EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Committee on Herbal Medicinal Products (HMPC)

# Assessment report on *Hypericum perforatum* L., herba

2nd Draft – Revision 1

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

Based on Article 16d(1), Article 16f and Article 16h of Directive 2001/83/EC as amended (traditional use)

| | |
|---|---|
| Herbal substance(s) (binomial scientific name of the plant, including plant part) | *Hypericum perforatum* L., herba |
| Herbal preparation(s) | **Traditional use**<br>a) Dry extract (DER 4-7:1), extraction solvent ethanol 38% (m/m) = 45% V/V<br>b) Liquid extract (DER 1:4-20), extraction solvent vegetable oil<br>c) Liquid extract (DER 1:13), extraction solvent maize oil or other suitable vegetable oil<br>d) Tincture (ratio herbal substance: extraction solvent 1:10), extraction solvent ethanol 45-50% (V/V)<br>e) Liquid extract (DER 1:2-7), extraction solvent ethanol 50% (V/V)<br>f) Expressed juice from the fresh herb (DER 1:0.5-0.9)<br>g) Comminuted herbal substance<br>h) Powdered herbal substance<br>**Well-established use**<br>a) Dry extract (DER 3-7:1), extraction solvent methanol (80% V/V)<br>b) Dry extract (DER 3-6:1), extraction solvent ethanol (80% V/V) |

| | |
|---|---|
| | c) Dry extract (DER 2.5-8:1), extraction solvent ethanol (50-68% V/V) |
| Pharmaceutical form(s) | **Traditional use**<br><br>Comminuted herbal substance as herbal tea for oral use.<br><br>Herbal preparations a, h in solid dosage forms for oral use.<br><br>Herbal preparations b, c, d, e, f in liquid dosage forms for oral use.<br><br>Herbal preparations b, d, e in liquid or semi-solid dosage forms for cutaneous use.<br><br>**Well-established use**<br><br>Herbal preparation in solid dosage forms for oral use. |
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Note: This draft assessment report is published to support the release for public consultation of the draft European Union herbal monograph on *Hypericum perforatum* L. herba. It should be noted that this document is a working document, not yet edited, and which shall be further developed after the release for consultation of the monograph public statement. Interested parties are welcome to submit comments to the HMPC secretariat, which the Rapporteur and the MLWP will take into consideration but no 'overview of comments received during the public consultation', will be prepared in relation to the comments that will be received on this assessment report. The publication of this draft assessment report has been agreed to facilitate the understanding by Interested Parties of the assessment that has been carried out so far and led to the preparation of the draft monograph.

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# 1. Introduction

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

- Herbal substance(s)

**Hyperici herba** (Pharm. Eur. 01/2017: 1438)

Hyperici herba consists of the whole or fragmented, dried flowering tops of *Hypericum perforatum* L., harvested during flowering time. It contains not less than 0.08% of total hypericins expressed as hypericin calculated with reference to the dried drug.

**Constituents** (Wichtl 2016; Bradley 2006, Hänsel & Sticher 2015)

Phloroglucinol derivates: 0.2-4%, depending on the age of the herbal drug, mainly hyperforin and its homologue adhyperforin, furanohyperforin

Naphtodianthrones: 0.03-0.4%, mainly pseudohypericin and hypericin, protohypericin, protopseudohypericin, cyclopseudohypericin, skyrinderivatives. The amount of pseudohypericin is about 2-4 times higher than that of hypericin.

Flavonoids: 2-4%, mainly glycosides of the flavonol quercetin: hyperoside, rutin, isoquercitrin, quercitrin; also biflavones (I3,II8-Biapigenin, Amentoflavone). Pilepic *et al.* (2013) determined in samples from Europe and Asia a content of total flavonoids of about 1.5%

Procyanidines: e.g. procyanidine $B_2$, tannins with catechin skeletal (6-15%). Hellenbrand *et al.* (2015) found that the content of oligomeric procyandines in Hyperici herba is highly variable and between 8 to 37 mg/g with the trimer as predominant compound.

Xanthones: in trace amounts

Essential oil: 0.07-0.25%; the essential oil of dried flowering tops contains as main compounds 2-methyloctane and α-pinene. In the essential oil of leaves of Indian origin 58 components were identified, α-pinene (67%) being dominant; the other components included caryophyllene, geranyl acetate and nonane (each about 5%). In samples collected in Turkey α-thujene, ß-caryophyllene, α- and ß- selinene, and caryophyllene oxide were found as main components (Bertoli *et al.* 2011). Pirbalouti *et al.* (2014) detected in samples collected in Iran as major compounds α-pinene, ß-pinene, 5-methyl-undecane, ß-ocimene, 2-methyl-decane, undecane, aromadendrene, germacrene-D and α-selinene. For samples collected in Greece a-pinene and 2-methyl-octane were reported as major compounds (Pavlovic *et al.* 2006). Helmja *et al.* (2011) concluded that the composition of the essential oil in samples collected in Estonia varies in a considerable range, no typical composition can be established.

Other constituents: include small amounts of chlorogenic acid and other caffeoylquinic and p-coumaroylquinic acids, and also free amino acids. According to Pilepic *et al.* (2013) the total content of phenolic acids in the dried aerial parts collected during the flowering season was about 10%.

- Herbal preparation(s)

**St. John's wort dry extract, quantified** (Pharm. Eur. 01/2017:1874)

Extraction solvents ethanol (50-80% v/v) or methanol (50-80% v/v). Content of total hypericins (expressed as hypericin) 0.10-0.30%, content of flavonoids (expressed as rutin) minimum 6.0%, content of hyperforin maximum 6.0% and not more than the content stated on the label.

Wurglics *et al.* (2001a, 2001b, 2002, 2003) report that in commercial batches the content of hypericin is between 0.16 and 0.32%, the content of hyperforin is <0.2% (Ze 117) or in the range between 1.5 and 4.4% (partly with considerable differences between batches). The content of total flavonoids is between 6 and 8%. Dissolution test revealed considerable differences in the dissolution of flavonoids among authorized products in Germany.

<u>Influence of the extraction solvent on the composition of the extract:</u>

When using extraction solvents containing more than 50% of ethanol or methanol in water the content of hypericin in the extract seems to be very similar independent of the actual concentration of the extraction solvent. In contrast the extraction of hyperforin and adhyperforin depends strongly on the concentration of the extraction solvent. Best yield (60% of the hyperforin in the herbal substance, 45% of adhyperforin in the herbal substance) is achieved with 70% (e.g. ethanol), while with 50% ethanol only 20% of hyperforin and no adhyperforin are extracted (Meier 1999).

Müller *et al.* (2004) reported for a liquid extract (DER 1:13), extraction solvent maize oil a content of hypericin of approximately 0.0013% and of hyperforin of approximately 0.01%. In the expressed juice 0.003% hypericin and 0.018% hyperforin were detected. The content in the powdered herbal substance was 0.1-0.5% hypericin and 0.5% hyperforin.

Anyzewska *et al.* (2010) found that approximately 16% of the hypericines contained in the herbal substance are extracted into a herbal tea (infusion).

Gao *et al.* (2010) determined the content of flavonoids in food supplements from the US market. While the major flavonoids were present in all samples significant quantitative differences between the products were identified.

Beside several liquid extracts and dry extracts prepared with ethanol/water or methanol water the so called ***Hypericum* oil** is in widespread medicinal use in Central Europe.

Preparation: According to the German "Ergänzungsbuch" to the German Pharmacopoeia 6 (Erg.-B6. 1941): The crushed fresh flowers of *H. perforatum* (25 parts) are doused with olive oil (100 parts) in a white glass. The mixture is allowed to ferment at a warm place. After completion of the fermentation the glass has to be sealed. It is then stored at a sunny place for about 6 weeks until the oil is bright red. The herbal substance has to be pressed out, the oil is dried with sodium sulphate (6 parts).

According to Swiss Pharmacopoeia (Pharm. Helv. 8 2001): The comminuted fresh flowering tops of *H. perforatum* are overflowed with 40.0 g refined sun flower oil. The mixture is reshuffled frequently; extraction and fermentation take place at a temperature of 15-30 °C. After 50-80 days the herbal substance is pressed out, the water layer is removed. It is allowed to dilute the oil to a maximum of 80 g of sunflower oil, the content of hypericin is at least 0.001% (spectrophotometric determination).

According to the company 'Caelo', Germany (Caelo, 2008): the dried herbal substance (according to Pharm. Eur.) is macerated with olive oil in a DER of 1:20. The mixture is agitated under light exposure for at least 40 hours. The content of hypericin is at least 0.005% (spectrophotometric determination).

Constituents: Maisenbacher & Kovar *(*1992) found that Hyperici oleum does not contain hypericin. By using the sunlight maceration method described in the supplement to German Pharmacopoeia 6 (Erg.-B6 1941), lipophilic breakdown products of this compound are obtained which lend the oil its red colour. The stability of hyperforin is limited; sufficient shelf-life could only be achieved by hot maceration of dried flowers with eutanol G and storage in the absence of air. The action of light during preparation of the oil led to a rise in the content of flavonoids. These findings are partially in contrast to more recent data, where hypericine (0.277-6.634 µg/g), pseudohypericine (0.135-3.28 µg/g) and hyperforin (0 – 2.399 µg/g) could be detected in commercial and homemade samples of *hypericum* oil

(Orhan *et al.* 2013). The composition of *hypericum* oil depends also on the kind of the fatty oil used for production (Heinrich *et al.* 2017).

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

Several combinations of herbal preparations of Hyperici herba with other medicinal plants are authorised or registered as medicinal products in member states. However, this assessment report deals with the medicinal use of Hyperici herba as single active ingredient only.

## 1.2. Search and assessment methodology

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

Scientific databases: Scifinder, Scopus, PubMed; search date 2.7.2015; key words: "*Hypericum perforatum*", "St. John's wort", "Hypericin", "Hyperforin"

Other resources: Library of the University of Vienna (Pharmacy and Nutritional Sciences library)

# 2. Data on medicinal use

## 2.1. Information about products on the market

### 2.1.1. Information about products on the market in the EU/EEA Member States

**Information on medicinal products marketed in the EU/EEA**

Table 1: Overview of data obtained from marketed medicinal products

| Active substance | Indication | Pharmaceutical form<br>Posology<br>Duration of use | Regulatory Status |
|---|---|---|---|
| Comminuted herbal substance | Transient mental exhaustion | Herbal tea<br>2-3 x 1.7 - 1.75 g<br>6 weeks | THMP AT 2011, 2015 |
| Comminuted herbal substance | Mild to moderate depressive states, psychovegetative disorders (tenseness, anxiety, mood changes of different origin, digestive disorders, climacteric disorders) | Herbal tea<br>2 x 1.5 g | TU 1996, 1998 CZ |
| Comminuted herbal substance | For the supportive treatment of nervous restlessness and sleep disorders | Herbal tea<br>1-2 teaspoons Hyperici | 1986, DE, Standard Marketing Authorisation according to section 36 |

| Active substance | Indication | Pharmaceutical form<br>Posology<br>Duration of use | Regulatory Status |
|---|---|---|---|
| | | herba /150 ml boiling water<br>2 times daily | of the German Medicinal Products Act |
| Comminuted herbal substance | Traditional herbal medicinal product used in case of slightly low mood and for minor nervous tension | Herbal tea<br>2 x 1.8 g | THMP 2004 SE |
| Comminuted herbal substance | Transient mental exhaustion | Coated tablets<br>3 x 300 mg<br>2 weeks | THMP 2011 AT |
| Comminuted herbal substance | Relief of symptoms of slightly low mood and mild anxiety | Capsules<br>3 x 300 mg | THMP UK |
| Comminuted herbal substance | For the improvement of condition in mental stress | Capsules, hard<br>2 x 500 mg | TU at least since 1976 DE |
| Dry extract, DER 4.6-6.5:1, extraction solvent ethanol 38% m/m (= 45% v/v) | Relief of temporary Mental exhaustion | Single dose: 60-180 mg<br>Daily dose: 180-360 mg | At least 30 years (DE)<br>Historically in combination products, but indication related solely to the content of Hypericum |
| Liquid extract, DER 1:4, ethanol 45% | Relief of symptoms of slightly low mood and mild anxiety. | 2 x 2.5 ml | THMP UK |

| Active substance | Indication | Pharmaceutical form<br>Posology<br>Duration of use | Regulatory Status |
|---|---|---|---|
| Liquid extract, DER 1:2, ethanol 50% V/V | Mild transient depressive conditions | 3 x 30 drops (= 3.6 ml) | DE, at least since 1978, no longer on the market |
| Liquid extract, DER 1:5-7, ethanol 50% V/V | For the improvement of condition in mental stress | 1-3 x 10-20 ml, max. daily dose 30 ml | TU at least since 1976 DE |
| Dry extract, DER 5-7:1, ethanol 50% V/V | Short-term treatment of reactive depressive symptoms and mild to moderate depressive symptoms after exclusion of typical major depressive episodes | Capsules, hard<br>2 x 300 mg | WEU 2004 BE |
| Dry extract, DER 5-7:1, ethanol 50% V/V | Short term treatment of reactive depressive status and mild depressive status after exclusion of a clearly severe depression | Capsules, hard<br>3 x 300 mg | WEU 2002 BE |
| Dry extract, DER 5-8:1, ethanol 50% V/V | Mild to moderate depressive episodes | Film-coated tablets<br>1 x 612 mg | WEU 2005 AT |
| Dry extract, DER 5-8:1, ethanol 50% V/V | Mild to moderate depressive conditions | Coated tablets<br>1 x 612 mg | WEU 2010 SK |
| Dry extract, DER 5-8:1, ethanol 50% V/V | Short term treatment of symptoms in mild depressive disorders | Film-coated tablets<br>1 x 612 mg | WEU 7.2008 HR |
| Dry extract, DER 5-8:1, ethanol 50% V/V | For the treatment of symptoms in mild depressive disorders | Film-coated tablets<br>1 x 612 mg | WEU 2008 LV |
| Dry extract, DER 5-8:1, ethanol 50% V/V | Mild temporary depressive disorders | Capsules, hard | WEU at least since 1976 DE |

