

# Investigation of the Bioavailability of Hypericin, Pseudohypericin, Hyperforin and the Flavonoids Quercetin and Isorhamnetin Following Single and Multiple Oral Dosing of a Hypericum Extract Containing Tablet

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## Summary

The objective of these two open phase I clinical trials was the investigation of the bioavailability of five constituents from a hypericum extract containing tablet, which are discussed as the components contributing to the antidepressant action. Each trial included 18 healthy male volunteers who received the test preparation, containing 612 mg dry extract of St John's wort (STW-3, Laif<sup>®</sup> 600), either as a single oral dose or as a multiple once daily dose over a period of 14 days. Concentration/time curves were determined for hypericin, pseudohypericin, hyperforin, the flavonoid aglycone quercetin, and its methylated form isorhamnetin for 48 h after single dosing and for 24 h on day 14 at the end of 2 weeks of continuous daily dosing. After single dose intake, the key pharmacokinetic parameters were determined as follows: hypericin: area under the curve ( $AUC_{(0-\infty)}$ ) = 75.96 h · ng/ml, maximum plasma concentration ( $C_{max}$ ) = 3.14 ng/ml, time to reach  $C_{max}$  ( $t_{max}$ ) = 8.1 h, and elimination half-life ( $t_{1/2}$ ) = 23.76 h; pseudohypericin:  $AUC_{(0-\infty)}$  = 93.03 h · ng/ml,  $C_{max}$  = 8.50 ng/ml,  $t_{max}$  = 3.0 h,  $t_{1/2}$  = 25.39 h; hyperforin:  $AUC_{(0-\infty)}$  = 1009.0 h · ng/ml,  $C_{max}$  = 83.5 ng/ml,  $t_{max}$  = 4.4 h,  $t_{1/2}$  = 19.64 h. Quercetin and isorhamnetin showed two

peaks of maximum plasma concentration separated by about 4 h. Quercetin:  $AUC_{(0-\infty)}$  = 318,7 h · ng/ml,  $C_{max}$  (1) = 47.7 ng/ml,  $t_{max}$  (1) = 1.17 h,  $C_{max}$  (2) = 43.8 ng/ml,  $t_{max}$  (2) = 5.47 h,  $t_{1/2}$  = 4.16 h; isorhamnetin:  $AUC_{(0-\infty)}$  = 98.0 h · ng/ml,  $C_{max}$  (1) = 7.6 ng/ml,  $t_{max}$  (1) = 1.53 h,  $C_{max}$  (2) = 9.0 ng/ml,  $t_{max}$  (2) = 6.42 h,  $t_{1/2}$  = 4.45 h. Under steady state conditions reached during multiple dose administration similar results were obtained. Further pharmacokinetic characteristics calculated from the obtained data were the mean residence time (MRT), the lag-time, the peak-trough fluctuation (PTF), the lowest observed plasma concentration ( $C_{min}$ ), and the average plasma concentration ( $C_{av}$ ). The data obtained for hypericin, pseudohypericin and hyperforin generally corresponded well with values previously published, with some deviations observed for the extent of absorption of hypericin and the time course of absorption and elimination of hyperforin. The kinetic characteristics of the hypericum flavonoids are reported here for the first time. The trial preparation was well tolerated.

## Key words

- Antidepressant
- Flavonoids
- Hyperforin
- Hypericin
- *Hypericum perforatum*
- Laif<sup>®</sup> 600
- STW-3, constituents, pharmacokinetics

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## Zusammenfassung

Untersuchung der Bioverfügbarkeit von Hypericin, Pseudohypericin, Hyperforin und den Flavonoiden Quercetin und Isorhamnetin nach einmaliger und wiederholter oraler Gabe einer Hypericum-Extrakt enthaltenden Tablette

Das Ziel dieser beiden offenen klinischen Studien der Phase I war die Untersuchung der Bioverfügbarkeit von fünf Bestandteilen einer Hypericum-Extrakt enthaltenden Tablette, die als Komponenten der antidepressiven Wirkung diskutiert werden. An jeder Studie nahmen 18 gesunde männliche Probanden teil, die das Testpräparat mit 612 mg Johanniskraut-Trockenextrakt (STW-3, Laif® 600) entweder als einzelne orale Dosis oder mehrfach als einmal tägliche Gabe über einen Zeitraum von 14 Tagen erhielten. Für Hypericin, Pseudohypericin, Hyperforin, das Flavonoid-Aglykon Quercetin und dessen Methylderivat Isorhamnetin wurden die Konzentrations-Zeit-Verläufe über 48 h nach der Einmalgabe sowie über 24 h am Tag 14 am Ende der zweiwöchigen kontinuierlichen täglichen Einnahme bestimmt. Nach der Einmalgabe wurden für die wichtigsten pharmakokinetischen Parameter folgende Werte bestimmt: Hypericin: Fläche unter der

