

Chapter 4

St. John's Wort: Quality Issues and Active Compounds

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St. John's wort (*Hypericum perforatum* L.) (SJW) contains numerous compounds with documented biological activity. Constituents that have stimulated the most interest include the naphthodianthrone, hypericin and pseudohypericin; a broad range of flavonoids, including rutin, quercetin, quercitrin, miquelianin, amentoflavone, and hyperoside; and the phloroglucinols hyperforin and adhyperforin. Although there are some contradictions, most data suggest that several groups of active compounds are responsible for the antidepressant efficacy of the plant extract. Thus, according to the current state of scientific knowledge, the total extract has to be considered as the active substance.

Data on efficacy and quality of SJW extracts have to be taken into consideration. Owing to the fast-growing SJW market in the United States, more and more SJW products (herb and extracts) are sold at varying levels of quality. Considerable differences exist in the composition of biologically active constituents among various commercially available preparations of SJW. Furthermore, the documented characterization of SJW preparations in published randomized controlled trials has been less than adequate. Future scientific and clinical publications about the efficacy of SJW would benefit from a full pharmaceutical and phytochemical description of the extract.

SJW is used for the treatment of mild to moderate depression. The antidepressant efficacy of SJW extracts has been confirmed in numerous clinical studies and was assessed in meta-analyses (1, 2). The pharmacological actions of SJW have likewise been extensively reviewed (3–6). Reports about the mechanism of antidepressant action of SJW extracts and their constituents both in vivo and in vitro have also been published. Antidepressant activity was reported for the phloroglucinol derivative hyperforin (for a review, see [7]), for the naphthodianthrone hypericin and pseudohypericin (8–10), and for several flavonoids (11–14). The role and the mechanisms of these different compounds are still a matter of debate. However, based on recent results, it appears that the prevailing simplistic view of one plant → one active compound → one mechanism of action is incorrect. It is more likely that the multiple bioactive compounds contribute to the antidepressant activity of the crude plant extract in a complex manner. This review focuses on the present knowledge about the active constituents of SJW and their contribution to antidepressant activity.

SJW CONSTITUENTS IN PRECLINICAL STUDIES

Flavonoids and Biflavonoids

Flavonolglycosides based on the aglycone quercetin represent up to 4% of the extract and form the largest group of secondary metabolites in *H. perforatum*. The major components are the flavonolglycosides rutin, hyperoside, isoquercitrin, quercitrin, and miquelianin and in smaller amounts the aglycon quercetin (Figure 4.1). Other flavonoid aglycones such as dihydroquercetin, luteolin, kaempferol, and myricetin are reported in the literature (15), but clear-cut data about their characterization are missing or the compounds occur in the plant only in trace amounts. When compared to other active ingredients, the flavonol glycosides show release rates from the powdered drug material and extract between 70% and 100% (16).

MAO Inhibition of Flavonoids

Several early in vitro experiments with SJW focused on pathways that alter monoamine neurotransmission in the central nervous system. Initial reports suggested that inhibition of monoamine oxidase (MAO)—the enzyme that is responsible for the catabolism of biogenic amines—is the main mechanism of antidepressant action of SJW extract. Studies performed by Sparenberg et al. showed most activity for the flavonoid aglycones—quercetin, kaempferol, and luteolin—whereas the glycosides were less active (15). However, the concentration in *Hypericum* of these substances, especially that of the aglycones, is too low to be responsible for the therapeutic efficacy of SJW extract (16).

Together with the ubiquitously existing monomeric flavonoids, the biflavonoids such as I3,II8-biapigenin and amentoflavone have been detected and exclusively occur in the flower part of SJW (17) (Figure 4.2).

Receptor Binding of the Flavonoids and Biflavonoids

Nielsen et al. and Baureithel et al. focused their investigations on the biflavone amentoflavone, which bound to the brain benzodiazepine receptors with an affinity comparable to diazepam (18, 19). However, the flavonoids rutin, hyperoside, and quercitrin did not inhibit benzodiazepine binding up to concentrations of 1 μ M. This result was recently confirmed by Butterweck and colleagues (20). In the same study, amentoflavone had remarkable affinity for the d-opioid receptor subtype in the nanomolar range ($K_i = 36.5$ nM) and significantly

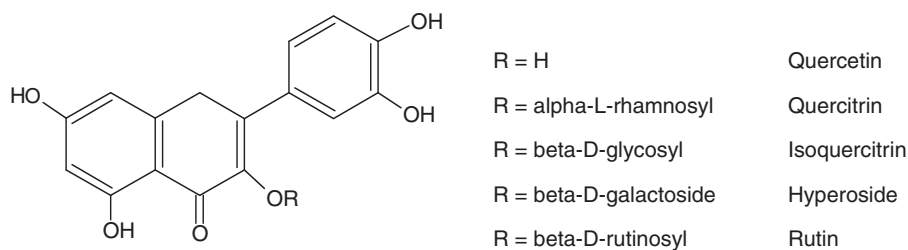


Figure 4.1. Flavonoids from SJW

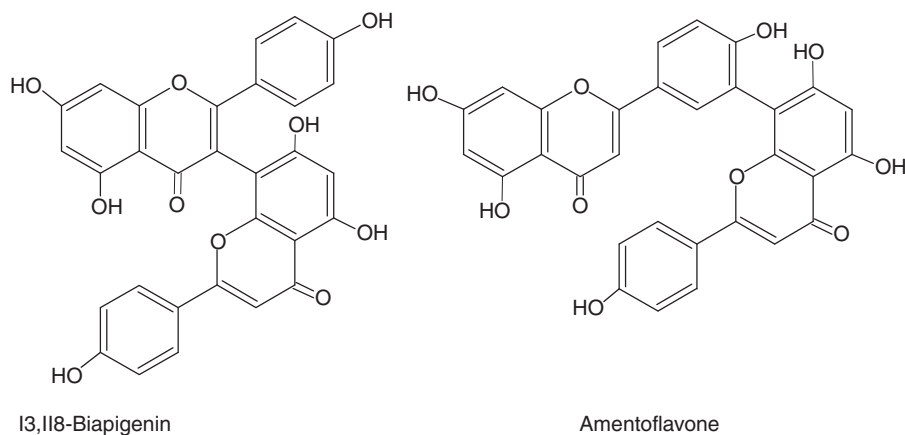


Figure 4.2. Biflavonoids from SJW

inhibited binding at 5-HT_{1D}, 5-HT_{2C}, and dopamine D₃ receptors. Further striking actions were observed for quercetin with an affinity to the D₄ receptor at Ki = 7.8 nM, miquelianin to the α_{2C} receptor at 4 nM, and rutin to the α_{2A} and α_{2C} receptor at 9 nM (20).