| Active substance | Indication | Pharmaceutical form<br>Posology<br>Duration of use | Regulatory Status |
|---|---|---|---|
| | | 2 x 306 mg | |
| Dry extract, DER 5-8:1, ethanol 50% V/V | Mild temporary depressive disorders | Film-coated tablets<br>1 x 612 mg | WEU at least since 1976 DE |
| Dry extract, DER 4-6:1, ethanol 60% m/m | Low mood, psychovegetative disorders | Coated tablets<br>1 x 180 mg<br>4 weeks | THMP 2009 AT |
| Dry extract, DER 4-6:1, ethanol 60% m/m | Relief of symptoms of slightly low mood and mild anxiety. | Coated tablets<br>1 x 180 mg | THMP UK |
| Dry extract, DER 3.5-6:1, ethanol 60% m/m | Relief of symptoms of slightly low mood and mild anxiety. | Tablets<br>1 x 250 mg | THMP UK |
| Dry extract, DER 3.5-6:1, ethanol 60% m/m | Mild temporary depressive disorders | Capsules, hard<br>2-3 x 250 mg | WEU 1998 DE, 1999 DE |
| Dry extract, DER 3.5-6:1, ethanol 60% m/m | Low mood, psychovegetative disorders | Capsules, hard, film-coated tablets<br>1 x 425 - 600 mg<br>6 weeks | Authorization 9.2010 AT, 10.2000 AT, 9.2010 AT |
| Dry extract, DER 3.5-6:1, ethanol 60% | Mild to moderate depressive episodes | Capsules, hard<br>1-2 x 425 mg | Authorization 8.2000 AT, 12.1999 AT |

| Active substance | Indication | Pharmaceutical form<br>Posology<br>Duration of use | Regulatory Status |
|---|---|---|---|
| m/m | | Minimum 4 weeks | |
| Dry extract, DER 3.5-6:1, ethanol 60% m/m | For the short term treatment of symptoms in mild depressive disorders | 1-2 x 425 mg | WEU CZ 2015 |
| Dry extract, DER 3.5-6:1, ethanol 60% m/m | Relief of symptoms of slightly low mood and mild anxiety. | Tablets<br>1 x 425 mg | THMP UK |
| Dry extract, DER 3.5-6:1, ethanol 60% m/m | Relief of symptoms of slightly low mood and mild anxiety. | Tablets<br>1 x 300 mg | THMP UK |
| Dry extract, DER 3.5-6:1, ethanol 60% m/m | Mild temporary depressive disorders | Capsules, hard<br>2 x 425 mg | WEU 1997 DE, 1998 DE, 2012 DE |
| Dry extract, DER 3.5-6:1, ethanol 60% m/m | Mild temporary depressive disorders | Capsules, hard<br>2 x 450 mg | WEU 1998 DE |
| Dry extract, DER 3.5-6:1, ethanol 60% m/m | Mild temporary depressive disorders | Film-coated tablets<br>2 x 325 mg | WEU 2003 DE |
| Dry extract, DER 3.5-6:1, ethanol 60% m/m | Mild temporary depressive disorders | Coated tablets<br>3 x 200 mg | WEU at least since 1976 DE |
| Dry extract, DER 3.5-6:1, ethanol 60% | Treatment of psychosomatic complaints, occurring as mild or moderate | Capsules, hard | WEU 2007 BE |

| Active substance | Indication | Pharmaceutical form<br>Posology<br>Duration of use | Regulatory Status |
|---|---|---|---|
| m/m | forms of depression, after exclusion of every severe pathology | 2 x 425 mg | |
| Dry extract, DER 6-7:1, ethanol 60% m/m | Symptomatic and short term treatment of decay and asthenia states, which occur with loss of interest, fatigue and sleep disturbances | Capsules<br>1-2 x 185 mg | WEU 10.1998 ES |
| Dry extract, DER 6-7:1, ethanol 60% m/m | Mild temporary depressive disorders | Capsules, hard; capsules, soft<br>2 x 185 mg<br>2-3 x 237.5 mg | WEU 1995 DE, WEU 1996 DE |
| Dry extract, DER 4-7:1, ethanol 57.9% V/V | Mild temporary depressive disorders | Film-coated tablets<br>1 x 250 mg | WEU 2003 DE |
| Dry extract, DER 3-6:1, ethanol 60% V/V | Short term treatment of symptoms in mild depressive disorders | Capsules<br>2 x 250 mg | WEU 6.1999 HR |
| Dry extract, DER 4.2-6.5:1, ethanol 60% V/V | Mild temporary depressive disorders | Coated tablets<br>3 x 160 mg | WEU 1995 DE |
| Dry extract, DER 5-7:1, ethanol 60% V/V | Relief of symptoms of slightly low mood and mild anxiety. | Tablets<br>3 x 150 mg | THMP UK |
| Dry extract, DER 5-7:1, ethanol 60% V/V | Relief of symptoms of slightly low mood and mild anxiety. | Tablets<br>1-2 x 170 mg | THMP UK |
| Dry extract, DER 5- | Relief of symptoms of slightly low mood and mild anxiety. | Tablets | THMP UK |

| Active substance | Indication | Pharmaceutical form<br>Posology<br>Duration of use | Regulatory Status |
|---|---|---|---|
| 7:1, ethanol 60% V/V | | 1-2 x 300 mg | |
| Dry extract, DER 5-7:1, ethanol 60% V/V | Relief of symptoms of slightly low mood and mild anxiety. | Tablets<br>1 x 317 mg | THMP UK |
| Dry extract, DER 5-7:1, ethanol 60% V/V | Relief of symptoms of slightly low mood and mild anxiety. | Tablets<br>1-2 x 334 mg | THMP UK |
| Dry extract, DER 5-7:1, ethanol 60% V/V | Relief of symptoms of slightly low mood and mild anxiety. | Tablets<br>1-2 x 370 mg | THMP UK |
| Dry extract, DER 5-7:1, ethanol 60% V/V | Relief of symptoms of slightly low mood and mild anxiety. | Tablets<br>1-2 x 425 mg | THMP UK |
| Dry extract, DER 2.5-5:1, ethanol 70% V/V | Treatment of psychosomatic complaints, occurring as mild or moderate forms of depression, after exclusion of every severe pathology | Film-coated tablets<br>2 x 140 mg | WEU 2002 BE |
| Dry extract, DER 5:1, ethanol 70% V/V | Symptomatic and short term treatment of decay and asthenia states, which occur with loss of interest, fatigue and sleep disturbances | Tablets<br>2-3 x 100 mg | WEU 5.2000 ES |
| Dry extract, DER 5-7:1, ethanol 70% V/V | Mild temporary depressive disorders | Capsules, soft<br>2 x 270 mg | WEU at least since 1976 DE |
| Dry extract, DER 6-7:1, methanol 70% V/V | Symptomatic and short term treatment of decay and asthenia states, which occur with loss of interest, fatigue and sleep disturbances | Tablets<br>1 x 300 mg | WEU 5.1998 ES |
| Dry extract, DER 3- | Treatment of mild to moderate depressive episodes | Film-coated tablets | WEU 7.2008 HR |

| **Active substance** | **Indication** | **Pharmaceutical form**<br>**Posology**<br>**Duration of use** | **Regulatory Status** |
|---|---|---|---|
| 6:1, ethanol 80% V/V | | 1 x 900 mg | |
| Dry extract, DER 3-6:1, ethanol 80% V/V | Moderate temporary depressive disorders (depressive episodes) | Coated tablets<br>3 x 300 mg | WEU 1998 DE |
| Dry extract, DER 3-6:1, ethanol 80% V/V | Mild temporary depressive disorders (depressive episodes) | Coated tablets<br>3 x 300 mg | WEU 1998 DE |
| Dry extract, DER 3-6:1, ethanol 80% V/V | For the treatment of mild to moderate depressive episodes (according to ICD-10) | Film-coated tablets<br>1 x 900 mg | WEU 2004 DE |
| Dry extract, DER 3.5-6:1, ethanol 80% m/m | Mild to moderate depressive episodes of different origin (menopause, convalescence) | Film-coated tablets<br>1 x 900 mg | WEU 2013 – 2016 CZ |
| Dry extract, DER 3-5:1, methanol 80% V/V | Short-term treatment of reactive depressive symptoms and mild to moderate depressive symptoms after exclusion of typical major depressive episodes | Coated tablets<br>3 x 300 mg | WEU 2003 BE, 2012 BE |
| Dry extract, DER 3-6:1, methanol 80% V/V | Mild to moderate depressive episodes | Film-coated tablets, coated tablets<br>2 x 450 mg<br>3 x 300 mg<br>Minimum 4 weeks | Authorization 7.2002 AT, 2.1998 AT |
| Dry extract, DER 3-6:1, methanol 80% V/V | Low mood | Film-coated tablets, coated tablets | Authorization 11.2004 AT, 7.1998 AT |

| Active substance | Indication | Pharmaceutical form<br>Posology<br>Duration of use | Regulatory Status |
|---|---|---|---|
| | | 1 x 450 mg<br>1-2 x 300 mg<br>6 weeks | |
| Dry extract, DER 3-6:1, methanol 80% V/V | Mild to moderate depressive episodes (anxiety, restlessness, sleep disorders) | Coated tablets<br>3 x 300 mg | WEU 1999 – 2015 CZ |
| Dry extract, DER 3-6:1, methanol 80% V/V | Treatment of psychosomatic complaints, occurring as mild or moderate forms of depression, after exclusion of all serious pathologies | Coated tablets<br>3 x 300 mg | WEU 2006 BE |
| Dry extract, DER 3-6:1, methanol 80% V/V | Mild to moderate temporary depressive disorders | Coated tablets<br>3 x 300 mg | WEU 2010 DE |
| Dry extract, DER 3-6:1, methanol 80% V/V | Relief of symptoms of slightly low mood and mild anxiety. | Tablets<br>1 x 450 mg | THMP UK |
| Dry extract, DER 3-6:1, methanol 80% V/V | Mild temporary depressive disorders | Film-coated Tablets<br>1-2 x 450 mg | WEU 2003 DE, 2004 DE |
| Dry extract, DER 3-6:1, methanol 80% V/V | Mild temporary depressive disorders | Coated Tablets<br>2 x 375 mg | WEU 2009 DE |
| Dry extract, DER 3-7:1, methanol 80% | Herbal medicinal product used in slightly low mood, mild anxiety and temporary difficulties in falling asleep | Coated tablets | WEU 1999 SE |

| Active substance | Indication | Pharmaceutical form<br>Posology<br>Duration of use | Regulatory Status |
|---|---|---|---|
| V/V | | 2 x 300 mg | |
| Dry extract, DER 3-7:1, methanol 80% V/V | Mild temporary depressive disorders | Film-coated tablets<br>2 x 300 mg<br>1 x 600 mg | WEU 2003 DE, at least since 1976 DE, 2005 DE |
| Dry extract, DER 3-7:1, methanol 80% V/V | Mild to moderate depressive episodes | Film-coated tablets<br>1 x 600 mg | WEU 2005 DE |
| Dry extract, DER 3-7:1, methanol 80% V/V | Moderate temporary depressive disorders | Film-coated tablets<br>1 x 600 mg | WEU 2006 DE |
| Dry extract, DER 4.1-7.1:1, methanol 80% V/V | For the treatment of mild to moderate depressive episodes | Film coated tablets<br>2-3 x 300 mg | WEU at least since 1976 DE |
| Dry extract from fresh herb, DER 3.1-4:1, ethanol 68% V/V<br><br>Dry extract, DER 3.1-4:1, ethanol 68% V/V = 60% m/m | Herbal medicinal product for the short term treatment of symptoms in mild depressive disorders.<br><br>Relief of symptoms of slightly low mood and mild anxiety. | Tablets<br>3 x 40-73 mg (corresponding to 0.33 mg total hypericines)<br><br>Tablets<br>3 x 40-73 mg | WEU 2.2012 FI<br><br>THMP UK |
| Expressed juice of fresh, flowering herb, | For the relief of temporary mental exhaustion | Expressed juice<br>2 x 10 ml | TU at least since 1976 DE |

| Active substance | Indication | Pharmaceutical form<br>Posology<br>Duration of use | Regulatory Status |
|---|---|---|---|
| DER 1:0.5-0.9 | | | |
| Liquid extract, DER 1:4-20, vegetable oil (Hypericum oil) | Symptomatic treatment of minor inflammations of the skin and as an aid in healing of minor wounds. | Apply in thin layer on the affected area | Available in pharmacies in AT since at least 30 years |
| Liquid extract (DER 1:13), extraction solvent maize oil or other suitable vegetable oil | For the relief of temporary mental exhaustion | Soft capsules<br>3 x 200 mg | TU at least since 1976<br>DE |

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

Several combinations of herbal preparations of Hyperici herba with other medicinal plants are authorised or registered as medicinal products in member states. However, this assessment report deals with the medicinal use of Hyperici herba as single active ingredient only.