Kurve ( $AUC_{(0-\infty)}$ ) = 75.96 h · ng/ml, maximale Plasmakonzentration ( $C_{max}$ ) = 3.14 ng/ml, Zeit bis zum Erreichen von  $C_{max}$  ( $t_{max}$ ) = 8.1 h und Eliminationshalbwertszeit ( $t_{1/2}$ ) = 23.76 h; Pseudohypericin:  $AUC_{(0-\infty)}$  = 93.03 h · ng/ml,  $C_{max}$  = 8.50 ng/ml,  $t_{max}$  = 3.0 h und  $t_{1/2}$  = 25.39 h; Hyperforin:  $AUC_{(0-\infty)}$  = 1009.0 h · ng/ml,  $C_{max}$  = 83.5 ng/ml,  $t_{max}$  = 4.4 h und  $t_{1/2}$  = 19.64 h. Quercetin und Isorhamnetin wiesen jeweils zwei Peaks maximaler Plasmakonzentration auf, die um etwa 4 h voneinander getrennt auftraten. Quercetin:  $AUC_{(0-\infty)}$  = 318,7 h · ng/ml,  $C_{max}$  (1) = 47.7 ng/ml,  $t_{max}$  (1) = 1.17 h,  $C_{max}$  (2) = 43.8 ng/ml,  $t_{max}$  (2) = 5.47 h und  $t_{1/2}$  = 4.16 h; Isorhamnetin:  $AUC_{(0-\infty)}$  = 98.0 h · ng/ml,  $C_{max}$  (1) = 7.6 ng/ml,  $t_{max}$  (1) = 1.53 h,  $C_{max}$  (2) = 9.0 ng/ml,  $t_{max}$  (2) = 6.42 h und  $t_{1/2}$  = 4.45 h. Unter Steady-State-Bedingungen nach wiederholter Verabreichung wurden ähnliche Ergebnisse erzielt. Weitere pharmakokinetische Kenngrößen, die aus den ermittelten Daten errechnet wurden, waren die mittlere Verweildauer (MRT), die Verzögerungszeit, die Peak-Trough Fluktuation (PTF), die minimale Plasmakonzentration ( $C_{min}$ ) und die durchschnittliche Plasmakonzentration ( $C_{av}$ ). Die für Hypericin, Pseudohypericin und Hyperforin

ermittelten Werte stimmten im Allgemeinen gut mit den bisher publizierten überein, wenn auch Abweichungen zu verzeichnen waren bezüglich des Ausmaßes der Absorption von Hypericin sowie hinsichtlich des zeitlichen Verlaufs der Absorption bzw. der Eliminierung von Hyperforin. Die kinetischen Kenngrößen der Flavonoide werden hier zum ersten Mal berichtet. Das Testpräparat wies eine gute Verträglichkeit auf.

## 1. Introduction

The aerial parts of the perennial herb St John's Wort (*Hypericum perforatum*) have been used over centuries in traditional medicine to cure conditions as diverse as gallbladder diseases, gastritis, bronchitis and asthma, diarrhea, rheumatism and gout, nocturnal incontinence, the infestation with worms, and wound healing. Most prominent, however, are its antidepressant properties, which have gained this herbal remedy an important role in modern medicine for the treatment of depressive states and somatoformic disturbances including symptoms such as restlessness, anxiety and irritability. Its clinical efficacy in comparison to placebo and standard synthetic antidepressants has been confirmed in a large array of clinical trials, conducted in compliance with modern GCP standards [1]. Lacking the cardiac and anticholinergic side-effects commonly observed with synthetic antidepressant medications, St John's wort can generally be regarded as a safe treatment option. However, like many other medicines, and also some foods, it influences the cytochrome P450 system, particularly the CYP 3A4 isoenzyme, and can therefore alter the metabolism of concomitantly administered drugs [2, 3].

Preparations from St John's wort are available in a wide variety differing in the kind of formulation used as well as in their dosages. Up to now only the antidepressant efficacy of ethanolic or methanolic herb extracts has been repeatedly confirmed in controlled clinical trials. It has not yet been conclusively elucidated which individual constituents of the extract are responsible for the psychovegetative effects, and in which way. The European Pharmacopoeia defines the naphthodianthrone hypericin – together with its 2'-hydroxymethyl derivative pseudohypericin – as the main substance, for which standardization of extracts is required [4]. The phloroglucinol derivative hyperforin, which is structurally related to the compounds humulon and lupulon from hops, has long been considered too unstable to significantly contribute to the pharmacological action of hypericum extracts. In the meantime, however, this hypothesis was disproved by the detection of intact hyperforin in human blood for as long as 72 h after drug intake. In vitro and animal tests have indeed shown some antidepressant activities of hyperforin [5, 6]. Very recently, such activities have also been detected for the flavonoid drug ingredients [5, 7], the most prominent of which are the quercetin glycosides hyperoside, quercitrin, isoquercitrin and miquelianin.

Pharmacokinetic investigations in humans have been reported for hypericin and pseudohypericin from several clinical trials involving single drug intake of different dosages as well as continuous administration three times a day over periods of 1–2 weeks [8–12]. Pharmacokinetic data for hyperforin have been published from one study, which also investigated both single and multiple drug administration [13]. Pharmacokinetic investigations of flavonoids have not been published for hypericum extracts yet. Generally, data for the pharmacokinetics of quercetin and its glycosides are complex and contradictory [14].

