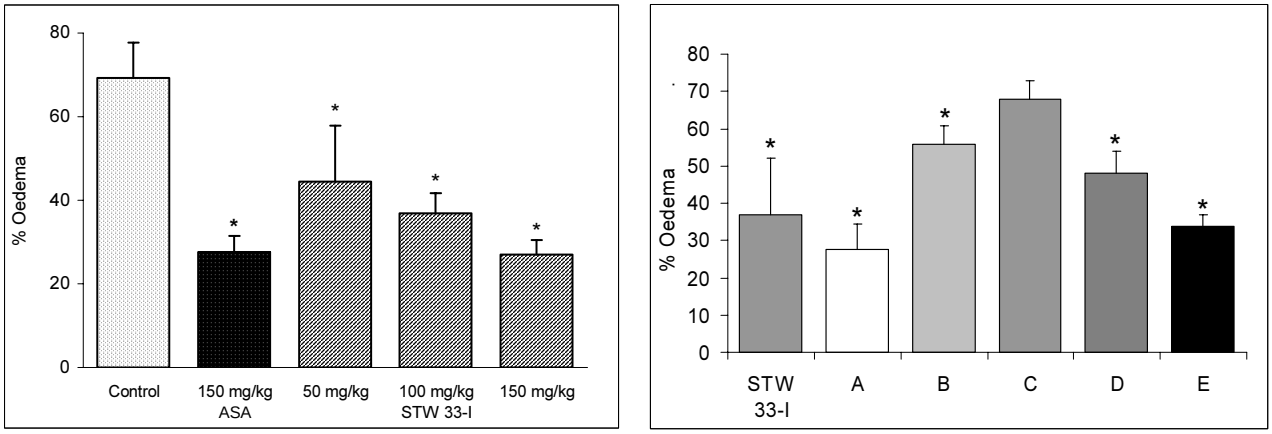

Fig. 2: Dose-dependent effects of STW 33-I (left) and its fractions (right) in the model of carrageenan-induced rat paw oedema. The effect of 150 mg of STW 33-I corresponded to the effect of 150 mg of ASA. Fractions were tested with 100 µg/ml, * $p \leq 0.05$ in Duncan´s test [15].

should be mentioned that in this system no metabolic activation to salicylic acid occurred.

Another enzyme system relevant for its involvement in inflammation is human leucocyte elastase (HLE). Again, the peak effect was found in fractions A and E (containing polyphenols and proanthocyanidins, respectively) [8].

Inhibition of gene expression of mediators of inflammation

Aqueous willow bark extract was shown to dose-dependently inhibit gene expression of COX-2 in activated chondrocytes and monocytes [9]. More detailed experiments were conducted by testing STW 33-I and its fractions in lipopolysaccharide and interferon-γ stimulated human monocytes. In comparison to diclofenac, which served as reference control, similar effects were found on mRNA and protein expression of COX-2 and TNF-α for STW 33-I and its hydrophilic fraction E, while fraction D, containing salicylic alcohol derivatives, was less effective [10, 11]. Using the same test model, STW 33-I and its fraction E significantly inhibited the reduced apoptosis rate of LPS-stimulated monocytes similar to diclofenac. In addition, the increased rate of NO synthesis in proinflammatorily activated monocytes, which is typically counteracted by anti-inflammatory drugs such as diclofenac, was also significantly inhibited by STW 33-I and its hydrophilic fraction E, but not by the other fractions [10, 11].

The aqueous extract and all the fractions of willow bark with different polarities influenced the rate of apoptosis significantly, whereas NO synthesis as well as COX-2 and TNF-α protein synthesis and their respective gene expressions were significantly influenced only by STW 33-I and by its polar fraction E. The results of fraction D, containing salicylic alcohol derivatives, suggest comparatively smaller effects on all analysed processes; but also in these *in vitro* systems no metabolic activation to salicylic acid is expected.

Free radical mediated processes

Free radicals are involved in the mediation of inflammatory processes such as the activation of nuclear factors NF-κB and TNF-α. Polyphenols from plants are known to act against these processes, at least partly due to their antioxidative potential [12, 13]. Typical models of anti-inflammatory effects involve the inhibition of formation of free radicals induced by AAPH (2,2′-azobis(2-amidino-propan)dihydrochloride), or by the xanthine / xanthine oxydase reaction. When STW 33-I and its fractions were tested in these models in a concentration of 2 µg/ml, the strongest antioxidative effect was observed with fractions A and B (polyphenols, flavonoids) [14].