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

Not relevant

### 2.1.2. Information on products on the market outside the EU/EEA

Hyperici herba and herbal preparations thereof are world-wide in popular use. However, in important markets outside the EU Hyperici herba is primarily classified as food supplement. Consequently, published information regarding efficacy and safety must be interpreted carefully as usually limited data on the quality and quantity of the herbal preparations are available.

## *2.2. Information on documented medicinal use and historical data from literature*

Table 2: Overview of historical data

| Herbal preparation | Documented use / Traditional use | Pharmaceutical form<br>Posology<br>Duration of use | Reference |
| --- | --- | --- | --- |
| Comminuted herbal substance | Among others: neurasthenia, sleep disorders, nervous complaints, depression<br><br>Nervous restlessness, sleep disorders<br><br>Psychovegetative disorders, mood depression, anxiety, nervous restlessness<br><br>Haemorrhoids, catarrhs of the gastrointestinal tract, disorders of the gall, kidney or bladder, heart insufficiency, ailments of the airways, enuresis nocturna even in children, depressive mood, sleep disorders, menstrual and climacteric complaints, diabetes<br><br>Mild depression, support of emotional balance<br><br>Mild gastrointestinal discomfort | Herbal tea, oral use<br><br>30 ml of infusion (1:20)<br><br>1.8 – 3.6 g, 2 times daily<br><br>1-2 g, 2 times daily<br><br>1 spoon, 3 times daily<br><br>2-4 g daily<br><br>2 g, 2 times daily | List & Hörhammer 1976<br><br>Wichtl 1984<br><br>Hänsel *et al.* 1993<br><br>Irion 1955<br><br>ESCOP 2003, Barnes *et al.* 2002<br><br>Ozarowsky *et al.* 1978 |
| Liquid extract (DER 1:4-20), extraction solvent vegetable oil | Dyspeptic complaints<br><br>Increase of bile flow | Oral use<br><br>Daily dose corresponding to 2-4 g herbal substance | Wichtl 1984<br><br>Hänsel *et al.* 1993 |

| Herbal preparation | Documented use / Traditional use | Pharmaceutical form<br>Posology<br>Duration of use | Reference |
|---|---|---|---|
| | Nervous stomach, gastritis<br><br>Gout, rheumatic complaints, bruises, sprains | (= 8-80 g *hypericum* oil)<br><br>6-8 drops | Böhme *et al.* 2006<br>Gerlach 2008<br>Madaus 1938 |
| Liquid extract (DER 1:4-20), extraction solvent vegetable oil | Injuries, myalgia, first grade burn wounds<br>Rheumatic complaints<br>Lumbago | Cutaneous use<br>Apply undiluted | Madaus 1938<br>Auster 1958<br>Wichtl 1984<br>Hänsel *et al.* 1993<br>Gerlach 2008 |
| Tincture (DER 1:10), extraction solvent ethanol 45-50% V/V | Oral use:<br>Relief of temporary mental exhaustion<br>Cutaneous use:<br>Symptomatic treatment of minor inflammations of the skin (such as sunburn) and as an aid in healing of minor wounds | 10-15 drops, 2-3 times daily<br><br>2-4 ml, 3 x daily<br><br>Apply undiluted to the affected area | Madaus 1983<br><br>Barnes *et al.* 2002<br><br>Irion 1955, Barnes *et al.* 2002, Gruenwald *et al.* 2004 |
| Tincture (DER 1:5), extraction solvent ethanol 50% -70%v/v | Oral use:<br>Relief of temporary mental exhaustion.<br>Cutaneous use:<br>Symptomatic treatment of minor inflammations of the skin (such as | 1-1.5 ml, 3 x daily<br><br>Apply undiluted to the | Irion 955, Hänsel *et al.* 1993, Bradley 2006 |

| Herbal preparation | Documented use / Traditional use | Pharmaceutical form<br>Posology<br>Duration of use | Reference |
|---|---|---|---|
| | sunburn) and as an aid in healing of minor wounds | affected area | |

## *2.3. Overall conclusions on medicinal use*

The historical data on specific extraction solvents and drug-extract-ratios are poor, also due to the fact that usually extracts have been defined by a certain standardised content of hypericin. From this content a calculation of a DER is not possible. Consequently, the proposed specifications of the traditionally used extracts mentioned below must be interpreted in a broader way. Traditional Herbal Medicinal Products (THMPs) nationally registered according to Dir. 2001/83 as amended are only considered for the monograph if the evidence of the traditional medicinal use is found in the public domain.

Infusions prepared with water are widely used in traditional medicine at least in Central Europe (Gerlach 2008). The indication 'depression' is unknown in traditional medicine; *Hypericum* is used in order to 'strengthen the nerves', to restore emotional balance. The wording which is found in literature reflects this fact, although put into different words.

Since for the indication of a THMP terms like 'depression' or 'depressed mood' are not suitable, special care is taken to the wording. The traditional use seems to be covered in a most suitable way by the definition of 'neurasthenia'. However, the very broad definition also includes symptoms which should be treated under medical supervision. Therefore the indication should be restricted to 'temporary mental exhaustion'.

The indications 'supportive treatment of nervous restlessness and associated sleep disorders', 'mild gastrointestinal discomfort' (both oral use) and 'symptomatic treatment of minor inflammations of the skin' (such as sunburn) and 'as an aid in healing of minor wounds' (cutaneous use) are supported by adequate references.

The numerous further traditional indications mentioned in the literature for liquid extracts and the herbal tea are not plausible due to the fact that no medicinal use is documented throughout 30 years back.

Table 3: Overview of evidence on period of medicinal use

| Herbal preparation Pharmaceutical form | Indication | Posology, Strength | Period of medicinal use |
|---|---|---|---|
| Traditional use | | | |
| Dry extract, DER 4.6-6.5:1, extraction solvent ethanol 38% m/m (= 45% v/v.<br><br>TU a) | Oral use<br><br>Relief of temporary mental exhaustion | Single dose: 60-180 mg<br><br>Daily dose: 180-360 mg | At least 30 years (DE), no concrete authorisation date available |
| Liquid extract (DER 1:4-20), extraction solvent vegetable oil (e.g. olive oil, sunflower oil, linseed oil, wheat germ oil) (Synonym: Hyperici oleum)<br><br>TU b) | Cutaneous use<br><br>Symptomatic treatment of minor inflammations of the skin (such as sunburn) and as an aid in healing of minor wounds | Apply undiluted to the affected area | At least 30 years (AT), no concrete authorisation date available<br><br>Wichtl 1984 |
| Liquid extract (DER 1:13), extraction solvent maize oil<br><br>TU c) | Oral use<br><br>Relief of temporary mental exhaustion | 3 x 200 mg | Since 1976 (DE) |
| Tincture (DER 1:10), extraction solvent ethanol 45-50% v/v<br><br>TU d) | Oral use:<br><br>Relief of temporary mental exhaustion<br><br>Cutaneous use:<br><br>Symptomatic treatment of minor inflammations of the skin (such as | 2-4 ml, 3 x daily<br><br>Apply undiluted to the | According to references Madaus (1983), Irion (1955), Barnes *et al.* (2002) and Gruenwald *et al.* (2004) |

| Herbal preparation Pharmaceutical form | Indication | Posology, Strength | Period of medicinal use |
|---|---|---|---|
| | sunburn) and as an aid in healing of minor wounds | affected area | |
| Tincture (DER 1:5), extraction solvent ethanol 50% -70%v/v<br>Included in TU e) | Oral use:<br>Relief of temporary mental exhaustion.<br>Cutaneous use:<br>Symptomatic treatment of minor inflammations of the skin (such as sunburn) and as an aid in healing of minor wounds | 1-1.5 ml, 3 x daily<br>Apply undiluted to the affected area | According to reference Bradley (2006)<br>Irion (1955) |
| Liquid extract DER 1:2, extraction solvent ethanol 50% v/v<br>Included in TU e) | Oral use:<br>Relief of temporary mental exhaustion | 0.8-1.2 ml, 3 x daily | Since 1978 (DE) |
| Liquid extract (DER 1:5-7), extraction solvent ethanol 50% v/v<br>Included in TU e) | Oral use:<br>Relief of temporary mental exhaustion | 1.3 ml, 3 x daily | Since 1976 (DE) |
| Expressed juice of fresh, flowering herb, DER 1:0.5-0.9 | Oral use:<br>Relief of temporary mental exhaustion | 10-20 ml, 1-3 x daily (max. 30 ml daily) | Since 1976 (DE) |

| Herbal preparation Pharmaceutical form | Indication | Posology, Strength | Period of medicinal use |
|---|---|---|---|
| TU f) | | | |
| Comminuted herbal substance as herbal tea<br><br>TU g) | Oral use:<br><br>Relief of temporary mental exhaustion<br><br>Oral use:<br><br>Symptomatic relief of mild gastrointestinal discomfort<br><br>Oral use:<br><br>For the supportive treatment of nervous restlessness and sleep disorders | 1.5-2 g, 2-3 x daily<br><br>2 g, 2 x daily<br><br>1-2 teaspoons (= 2-3 g) / 150 ml boiling water, 2 times daily | Irion (1955), List & Hörhammer (1976)<br><br>Ozarowsky et al. 1978<br><br>Since 1986 (DE) |
| Powdered herbal substance<br><br>TU h) | Oral use:<br><br>Relief of temporary mental exhaustion | 300 – 500 mg, 2-3 x daily (max. 1000 mg daily) | Since 1976 (DE) |
| Well-established use | | | |
| Dry extract (DER 3-7:1), extraction solvent methanol (80% v/v)<br><br>WEU a) | Herbal medicinal product for the treatment of mild to moderate depressive episodes (according to ICD-10) | 300 – 600 mg, daily dose 600 – 1800 mg | Several member states, at least since 1976 |
| Dry extract (DER 3- | Herbal medicinal product for the treatment of mild to moderate | Single = daily dose 900 | Several member |

| Herbal preparation Pharmaceutical form | Indication | Posology, Strength | Period of medicinal use |
|---|---|---|---|
| 6:1), extraction solvent ethanol (80% v/v)<br><br>WEU b) | depressive episodes (according to ICD-10) | mg | states, at least since 1998 |
| Dry extract (DER 2.5-8:1), extraction solvent ethanol (50-68% v/v)<br><br>WEU c) | Herbal medicinal product for the short term treatment of symptoms in mild depressive disorders | 600 or 612 mg 1 x daily<br><br>Or<br><br>250 – 600 mg, 2-3 x daily<br><br>Daily dose 500-1200 mg | Several member states, at least since 1976 |

# 3. Non-Clinical Data

## *3.1. Overview of available pharmacological data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof*

### 3.1.1. Primary pharmacodynamics

#### *3.1.1.1. Primary pharmacodynamics related to the treatment of depression*

Many pharmacological studies have been conducted with extracts and isolated constituents of *Hypericum perforatum in-vivo* and *in-vitro*.

The mechanisms of action as well as the responsible compounds of *Hypericum* extracts are still under discussion. Several actions contributing to clinical efficacy are reported (overview e.g. in the reviews of Butterweck & Nahrstedt 2003, Schmidt & Butterweck 2015): Blockade of the reuptake of serotonin (5-HT), noradrenaline and dopamine; upregulation of postsynaptic 5-HT1 and 5-HT2 receptors and of dopaminergic receptors; increased affinity for GABAergic receptors. Constituents which may contribute to the activity are hypericin, pseudohypericin, flavonoids, and oligomeric procyanidins. The relevance of hyperforin is discussed controversially. As a consequence the entire extract has to be considered as the active substance.