Therefore, two phase I clinical trials have been performed to investigate oral bioavailability of five constituents of a hypericum extract containing Tablet and to obtain basic pharmacokinetic data for hypericin, pseudohypericin, hyperforin and the flavonoid aglycone quercetin and its methylated form isorhamnetin after single dose administration and after multiple once daily intake on 14 continuous days.

## 2. Subjects, materials and methods

### 2.1. General

The two open phase I clinical trials were conducted at LAFAA Laboratory for Contract Research in Clinical Pharmacology and Biopharmaceutical Analytics GmbH, Bad Schwartau, Germany, in August 2001 (single dose) and November/December 2001 (multiple dose), respectively. Trial medication was STW-3<sup>1)</sup>, 1 tablet containing 612 mg dry extract of St John's Wort (*Hypericum perforatum*), drug extract ratio 5–8:1, extraction solvent 50 % (v/v) ethanol. Each tablet contains about 600 µg hypericin, 1200 µg pseudohypericin, 13.5 mg hyperforin, and 73.2 mg flavonoids (ratio ≈ 1:2:22.5:122). Both trials were performed in compliance with the European recommendations of Good Clinical Practice guidelines, ICH-Guidelines, the declaration of Helsinki, national regulatory requirements and approved by a local ethics committee. In both trials, the pharmacokinetic characteristics for hypericin, pseudohypericin, hyperforin, quercetin and isorhamnetin were calculated by means of the biostatistics program BIOQ V3 (Byk Gulden Pharmaceuticals, Konstanz, Germany).

### 2.2. Subjects

Each trial recruited 18 subjects from a pool of male volunteers of Caucasian origin and in the age range 18–45 years, who were assessed as healthy based on physical examination, medical history, and clinical laboratory tests, who were of normal weight (Broca) ± 20 %, who had not been smoking within at least 2 years prior to the study, and who gave their written informed consent. Exclusion criteria were hypersensitivity against St John's Wort, all serious and acute diseases, chronic alcohol or drug abuse, HIV or hepatitis infection, surgery of the gastrointestinal tract, chronic medication within 4 weeks before the study, or any other medication within 10 days before the study. Participants had to refrain from the consumption of

grapefruits, grapefruit juice, St John's Wort tea, methylxanthine containing beverages and food, and alcohol within 48 h prior to and during the study period.

### 2.3. Study protocol

#### 2.3.1. Single dose study

Subjects were hospitalized from 8:30 p.m. on the day before study day 1 until up to 48 h post drug administration. Fasting conditions were required from 9:00 p.m. on the day before study begin until 12:00 a.m. on study day 1. The trial medication of 1 tablet containing 612 mg Hypericum extract was taken with 200 ml tap water at 7:00 a.m. on study day 1. Blood samples (2 × 5.6 ml) were taken predose and at the following intervals post drug administration: 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 16, 20, 24, 30, 36, 42, and 48 h. After centrifugation, the plasma samples were kept frozen (–20 °C) until analysis for hypericin/pseudohypericin, hyperforin and quercetin/isorhamnetin. Primary pharmacokinetic characteristic to be calculated from plasma concentration/time data was AUC<sub>(0-∞)</sub> (total area under the plasma concentration/time curve). Secondary pharmacokinetic characteristics were: AUC<sub>(0-tz)</sub> (area under the concentration/time curve from zero to the last time point), C<sub>max</sub> (maximum plasma concentration), t<sub>max</sub> (time to reach maximum plasma concentration), t<sub>1/2</sub> (terminal elimination half-life), MRT (mean residence time), and lag-time (time to attain the first measurable plasma concentration). Tolerability and safety of the trial medication were assessed by monitoring adverse events, vital signs, ECG parameters, clinical laboratory examinations, and urine status.

#### 2.3.2. Multiple dose study

Subjects were ambulatory during days 1 through 13 and advised to take 1 tablet of the trial medication at 7:00 a.m. after breakfast every day. During this period, blood samples were obtained on days 1, 4, 11, 12, and 13 before drug intake in the morning. For profiling, the subjects were hospitalized from 8:30 p.m. on day 13 until up to 24 h following the last drug administration at 7:00 a.m. on study day 14. Fasting conditions were applied from 9:00 p.m. on day 13 until 12:00 a.m. on day 14. Blood samples (2 × 5.6 ml) were taken predose and after 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 10, 12, 16, 20, and 24 h. The plasma samples were stored deep-frozen as described for the single dose study. Primary pharmacokinetic characteristic was AUC<sub>(0-24)</sub> (area under the concentration/time curve during the profiling day 14). Secondary characteristics were PTF (peak-trough fluctuation), C<sub>min</sub> (lowest observed plasma concentration), C<sub>max</sub> (highest observed plasma concentration), C<sub>av</sub> (average plasma concentration), and t<sub>max</sub> (time to reach maximum plasma concentration). Tolerability and safety were monitored as described for the single dose study.