These *in vitro*-findings were confirmed in a model of activated alveolar macrophages from the lungs of pigs. Again, the antioxidative effect was correlated with the fractions of polyphenols and flavonoids [14].

In vivo *models of inflammation*

The pharmacological profile of aqueous willow bark extract, STW 33-I, was also compared with ASA in various *in vivo* models. Dose-dependent effects of the extract corresponding to those of the same dose of ASA were found in models of acute and chronic inflammation (paw oedema and air pouch, adjuvant-induced arthritis in rats), analgesia (writhing test in mice, Randall-Sellito test in rats), and antipyretic effect (brewer's yeast-induced fever reaction (in rats), and the air pouch model (in rats) [15, 16]. Fractions of STW 33-I were also tested in the carrageenan-induced rat paw oedema, a model of acute inflammation [15, 16] (Fig. 2).

In the Randall-Sellitto-test an oral dose of 150 mg extract per kg body weight had approximately the same effect as the same dose of ASA, and the onset of its effect was just as rapid (Fig. 3). This finding does not support assumptions of a delayed onset of action in therapy. The observation of an acute effect similar to that of ASA is clearly relevant for the therapeutic usefulness in indications such as back pain.

In addition, the equipotent effect of similar doses of the extract and ASA is likewise noteworthy. It clearly demonstrates that the effect of the total extract cannot

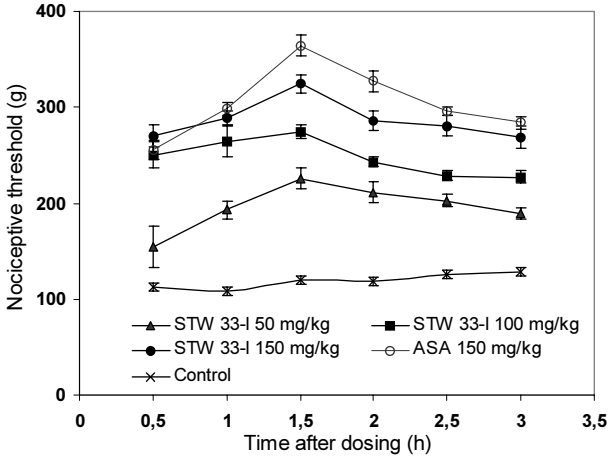

Fig. 3: Time course of the anti-nociceptive effect of willow bark extract on yeast-induced pain of rat paw (according to Randall-Sellitto), $p \leq 0.05$ in Duncan´s test for all time points in all four treated groups [16].

have been caused by the fraction of salicins alone, as salicins constitute only about 25% of the extract. In fact, the testing of the fractions resulted in the finding of the most pronounced effect in fractions A (polyphenols) and E (proanthocyanidins).

Conclusions

In summary, in the *in vitro*-models applied in the screening program the effect of the total extract could essentially be attributed to the fractions of polyphenols, flavonoids and proanthocyanidins, whereas the contribution of salicin derivatives was regularly found to be only minor. However, it has to be taken into account that a metabolic activation of the salicins cannot be expected to take place in the *in vitro* test models. Still, the willow bark extract STW 33-I was comparatively as potent as the same dose of ASA, both in terms of strength and rapidity of onset of effect.

With a content of total salicin of 23–26%, 100 mg STW 33-I contain approximately 25 mg of total salicin derivatives. Thus, the results from *in vivo* testing clearly suggest that the overall effect cannot exclusively be explained through the fraction of salicins. Other fractions of the total extract distinctly contribute to the efficacy and practical applicability of aqueous willow bark extract observed in clinical trials [7, 17].

With polyphenols (e. g. proanthocyanidins) and flavonoids identified as fractions contributing to the efficacy of willow bark, future research should be directed towards the identification of the constituents or combination of constituents mainly responsible for the observed effects. Polyphenols such as flavonoids and proanthocyanidins also seem to contribute to effects of willow bark hitherto not used therapeutically, such as antiproliferative and apoptosis-inducing activity in cancer cells [18].

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