Table 4: Overview of the non-clinical data related to indication 'depression'

| **Herbal preparation tested** | **Strength**<br>**Dosage**<br>**Route of administration** | **Experimental model**<br>***In vivo/***<br>***In vitro*** | **Reference**<br>**Year of publication** | **Main non-clinical conclusions of the authors** |
|---|---|---|---|---|
| Herbal substance | 350 mg/kg/day for 3 weeks | *In vivo* (rats)<br>Chronic restraint stress, prolonged corticosterone administration<br>Spatial working memory tested in Barnes maze and Morris water maze | Trofimiuk *et al.* 2008 | H. perforatum prevented the deleterious effects of both chronic restraint stress and prolonged corticosterone on working memory measured in both tests. The herb significantly improved hippocampus dependent spatial working memory in comparison with control ($p < 0.01$) and alleviated some other negative effects of stress on cognitive functions |
| Comminuted herbal substance | 4.3 and 13 µg/kg<br>8 weeks of treatment | *In vivo*<br>Behavioural testing of rats in the circular water maze | Widy-Tyszkiewicz *et al.* 2002 | The mean escape latency was significantly reduced with the dosage containing 13 µg/kg hypericin. After completion of the behavioural experiments significantly increased 5-HT levels in the prefrontal cortex were found |
| Hyperici herba, 0.3% hypericin | 4.3 and 13 µg/kg for 9 weeks, oral | *In vivo*<br>Water maze | Widy-Tyszkiewicz *et al.* 2002 | The mean escape latency over 4 days for the control group (21.9 s) and HP 4.3 group (21.7 s) was significantly greater than the latency of the HP 13 group (15.8s). In the probe trial on day 5, the HP 13 group crossed the correct annulus in the SE quadrant more often (4.5) than the other groups: Con (2.4) and HP 4.3 (3.1). |

| Herbal preparation tested | Strength<br>Dosage<br>Route of administration | Experimental model<br>***In vivo/***<br>***In vitro*** | Reference<br>Year of publication | Main non-clinical conclusions of the authors |
|---|---|---|---|---|
| | | | | Significant differences in the content of monoamines and metabolites in several brain regions between treatment groups compared to control were detected. Increased 5-HT levels in the prefrontal cortex correlated with the retention of spatial memory |
| Dry extract (DER 4-7:1, methanol 80% V/V) | 240 mg/kg orally for 14 days | *In vitro*<br>MAO-A and MAO-B assays<br>Synaptosomal uptake assays<br><br>*In vivo*<br>Receptor binding test with rats | Müller *et al.* 1997 | *Hypericum* extract was a weak inhibitor of MAO-A and MAO-B activity. At 2µg/ml the synaptosomal uptake of serotonin, dopamine and norepinephrine was inhibited equally.<br>Subchronic treatment of rats led to a significant down-regulation of ß-receptors and to a significant up-regulation of 5-HT2 receptors in the frontal cortex |
| Dry extract (DER 4-7:1, methanol 80% V/V) | 1 – 50 µg/ml | *In vitro*<br>Astrocyte cultures from neonatal rat cerebral cortices | Neary & Bu 1999 | *Hypericum* extract inhibited serotonin and norepinephrine uptake in a dose-dependent manner. |
| Dry extract (DER 4-7:1, methanol 80% V/V, 4.67% | Methanol extract: 31.25 – 125 mg/kg<br>$CO_2$ extract: 2.42-9.86 mg/kg | *In vivo*<br>Rats<br>*In vivo* microdialysis in shell region of nucleus | Rommelspacher *et al.* 2001 | Both extracts induced after acute administration an increase in dopamine and 5-HT levels. The dose-response followed an inverse U-shape. Repeated application caused a rapid tolerance of dopamine but not |

| Herbal preparation tested | Strength<br>Dosage<br>Route of administration | Experimental model<br><br>*In vivo/*<br>*In vitro* | Reference<br><br>Year of publication | Main non-clinical conclusions of the authors |
|---|---|---|---|---|
| hyperforin)<br><br>$CO_2$-extract (30.14% hyperforin) | Oral | accumbens | | of 5-HT neurons. |
| Dry extract (DER 4-7:1, methanol 80% V/V), 1.5% hyperforin<br><br>CO2 extract, 38.8% hyperforin<br><br>Pure hyperforin | 240 mg/kg orally for 14 days | *In vitro*<br><br>MAO-A and MAO-B assays<br><br>Synaptosomal uptake assays<br><br>*In vivo*<br><br>Receptor binding test with rats | Müller *et al.* 1998 | Hyperforin was identified as reuptake inhibitor for the synaptosomal uptake of serotonin, norepinephrine and dopamine with half-maximal inhibitory concentrations between 80 and 200 nmol/l. After subchronic treatment the CO2 extract led to a significant down-regulation of ß-receptors. |
| Dry extract (ethanol, no further details) | 0.4-400 µg/ml | *In vitro*<br><br>Effects of extracts on naloxone binding to the µ and κ-opioid receptors<br><br>Semliki Forest virus system | Simmen *et al.* 1998 | $IC_{50}$ values of *Hypericum* extract 25 µg/ml at the µ-receptor, 90 µg/ml at the κ-receptor. Isolated flavonoids did not inhibit binding upt o a concentration of 10 µM. |
| Dry extracts (DER 3-7:1, | 6.25 – 100 µg/ml | *In vitro* | Enning *et al.* 2011 | In a small concentration range the glucocorticoid receptor α mRNA was primarily and transiently up- |

| Herbal preparation tested | Strength<br>Dosage<br>Route of administration | Experimental model<br>*In vivo/*<br>*In vitro* | Reference<br>Year of publication | Main non-clinical conclusions of the authors |
|---|---|---|---|---|
| methanol 80% V/V) | 300 µl per plate | Human monocytic U-937 cells<br>Corticosteroid receptor mRNA expression | | regulated, after 16 hours of treatment the mRNA for the ß-receptor was down-regulated. The sodium channel is involved in these effects. |
| Dry extract (Ze 117, DER 4-7:1, ethanol 50% m/m); hyperforin <0.02%, hypericins 0.212%, rutin 0.93%, hyperosid 0.69%, quercetin 0.13%, quercitrin 0.11%, biapigenin 0.048% | Equivalent to 5µM hypericin in the media | *In vitro*<br>C6 cells tissue culture | DeMarchis *et al.* 2006 | Receptor down-regulation by the extract was inhibited in the presence of α-Tocopherol suggesting a a mode of action of *Hypericum* comparable to synthetic antidepressants |
| Dry extract (Ze 117, DER 4-7:1, ethanol 50% m/m); hyperforin | | *In vitro*<br>Rat brain slices<br>C6 cells tissue culture | Kientsch *et al.* 2001 | A dose-dependent inhibition was seen on norepinephrine (NE) and serotonin (5-HT) uptake into brain slices. The Ze 117 extract was more selective for the uptake of NE than for that of 5-HT. The maximal extent of uptake inhibition by Ze 117 extract was comparable to that of |

| Herbal preparation tested | Strength<br>Dosage<br>Route of administration | Experimental model<br>*In vivo/*<br>*In vitro* | Reference<br>Year of publication | Main non-clinical conclusions of the authors |
|---|---|---|---|---|
| <0.02% | | | | imipramine (IMI), desipramine (DMI) or fluvoxamine for 5-HT, but lower for NE transport, than that of the synthetic antidepressants. Chronic exposure (8 days) of confluent C 6-cell cultures to Ze 117 extract resulted in a dose-dependent β-adrenoceptor downregulation equal to that induced by DMI. None of these effects could be achieved with either hypericin or hyperforin alone in a relevant dose range |
| Dry extract (ethanol 50%, DER 4-7:1), 0.2% hypericin, less than 0.1% hyperforin | $10 – 10^4$ µg/ml | *In vitro*<br>Rat cortical brain slices | Ruedeberg *et al.* 2010 | Dopamin uptake was inhibited in a dose dependent manner<br>The uptake inhibition of noradrenalin was strongest, for serotonin lowest, for dopamine in the middle range |
| Dry extracts (DER 4.6-6.5:1, ethanol 38% m/m) | *In vivo*: 180 and 360 mg/kg<br>Also in combination with a dry extract of Passiflorae herba (DER 6.25-7.1:1, ethanol 60% m/m) | *In vitro*<br>Serotinin re-uptake<br>*In vivo*<br>Sprague-Dawley rats<br>Forced swimming test<br>Open field test | Fiebich *et al.* 2011 | $IC_{50}$ values for serotononin uptake:<br>Hyperforin rich extract (2.7%): 13.0 µg/ml<br>Hyperforin poor extract (1.1%): 88.2 µg/ml<br>Combination with passionflower extract reduces the $IC_{50}$ to 9.7 and 14.0 µg (ml respectively).<br>*Hypericum* extracts (180 mg/kg, 360 mg/kg) exerted similar effects *in vivo* compared to imipramine (30 mg/kg). The effects were more pronounced when *Hypericum* was combined with Passiflora |

| **Herbal preparation tested** | **Strength**<br>**Dosage**<br>**Route of administration** | **Experimental model**<br><br>***In vivo/***<br>***In vitro*** | **Reference**<br><br>**Year of publication** | **Main non-clinical conclusions of the authors** |
|---|---|---|---|---|
| Dry extract (ethanol 80%, no further information)<br><br>Hypericin<br><br>Hyperforin | Extract: 50 µg/ml<br><br>Hyperforin 10 µM<br><br>Hypericin 1 µM | *In vitro*<br><br>Live cell calcium imaging<br><br>Glutamatergic neurotransmission within the neurons of the solitary tract | Vance *et al.* 2014 | The extract increased the intracellular calcium levels of stimulated vagal afferent terminals compared with the bath control. This increase in presynaptic vagal afferent calcium by the extract coincided with an increase in neurotransmitter release within the nucleus of the solitary tract, as the frequency of mEPSCs was significantly higher in the presence of the extract compared with the control. Hyperforin also significantly increased terminal calcium levels. |
| Dry extract (DER 4-7:1, methanol 80% V/V) | Oral administration by gavage | *In vivo*<br><br>Male and female mice<br><br>Male rats<br><br>Induced sleeping time<br><br>Forced swimming test<br><br>Tail suspension test | Butterweck *et al.* 1997 | 500 mg/kg *Hypericum* extract reduces the ketamine induced sleeping time in the same extent as 20 mg/kg bupropion.<br><br>500 mg/kg *Hypericum* extract causes a significant decrease of immobility time in the tail suspension test.<br><br>125-1000 mg/kg *Hypericum* extract reduces the period of immobility in the forced swimming test similar to 20 mg/kg bupropion. This effect was countermanded completely by addition of haloperidol or sulpiride indicating the involvement of the dopaminergic system. |
| Flavonoid rich fractions | 0.9-10.05 mg/kg, oral<br><br>Acute treatment, 12 days treatment | *In vivo*<br><br>Forced swimming test<br><br>Open field test | Butterweck *et al.* 2000 | Fractions containing mainly hyperoside, isoquercitrin, miquellianin, quercitrin and astilbin were active in the forced swimming test after acute treatment. Isolated hyperoside and miquellianin were also active. Validity of results was confirmed in open field experiments and in the forced swimming test after 12 days treatment |

| Herbal preparation tested | Strength<br>Dosage<br>Route of administration | Experimental model<br>*In vivo/*<br>*In vitro* | Reference<br>Year of publication | Main non-clinical conclusions of the authors |
|---|---|---|---|---|
| Dry extract (DER 4-7:1, methanol 80% V/V)<br>Hypericin | Extract: 500 mg/kg p.o.<br>Hypericin: 0.2 mg/kg p.o.<br>2 weeks, 8 weeks | *In vivo*<br>Serotonin, norepinephrine and dopamine levels in rat brain | Butterweck *et al.* 2002a | Imipramine (15 mg/ kg, p.o.), *Hypericum* extract (500 mg/ kg, p.o.), and hypericin (0.2 mg/ kg, p.o.) given daily for 8 weeks significantly increased 5-HT levels in the hypothalamus (P,0.05). The 5-HT turnover was significantly lowered in both brain regions after 8 weeks of daily treatment with the *Hypericum* extract (both P,0.05). Consistent changes in catecholamine levels were only detected in hypothalamic tissues after long-term treatment. Comparable to imipramine, *Hypericum* extract as well as hypericin significantly decreased 3,4-dihydroxyphenylacetic acid and homovanillic acid levels in the hypothalamus (P,0.01) |
| Dry extracts (DER 3-7:1, methanol 80% V/V, DER 2.5-5:1, ethanol 60% V/V)<br>Rutin | Oral administration by gavage | *In vivo*<br>Forced swimming test with male rats<br>Locomotor activity in rats | Nöldner & Schötz 2002 | Several extracts showed efficacy in the forced swimming test. Only an extract with a reduced content of rutin had no effect. Addition of rutin to this extract resulted in a strong pharmacological effect. This effect is not dose-dependent. Rutin must be present above a threshold limit. A concentration of 3% in the extract was sufficient, while 1% did not alter the pharmacological effect |
| Dry extracts (DER 3-7:1, methanol 80% V/V) | 2700 mg/kg orally for 26 weeks | *In vivo*<br>Receptor binding assay in rat brain | Teufel-Mayer & Gleitz 1997 | Number of 5-HT2A and 5-HT2B receptors significantly increased by 50% compared to control. The affinity of both receptors remained unaltered |
| Dry extracts (DER 3-7:1, | 150 – 500 mg/kg, oral, in | *In vivo* | Beijamini & | All doses significantly reduced the immobility time. |

| Herbal preparation tested | Strength<br>Dosage<br>Route of administration | Experimental model<br><br>*In vivo/*<br>*In vitro* | Reference<br><br>Year of publication | Main non-clinical conclusions of the authors |
|---|---|---|---|---|
| methanol 80% V/V) | 3 portions<br><br>Elevated T-maze: up to 300 mg/kg oral, 7 days | Forced swimming test<br><br>Elevated T-maze<br><br>Open field test | Andreatini 2003 | Subacute treatment (300 mg/kg) exerts a partial anxiolytic-like effect in the inhibitory avoidance task. Repeated administration of 300 mg/kg induced an anxiolytic effect (decreased inhibitory avoidance) and an antipanic effect (increased one-way escape). No effect on locomotor activity was found with any treatment |
| Dry extracts (DER 4-7:1, methanol 80% V/V) | 62.5-500 mg/kg, oral<br><br>Acute treatment<br><br>62.5- 250 mg/kg, oral<br><br>14 days | *In vivo*<br><br>Elevated T-maze<br><br>Light/dark transition<br><br>Cat odour test | Flausino *et al.* 2002 | Acute treatment (125 mg/kg) impaired elevated T-maze inhibitory avoidance, an anxiolytic effect, without altering escape performance. Chronic treatment (250 mg/kg, 14 days) enhanced avoidance latencies only in animals that were preexposed to the open arms of the maze. Preexposure shortens escape latency, improving it as an escape index. Differently from the reference drug imipramine (15 mg/kg), chronic *Hypericum* treatment did not impair escape from the open arms of the maze. The extract increased the number of transitions between the two compartments in the light/dark transition model |
| Dry extracts (DER 4-7:1, methanol 80% V/V), 5.3% hyperforin<br><br>$CO_2$ extract (26.2% | Oral<br><br>Methanolic extract: 500 mg/kg<br><br>Hypericin 0.2 mg/kg<br><br>Hyperoside 0.6 mg/kg<br><br>CO2 extract: 27 mg/kg | *In vivo*<br><br>Radioligand receptor binding studies<br><br>2 weeks, 8 weeks | Simbrey *et al.* 2004 | The $CO_2$ extract decreased beta-AR-binding (13%) after two weeks and slightly increased the number of β-receptors after 8 weeks (9%). Short-term treatment with the methanolic *Hypericum* extract decreased β -receptor-binding (14%), no effects for this extract were observed after 8 weeks. Treatment with hypericin led to a significant down-regulation (13%) of β -receptors in the frontal cortex after 8-weeks, but not after 2 weeks, |