### 2.4. Principles of the analytical methods

#### 2.4.1. Hypericin/pseudohypericin

Hypericin and its 2'-hydroxymethyl derivative pseudohypericin were determined simultaneously. Quantification was achieved after repeated extractions of a mixture consisting of plasma (300 µl of the unknown sample, calibration standard or quality control sample), dimethylsulfoxide, acetonitrile, 2-butoxyethanol and dansylamide as internal standard. The repeated extractions were performed with ethyl acetate at 37 °C for 15 min each. The evaporated extracts were reconstituted in a meth-

<sup>1)</sup> Laif<sup>®</sup>; manufacturer: Steigerwald Arzneimittelwerk GmbH, Darmstadt (Germany).

anol/tetrahydrofurane/phosphate buffer solution (pH 4). Chromatographic separation and detection was carried out by high-performance liquid chromatography using a reversed-phase system and fluorescence detection with excitation at 315 nm and emission at 598 nm.

For hypericin and pseudohypericin the intra-batch and the inter-batch precision and accuracy of the method did not exceed the limits accepted by the FDA [15] (precision:  $CV \leq 15\%$ , at LOQ:  $CV \leq 20\%$ ; accuracy  $\leq \pm 15\%$ , at LOQ: accuracy  $\leq \pm 20\%$ ).

### 2.4.2. Hyperforin

Hyperforin was analyzed after extraction of the plasma (1000  $\mu$ l of the unknown sample, calibration standard or quality control sample) with an ethyl acetate/isooctane/acetonitrile mixture. The evaporated extracts were reconstituted in an acetonitrile/water/methanol mixture. Chromatographic separation and quantification was achieved by HPLC using a reversed phase system, UV-detection at 273 nm and external standardization. All operations must be carried out under protection from direct light.

The intra-batch and the inter-batch precision was characterized by coefficients of variation never exceeding 4.6%. The intra-batch and the inter-batch accuracy did not exceed  $\pm 7.3\%$ .

### 2.4.3. Quercetin/isorhamnetin

Quercetin and its methylated derivative isorhamnetin were analyzed simultaneously after enzymatic hydrolysis of the plasma (300  $\mu$ l of the unknown sample, calibration standard or quality control sample) with purified  $\beta$ -glucuronidase solution for 2 h at 37 °C. Naringenin was used as internal standard. Borate buffered (pH 8.1) hydrolysed samples were extracted with ethyl acetate. The evaporated extracts were reconstituted in acidified acetonitrile/methanol. High-performance liquid chromatographic separation worked with a reversed phase system and UV-detection at 360 nm.

For quercetin and isorhamnetin the intra-batch and the inter-batch precision and accuracy of the method did not exceed the limits accepted by the FDA [15] (precision:  $CV \leq 15\%$ , at LOQ:  $CV \leq 20\%$ ; accuracy  $\leq \pm 15\%$ , at LOQ: accuracy  $\leq \pm 20\%$ ).

## 3. Results

Demographic data of the trial participants are summarized in Table 1. All subjects completed the study according to the protocol. The concentration/time curves for hypericin, pseudohypericin, hyperforin, quercetin and isorhamnetin are shown in Fig. 1A (single dose) and Fig. 1B (multiple dose). The pharmacokinetic data obtained after single and multiple dosing are summarized in Tables 2 and 3, respectively.

### 3.1. Hypericin

After single dose administration, the mean value curve of plasma hypericin concentrations was characterized by a slow increase followed by a very slow decline. Of all investigated compounds, hypericin showed the lowest mean value of  $C_{max}$  (3.1 ng/ml) but by far the highest

**Table 1: Demographic characteristics of study participants.**

	Single dose study	Multiple dose study
N (gender)	18 (male)	18 (male)
Median age, years (range)	28 (20–43)	27 (23–37)
Median weight, kg (range)	80 (72–90)	79 (71–90)
Median height, cm (range)	180 (170–191)	180 (173–189)

one for  $t_{max}$  (8.1 h), as well as a remarkably long mean lag-time of 2.3 h before it could be measured. The mean elimination half-life was 23.8 h, with a mean residence time of 34.7 h. The mean  $AUC_{(0-\infty)}$  value, as a measure of the extent of absorption, was 75.96 h · ng/ml.

In the multiple dosing study, trough levels were determined on study days 1, 4, 11, 12, 13, and 14 in the morning prior to drug intake. The results showed that steady state was reached before the profiling day 14 (Fig. 2). The mean plasma concentration/time curve closely resembled the one after single dose administration, as did the pharmacokinetic data. Hypericin, again, had the highest mean  $t_{max}$  value of all substances (6.8 h) which was nevertheless shorter than after single dose intake. The mean maximum plasma concentration was slightly increased (4.43 ng/ml) but still the lowest of all. With a mean  $C_{min}$  value of 2.18 ng/ml, hypericin also showed the lowest mean peak-trough fluctuation (70%). The mean  $AUC_{(0-24h)}$  value of 76.5 h · ng/ml was very similar to the mean  $AUC_{(0-\infty)}$  value in the single dose study.