In Vivo Tests on Flavonoids and Biflavonoids

Studies show that amentoflavone was able to pass the blood-brain barrier in vitro by passive diffusion (21). The potential antidepressant activity of some SJW flavonoids was confirmed in the forced swimming test, a well-established screening model for antidepressant activity (22). During bioassay-guided fractionation of SJW extract, a fraction was obtained that was mainly characterized by its high content of flavonoids. The fraction significantly reduced immobility time in the forced swimming test, and the effect was comparable to that of imipramine (12). The flavonoid fraction was further purified, and some flavonol glycosides such as hyperoside, quercitrin, isoquercitrin, and miquelianin, as well as the flavone glycoside astilbin, were isolated (16) and tested for activity in the forced swimming test at doses comparable to their amounts present in the crude drug material. Except for quercitrin and astilbin, all flavonoids (hyperoside, isoquercitrin, and miquelianin) were significantly active in the forced swimming test after acute, as well as after repeated, oral treatment.

The data obtained by Butterweck et al. (12) indicate certain flavonol-3-O-glycosides as active constituents of *Hypericum* extract. The activity seems to be bound to the sugar moiety of the aglycone quercetin, in that the glucoside, galactoside, and glucuronide forms are active compounds. It may be that these particular glycosides are absorbed from the intestine, whereas others are not.

Further evidence that flavonoids contribute to the antidepressant activity of SJW was recently provided by Butterweck et al. (11). The authors could show that hyperoside, isoquercitrin, and miquelianin significantly down-regulated plasma adrenocorticotrophic hormone (ACTH) and corticosterone levels after two weeks of daily treatment. The effect was similar to the changes elicited by the prototypic synthetic antidepressant imipramine. Hypersecretion of ACTH and plasma cortisol have been reported in 40% to 50% of patients

suffering from depression (23, 24). Normalization of the hyperactive hypothalamic-pituitary-adrenal (HPA) system occurs during successful antidepressant pharmacotherapy of depressive illness (25). These data clearly show that flavonoids represent a possible major active principle that may in turn contribute to the beneficial effect of SJW extract after oral dosing.

Potential Synergism of Flavonoids and Biflavonoids

It also could be shown that the flavonol-3-O-rutinoside rutin, which was inactive in the forced swimming test when administered orally as a pure compound, seems to play an important role in the SJW extract. Thus the addition of rutin to inactive extracts resulted in a strong pharmacological effect comparable to that of other extracts that contained a sufficient amount of rutin (14). Although the study provides sufficient quantitative data about the amount of flavonoids in the different SJW extracts, unfortunately no quantitative data are given for hypericin and hyperforin. Nevertheless, the experiments show that the pharmacological effects of an extract cannot directly be compared with the effects of single compounds. The authors suggest a modulation of phase I proteins for rutin, which would lead to an increased bioavailability of simultaneously administered active SJW compounds (14). However, this might also be achieved by an increased solubility of hypericin by rutin (see the following section on naphthodianthrones).

In a recent *in vitro* study, it could be demonstrated that miquelianin—besides crossing walls from the small intestine—was able to cross the blood-brain barrier, as well as the blood-cerebrospinal fluid barrier (26). This finding gives further evidence for the assumption that flavonoids are able to reach the central nervous system after oral administration. There might be synergistic or additive effects of single flavonoids, but this needs to be established in future investigations.

Naphthodianthrones

The naphthodianthrones hypericin and pseudohypericin occur in the flowers and leaves of the crude drug material in concentrations of 0.03% to 0.3%, depending on the developmental stage of the plant with significant variation (27) (Figure 4.3). The amount of pseudohypericin in SJW is approximately two to four times higher than that of hypericin (28). In some extracts this ratio may even be 10:1.

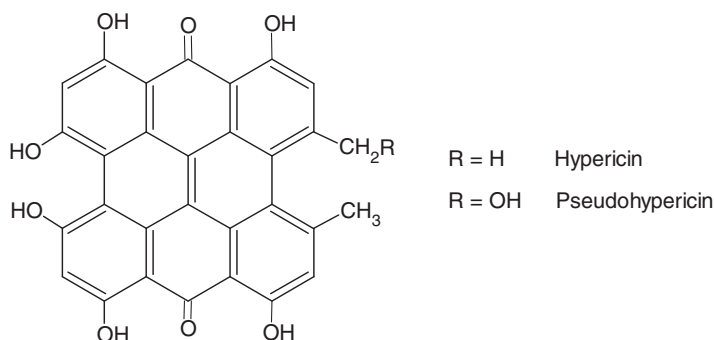


Figure 4.3. Naphthodianthrones from SJW

The naphthodianthrones show a restricted solubility in almost all solvents; the pure compounds, especially hypericin, are almost insoluble in water at ambient temperature. Nevertheless, more than 40% of the naphthodianthrone amount is extractable from the crude drug when preparing a tea with water at 60 to 80 °C (approx. 35% pseudohypericin and 6% hypericin) (29). The increase in solubility suggests the presence of coeffectors in the drug material that modify the solubility of the naphthodianthrones. The potassium salts of hypericin and pseudohypericin have been identified as “soluble” pigments of the *Hypericum* species (30).

Phototoxicity of the Naphthodianthrones

The naphthodianthrones attracted the attention of phytochemists early on because of their intense red color and their phototoxic properties (31, 32). Thus, toxicological concerns have centered on the potential photosensitizing effects of humans ingesting SJW extract, since this is a well-known toxic effect in animals consuming large amounts of the fresh plant material (1–4% of body weight). Calves receiving up to 3 g/kg of SJW herb have demonstrated no photosensitivity (33).

Bernd et al. (34) investigated the phototoxic activity of SJW extract (0.3% hypericin) using cultures of human keratinocytes. The authors cultivated human keratinocytes in the presence of different SJW extract concentrations and irradiated the cells with 150 mJ/cm² UVB, 1 J/cm² UVA, or 3 h with a white light of photon flux density 2.6 mmol m⁻² s⁻¹. The determination of the bromodeoxyuridine incorporation rate showed a concentration- and light-dependent decrease in DNA synthesis with high hypericin concentrations ($\geq 50 \mu\text{g}/\text{ml}$) combined with UVA or visible light radiation. In the case of UVB irradiation, a clear phototoxic cell reaction was not detected. The researchers concluded that phototoxic reactions of the skin would not be expected from the amounts of SJW extract used in the treatment of depression because blood levels of hypericin would be below the phototoxic dose (34). Based on the hypericin content, others have estimated that the amount of a commercial extract of SJW required to increase the risk of phototoxicity is approximately 2 to 4 g/d, or 5 to 10 mg/d of hypericin (35, 36).