| Herbal preparation tested | Strength<br>Dosage<br>Route of administration | Experimental model<br>***In vivo/***<br>***In vitro*** | Reference<br>Year of publication | Main non-clinical conclusions of the authors |
|---|---|---|---|---|
| hyperforin)<br>Hypericin<br>Hyperoside<br>Hyperforin-trimethoxybenzoate | Hyperforin-trimethoxybenzoate 8 mg/kg | | | while hyperforin (used as trimethoxybenzoate), and hyperoside were ineffective in both treatment paradigms. Compared to the *Hypericum* extracts and single compounds the effect of imipramine on b -receptor-binding was more pronounced in both treatment paradigms |
| Dry extracts (DER 3-7:1, methanol 80% V/V)<br>Dry extracts (DER 4-7:1, ethanol 50% m/m) | i.p.<br>Acute administration:<br>Imipramine 0-30 mg/kg<br>Fluoxetine 0-30 mg/kg<br>Extracts 0-40 mg/kg<br>Subacute administration:<br>Up to 10 mg/kg | *In vivo*<br>Rat forced swimming model<br>cAA rat model of alcoholism | De Vry *et al.* 1999 | Rat forced swimming model:<br>Minimal effective dose: imipramine 30 mg/kg; fluoxetine 10 mg/kg; *Hypericum* extracts 20 mg/kg.<br>The anti-immobility effects were more pronounced after subacute treatment.<br>cAA model of alcoholism:<br>For all substances a dose dependent reduced alcohol intake was observed.<br>Minimal effective dose: imipramine 30 mg/kg; fluoxetine 5 mg/kg; *Hypericum* extracts 20 mg/kg |
| Dry extracts (DER 3-7:1, methanol 80% V/V) containing | Oral<br>Acute administration<br>Comparison with fluoxetine | *In vivo*<br>Male Sprague-Dawley rats Levels of neurotransmitters in | Calapai *et al.* 1999 | *Hypericum* extracts (25-500 mg/kg) and fluoxetine (10-80 mg/kg) induced a significant increase of 5-hydroxytraptamine content in the cortex. Both *Hypericum* extracts increased noradrenaline and dopamine in the diencephalon. In the brainstem only the |

| Herbal preparation tested | Strength<br>Dosage<br>Route of administration | Experimental model<br>*In vivo/*<br>*In vitro* | Reference<br>Year of publication | Main non-clinical conclusions of the authors |
|---|---|---|---|---|
| 6% flavonoids<br>Dry extracts (DER 4-7:1, ethanol 50% m/m) containing 50% flavonoids | | different regions of the brain | | extract rich in flavonoids was able to increase the noradrenaline content.<br>The authors conclude that extracts with a higher content of flavonoids may act in a broader sense |
| Dry extract (DER 3-7:1, methanol 80% V/V)<br>Liquid extract (DER 1:2, ethanol 50% [no further information]) | Dry extract 15-600 mg/kg, p.o.<br>Liquid extract 0.5-4.0 ml, i.v. | *In vivo*<br>Male cats<br>Recording of electric potentials | Fornal *et al.* 2001 | *Hypericum* extracts had no effect on the neuronal activity while 2 mg/kg p.o. of fluoxetine and sertraline markedly depressed the neuronal activity |
| Dry extract (DER 3-7:1, methanol 80% V/V)<br>Dry extract (DER 2.5-5:1, ethanol 60% V/V) | 30 – 300 mg/kg, 7 days, oral | *In vivo*<br>Forced swimming test in rats | Nöldner & Schötz 2002 | Ethanolic extract: dose dependent reduction of immobility time.<br>Methanolic extract: inactive; after addition of rutin comparable effect to the ethanolic extract |

| Herbal preparation tested | Strength<br>Dosage<br>Route of administration | Experimental model<br>*In vivo/*<br>*In vitro* | Reference<br>Year of publication | Main non-clinical conclusions of the authors |
|---|---|---|---|---|
| Dry extract (DER 2.5-5:1, ethanol 60% V/V) | Oral administration 9 days<br>12.5-800 mg/kg extract<br>3-30 mg/kg rutin<br>3-10 mg/kg isorhamnetin | *In vivo*<br>Forced swimming test in rats | Paulke *et al.* 2008 | From 200 mg/kg up the extract showed the same activity as 30 mg/kg imipramine.<br>48 mg/kg rutin showed the same activity as 30 mg/kg imipramine.<br>The metabolite isorhamentin showed the strongest activity. 3-10 mg/kg were more active than the same dosage of imipramine |
| Dry extract (DER 3-6:1, ethanol 80% V/V), 0.3% hypericin, 4% hyperforin, 9.4% flavonoids | Oral by feeding needle<br>125 – 1000 mg/kg | *In vivo*<br>Induced stress in mice | Grundmann *et al.* 2006 | *Hypericum* extract 250 and 500 mg/kg reduced the body temperature during the test significantly. Synthetic antidepressants did not show an effect in contrast to synthetic anxiolytics. Hypericin (0.1 mg/kg) was also active while hyperforin (1-10 mg/kg) had no effect. From the flavonoids miquelianin (1.2 mg/kg, p.o.) was the most potent compound |
| Dry extract (DER 3-6:1, ethanol 80%, STW3-VI) | 125 – 750 mg/kg p.o. | *In vivo*<br>Male Sprague-Dawley rats<br>Restrained stress conditions<br>Open field test | Grundmann *et al.* 2010 | Stressed animals decreased in open field activity compared to unstressed animals, which could be reversed by fluoxetine (10 mg/kg, p.o.) and *Hypericum* extract (125-750 mg/kg, p.o.) treatment. In addition, chronic restraint stress significantly decreased thymus and spleen indices in the stressed control group. However, treating stressed rats with fluoxetine or *Hypericum* extract produced a significant and dose dependent increase in both thymus and spleen indices compared to stressed controls. Additionally, *Hypericum* and fluoxetine significantly reduced stressinduced |

| Herbal preparation tested | Strength<br>Dosage<br>Route of administration | Experimental model<br>*In vivo/*<br>*In vitro* | Reference<br>Year of publication | Main non-clinical conclusions of the authors |
|---|---|---|---|---|
| | | | | increases in plasma ACTH and corticosterone levels. Furthermore, the administration of *Hypericum* extract significantly reduced the stress-induced increase in TNF-α levels |
| Dry extract (ethanol 80%), 0.2% hypericin, 2% hyperforin, 13.3% folavonoids | 250, 500 mg/kg, p.o.<br>Fluoxetine 10 mg/kg p.o. | *In vivo*, rats<br>Gene expression in hypothalamic and hippocampal tissues | Jungke *et al.* 2011 | Similarities and differences between *Hypericum* and fluoxetine are described |
| Dry extract (ethanol 80% V/V, 12:1)<br>Hypericin<br>Hyperforin | Extract: 500 mg/kg p.o.<br>Hypericin: 1.25 mg/kg p.o.<br>Hyperforin: 2.14 mg/kg p.o. | *In vivo* (rats, mice)<br>Acoustic startle response test | Tadros *et al.* 2009a | Prepulse inhibition (PPI) disruption was prevented after blocking the serotoninergic 5-HT1A and 5-HT2A, alpha-adrenergic and dopaminergic D1 receptors. Results also demonstrated a significant PPI deficit after acute treatment of rats with hyperforin, and not hypericin. In some conditions manifesting disrupted PPI response, apoptosis coexists. Electrophoresis of DNA isolated from brains of hyperforin-treated animals revealed absence of any abnormal DNA fragmentation patterns. It is concluded that serotoninergic 5-HT1A and 5-HT2A, alpha-adrenergic and dopaminergic D1 receptors are involved in the disruptive effect of St. John's wort extract on PPI response in rats. Hyperforin, and not hypericin, is one of the active ingredients responsible for St. John's wort-induced PPI disruption with no relation to apoptotic processes |

| Herbal preparation tested | Strength<br>Dosage<br>Route of administration | Experimental model<br><br>***In vivo/***<br>***In vitro*** | Reference<br><br>Year of publication | Main non-clinical conclusions of the authors |
|---|---|---|---|---|
| Aqueous extract (no further information); extract free of hyperforin, total hypericins 0.16% m/m | 5-100 mg/kg | *In vivo*<br><br>Male Charles River mice<br><br>Elevated plus maze test, open field test, horizontal wire test | Coleta *et al.* 2001 | Significant raise in immobility time |
| Methanolic extract (no further information); 0.34% hypericin, 4.1% hyperforin, 5% flavonoids | 30 mg/kg | *In vivo*<br><br>Male CD1 mice<br><br>7 weeks corticosterone administration (5 mg/kg/day); after 4 weeks one group received *Hypericum* i.p. for further 3 weeks | Crupi *et al.* 2011 | The anxiety/depressive-like state due to chronic corticosterone treatment was reversed by administration of Hypericum perforatum; the proliferation of progenitor cells in mice hippocampus was significantly reduced under chronic corticosterone treatment, whereas a long term treatment with *Hypericum perforatum* prevented the corticosterone-induced decrease in hippocampal cell proliferation. Corticosterone-treated mice exhibited a reduced spine density that was ameliorated by *Hypericum perforatum* administration. |
| Hydromethanolic extract (no details), $CO_2$ extract, hypericin, hyperforin, biapigenin | 10 µg/ml | *In vitro*<br><br>Binding assay | Gobbi *et al.* 2001 | The methanolic extract (4.5% hyperforin) interacted with a GABA A receptor, an extract rich in hyperforin did not show an interaction. Data on the inhibition of specific bindings to the dopamine transporters indicate that the hyperforin content cannot explain effects of extracts on receptors. |
| Ethanolic extract (no | 0.1-1000 µg/ml | *In vitro* | Simmen *et al.* 1998 | *Hypericum* extract inhibited the binding of naloxone to the µ- and k-opioid receptor ($IC_{50}$ values 25 and 90 |

| Herbal preparation tested | Strength<br>Dosage<br>Route of administration | Experimental model<br><br>*In vivo/*<br>*In vitro* | Reference<br><br>Year of publication | Main non-clinical conclusions of the authors |
|---|---|---|---|---|
| further details) | | Human μ- and k-opioid receptors | | μg/ml). Isolated flavonoids like quercetin, kaempferol as well as quercitrin did not inhibit naloxone binding. |
| Ethanolic extracts (no further details), 4.5% hyperforin and 0.5% hyperforin | 4.5% extract: 1.56 – 6.25 mg/kg i.p.<br><br>0.5% extract: 3.12-12.5 mg/kg i.p. | *In vivo*<br><br>Forced swimming test | Cervo *et al.* 2002 | The extract containing 4.5% hyperforin, but not the 0.5% extract, reduced immobility time. Hyperforin in concentrations reaching similar plasma concentrations compared to the 4.5% extract yielded similar effects. |
| Dry extract (no further details), 50% flavonoids, 0.3% hypericin, 4.5% hyperforin | Oral administration by gavage, 2 administrations<br><br>62.5-500 mg/kg | *In vivo*<br><br>Male rats<br><br>Brain content of tryptophan, 5-hydroxytryptamine, 5-hydroxyindoleacetic acid, norepinephrine, dopamine<br><br>Forced swimming test | Calapai *et al.* 2001 | After acute oral administration (250 – 500 mg/kg) dose-dependently the contents of 5-HT and 5-hydroxyindolacetic acid (5-HIAA) were significantly enhanced in all brain regions examined. Noradrenaline and dopamine levels were significantly increased in the diencephalon; in the brainstem only noradrenaline was significantly enhanced. |
| Ethanolic extract (4.5% hyperforin); $CO_2$ extract (devoid of hypericins, 38.8% | Oral administration | *In vivo*<br><br>Adult male rats and mice<br><br>Behavioral despair test in rats<br><br>Muricidal behaviour in | Bhattacharya *et al.* (1998) | The antidepressant activities of 50, 150 and 300 mg/kg/day ethanolic extract were similar to those of 5, 15 and 30 mg/kg/day of the $CO_2$ extract. The ethanol extract potentiated dopaminergic behavioural responses, whereas these effects were either absent or less pronounced in the $CO_2$ extract treated groups. Serotonergic effects were more pronounced in the $CO_2$ |