### 3.2. Pseudohypericin

After single dose intake, the mean concentration/time curve of pseudohypericin was characterized by a rapid increase followed by an exponential decline. The mean maximum concentration of 8.50 ng/ml was reached after 3.0 h. Only a short lag-time of 0.7 h was observed. The mean values of elimination half-life and mean residence time were 25.4 h and 28.2 h, respectively. Mean  $AUC_{(0-\infty)}$  was determined as 93.03 h · ng/ml.

Multiple dosing resulted in a very similar pharmacokinetic profile with mean  $C_{max} = 8.51$  ng/ml and  $t_{max} = 3.3$  h. The mean  $C_{min}$  value of 1.32 ng/ml was the lowest of all substances; the mean peak-trough fluctuation was 199%. The mean  $AUC_{(0-24h)}$  value (87.63 h · ng/ml) was within the same order of magnitude as the mean  $AUC_{(0-\infty)}$  in the single dose study.

### 3.3. Hyperforin

Out of all investigated compounds, hyperforin showed by far the highest  $C_{max}$  values. In the single dose study, a mean value of 83.5 ng/ml was reached. The mean  $t_{max}$  value of 4.4 h was in between the mean values observed for the hypericins, as was the mean lag-time of 1.26 h. Elimination was faster than for the hypericins with mean  $t_{1/2} = 19.64$  h and mean MRT = 21.77 h. In accord-

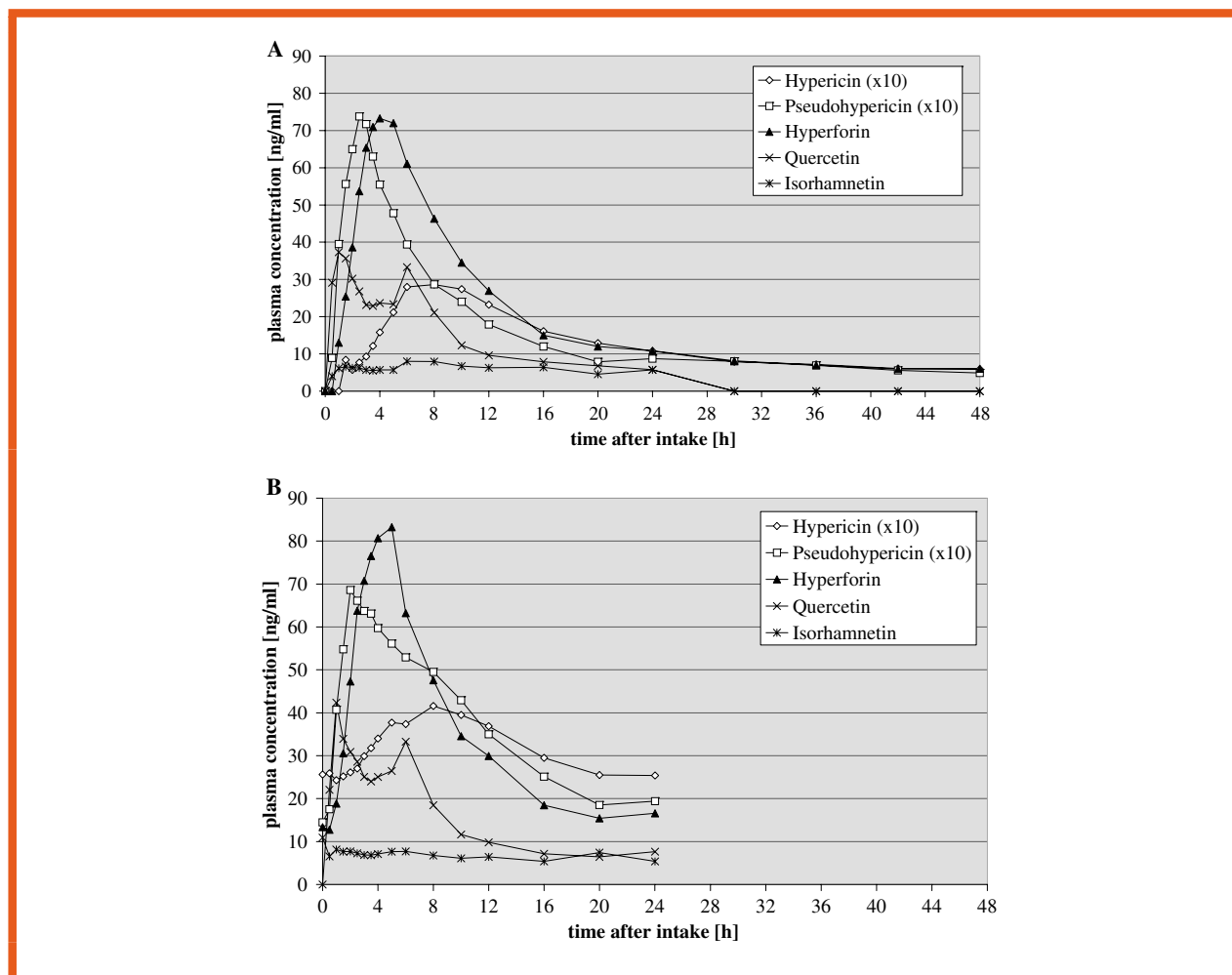


Fig. 1: Pharmacokinetics of ingredients of Hypericum extract in plasma after intake of Hypericum extract STW-3 once daily. (A) After single dosing, (B) after multiple dosing (14 days). Data points are expressed as arithmetic means. Note that concentrations of hypericin and pseudohypericin are multiplied by 10.