Receptor Interactions of the Naphthodianthrones

From a pharmacological standpoint, the hypericins are at present the most interesting compounds of SJW. Inhibition of monoamine oxidase (MAO) was considered as one of the key mechanisms used in conventional therapy for depression. Suzuki et al. (37) were the first to find that micromolar concentrations of hypericin could irreversibly inhibit MAO-A and MAO-B activity in vitro. However, the progress made in preparation and analytical techniques since 1984 has shown that the hypericin used in these experiments was impure and contained at least 20% of other constituents of the extracts—among these, the flavonoids are most noticeable. In fact, the MAO inhibitory effects of hypericin alone could not be confirmed in subsequent studies (38, 39).

The effects of hypericin in various receptor-screening models provide the most contradictory data: Raffa (40) found that hypericin had no affinity for traditional monoamine receptors or for adrenergic, gamma-aminobutyric acid (GABA), adenosine, or benzodiazepine receptors. The naphthodianthrone had modest affinity for muscarinic cholinergic receptors (subtype not measured) and similar affinity to σ receptors. Gobbi et al. (41) showed that hypericin inhibited ligand binding to NPY₁-, NPY₂-, and σ receptors. The authors found that these inhibitory effects were light dependent because they decreased or disappeared when binding assays were carried out in the dark.

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Cott tested hypericin in a battery of 39 *in vitro* receptor assays, and hypericin showed affinity only for the N-methyl D-aspartate (NMDA) receptor ($K_i \sim 1 \mu\text{M}$) (39). These results could not be confirmed by Butterweck et al. (20). In Cott's study, hypericin showed high affinity for the D_3 -dopamine receptor ($K_i = 34.5 \text{ nM}$) and negligible affinities ($K_i > 1000 \text{ nM}$) for nearly all other tested receptors and transporters. Interestingly, the affinity of hypericin for the D_3 -receptor subtype was much higher than that of the atypical antipsychotic clozapine ($K_i = 372.3 \text{ nM}$).

Simmen et al. showed that hypericin had the most potent binding inhibition of all tested constituents to human corticotropin-releasing factor 1 (CRF_1) receptor with an IC_{50} value of 300 nM (42). In a follow-up study, the same authors investigated the CRF-binding properties of both naphthodianthrones in greater detail by measuring their effect on CRF-stimulated cAMP formation in recombinant Chinese hamster ovary (CHO) cells (43). The authors found that only pseudohypericin selectively antagonized CRF ($K(B) 0.76 \mu\text{M}$).

In summary, the data from *in vitro* studies allocate interesting pharmacological properties to hypericin and similarly to the flavonoids. The therapeutic relevance of these findings needs verification by *in vivo* experiments. However, with regard to the contradictory effects of hypericin in various test models, it should be pointed out that the pharmacological evaluation of hypericin in most *in vitro* and *in vivo* studies is hampered by its poor solubility in aqueous solutions.

In Vivo Tests on the Naphthodianthrones

The *in vivo* effects of hypericin and pseudohypericin have been investigated by Butterweck et al. (10, 44, 45). During a bioassay-guided fractionation of a methanolic SJW extract, hypericin and pseudohypericin were detected as compounds that exerted antidepressant activity in the forced swimming test. Interestingly, pure hypericin and pseudohypericin did not reduce immobility time after acute pretreatment at doses comparable to the total extract, and only the exceptionally high dose of 0.23 mg/kg orally (p.o.) of hypericin was significantly active, whereas pseudohypericin indicated some nonsignificant activity at about 0.5 mg/kg (p.o.) (44).

A common biological alteration in patients with major depression is the activation of the HPA axis, manifested as hypersecretion of ACTH and cortisol. The hyperactivity of the HPA axis in depressed patients can be corrected during clinically effective therapy with standard antidepressant drugs such as imipramine, indicating that the HPA axis may be an important target for antidepressant action. Oral treatment of rats for two weeks with either SJW extract or hypericin significantly reduced plasma ACTH and corticosterone levels, suggesting a decrease in functional activity of the HPA axis (9).

In a more detailed study using *in situ* hybridization technique, it was shown that hypericin (0.2 mg/kg) given daily by gavage for eight weeks but not for two weeks significantly decreased levels of corticotropin-releasing hormone (CRH) mRNA by 16% to 22% in the hypothalamic paraventricular nucleus (PVN) and serotonin 5-HT_{1A} receptor mRNA by 11% to 17% in the hippocampus (45). Thus, these results are of major interest because CRF_1 receptor antagonists as potential pharmacotherapies for depression and anxiety disorders are under current development (46).

Synergism Between Hypericins and Other Constituents

When a fraction of procyanidins, which was itself not active in the forced swimming test, was recombined with the naphthodianthrones, hypericin was significantly active at

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0.028 mg/kg (p.o.), and pseudohypericin was active at 0.166 mg/kg (p.o.). The procyanidin fraction as well as the pure procyanidins B2 and C1 increased the water solubility of both hypericins, which may lead to a better bioavailability (10). When the octanol/water partition coefficient was being determined, it became obvious that the solubility of pure hypericin in water increased upon addition of some phenolic constituents typical for SJW extracts as well. Most effective in solubilizing hypericin was hyperoside (quercetin 3-O-beta-D-galactoside), which increased the concentration of hypericin in the water phase up to 400-fold in this model (26).

However, an improved solubility does not necessarily result in an improved bioavailability. In a pharmacokinetic study, Butterweck et al. investigated whether the improved water solubility in the presence or absence of procyanidin B2 or hyperoside is correlated to increased plasma levels of hypericin in rats, determined by reversed-phase high-performance liquid chromatography (HPLC) using fluorimetric detection (47). Both compounds increased the oral bioavailability of hypericin by ca. 58% (B2) and 34% (hyperoside). The authors concluded that a significant accumulation of hypericin in rat plasma in the presence of both polyphenols might be responsible for the observed increased *in vivo* activity. These interesting pharmacokinetic properties would also explain why hypericin was inactive or why conflicting data were obtained in most *in vitro* experiments. These results are also of general importance for the concept of phytotherapy because they qualitatively and quantitatively describe the superiority of herbal extracts over single active ingredients.

Phloroglucinols

The main representative of the phloroglucinol group of constituents is hyperforin. Together with adhyperforin, hyperforin occurs exclusively in the generative parts of the plant, especially in the unripe fruits (Figure 4.4). Consequently, plant material collected at the end of the flowering period, when fruits are developing, will contain more hyperforin than material collected during the flowering time (as requested by monographs) (48). Hyperforin occurs in amounts up to 2% to 5% in the crude drug material and can reach about 6% in some extracts.

Although this compound is quite unstable—especially in aqueous solutions and when exposed to light and heat—it is present in many commercial extracts but at highly varying concentrations of 0% to 6% (49). Products of degradation are 2-methyl-3-buten-2-ol and two oxidation products with an intact hyperforin carbon skeleton (50, 51).