| **Herbal preparation tested** | **Strength**<br>**Dosage**<br>**Route of administration** | **Experimental model**<br>***In vivo/***<br>***In vitro*** | **Reference**<br>**Year of publication** | **Main non-clinical conclusions of the authors** |
|---|---|---|---|---|
| hyperforin) | | rats<br>5-hydroxytryptophan-induced head twitches in mice<br>L-DOPA-induced behaviour in mice<br>Apomorphine-induced stereotypy in rats<br>Post-swim grooming response in mice<br>Elevated plus-maze in mice | | extract treated groups. |
| Amentoflavone | Concentrations between $10^{-1}$ and $10^{3}$ nM | *In vitro*<br>Rat brain membranes | Baureithel *et al.* 1997 | Amentoflavon inhibited binding of flumazenil to the rat brain benzodiazepine site of the $GABA_A$ receptors with an $IC_{50}$ of 14.9±1.9 nM. Hypericin and other flavonoids did not show an effect |
| Hypericin, pseudohypericin | | *In vivo*<br>Forced swimming test in rats. Oral administration by gavage | Butterweck *et al.* 1998 | Isolated hypericin and pseudohypericin suspended in water were inactive in the test. The solubility is increased in the presence of procyanidins. Such solubilised hypericin and pseudohypericin were active in the test in concentrations ranging 0.009-0.9 mg/kg BW and 0.044 to 2.5 mg/kg BW respectively. The effect size was comparable to 2.0 mg/kg BW of bupropion. The effect was antagonised by the dopamine antagonist |

| Herbal preparation tested | Strength<br>Dosage<br>Route of administration | Experimental model<br>*In vivo/*<br>*In vitro* | Reference<br>Year of publication | Main non-clinical conclusions of the authors |
|---|---|---|---|---|
| | | | | sulpiride (100 mg/kg BW i.p.). |
| Hypericin | 1.56 mg/kg<br>Comparator: venlafaxine 7.81 mg/kg | *In vivo*<br>Chronic unpredictable mild stress model in rats | Zhai *et al.* 2015 | Changes in the classic behavioral tests and pharmacological biochemical indices reflected that hypericine (HY) alleviated the symptoms of depression in a shorter period than the active comparator. Metabolites analysis of urine revealed that HY affected excitatory amino acids and monoamine neurotransmitter metabolites. Remarkably, urinary valine was increased remarkably by HY |
| Hypericin, pseudohypericin , hyperforin, several flavonoids | | *In vitro*<br>42 biogenic amine receptors and transporters | Butterweck *et al.* 2002b | Amentoflavone significantly inhibited binding at serotonin receptor (85%), $D_3$-dopamine receptor (112%), δ-opioid receptor (75%), benzodiazepine receptor (98%), bDAT transporter (70%). Hypericine showed a significant inhibition at $D_3$- and $D_4$-dopamin receptor (83%, 70%) and ß-adrenergic receptor (92%). Hyperforin was less active |
| Quercetin | Oral; 10 – 40 mg/kg | *In vivo*<br>Adult rats; electrodes in the frontal cortex, hippocampus, striatum, reticular formation. Changes of field potential 5 h after oral administration | Dimpfel 2008 | Dose-dependent decrease of spectral power mainly in alpha2 and beta1 range, predominantly in the hippocampus. The effect increased with time. The overall changes resembled that obtained after i.p. administration of moclobemide, paroxetine and imipramine |

| **Herbal preparation tested** | **Strength**<br>**Dosage**<br>**Route of administration** | **Experimental model**<br><br>***In vivo/***<br>***In vitro*** | **Reference**<br><br>**Year of publication** | **Main non-clinical conclusions of the authors** |
|---|---|---|---|---|
| Hyperosid | 1 µM hyperforin; 1 µM hyperosid | *In vitro*<br><br>C6 glioblastoma cells | Häberlein *et al.* 2008 | After 6 days of treatment an endocytotic decrease of ß1-adrenergic receptors in the cell membrane due to inhibition of receptor recycling and interference with the mobility of ß1AR-GFP proteins |
| Hyperosid | 10 – 50 mg/kg i.p.<br><br>20-40 mg/kg p.o. | *In vivo*, mice, rats<br><br>Open field test<br><br>Pentobarbital sleeping time<br><br>Hot-plate test<br><br>Acetic-acid induced writhing test<br><br>Forced swimming test | Schulte Haas *et al.* 2011 | 20-40 mg/kg i.p.: exploratory behaviour in the open-field test reduced<br><br>20 mg/kg i.p.: pentobartbital sleeping time increased, but not sleeping latency.<br><br>No activity in the hot-plate test and acetic acid-induced writhing test<br><br>Antidepressant-like effect in forced swimming test (10-20 mg/kg i.p. in mice; 1.8 mg/kg/day p.o. in rats) |
| Dry extract (ethanol), 4.5% hyperforin<br><br>$CO_2$ extract, 38.8% hyperforin | Oral administration for 3 consecutive days<br><br>Ethanolic extract 50-300 mg/kg<br><br>$CO_2$ extract 5-30 mg/kg | *In vitro*<br><br>*In vivo*<br><br>Synaptosomal preparations of rat striatum<br><br>MAO-A and MAO-B in the whole mouse brain<br><br>Behavioural despair in rats | Chatterjee *et al.* 1998 | Hyperforin inhibits serotonin, dopamine, noradrenaline, GABA and L-glutamate with $IC_{50}$ values of about 0.05-0.50 µg/ml in synaptosomal preparations.<br><br>The effects in the *in vivo* test systems correlated with the content of hyperforin in the extracts. |

| **Herbal preparation tested** | **Strength**<br>**Dosage**<br>**Route of administration** | **Experimental model**<br>***In vivo/***<br>***In vitro*** | **Reference**<br>**Year of publication** | **Main non-clinical conclusions of the authors** |
|---|---|---|---|---|
| | | Learned helplessness in rats | | |
| Hypericin, dry extract (no further details) | 5 µg/ml | *In vitro*<br>Receptor binding studies | Cott 1997 | Hypericin affinity only to NMDA receptor.<br>Extract significant receptor affinity to adenosine, GABAA. GABAB, benzodiazepine, inositol triphosphate, MAO A and MAO B receptors. With the exception of GABAA and GABAB the concentrations of extract required are unlikely to be attained after oral administration |
| Hypericin | 1 µM | *In vitro*<br>Receptor binding studies | Raffa 1998 | 30 receptors or reuptake sites<br>Modest affinity (49% inhibition) for muscarinic cholinergic receptors and similar affinity (48% inhibition) for σ receptors |
| Hypericin | 1-100 µM | *In vitro*<br>Nerve terminals from cerebral cortex from male Sprague-Dawley rats | Chang & Wang 2010 | Hypericin inhibited the release of glutamate evoked by 4-aminopyridine in a concentration-dependent manner by reduction of vesicular exocytosis |
| Hypericin | Up to 1 µM | *In vitro*<br>Isolated hippocampal neurons | Wang *et al.* 2010 | Extracellularly applied hypericin dose-dependently increased action potential duration but barely affected its amplitude. Further analysis revealed that hypericin inhibited both transient I(A) and delayed rectifier I(K) potassium currents. In contrast, hypericin exerted no significant effect on both Na(+) peak current and its |

| **Herbal preparation tested** | **Strength**<br>**Dosage**<br>**Route of administration** | **Experimental model**<br>***In vivo/***<br>***In vitro*** | **Reference**<br>**Year of publication** | **Main non-clinical conclusions of the authors** |
|---|---|---|---|---|
| | | | | decay kinetics |
| Hyperforin | 10 mg/kg, i.p. | *In vivo*<br>Push-pull superfusion technique<br>Rat locus caeruleus | Kaehler *et al.* 1999 | Hyperforin enhanced the extracellular levels of dopamine, noradrenaline, serotonin and glutamate. The levels of the serotonine metabolite 5-hydroxyindolacetic acid, of GABA, taurine, aspartate, serine and arginine were not influenced |
| Hyperforin | 2 µM | *In vitro*<br>Synaptosomal preparations from the frontal cortex of female mice<br>Blood from human volunteers | Singer *et al.* 1999 | Hyperforin inhibits serotonin uptake by elevating free intracellular $Na^+$ |
| Hyperforin | | *In vitro*<br>*In vivo*<br>Choline (CH) uptake<br>Rat brain synaptosomes<br>Striatal Acetylcholine (ACh) release | Buchholzer *et al.* 2002 | In rat brain synaptosomes, hyperforin inhibited high-affinity choline uptake with an $IC_{50}$ of 8.5 µM, whereas low-affinity uptake was not affected. Local infusion of hyperforin (100 µM) via the dialysis probe caused a delayed reduction of ACh release and a concomitant increase of Choline levels. Infusion of a lower concentration of hyperforin (10 µM) increased striatal ACh release and lowered Choline levels.<br>Systemic administration of hyperforin (1–10 mg/kg i.p.) led to therapeutic plasma levels of hyperforin and caused a significant elevation of striatal ACh release |

| Herbal preparation tested | Strength<br>Dosage<br>Route of administration | Experimental model<br>*In vivo/*<br>*In vitro* | Reference<br>Year of publication | Main non-clinical conclusions of the authors |
|---|---|---|---|---|
| Hyperforin | *In vivo*: 4 mg/kg i.p. | *In vitro*<br>Primary cultures of cortical neurons<br>*In vivo*<br>Male C57Bl6/J mice, 4 weeks treatment | Gibon *et al.* 2013 | Hyperforin stimulated the expression of TRPC6 channels and cortical brain-derived neurotrophic factor receptor TrkB via SKF-96365-sensitive channels controlling a downstream signalling cascade involving $Ca^{2+}$, protein kinase A, CREB and p-CREB. *In vivo*, hyperforin augmented the expression of TrkB in the cortex but not in the hippocampus where hippocampal neurogenesis remained unchanged. Hyperforin acts on the cortical brain-derived neurotrophic factor/TrkB pathway leaving adult hippocampal neurogenesis unaffected |
| Hyperforin | 10 µM<br>10 mg/kg i.p. | *In vitro*<br>*In vivo*<br>Models of N-methyl-D-aspartate receptor antagonism and neuroprotection | Kumar et al 2006 | *In vitro*:<br>Inhibition of N-methyl-D-aspartate (NMDA) induced calcium influx into cortical neurons.<br>Inhibition of NMDA-receptor mediated release of choline from phospholipids<br>*In vivo*:<br>Inactive in models of brain edema formation, middle cerebral artery occlusion, water intoxication |
| Hyperforin | 0.3-10 µM | *In vitro*<br>CA1 and CA3 pyramidal neurons of hippocampal slices | Leuner *et al.* 2013 | Hyperforin modulates dendritic spine morphology in CA1 and CA3 pyramidal neurons of hippocampal slice cultures through the activation of TRPC6 channels. Hyperforin evoked intracellular Ca(2+) transients and depolarizing inward currents sensitive to the TRPC channel blocker La(3+) , thus resembling the actions of |

| **Herbal preparation tested** | **Strength**<br>**Dosage**<br>**Route of administration** | **Experimental model**<br>***In vivo/***<br>***In vitro*** | **Reference**<br>**Year of publication** | **Main non-clinical conclusions of the authors** |
|---|---|---|---|---|
| | | | | the neurotrophin brain-derived neurotrophic factor (BDNF) in hippocampal pyramidal neurons |
| Hyperforin | 1-10 µM | *In vitro*<br>HEK-293 cells<br>Transfected HEK-293 cells expressing TRPC6 cDNA<br>Primary mouse cortical microglia cells | Sell at al. 2014 | Hyperforin induces TRPC6-independent H(+) currents in HEK-293 cells, cortical microglia, chromaffin cells and lipid bilayers. Hyperforin acts as a protonophore. The protonophore activity of hyperforin causes cytosolic acidification, which strongly depends on the holding potential, and which fuels the plasma membrane sodium-proton exchanger. The free intracellular sodium concentration increases and the neurotransmitter uptake by Na(+) cotransport is inhibited. Hyperforin depletes and reduces loading of large dense core vesicles in chromaffin cells, which requires a pH gradient in order to accumulate monoamines |
| Hyperforin, hyperoside | 1 µM hyperforin<br>1 µM hyperoside<br>6 days | *In vitro*<br>Rat C6 glioblastoma cells | Jakobs *et al.* 2013 | Reduced ß2-adrenergic receptor density in plasma membranes<br>Reduced downstream signalling |
| Hyperforin, adhyperforin | $10^{-4}$ – $10^{2}$ M | *In vitro*<br>Synaptosomal uptake assay in rat brain tissue<br>WIN 35,428 binding assay in rat striata | Jensen *et al.* 2001 | In contrast to imipramine, nomifensin and fluoxetine, hyperforin and adhyperforin did not inhibit the binding of the cocain analogue WIN 35,428. Hyperforin and adhyperforin did not prevent dopamine binding but inhibited dopamine translocation |
| Adhyperforin | 8-16 mg/kg | *In vivo* | Tian et a. 2014 | Adhyperforin reduced the immobility time of mice in the |

| **Herbal preparation tested** | **Strength**<br>**Dosage**<br>**Route of administration** | **Experimental model**<br>***In vivo/***<br>***In vitro*** | **Reference**<br>**Year of publication** | **Main non-clinical conclusions of the authors** |
|---|---|---|---|---|
| | | Forced swimming test<br>Tail suspension assay<br>Open field test<br>*In vitro*<br>Synaptosomes from frontal cortex | | forced swimming test and tail suspension assay, antagonized the behaviors induced by reserpine, and had no effect on locomotor activity. Adhyperforin increased the number of crossings and rearings in rats in the open field test and increased the sucrose consumption. It inhibited uptake of serotonin, norepinephrine, and dopamine, and displayed robust binding affinities for the serotonin and norepinephrine transporters |
| Amentoflavon | $10^{-10}$ – $10^{-3}$ M | *In vitro*<br>Radioligand binding studies | Hansen *et al.* 2005 | Interaction of amentoflavon at $GABA_A$ receptor follows a complex mechanism |
| Review | | | Crupi et al 2013 | *Hypericum perforatum*, like conventional antidepressants, is involved in the regulation of genes that control hypothalamic-pituitary-adrenal axis function and influences, at least in part, stress-induced effects on neuroplasticity and neurogenesis. Results from experiments carried out with extracts or pure compounds do not always resemble biochemical and pharmacological profile characteristic of synthetic antidepressants. In particular, the majority of findings in preclinical studies have been obtained with high doses of pure compounds and extracts that are not comparable to the concentrations of single active constituents after oral administration in humans |