Table 2: Pharmacokinetic parameters after single dose administration. Values are given as means  $\pm$  SD (range).

	Hypericin	Pseudohypericin	Hyperforin	Quercetin	Isorhamnetin
AUC <sub>(0-∞)</sub> [h · ng/ml]	75.96 $\pm$ 23.52 (43.86–128.30)	93.03 $\pm$ 29.40 (60.67–163.52)	1009.0 $\pm$ 203.4 (687.1–1430.6)	318.70 $\pm$ 130.82 (148.49–558.18)	97.99 $\pm$ 107.28 (27.11–478.54)
AUC <sub>(0-tz)</sub> [h · ng/ml]	57.21 $\pm$ 21.50 (30.76–107.34)	74.43 $\pm$ 23.61 (41.64–125.30)	862.0 $\pm$ 172.2 (596.6–1158.9)	283.43 $\pm$ 120.66 (136.31–510.88)	77.85 $\pm$ 95.87 (17.23–415.60)
C <sub>max</sub> [ng/ml]	3.14 $\pm$ 1.57 (1.36–7.58)	8.50 $\pm$ 4.35 (3.93–20.60)	83.5 $\pm$ 27.8 (45.8–156.6)	1) 47.7 $\pm$ 22.5 (12.7–97.6) 2) 43.8 $\pm$ 23.1 (12.7–104.0)	1) 7.6 $\pm$ 2.8 (3.8–13.5) 2) 9.0 $\pm$ 6.1 (3.0–24.3)
t <sub>max</sub> [h]	8.1 $\pm$ 1.8 (5.0–10.0)	3.0 $\pm$ 1.4 (1.5–6.0)	4.4 $\pm$ 1.5 (2.5–8.0)	1) 1.17 $\pm$ 0.52 (0.50–2.50) 2) 5.47 $\pm$ 1.38 (3.00–8.00)	1) 1.53 $\pm$ 0.67 (0.75–2.50) 2) 6.42 $\pm$ 1.90 (3.50–10.00)
t <sub>1/2</sub> [h]	23.76 $\pm$ 5.46 (16.06–35.24)	25.39 $\pm$ 10.18 (14.12–54.18)	19.64 $\pm$ 6.35 (7.52–36.09)	4.16 $\pm$ 2.97 (1.76–11.86)	4.45 $\pm$ 3.27 (1.48–16.50)
MRT [h]	34.74 $\pm$ 8.86 (21.24–54.34)	28.17 $\pm$ 11.99 (14.52–62.68)	21.77 $\pm$ 5.69 (8.03–29.83)	7.44 $\pm$ 3.15 (3.62–14.94)	8.86 $\pm$ 4.44 (4.39–24.13)
lag-time [h]	2.33 $\pm$ 0.86 (1.00–5.00)	0.69 $\pm$ 0.27 (0.50–1.50)	1.26 $\pm$ 0.67 (0.75–3.50)	0.58 $\pm$ 0.21 (0.00–1.00)	0.68 $\pm$ 0.45 (0.00–2.00)

1): first maximum, 2): second maximum.



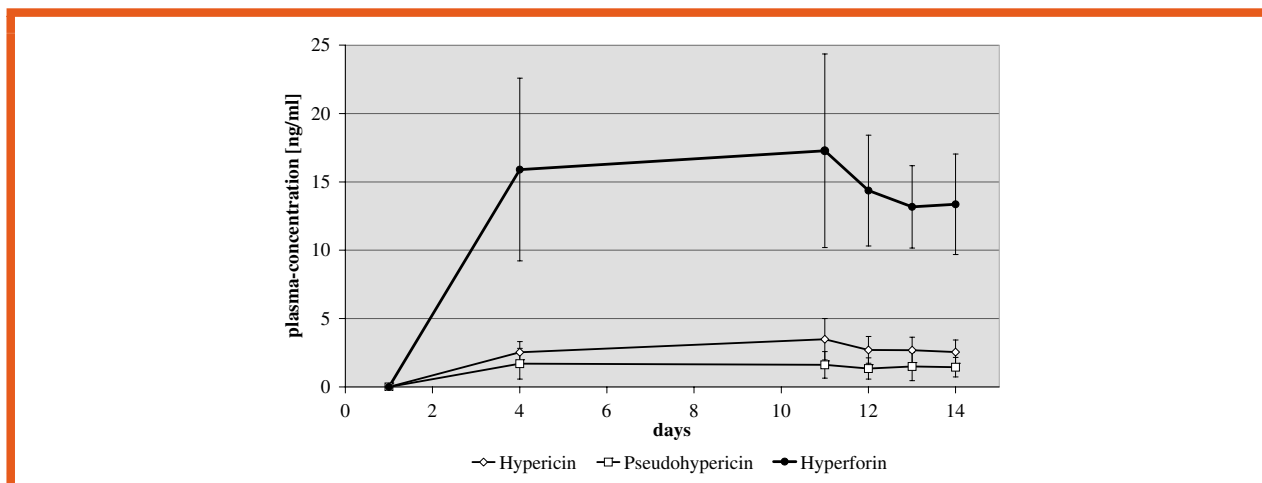


Fig. 2: Trough plasma concentrations of the hypericins and hyperforin during 14 days of multiple dosing. Data points are expressed as arithmetic means ( $\pm$  SD).

ance with the high maximum concentration, the overall absorption of hyperforin was also very high with a mean  $AUC_{(0-\infty)}$  of 1009.0 h · ng/ml.