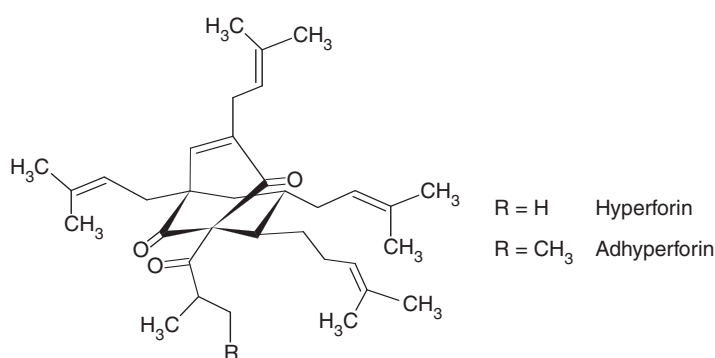


Figure 4.4. Phloroglucinols from SJW

Mechanisms of Action of Phloroglucinols Related to Neurotransmitters

Chatterjee and coworkers were the first who pointed to hyperforin as the major non-nitrogenous metabolite of SJW (52, 53). The authors have shown that a lipophilic CO₂-extract enriched in hyperforin as well as pure hyperforin in vitro inhibited serotonin-induced responses and uptake of neurotransmitters in peritoneal cells. The pharmacological work was continued by Müller et al., who detected that hyperforin was capable of inhibiting the reuptake of serotonin (5-HT), norepinephrine (NE), and dopamine (DA) at a potency comparable to that of conventional 5-HT and NE inhibitors (54). Moreover, the authors noted that the ability of hyperforin to inhibit the reuptake of 5-HT, NE, and DA at a nanomolar concentration sets it apart from any known synthetic antidepressant. Subsequent papers could confirm that hyperforin is a potent monoamine reuptake inhibitor, but all authors reported micromolar concentrations (55–57).

Buchholzer et al. first presented data on interactions of hyperforin with the cholinergic system (58). The authors found that hyperforin inhibited high-affinity choline uptake in rat synaptosomes. These results are of special interest and could form the basis for the use of hyperforin in cognitive disorders. To explain the mechanism of synaptosomal reuptake, several researchers focused therefore on the effects of SJW on monoamine transporters in intact neural cells. The neurotransmitter transporters have been proven to be important targets for drug discovery in the central nervous system, particularly for antidepressants (59). Gobbi et al. (55) reported that inhibition of 5-HT uptake by a methanolic hypericum extract and hyperforin is not due to a direct interaction with, and blockade of, the 5-HT transporters because both of them had no or only very slight inhibitory effect on [³H]citalopram binding. The authors speculated that the inhibitory effects on synaptosomal 5-HT accumulation might be caused by a reserpine-like mechanism because similar results were obtained with Ro-04-1284, a reserpine-like compound, that also did not inhibit [³H]citalopram binding. Their hypothesis was confirmed by the finding that SJW extract and hyperforin induced marked release from synaptosomes preloaded with [³H]5-HT. The authors conclude that the apparent inhibition of uptake is an artifact from the interaction of the high concentration of the tested compounds with monoamine storage vesicles.

Jensen et al. found that the potency of hyperforin and adhyperforin on monoamine reuptake was comparable to that seen for imipramine, nomifensine, and fluoxetine (60). But contrary to the antidepressant drugs, the phloroglucinols potently inhibited all three transporter systems. Interestingly, the inhibition of DA-uptake by hyperforin and adhyperforin was not due to a direct interaction with the [³H] WIN 35,428 (a cocaine analogue) binding site on the DA transporter as seen for imipramine, nomifensine, and fluoxetine. Therefore, the authors suggest a noncompetitive interaction of hyperforin and adhyperforin with the DA transporter.

Further Mechanistic Studies on Phloroglucinols

Another interesting hypothesis regarding the mechanism of synaptosomal reuptake is presented by Müller's group (57). The apparent inhibition of serotonin uptake observed with SJW extract and hyperforin in vitro might be caused by an increase in free intracellular sodium concentrations (57). Additional data suggested interactions of the compound with Na⁺ channels or Na⁺/H⁺ exchangers (61). Such nonselective effects might explain

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why SJW extracts and hyperforin blocked the synaptosomal uptake of multiple neurotransmitters (52, 55, 57, 61).

Recent work indicated that hyperforin may also influence calmodulin-dependent mechanisms (62). Finally, hyperforin was reported to modulate several ionic conductances in cerebellar Purkinje cells including a P-type calcium channel that is known to be involved in neurotransmitter release (63). The authors also screened an ethanolic hypericum extract (hyperforin amount approx. 5%) on various voltage- and ligand-gated conductances (64). The plant extract inhibited almost all the ligand-gated ion channels; quercitrin was detected as a potent inhibitor of adenosine triphosphate (ATP)-induced conductance; biapigenin inhibited both the acetylcholine- and ATP-induced conductance, whereas hyperoside blocked currents activated by ATP and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA). The naphthodianthrone hypericin was inactive in all cases. The authors concluded that hyperforin is not the only active substance of SJW and that the extract contains several potentially neuroactive molecules, such as biapigenin, hyperoside, and quercitrin.

Relevance for the Situation In Vivo of Phloroglucinols

However, the concentration of hyperforin (micromolar range) used in these studies (64–66) was far higher than plasma concentrations reached in vivo. In human volunteers receiving daily doses of 900 mg (3 × 300 mg) SJW extract, plasma steady-state concentrations of approximately 180 nM were measured (65). Reported IC₅₀ values for hyperforin as an inhibitor of synaptosomal uptake of serotonin have ranges from 120 nM to 3.300 nM. According to the results of the studies mentioned above, the blood levels of hyperforin in human volunteers after a daily dose of 900 mg hypericum extract are within a concentration range needed to inhibit serotonin uptake in vitro, but the free drug concentration available for action at central ion channels in vivo is unclear.

In Vivo Tests on Phloroglucinols

The in vivo antidepressant effects of hyperforin, a hyperforin-enriched CO₂ extract, or more stable salts such as dicyclohexylammonium (DCHA), acetate, or sodium hyperforin were demonstrated in different animal models of depression. In the forced swimming test, triple administration of hyperforin acetate (5–20 mg/kg) significantly reduced the immobility time of rats, while in the learned helplessness test a daily treatment of 10 mg/kg for seven consecutive days was necessary to elicit an antidepressant effect (66). In the tail suspension test in mice, pure hyperforin significantly reduced immobility time in a dosage of 4 and 8 mg/kg, whereas it was inactive in dosages below or above that (67). In the same study, it was also shown that step-by-step removal of either hyperforin or hypericin did not result in a loss of pharmacological activity. The results clearly show that the crude SJW extracts contain several constituents with antidepressant activity.