### *3.1.1.2. Primary pharmacodynamics related to wound healing*

Table 5: Overview of the non-clinical data related to indication 'wound healing'

| **Herbal preparation tested** | **Strength Dosage Route of administration** | **Experimental model** <br> ***In vivo/ In vitro*** | **Reference** <br> **Year of publication** | **Main non-clinical conclusions of the authors** |
|---|---|---|---|---|
| Laboratory extracts prepared with methanol/acetone 1:1 <br> *Hypericum perforatum* subsp. perforatum and subsp. veronense | Up to 10 µg/ml | *In vitro* <br> Cultured fibroblasts | Dikmen *et al.* 2011 | Both extracts increased the percentage of polygonal fibroblasts and the number of collagen granules in fibroblasts, which is interpreted as parameters related to wound healing. Differences between the two subspecies were observed. |
| Oil macerate (several home-made and commercial samples) | 1000 µg/ml | *In vitro* <br> Transiently transfected K562 cells | Orhan *et al.* 2014 | Some of the tested oil macerates reduced TNF$\alpha$-induced NF-$\kappa$B activation in a concentration dependent manner. |
| Oil macerate (1:10) from dried *Hypericum perforatum* <br> Ethanolic extract (ethanol 70%) (1:10). Ointment containing 15% of oil macerate and 15% of ethanolic extract | Ointment applied once a day for 21 days | *In vivo*, rats <br> Artificial wounds | Prisacaru *et al.* 2013 | Significant wound healing effects. |
| Oil extract (1:10), dry ethanolic extract (app. 3:1, ethanol 96%) | Capillary permeability: 0.2 ml/20 g body weight p.o. <br> Cutaneous | *In vivo* <br> Linear incision wound model (rats) <br> Circular incision wound model (rats) | Süntar *et al.* 2010 | Statistically significant faster wound healing compared to placebo. <br> Bioassay-guided fractionation led to the suggestion that the flavonoids play a major role in wound healing. <br> The ethanolic extract exhibited a dose-dependent anti- |

| Herbal preparation tested | Strength<br>Dosage<br>Route of administration | Experimental model<br>*In vivo/*<br>*In vitro* | Reference<br>Year of publication | Main non-clinical conclusions of the authors |
|---|---|---|---|---|
| | application: in ointment base (no data o concentration) | Excision wound model (rats)<br>Acetic acid-induced increase in capillary permeability (mice) | | inflammatory activity. |
| Oil extract (1:5), sunflower oil<br>Oil extract (1:10), sunflower oil<br>Oil extract (first extraction solvent ethanol 96%, second extraction solvent sunflower oil)<br>Quercetin and I3,II8-biapigenin | 1.25 ml/kg p.o. | *In vivo*<br>Rat paw edema<br>Indomethacin induced model of acute gastric mucosa damage | Zdunic *et al.* 2009 | The oil extract prepared by maceration with 96% ethanol, followed by extraction with sunflower oil exhibited the highest antiinflammatory effect (95.24 +/- 11.66%) and gastroprotective activity (gastric damage score of 0.21 +/- 0.12). The same oil extract had the highest content of quercetin and I3,II8-biapigenin (129 +/- 9 microg/mL and 52 +/- 4 microg/mL, respectively). Quercetin and I3,II8-biapigenin exhibited antiinflammatory activity similar to those of indomethacin as well as significant gastroprotective activity |
| Hyperforin | 0-100 µg/ml | *In vitro*<br>Human epidermal cell suspensions<br>Peripheral blood mononuclear cells | Schempp *et al.* 2000b | Hyperforin inhibits the allostimulatory capacity of epidermal cells and inhibits the proliferation of peripheral blood mononuclear cells |
| Methanolic extract (80%) | | *In vivo* | Sosa *et al.* | Dose-dependent oedema reduction. |

| **Herbal preparation tested** | **Strength Dosage Route of administration** | **Experimental model** <br>***In vivo/ In vitro*** | **Reference** <br>**Year of publication** | **Main non-clinical conclusions of the authors** |
|---|---|---|---|---|
| Lipophilic extract (CO2)<br>Ethylacetic fraction of an ethanolic extract (ethanol 70%)<br>hypericin, adhyperforin, amentoflavone, hyperoside, isoquercitrin, hyperforin dicyclohexylammonium (DHCA) salt and dicyclohexylamine | | Croton oil induced ear oedema in mice | 2007 | Lipophilic extract > ethylacetic fraction > hydroalcoholic extract (ID50 220, 267 and >1000 µg cm-2, respectively). Amentoflavone (ID50 0.16 µmol cm-2), hypericin (ID50 0.25 µmol cm-2), hyperforin DHCA salt (ID50 0.25 µmol cm-2) and adhyperforin (ID50 0.30 µmol cm-2) had anti-inflammatory activity that was more potent or comparable to that of indometacin (ID50 0.26 µmol cm-2), whereas isoquercitrin and hyperoside were less active (ID50 about 1 µmol cm-2) |
| Dry extract (ethanol 50%, | 1-100 mg/ml | *In vitro*<br>Chicken embryo fibroblasts | Öztürk *et al.* 2006 | Increase in the stimulation of fibroblast collagen production and the activation of fibroblast cells in polygonal shape |

### 3.1.2. Secondary pharmacodynamics

Table 6: Overview of the non-clinical data secondary pharmacology

| **Herbal preparation tested** | **Strength Dosage Route of administration** | **Experimental model** <br>***In vivo/ In vitro*** | **Reference** <br>**Year of publication** | **Main non-clinical conclusions of the authors** |
|---|---|---|---|---|
| Fractionated ethanolic extract (80%) and | Results calculated on the actual amount of a | *In vitro*<br>Amyloid-β-peptide induced cell death in rat cultured | Silva *et al.* 2004 | Induced lipid peroxidation was significantly inhibited by fractions containing flavonol glycosides, flavonol and biflavone aglycones, and by a fraction containing several |

| **Herbal preparation tested** | **Strength**<br>**Dosage**<br>**Route of administration** | **Experimental model**<br>***In vivo/***<br>***In vitro*** | **Reference**<br>**Year of publication** | **Main non-clinical conclusions of the authors** |
|---|---|---|---|---|
| isolated constituents | constituent in the fractions | hippocampal neurons.<br>Lipid peroxidation in rat cortical synaptosomes | | phenols, mainly chlorogenic acid-type phenolics (21%, 77% and 98%, respectively).<br>The total ethanolic extract (TE) and fractions containing flavonol glycosides, flavonol and biflavone aglycones, reduced Amyloid-β-peptide induced cell death (65%, 58% and 59%, respectively).<br>Total extract as well as fractions containing hypericin and flavonoids inhibited Amyloid-β-peptide induced decrease in cell volume, chromatin condensation and nuclear fragmentation |
| Hyperforin | Rats injected with amyloid-β-fibrils alone or together with 6 µM hyperforin in the hippocampus | *In vivo*<br>Circular water maze<br>Brain slices | Dinamarca *et al.* 2006 | Hyperforin decreased amyloid deposit formation, decreased the neuropathological changes and behavioural impairments in a rat model of amyloidosis, and prevented Amyloid-β-induced neurotoxicity in hippocampal neurons both from amyloid fibrils and Amyloid-β oligomers |
| Hyperforin | 50 nM – 10 µM | *In vitro*<br>P12 cells transfected with human wildtype amyloid precursor protein APP | Froestl *et al.* 2003 | Increased release of secretory APP fragments upon hyperforin treatment |
| Extract (5% hyperforin, no further | 1250 mg/kg<br>60 or 120 days | *In vivo*<br>C57BL/6J-APP/PS+/− mice | Brenn *et al.* 2014 | Mice receiving *Hypericum* extract showed (i) significant reductions of parenchymal beta-amyloid 1–40 and 1–42 accumulation; and (ii) moderate, but statistically significant increases in cerebrovascular P-glycoprotein |

| Herbal preparation tested | Strength<br>Dosage<br>Route of administration | Experimental model<br>*In vivo/*<br>*In vitro* | Reference<br>Year of publication | Main non-clinical conclusions of the authors |
|---|---|---|---|---|
| information) | | | | expression |
| Dry extract (acidified methanol, no further information)<br>Hyperforin<br>Hypericin<br>Pseudihypericin | Up to 4.66 mg/ml culture | *In vitro*<br>Mycobacterium cultures (M. JLS, M. KMS, M. CMS, M. smegmatis, M. phlei) | Mortensen *et al.* 2012 | The extract was effective at inhibiting five nonpathogenic Mycobacterium isolates and Bacillus subtilis, but not Escherichia coli. Quantitative studies of concentration sensitivity to the *Hypericum* extract were performed with minimal bactericidal concentrations (MBC) ranging from 0.33 to 2.66 mg extract/mL. The *Hypericum* constituents hyperforin (Hfn), hypericin (Hpn), and pseudohypericin (Phn) were quantified in the extract using. Purified Hfn, Hpn, and Phn were tested for inhibitory activity against Mycobacterium JLS (M. JLS) at similar concentrations used in the crude extract. While Hfn was inhibitory at 46 µg/mL, none of the purified SJW constituents were bactericidal at concentrations corresponding to the extract |
| Dry extract prepared from fresh aerial parts extracted with methanol | Up to 300 µg/ml | *In vitro*<br>Human cytomegalovirus (HCMV) strain AD-169 cultivated in human diploid embryonic lung fibroblasts (MRC-5) | Axarlis *et al.* 1998 | Up to 100 % antiviral activity |
| Pruified fractions of a chloroform extract | Up to 100 µg/ml | *In vitro*<br>HeLa 37 cells for HIV studies<br>Equine dermal cells for EIAV | Maury *et al.* 2009 | Antiviral activity was associated with more polar subfractions. GC/MS analysis of the two most active subfractions identified 3-hydroxy lauric acid as predominant in one fraction and 3-hydroxy myristic acid |

| **Herbal preparation tested** | **Strength Dosage Route of administration** | **Experimental model** *In vivo/ In vitro* | **Reference Year of publication** | **Main non-clinical conclusions of the authors** |
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| | | studies | | as predominant in the other. Synthetic 3-hydroxy lauric acid inhibited HIV infectivity without cytotoxicity, suggesting that this modified fatty acid is likely responsible for observed antiviral activity present in that fraction. As production of 3-hydroxy fatty acids by plants remains controversial, H. perforatum seedlings were grown sterilely and evaluated for presence of 3-hydroxy fatty acids by GC/MS. Small quantities of some 3-hydroxy fatty acids were detected in sterile plants, whereas different 3-hydroxy fatty acids were detected in the chloroform extracts or field-grown material |
| Dry extract (4.73% hypericin, no further information) | 50 – 200 mg/kg, 2 x daily, 5 days, oral | *In vivo*<br>Mice infected with influenza A virus | Pu *et al.* 2012 | The administration of *Hypericum* extract reduced the ling index and the viral titer, decreased mortality and prolonged the mean survival time. *Hypericum* decrease the concentration of IL-6 and TNF-$\alpha$ in lung tissue. In contrast it enhanced the lung and serum levels of IL-10 and INF-$\gamma$. |
| Hypericin | 2, 10 and 50 µl/ml | *In vitro*<br>MCF7 breast cancer cells | Ocak *et al.* 2013 | ADAMTS9 expression in MCF7 cells was increased 1.8 and 3.6 fold with the use of 2 and 10 µl/mL of hypericin, respectively; and decreased 0.7 fold with the use of 50 µl/mL of hypericin. There was no significant change in the ADAMTS8 expression. Rapid cell death was observed in the cancer cells when hypericin was used at a dose of $\geqslant$ 50 µl/mL. |
| Hyperforin | Up to 50 µM | *In vitro* | Rothley *et al.* 2009 | At concentrations less than 10 µM, hyperforin and |