The mean concentration/time curve after multiple dosing was again very similar. With 4.3 h, the mean  $t_{max}$  value was almost identical. The mean  $C_{max}$  of 97.4 ng/ml was even higher than after single dosing; the mean  $C_{min}$  was 12.4 ng/ml, and the mean peak-trough fluctuation of 246 % was the highest of all substances. The mean  $AUC_{(0-24\ h)}$  value (825.5 h · ng/ml) was somewhat below the mean  $AUC_{(0-\infty)}$  in the single dose study.

### 3.4. Flavonoids

Following single and multiple dose administration, the flavonoid concentrations in the blood increased rapidly, to decline after a first maximum and rose again to give a second maximum. Thus, two  $C_{max}$  and  $t_{max}$  values could be observed.

#### 3.4.1. Quercetin

Following single dose administration, the mean quercetin concentration in the blood fell continuously after the second maximum. 20 h post dosing the most individual quercetin concentrations were below the limit of quantitation, only 2 of the 18 were measurable at 24 h p.a. The first mean  $C_{max}$  value [ $C_{max} (1) = 47.7$  ng/ml] was reached very early [ $t_{max} (1) = 1.2$  h] and the second one [ $C_{max} (2) = 43.8$  ng/ml] after 5.5 h. The mean lag-time was low (0.6 h). The mean  $t_{1/2}$  value (4.2 h) and the MRT (7.44 h) were short compared to the other ingredients. The  $AUC_{(0-\infty)}$  value of 318.70 h ng/ml was considerably higher than that of the other substances, with the exception of hyperforin.

During multiple dosing, no trough levels could be determined for the flavonoids as the majority of the trough concentrations were below the limits of determination. As in the single dose study, quercetin showed

Table 3: Pharmacokinetic parameters after multiple dose administration, obtained on day 14. Values are given as means  $\pm$  SD (range).

	Hypericin	Pseudohypericin	Hyperforin	Quercetin	Isorhamnetin
$AUC_{(0-24)}$ [h · ng/ml]	76.50 $\pm$ 24.74 (48.26–134.55)	87.63 $\pm$ 30.33 (27.55–141.69)	825.5 $\pm$ 176.4 (560.8–1151.4)	272.34 $\pm$ 157.32 (18.45–582.78)	84.96 $\pm$ 106.89 (9.85–478.20)
PTF [%]	70.45 $\pm$ 15.78 (39.93–97.61)	199.07 $\pm$ 47.49 (141.26–306.72)	246.2 $\pm$ 67.5 (131.7–335.6)	n.a.	n.a.
$C_{min}$ [ng/ml]	2.18 $\pm$ 0.72 (1.47–4.30)	1.32 $\pm$ 0.67 (0.25–2.34)	12.4 $\pm$ 3.4 (5.8–18.2)	n.a.	n.a.
$C_{max}$ [ng/ml]	4.43 $\pm$ 1.49 (2.90–8.21)	8.51 $\pm$ 3.29 (2.49–15.61)	97.4 $\pm$ 30.0 (46.8–146.1)	1) 45.1 $\pm$ 21.1 (12.4–88.0) 2) 59.1 $\pm$ 27.2 (21.9–126.8)	1) 8.7 $\pm$ 5.5 (4.2–27.8) 2) 9.8 $\pm$ 8.1 (3.3–38.7)
$C_{av}$ [ng/ml]	3.19 $\pm$ 1.03 (2.01–5.61)	3.65 $\pm$ 1.26 (1.15–5.90)	34.4 $\pm$ 7.3 (23.4–48.0)	11.3 $\pm$ 6.6 (0.8–24.3)	3.5 $\pm$ 4.5 (0.4–19.9)
$t_{max}$ [h]	6.8 $\pm$ 1.8 (4.0–10.0)	3.3 $\pm$ 1.7 (1.0–7.5)	4.3 $\pm$ 1.0 (2.5–5.5)	1) 1.33 $\pm$ 0.59 (1.00–2.50) 2) 5.33 $\pm$ 1.25 (3.00–7.50)	1) 1.47 $\pm$ 0.67 (1.00–3.00) 2) 5.82 $\pm$ 1.19 (3.00–7.50)

n.a.: not available. 1): first maximum, 2): second maximum.

two distinct concentration maxima: the first mean value [ $C_{\max}(1) = 45.1$  ng/ml] at  $t_{\max}(1) = 1.3$  h, the second one [ $C_{\max}(2) = 59.1$  ng/ml] at  $t_{\max}(2) = 5.3$  h. Especially the second  $C_{\max}$  mean value was increased compared to the single dose study. The mean  $AUC_{(0-24\text{ h})}$  value of 272.34 h ng/ml was again between the one of hyperforin and the ones of the other substances.