In addition to the antidepressant effects, other behavioral effects of hyperforin have been described. Chatterjee and colleagues were the first to investigate two different SJW extracts in the learned helplessness paradigm (52). According to this paradigm, the exposure to uncontrollable stress produces performance deficits in subsequent learning tasks, which can be reversed by subchronic treatment (4–7 days) with a variety of antidepressants including tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), and atypical antidepressants, and in treatments related to electroconvulsive seizures (ECS) (68, 69). In the group treated with ethanolic extract (hyperforin amount 4.5%), escape deficits

significantly and dose dependently decreased after 150 and 300 mg/kg/day (p.o.). Comparable effects were observed for the CO₂ extract (hyperforin amount 38.8%) in doses of 15 and 30 mg/kg/day (p.o.) (52).

A similar study was performed by Gambrana et al. (70), who used the escape deficit paradigm as a test model. When tested in the escape deficit paradigm, 25 mg, 50 mg, and 75 mg/kg (i.p.) of hyperforin significantly counteracted the effects of acute stress. Taken together, the results in the learned helplessness paradigm show that different SJW extracts (hydroalcoholic, CO₂) as well as hyperforin (pure or in salt form) could reduce behavioral deficits induced by uncontrollable shock. How far other constituents of SJW are active in this model needs to be further elucidated.

SJW CONSTITUENTS IN CLINICAL STUDIES

Most hypericum products were—and still are—standardized on the key compound hypericin. In most products, hypericin doses are applied in a range between 1 and 2 mg per day. A German study that analyzed 33 different SJW products showed that many extracts with a well-balanced ratio of the three main groups of components are standardized to an even higher upper limit of hypericin than that allowed by the proposed range of 0.1% to 0.3% (49). This upper limit can also not be derived from a photosensitizing potential associated to hypericin, as photosensitizing effects of SJW extracts are rare in clinical practice (35).

The flavonoids are mentioned as relevant for clinical efficacy in some clinical studies, but in most clinical studies, no information regarding the quantitative amount of this substance group in extracts of commercial preparations is found.

Several clinical studies with SJW extract were screened for information on the hyperforin content of the tested products. Unfortunately, information regarding the hyperforin content in all these publications is rare. Interestingly, in cases where data about the hyperforin content were provided, they were contradictory for the same extract in different studies. For example, Kalb et al. (71) indicate a hyperforin content of 1.5% for the extract with the brand name WS 5572 (Dr. Willmar Schwabe & Co. KG, Karlsruhe, Germany), whereas Laakmann et al. (72) declare a hyperforin content of 5% for the extract with the identical brand name, WS 5572. This is somewhat confusing, especially because the latter authors argue that hyperforin is important for the efficacy of SJW extract. For the extract WS 5570, Lecrubier et al. (73) report a hyperforin content of 3% to 6%. For another extract from the same manufacturer (WS 5573) that has been used as a reference extract by Laakmann et al. (72), a hyperforin content of 0.5% is stated in the publication. However, insufficient information is provided about the amount of other constituents (especially hypericin and flavonoids) in this specific extract. Thus, based on this study, no definite conclusion regarding the role of hyperforin for clinical efficacy can be drawn.

Further, in published studies using the product Neuroplant (Dr. Willmar Schwabe & Co. KG), neither the extract code name nor the hyperforin content is provided. In the case of older studies (74), it was still unclear which compound might play a role in clinical efficacy of SJW. Since then, many pharmacological studies have been performed, showing that hypericin, hyperforin, and flavonoids play a crucial role. Therefore, in ongoing current studies, it is imperative that the amount of those compounds be stated, in order to avoid contradictory and confusing results. In a recent study, Szegedi et al. (75) used the extract

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WS 5570, but unfortunately, information regarding the hyperforin content is missing. Interestingly, heterogeneities regarding Neuroplant can be found in the phytochemical literature. While Melzer et al. (49) stated a hyperforin content of 5.0%, in a later publication (describing the extract as having a stabilized hyperforin content), only 4.1% hyperforin was found (76).

The largest number of clinical studies exists for the extract LI 160 (Lichtwer GmbH & Co. KG). However, even though some of these studies have been published up to four times, none of these publications contain any information about the hyperforin content of the extract.

In summary, the information provided about these extracts does not allow any conclusion about the relevance of the hyperforin content for clinical efficacy.

Data from Phytochemical Studies

Interestingly, whereas most clinical studies do not provide data about the hyperforin content, this information can be accessed from several phytochemical screening studies. A German study including 33 different commercial SJW products showed that their hyperforin contents varied between <0.02% and 5.85% of extract (49); several products are within the range of 1.5% and 2.5%, which would be within the limit of 2% proposed in the draft monograph:

BardoH: 1.5%
Helarium: 2.5%
Hypericum Stada: 1.9%
Johanniskraut-Dragees SN: 2.3%
Neurovegetalin 425: 2.5%
Texx 300: 2.5% (span according to Wurglics et al.: 1.7% to 3.2%)
Tonizin forte: 1.7%

Another screening of different batches of several products was published by Wurglics et al. (76, 77). The measurements in some cases confirm the data of Melzer et al. (49); in other cases, the authors find different values. This difference is most obvious in the case of the extract LI 160, for which Melzer et al. state a hyperforin content of 4.3%, while Wurglics et al. found only 2.2% to 3.1%. A similar tendency is found in the product Felis, where Melzer et al. (49) found 4.9% hyperforin, while Wurglics et al. found only 1.9% to 3.5%. The reason for this discrepancy remains unclear.

For the extract STW3-VI, for which clinical data have been published (78), a hyperforin amount of 1.8% is stated, which is well within the range of 1.5% to 2.4% found by Wurglics et al. (76, 77). However, these values are also not compatible with the classes of hyperforin content proposed by the draft monograph.

Hyperforin Levels for Clinical Efficacy

Based on the present data, the question arises, is hyperforin necessary for the antidepressant activity of SJW? Data from in vitro or animal studies are either pro or contra hyperforin

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and do not help answer the question appropriately. Interestingly, a recent study showed that step-by-step elimination of hyperforin and hypericin from a hydroalcoholic SJW extract did not result in any loss of pharmacological activity (67). An extract free of hyperforin and hypericin but enriched in flavonoids (~12%) showed antidepressant activity in valid animal models. The results indicate that flavonoids are also involved in the therapeutic efficacy of SJW.

This question can be answered only by clinical studies. As mentioned above, a study approach to compare extracts directly with different hyperforin contents was conducted by Laakmann et al. (72). On the one hand, a statistically significant superiority between an extract with 5% hyperforin (WS 5572) in reducing the Hamilton depression score and placebo was demonstrated; on the other hand, no statistical differences were found between an extract with 0.5% hyperforin (WS 5573) and placebo and between the two extracts WS 5572 and WS 5573. Because of this result, it has been suggested that hyperforin may be relevant for the clinical efficacy of the extract, but this is not convincing because of (a) the lack of statistical power and (b) the lack of quantitative data for additional constituents (such as flavonoids and biflavones). Thus, it cannot be excluded that constituents other than hyperforin may have caused the small differences in efficacy of both extracts. Therefore, the outcome does not give convincing proof for the activity of hyperforin but indicates that there are extracts that show clinical efficacy and others that are inactive.

However, three clinical trials performed using an SJW extract with a low hyperforin amount (<0.2%) showed that the efficacy of this SJW extract was superior to placebo and as effective as imipramine and fluoxetine (79–81), thus showing that hyperforin is not necessarily needed for the antidepressive efficacy of SJW.

Taken together, the collected data from the clinical studies with SJW extracts allow only the conclusion that hyperforin is no convincing parameter for a prediction of the clinical efficacy.

The available analytical data show a large range of different hyperforin content for different extracts with clinically proven efficacy. Even for the same extract, the hyperforin content can differ considerably, as exemplified by the extract WS 5572, for which in different publications values of 5% (72) and 1.5% (71) are given, as already mentioned.

SJW SAFETY ISSUES

SJW and Drug Interactions

Besides the data on clinical efficacy, data on clinical safety of SJW extracts have to be considered. Recently, interactions of herbal medicines with synthetic drugs have come into focus. In the past three years, more than 50 papers were published regarding interactions between SJW and prescription drugs. Comedication with SJW resulted in decreased plasma concentrations of a number of drugs including amitriptyline, cyclosporine, digoxin, indinavir, irinotecan, warfarin, phenprocoumon, alprazolam, dextromethorphan, simvastatin, and oral contraceptives. Sufficient evidence from interaction studies and case reports indicate that SJW is a potent inducer of cytochrome P450 enzymes (particularly CYP3A4) and/or P-glycoprotein. Recent studies could show that the degree of enzyme induction by SJW correlates strongly with the amount of hyperforin found in the product. Products that do not contain substantial amounts of hyperforin (<1%) have not been

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shown to produce clinically relevant enzyme induction. As mentioned above, a German study that analyzed 33 different SJW products showed that the hyperforin content varied from <0.5 mg per unit (<0.02% of extract) to 24.87 mg per unit (5.85% of extract) (49). Data for the extract ZE 117, marketed as Remotive, indicate hypericin in an amount of 0.2% and negligible amounts of hyperforin (0.2%) (82). In the past, this also was most likely the case for extract LI 160, marketed as Jarsin or a similar extract, marketed as Neuroplant, before the extraction process was modified (83, 84). With the modified method, a hyperforin content of 4% to 5% has been reported for products containing LI 160 (49). This is equivalent to a daily dose of approximately 50 mg of hyperforin, based on a 300 mg extract administered three times daily. The widely differing amounts of hyperforin in SJW preparations should be taken into account when drug interactions with SJW are discussed. Most of the current cases that report a specific product involved SJW products that are rich in hyperforin (up to 5%). No drug interaction has been reported with products that have low contents of hyperforin (82, 85, 86). Furthermore, it is interesting to note that reports of drug interactions with SJW have not been reported before 1998, when a modified extraction method was introduced that led to products with a higher content of hyperforin (83, 84).

The most frequently documented drug interactions of SJW extract, which have been extensively reviewed in recently published articles (87, 88), are initially discussed in brief before dealing in detail with the role of hyperforin in these interactions.

Influence of SJW on P-glycoprotein and Several Cytochrome P-450 Enzyme Activities in Humans

CYP3A4, the most abundant cytochrome P-450 (CYP450) isoenzyme, is responsible for the metabolism of more than 73 medications and numerous endogenous compounds (89–91). Substrates for this isoenzyme include protease inhibitors, nonsedating antihistamines, calcium channel blockers, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, benzodiazepines, estrogens, macrolide antibiotics, cyclosporine, carbamazepine, ketoconazole, and corticosterone (89–91). One method to determine the *in vivo* effect of medications on CYP3A4 activity is through the evaluation of the urinary 6- β -hydroxycortisol/cortisol ratio. 6- β -hydroxycortisol has been shown to be a nonspecific marker of CYP3A4 activity (92–94). Roby et al. (95) could show that reagent-grade SJW taken for 14 days (Hypericum Byers Club, Lot 180632, 0.3% hypericin, 3 \times 300 mg/d, hyperforin amount not provided) at the dose recommended for the treatment of mild to moderate depression was associated with a significant increase in the mean urinary 6- β -hydroxycortisol/cortisol ratio. The ratio increased from 7.1 to 13.0, suggesting a CYP3A4 induction after intake of SJW at the recommended dosage.

Besides an induction of CYP3A4 enzyme activities in the liver and small intestine, the involvement of the P-glycoprotein/MDR1 system in the intestine was also discussed. P-glycoprotein (P-gp), an ATP-dependent primary active transporter belonging to the ABC transporter superfamily, occurs in plasma membranes of many tissues, where it serves as an efflux transporter of xenobiotics (96). In the intestine, P-gp is located at the apical surface of epithelial cells and interferes with drug absorption by pumping out a variety of orally administered drugs, such as cyclosporine, into the intestinal lumen (97). After uptake by the enterocyte, many lipophilic drugs are either metabolized by CYP3A4 or pumped back into

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Table 4.1. Reported Drug Interactions with St. John's Wort Preparations

Reported Pharmacokinetic Interactions		
<i>Drug Category</i>	<i>Drug(s)</i>	<i>Possible Mechanism of Interaction</i>
Cystostatic drugs	Imatinib, Irinotecan	CYP3A4 induction
HIV drugs	Indinavir, Nevirapine	CYP3A4 and MDR1 induction
Immunosuppressants	Cyclosporine, Tacrolimus	CYP3A4 and MDR1 induction
Anticoagulants	Warfarin, Phenprocoumon	CYP2C9 induction
Cardiovascular drugs	Digoxin	MDR1 efflux activity induced
Opiates	Methadone	CYP3A4 induction
Oral contraceptives	Ethinylestradiol (EE) / Desogestrel, EE/Norethisterone, Levonorgestrel, Norethisterone*	CYP3A4 induction
Antiepileptic drugs	Carbamazepine*	CYP3A4 induction
Anti-asthma drugs	Theophylline *	CYP1A2 induction
HMG-CoA reductase inhibitors	Simvastatin, Atorvastatin*	Intestinal CYP3A4 induction

the lumen by the P-gp transporter. Therefore, CYP3A4 and P-gp may act in tandem as a barrier to oral delivery of many drugs.