### 3.4.2. Isorhamnetin

The shape of the mean isorhamnetin curve was similar to the one of quercetin. However, the concentration level was low compared with the mean quercetin curve. After single dose administration two maximum peaks were discernible: the mean  $C_{\max}(1) = 7.6$  ng/ml at  $t_{\max}(1) = 1.5$  h and  $C_{\max}(2) = 9.0$  ng/ml at  $t_{\max}(2) = 6.4$  h. Comparable to quercetin the most individual isorhamnetin concentrations fell below the limits of quantitation within 24 h, only 1 of the 18 was detectable over the total period of 48 h. Similar to quercetin, the lag-time (0.7 h) and  $t_{1/2}$  (4.5 h) of isorhamnetin were short, whereas  $t_{\max}(2) = 6.4$  h and  $MRT = 8.9$  h were higher than the respective values of quercetin.

Multiple dosing, too, resulted in two concentration peaks –  $C_{\max}(1) = 8.7$  ng/ml at  $t_{\max}(1) = 1.5$  h and  $C_{\max}(2) = 9.8$  ng/ml at  $t_{\max}(2) = 5.8$  h – but these were less distinct because the concentrations remained in the same range over a long period of time. The mean  $AUC_{(0-24\text{ h})}$  value of 84.96 h ng/ml lay within the same order of magnitude as the ones of the hypericins.

### 3.5. Tolerability

In the single dose trial, no adverse events occurred. In the multiple dose trial, one participant experienced a mild headache on study day 14. No clinically significant changes in the vital signs or the laboratory values were observed in any of the subjects.

## 4. Discussion

Pharmacokinetic properties of hypericins and hyperforin are investigated in several publications. But, till now there have not been published an investigation of the pharmacokinetics of different constituents of a hypericum extract in one clinical trial. The pharmacokinetic data obtained from these two studies represent the most complete pharmacokinetic profile published for a hypericum preparation to date. It considers all components of the extract that have been discussed to contribute to the antidepressant action of St John's wort, confirming the data known for the hypericins and hyperforin and including flavonoid aglycones quercetin and isorhamnetin.

The shape of the concentration/time curves are in approximate accordance with those published earlier for the hypericins [8, 9, 10] and for hyperforin [13]. As for the pharmacokinetic data, the absorption of hypericin judged by both  $C_{\max}$  and  $AUC_{(0-\infty)}$  appears to be

lower in these studies compared with others while the time course gave very similar values for lag time,  $t_{\max}$  and  $t_{1/2}$ . The results obtained for pseudohypericin in these studies in general are in good agreement with previously published results. The kinetic variables of hyperforin, on the other hand, differed considerably from those reported before [13]:  $t_{\max}$  was slightly prolonged, after single dose administration (4.4 h compared to 2.8–3.6 h) as well as during continuous daily intake (4.3 h compared to 3.0–3.1 h), but the most pronounced deviation was noted for the elimination half-life and the mean residence time, both of which were substantially increased compared to the literature values with  $t_{1/2} = 19.6$  h vs. 8.5–9.7 h and  $MRT = 21.8$  h vs. 11–12.6 h. Interestingly enough Biber et al. [13] reported, that after multiple-dose administration at day 8  $t_{1/2}$  was significantly prolonged compared to day 1, up to maximal  $16 \pm 3.5$  h. An explanation for this cannot readily be found at the present.

The relation of the plasma concentrations observed for these substances reflect their respective quantities present in the drug preparation. Each tablet contains hypericin, pseudohypericin and hyperforin in a ratio of about 1:2:22. The  $C_{\max}$  values of hypericin, pseudohypericin and hyperforin after single and after multiple dosing are indeed in a similar ratio.

No pharmacokinetic data have been published for the flavonoid components of hypericum preparations before. The concentration/time curves of quercetin and isorhamnetin observed in this study differ from those of the other substances as far as they show two peaks of maximum concentration instead of only one. This finding confirmed with some concentration/time curves of quercetin published after oral intake of quercetin aglycone or rutin [14, 16]. However, both quercetin and isorhamnetin are not present in the drug preparation as such but in the form of glycosides with various sugar moieties. Their appearance in the blood depends on the enzymatic hydrolysis of those glycosides in the small intestine. The rate of hydrolysis may well be different for the different compounds which would result in the flavonoid aglycones being released at different times. It has been reported before that the absorption rate of quercetin varies greatly depending on the form in which it is taken up [16, 17].

In conclusion, the results of the two studies demonstrate that for hypericin, pseudohypericin and hyperforin a once daily multiple oral administration is sufficient to reach steady state conditions without accumulation of the ingredients. Furthermore, over a period of 14 days medication was well tolerated by all subjects.

## 5. References

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