This hypothesis could be confirmed in animal experiments as well as in clinical studies (98). The administration of SJW extract to rats during 14 days resulted in a 3.8-fold increase of intestinal P-glycoprotein/MdR1 expression and in a 2.5-fold increase in hepatic CYP3A4 expression (98). In a clinical study, the administration of SJW extract resulted in 1.4- and 1.5-fold increased expressions of duodenal P-glycoprotein/MDR and CYP3A4, respectively (98). These results indicate direct inducing effects of SJW on intestinal P-glycoprotein/MDR1 and intestinal and hepatic CYP3A4. In both studies, the extract LI 160 (80% methanol v/v, 0.3% hypericin, batch 99100400, 3×300 mg/d, unknown amount of hyperforin) was used.

In contrast to short-term administration (1×900 mg SJW in 24h), long-term SJW administration (3×300 mg/d for 14 days) resulted in a significant and selective induction of CYP3A4 (midazolam) activity in the intestinal wall (99). There was no change in CYP2C9 (tolbutamide), CYP1A2 (caffeine), or CYP2D6 (dextromethorphan) activities as a result of SJW administration. In contrast to the >50% decrease in the area under the plasma-concentration time curve (AUC) when midazolam was administered orally, long-term SJW administration caused a 20% decrease in AUC when midazolam was given intravenously. This result confirms the involvement of intestinal as well as hepatic CYP3A4. In this study, an SJW extract from Rexall Sundown Pharmaceuticals was used (0.3% hypericin, unknown hyperforin content, 3×300 mg) (99). The authors concluded that the reduced therapeutic efficacy of drugs metabolized by CYP3A4 should be anticipated during long-term administration of SJW.

Several reports have documented clinically relevant drug interactions between SJW and coadministered drugs such as indinavir, cyclosporine, and digoxin (100–102), attributing

induction of hepatic CYP3A4 as the likely mechanism (95, 103). However, interactions with digoxin and indinavir are unlikely to be fully explained by this mechanism, as they are not only a CYP3A4 substrate but also a substrate for P-gp. Hennessy and coworkers (104) could show that SJW increased expression and enhanced the drug efflux function of P-gp in peripheral blood lymphocytes of healthy volunteers. P-gp expression increased 4.2-fold from baseline in subjects treated with SJW (Good n' Natural, 0.15% hypericin, 600 mg extract 3×daily) over a period of 16 days; there was no effect observed in patients receiving placebo.

In another pharmacokinetic study in healthy volunteers, changes in plasma pharmacokinetics of alprazolam as a probe for CYP3A4 activity and the ratio of dextromethorphan to its metabolite dextrorphan were measured after 14 days of comedication with SJW extract (LI 160, 2×300 mg/d; each tablet contained 1398 µg hyperforin, 151 µg hypericin, and 279 µg pseudohypericin) (105). A twofold decrease in the area under the curve for alprazolam plasma concentration versus time and a twofold increase in alprazolam clearance were observed following SJW administration. Alprazolam elimination half-life was shortened from a mean (SD) of 12.4 (3.9) hours to 6.0 (2.4) hours. The mean (SD) urinary ratio of dextromethorphan to its metabolite was 0.006 at baseline and 0.014 after SJW administration (105). These findings indicate that long-term administration of SJW may result in diminished clinical efficacy or increased dosage requirements for CYP3A4 substrates.

Recently, the authors Rengelshausen et al. (106) investigated the short-term and long-term effects of SJW on the pharmacokinetics of voriconazole. The metabolism of this new antifungal triazole is mediated by CYP2C19 and CYP3A4, as well as by CYP2C9 to a lesser extent. The authors could show that coadministration of the hyperforin-rich methanolic extract LI 160 with voriconazole increased the plasma AUC of the antifungal drug (by 22%) during the first ten hours of the first day of SJW administration when compared to the control. After 15 days of SJW intake, the AUC from hour 0 to infinity was reduced by 59% compared with control with a corresponding increase in oral voriconazole clearance. Thus, it is reasonable that SJW might induce the metabolism of voriconazole, depending on both CYP3A4 and CYP2C19.

Based on the studies listed above, it can be concluded that SJW induces hepatic and intestinal CYP3A4 and intestinal P-gp. Thus, it is likely that SJW will interact with drugs that are metabolized via CYP3A4 or P-gp. Interactions (until 2004) of SJW and synthetic drugs have been systematically reviewed (87, 88, 107, 108). These reviews clearly show that interactions between xenobiotics and the plant extract particularly occur with drugs that are metabolized and eliminated by both CYP3A4 and P-gp. It also becomes evident that not all drugs have the potential to interact with SJW. Table 4.1 gives an overview about drugs with which an interaction with SJW is likely to occur.

Pharmacological Evaluation of Interactions with Hyperforin

The mechanism for the apparent increase in drug metabolism by SJW extracts was examined by Moore et al. (109). They showed that hyperforin, a constituent of SJW, is a potent ligand ($K_{(i)} = 27$ nM) for the pregnane X receptor, an orphan nuclear receptor that regulates expression of the cytochrome P450 (CYP) 3A4 monooxygenase. Treatment of primary human hepatocytes with hypericum extracts or hyperforin resulted in a marked induction of CYP3A4

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expression. The authors cautioned that because CYP3A4 is involved in the oxidative metabolism of >50% of all drugs, their findings provide a molecular mechanism for the interaction of SJW with drugs and suggest that hypericum extracts would be likely to interact with numerous drugs (109). Interestingly, a study using alcoholic extracts of SJW prepared with methods that did not stabilize hyperforin reported CYP3A4 inhibition (103).

A study in mice investigated the role of components of hypericum extracts on CYP3A induction (110). This study explored whether hyperforin accounts for the inductive effects on CYP3A enzymes of SJW extracts. A hydroalcoholic extract containing 4.5% hyperforin was given at a dose of 300 mg/kg twice daily for 4 and 12 days. Hyperforin was given as dicyclohexylammonium (DCHA) salt (18.1 mg/kg) on the basis of its content in the extract, to ensure comparable exposure to hyperforin. The extract increased hepatic erythromycin-N-demethylase (ERND) activity, which is cytochrome P450 enzyme (CYP) 3A-dependent, about 2.2-fold after 4 days of dosing, with only slightly greater effect after 12 days (2.8 times controls). Hyperforin similarly increased ERND activity within 4 days, to 1.8 times the activity of controls, suggesting that hyperforin behaves qualitatively and quantitatively like the extract as regards induction of CYP3A activity. This effect was confirmed by Western blot analysis of hepatic CYP3A expression. Exposure to hyperforin at the end of the 4-day treatment was still similar to that with SJW extract, although it was variable and lower than after the first dose in both cases, further suggesting that hyperforin plays a key role in CYP3A induction by the SJW extract in the mouse. The authors proposed standardizing the extracts based on the hyperforin content in addition to hypericin content.

Clinical Evaluation of Interactions with Hyperforin

A recently completed four-period study in ten stable kidney transplant patients examined the influence of two SJW preparations with different hyperforin content on cyclosporine pharmacokinetic parameters (86). Test periods included baseline with cyclosporine alone, 14 days of treatment with low-hyperforin SJW comedication (< 1 mg hyperforin/day), a 4-week washout with cyclosporine doses alone, and 14 days of treatment with high-hyperforin SJW comedication (> 40 mg hyperforin/day). Cyclosporine kinetic parameters (AUC, C_{max} , and t_{max}) were determined at the end of each period.

Pharmacokinetic parameters were adjusted for cyclosporine dose, and plasma creatinine was monitored for safety. As shown in Table 4.2, there was no significant effect of low hyperforin on cyclosporine. However, high-hyperforin SJW decreased dose-adjusted exposure to cyclosporine, and increased doses of the immunosuppressant were required to maintain plasma creatinine. Serum creatinine was not different among treatment periods.

The results are consistent with a role for hyperforin in decreasing cyclosporine exposure. Induction of the intestinal drug transporter, P-glycoprotein, as well as induction of CYP3A4 could explain decreased bioavailability of cyclosporine in the presence of hyperforin-rich SJW preparations. The increased mean cyclosporine daily dose from 225 to 362 mg when patients were comedicated with the high-hyperforin preparation indicates that the change in cyclosporine levels was considered clinically significant by the medical investigator and required a dose increase. Thus, the dose-adjusted decrease in cyclosporine exposure of

Table 4.2. Influence of Different Preparations of St. John's Wort on Cyclosporine Dose-Corrected Pharmacokinetic Parameters in Ten Renal Transplant Patients (86)

<i>Parameter</i>	<i>Baseline</i>	<i>Low:Hyperforin</i>	<i>Washout</i>	<i>High:Hyperforin</i>
Dose (mg/day)	216±58	223±54	225±54*	362±63**
AUC (ng*h/mL)	3663±678	3109±527	3442±741	1671±313**
C _{max} (ng/mL)	995±284	894±187	986±201	532±117**

p<0.05 vs. baseline; **p<0.01 vs. baseline

approximately 50% that is reflected by AUC and C_{max} values should be considered clinically significant.

Another study (111) compared effects of different SJW preparations containing different amounts of hyperforin on CYP3A4 activity using a midazolam probe (Study No. 786, Pilotstudie1: Midazolam-Interaktion) (Table 4.3). The approach provides a means to evaluate whether drugs affect CYP3A enzymes and thus would be likely to alter the clearance of other substances metabolized by this important isoenzyme. Hydroxylation of the benzodiazepine, midazolam, to 1-OH-midazolam is mediated almost exclusively by CYP3A isoenzymes. Midazolam plasma clearance that is reflected from plasma profiles of the benzodiazepine can be used as a marker for CYP3A activity. Drugs that are substrates, inducers, or inhibitors of CYP3A enzymes would be expected to alter the plasma profiles of midazolam.

The study included 42 healthy subjects randomized to six groups of seven subjects each (111). Midazolam plasma profiles were characterized following a 7.5 mg oral dose on day 1 prior to SJW exposure and 14 days later after repeated daily dosing with one of six SJW preparations. The test groups included the following different SJW preparations: Extract LI160 (drug-extract ratio 4–7:1; hyperforin content 4.6%, Lichtwer Berlin, Germany); hypericum herb powder A, which was used to explore dose-response relationship with hyperforin (hyperforin content 0.4%, Kneipp-Werke, Wuerzburg, Germany); and hypericum herb powder B with a very low hyperforin content (<0.1%, Kneipp-Werke, Wuerzburg, Germany). The decrease in midazolam AUC was highest in the LI160-treated group exposed to the highest hyperforin concentrations with a mean decrease of 79%, indicating induction of CYP3A enzymes. Using the herb powder A, the highest concentration (2700 mg/d,

Table 4.3. Effect of 14 Days of Different St. John's Wort Preparations on Percent Change in Midazolam AUC_{0-12h} Compared to Baseline (111)

<i>SJW Preparation</i>	<i>Extract Dose (mg/d)</i>	<i>Hyperforin Dose (mg/d)</i>	<i>Mean % Change</i>	<i>95%-CI</i>
LI 160 extract	900	41.25	-79.4	-88.6; -70.1
Hypericum powder A	2700	12.06	-47.9	-59.7; -36.2
Hypericum powder A	1800	8.04	-37.0	-58.2; -15.8
Hypericum powder A	1200	5.36	-31.3	-45.3; -17.3
Hypericum powder A	600	2.68	-20.4	-40.0; -0.8
Hypericum powder B	2700	0.13	-21.1	-33.9; -8.3

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hyperforin amount 12.1 mg/d) showed the strongest decrease on midazolam AUC (-47.9%), when compared to the further dilutions of the powdered herb. A limited effect was noted after 14 days of dosing with the SJW herb powder B with a mean decrease in midazolam AUC values of 20.4% compared to baseline. Interestingly, the upper limit of the calculated 95% confidence intervals approached 0 for the two lowest hyperforin preparations, indicating little clinical significance. The results indicate induction of CYP3A4 varies between SJW products. SJW products with low hyperforin content induce CYP3A4 significantly less than those preparations with a high hyperforin amount. The degree of induction depends on the hyperforin dose.

CONCLUSIONS

Considerable differences exist in the composition of biologically active constituents among various commercially available preparations of SJW. Although several reports fail to rigorously define the specific herbal product used in clinical studies, investigators are increasingly aware that significant differences in outcome are likely to be product specific. However, a major change was made since 1998, when the quite unstable component of SJW, hyperforin, became stabilized in many products, leading to a 10- to 20-fold amount of hyperforin in the product. Moreover, the first reports of clinically significant drug interactions of SJW coincided with availability of hyperforin-enriched products. Further laboratory investigations demonstrated that CYP3A4 induction with SJW preparations was associated with hyperforin content, suggesting that this component plays a major role in clinically significant drug interactions. Clinical studies with traditional SJW extracts and products with low hyperforin content found low enzyme induction using midazolam probes that consistently had AUC decreases that were less than 50% compared to baseline. Since “hyperforin-free” extracts have been proven to be effective in clinical therapy, it is recommended to set an upper limit (1%) for the amount of hyperforin in SJW extracts in order to prevent clinically significant interactions with other comedicated drugs. It is further suggested that products used for clinical studies should be characterized by the extraction solvent, the drug-extract ratio, and the amount of hyperforin, hypericin, and total flavonoids. This information should allow a more substantial discussion of the data and would help to better explain discrepancies between studies.

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