| **Herbal preparation tested** | **Strength Dosage Route of administration** | **Experimental model** <br><br> ***In vivo/ In vitro*** | **Reference** <br><br> **Year of publication** | **Main non-clinical conclusions of the authors** |
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| Aristoforin | <br><br><br><br><br><br><br>2 mM daily for 2 weeks, peritumoral injections | Primary human lymphatic endothelial cells (LEC) <br><br> Human umbilical vein endothelial cells (HUVEC) <br><br><br> *In vivo* <br><br> Tumor induced lymphangiogenesis in rats | | aristoforin induced cell cycle arrest of LECs, and at higher concentrations induce apoptosis. The loss of mitochondrial membrane potential and the activation of caspase-9 during the induction of apoptosis indicate that the intrinsic pathway of apoptosis is stimulated by these compounds, similar to the situation in tumor cells. In thoracic duct ring outgrowth assays, hyperforin and aristoforin both inhibited lymphangiogenesis, as evidenced by the suppression of lymphatic capillary outgrowth. *In vivo* both substances were able to inhibit tumor-induced lymphangiogenesis. |
| Hyperforin | | *In vitro* <br><br> Chronic lymphoid leukemia cells (CLL) <br><br> Acute myeloid leukeima cells (AML) | Billard *et al.* 2013 | Review article <br><br> In AML cell lines and primary AML cells, hyperforin directly inhibits the kinase activity of the serine/threonine protein kinase B/AKT1, leading to activation of the pro-apoptotic Bcl-2 family protein Bad through its non-phosphorylation by AKT1. In primary CLL cells, hyperforin acts by stimulating the expression of the pro-apoptotic Bcl-2 family member Noxa (possibly through the inhibition of proteasome activity). Other hyperforin targets include matrix metalloproteinase-2 in AML cells and vascular endothelial growth factor and matrix metalloproteinase-9 in CLL cells - two mediators of cell migration and angiogenesis. In summary, hyperforin targets molecules involved in signaling |

| Herbal preparation tested | Strength<br>Dosage<br>Route of administration | Experimental model<br>*In vivo/*<br>*In vitro* | Reference<br>Year of publication | Main non-clinical conclusions of the authors |
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| | | | | pathways that control leukemic cell proliferation, survival, apoptosis, migration and angiogenesis. Hyperforin also downregulates the expression of P-glycoprotein, a protein that is involved in the resistance of leukemia cells to chemotherapeutic agents. |
| Hyperforin | Up to 1 µM | *In vitro*<br>Human medulloblastoma (DAOY) and human glioblastoma (A172 and U87) cells | Tassone *et al.* 2011 | Real-time PCR and ELISA revealed that under hyperforin vascular endothelial growth factor VEGF expression increased more than three fold in DAOY medulloblastoma cells; while, U87 glioblastoma cells – constitutively expressing high VEGF levels – showed no significant differences. Hyperforin induced endothelial pro-angiogenic behaviour in a multi-parametric Matrigel colonisation assay, and down-modulation of pro-MMP-2 and pro-MMP-9 activities as measured by gelatin zymography. |
| Hyperforin | 18 µM, up to 12 µg/ml | Ex vivo<br>Human chronic lymphocytic leukemia cells (CLL)<br>MEC-1 cells | Zaher *et al.* 2012 | The increase in Noxa (a BH3-only protein of the Bcl-2 family ) expression is a time- and concentration-dependent effect of hyperforin occurring without change in Noxa mRNA levels. A post-translational regulation is suggested by the capacity of hyperforin to inhibit proteasome activity in CLL cells. Noxa silencing by siRNA reduces partially hyperforin-elicited apoptosis. Treatment with hyperforin, which has no effect on the expression of the prosurvival protein Mcl-1, induces the interaction of Noxa with Mcl-1 and the dissociation of Mcl-1/Bak complex, revealing that upregulated Noxa |

| Herbal preparation tested | Strength Dosage Route of administration | Experimental model *In vivo/ In vitro* | Reference Year of publication | Main non-clinical conclusions of the authors |
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| | | | | displaces the proapoptotic protein Bak from Mcl-1. |
| Hyperforin | Up to 10 µM | *In vitro* MCF-7 human breast cancer cells transfected with estrogen receptor | Kwon *et al.* 2016 | Compared to 17β-estradiol, hyperforin showed significantly lower estrogenic activity and cell proliferation. A total of 453 proteins were identified, of which 282 proteins were significantly modulated in hyperforin-treated cells compared to 17β-estradiol-treated cells. Ingenuity pathway analysis also demonstrated that hyperforin treatment induced less cell proliferation than 17β-estradiol by downregulating estrogen receptor 1. Protein network analysis showed that cell proliferation was regulated mainly by cyclin D1 and extracellular signal-regulated kinases. |
| Dry extract (ethanol 50% V/V, DER 1:16) | 0.02-0.2% extract | *In vitro* Nerve cells derived from mouse hippocampus (HT22 cells) | Breyer *et al.* 2007 | At a concentration of 0.05% the extract showed cytoprotective effects by attenuation of calcium fluxes. |
| Dry extract (no further details), 0.34% hypericin, 4.1% hyperforin,5% flavonoids, 10% tannins | 30 mg/kg, o.s. | *In vivo* Cerulein-induced acute pancreatitis Serum levels of lipase, amylase, pancreas injury, adhesion molecule expression, nitration of cellular proteins, activation of | Genovese *et al.* 2006a | Cerulein-induced damages were markedly reduced by *Hypericum* extract. Mortality at day 5 after cerulean administration was siginificantly reduced. |

| **Herbal preparation tested** | **Strength<br>Dosage<br>Route of administration** | **Experimental model**<br>***In vivo/ In vitro*** | **Reference**<br>**Year of publication** | **Main non-clinical conclusions of the authors** |
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| | | the nuclear enzyme PAR synthetase | | |
| Dry extract (methanol, no further details)<br>Hypericin | 1 hour exposure<br>0.5-5.0 µg/ml hypericin as isolated substance or corresponding amounts of the extract | *In vitro*<br>Cytotoxic properties<br>Viable cell count, flow cytometry fluorescence microscopy | Roscetti *et al.* 2004 | Hypericin had a weak effect on cell growth and no effect on inducing apoptosis.<br>The extract showed a significant concentration-dependent and long-lasting inhibition of cell growth and induced apoptotic cell death. |
| Dry extract (no further information) | 10 – 100 µg/ml | *In vitro*<br>P12 cells | Lu *et al.* 2004 (abstract only) | Protective effect against trauma of P12 cells induced by H2O2.<br>Reactive oxygen species levels decreased significantly.<br>The extract blocked DNA fragmentation. |
| Hypericin | 0.1-10 µM | *In vitro*<br>Cerebellar granule cells | Kaltschmidt *et al.* 2002 | Hypericin induced short-time activation of NF-kB. Cell death was induced at 10 µM.<br>Hypericin in low concentrations partly prevented cell death induced by amyloid-β-peptide. At 10 µM it synergistically enhanced amyloid-β-peptide toxicity. |
| Dry extract (petroleum ether, no further details) | 50 mg/kg BW, i.p. | *In vivo*<br>Hepatic ischaemia / reperfusion model in rats | Bayramogly et al 2014 | Treatment with *Hypericum* extract significantly decreased the alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase activities and malondialdehyde levels, and markedly increased activities of catalase and glutathione peroxidase in |

| Herbal preparation tested | Strength<br>Dosage<br>Route of administration | Experimental model<br>*In vivo/*<br>*In vitro* | Reference<br>Year of publication | Main non-clinical conclusions of the authors |
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| | | | | tissue homogenates compared to ischaemia/reperfusion-induced rats without treatment-control group ($p < 0.05$). |
| Dry hydroethanolic extract (no further information)<br>Hyperforin | Extract: 25 µg/ml<br>Hyperforin: 1-5 µM | *In vitro*<br>Rat insulinoma cells INS-1E<br>Pancreatic islands of rats<br>Human pancreatic islets | Menegazzi *et al.* 2008 | *Hypericum* extract and hyperforin (at 1-3 µM) prevented cytokine-induced impairment in glucose-stimulated insulin secretion and protected cells against apoptosis in a dose-dependent fashion. Inducible-NO-synthase expression was also hindered. Cytokine-induced activations of the signal-transducer-and-activator-of-transcription-1 (STAT-1) and the nuclear-factor-kappaB (NF-kappaB) were both down-regulated by *Hypericum* extract or hyperforin (range 0.5-5 µM) when evaluated by electrophoretic-mobility-shift-assay. Other transcription factors (CBF-1, SP-1) were unaffected. Components of *Hypericum* extract other than hyperforin were much less effective in down-regulating cytokine signalling. Inhibition of cytokine-elicited STAT-1 and NF-kappaB activation was confirmed in isolated rat and human islets incubated in the presence of *Hypericum* extract or hyperforin. |
| Dry extract (4.1% hyperforin, no further information)<br>Hyperforin | Extract: 200 µg/ml<br>Hyperforin: 2 µM | *In vitro*<br>Pancreatic islands of rats<br>Human pancreatic islets | Novelli *et al.* 2014 | In both rat and human islets, the extract and hyperforin counteracted cytokine-induced functional impairment and down-regulated mRNA expression of pro-inflammatory target genes, such as iNOS, CXCL9, CXCL10, COX2. Cytokine-induced NO production from cultured islets, evaluated by nitrites measurement in the |

| Herbal preparation tested | Strength<br>Dosage<br>Route of administration | Experimental model<br><br>*In vivo/*<br>*In vitro* | Reference<br><br>Year of publication | Main non-clinical conclusions of the authors |
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| | | | | medium, was significantly reduced. The increase in apoptosis and necrosis following 48-h exposure to cytokines was fully prevented by the extract and partially by hyperforin. Ultrastructural morphometric analysis in human islets exposed to cytokines for 20 h showed that th extract or hyperforin avoided early β-cell damage (e.g., mitochondrial alterations and loss of insulin granules). |
| Dry extract (no further information) | 31.25 – 5000 ng/ml | *In vitro*<br><br>Human SH-SY5Y neuroblastoma cells | Schmidt *et al.* 2010 | *Hypericum perforatum* significantly decreased the survival of cells after treatment with a concentration of 5000 ng/mL. The same concentration led to a significant increase of ATP levels, whereas treatment with a concentration of 500 ng/mL had no significant effect |
| Hyperforin<br><br>Aristoforin | 0.01-10 mM | *In vitro*<br><br>Plasmid DNA<br><br>DPPH-test | Ševčovičová *et al.* 2015 | The DNA-topology assay revealed partial DNA-protective activities of hyperforin and aristoforin against Fe(2+)-induced DNA breaks. The reduction in the fluorescence of hyperforin indicated an interaction between hyperforin and DNA with a binding constant of 0.2×10(8)M(-1). |
| Quercetin<br><br>Kaempferol<br><br>Biapigenin | 10 µM | *In vitro*<br><br>Hippocampal neurons of rat embryos | Silva *et al.* 2008 | Quercetin, kaempferol and biapigenin significantly reduced neuronal death caused by 100 µM kainate plus 100 µM N-methyl-D-aspartate. The observed neuroprotection was correlated with prevention of delayed calcium deregulation and with the maintenance of mitochondrial transmembrane electric potential. The |

| **Herbal preparation tested** | **Strength Dosage Route of administration** | **Experimental model** <br> ***In vivo/ In vitro*** | **Reference** <br> **Year of publication** | **Main non-clinical conclusions of the authors** |
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| | | | | three compounds were able to reduce mitochondrial lipid peroxidation and loss of mitochondrial transmembrane electric potential caused by oxidative stress induced by ADP plus iron. Biapigenin was also able to significantly affect mitochondrial bioenergetics and decrease the capacity of mitochondria to accumulate calcium. |
| Flavonoid rich extract | Up to 50 µg/ml | *In vitro* <br> PC12 cells | Zou *et al.* 2010 | Following a 4 h exposure of PC12 cells to H2O2, a significant decrease in the cell viability and increased levels of lactate dehydrogenase (LDH) release were observed. Pretreatment of PC12 cells with *Hypericum* prior to H2O2 exposure elevated the cell viability, decreased the levels of LDH release and decreased the occurrence of apoptotic cells. Also, the intensity of H2O2-induced DNA laddering was inhibited in a dose-dependent fashion by a DNA fragmentation assay. |
| Dry extract (methanol, no more details), 0.3% hypericin, 3.8% hyperforin <br> Dry extract ($CO_2$), 24.33% hyperforin | Ethanolic extract 62.5-500 mg/kg i.g. <br> $CO_2$ extract 7.8-250 mg/kg i.g. | *In vivo* <br> msP rats <br> Intake of ethanol <br> Effect on blood alcohol level | Perfumi *et al.* 2001 | Both extracts reduced dose-dependently the ethanol intake. The $CO_2$ extract was about 8 times more potent. The $CO_2$ extract reduced the blood alcohol levels. |
| Dry extracts (DER 3-7:1, methanol | Oral <br> Acute: | *In vivo* <br> Rats accustomed to ethanol | Rezvani *et al.* 1999 | Hypericum significantly reduced alcohol intake in both types of rats. |

| **Herbal preparation tested** | **Strength**<br>**Dosage**<br>**Route of administration** | **Experimental model**<br><br>***In vivo/***<br>***In vitro*** | **Reference**<br><br>**Year of publication** | **Main non-clinical conclusions of the authors** |
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| 80% V/V) | Fawn-hooded (FH) rats: 100-800 mg/kg<br><br>High-alcohol drinking (HAD) rats: 100-600 mg/kg<br><br>Chronic:<br><br>FH rats 400 mg/kg, 15 days | | | FH rats did not develop tolerance to the effects of *Hypericum* in chronic treatment |
| Dry extract (no further details), 0.3% hypericin | 125-500 mg/kg, intragastral, acute | *In vivo*<br><br>msP rats with preference to alcohol<br><br>Forced swimming test<br><br>Open field test | Perfumi *et al.* 1999 | 125 and 250 mg/kg *Hypericum* induced a 30-40% reduction of ethanol intake in rats offered 10% v/v ethanol for 2h/day. These doses did not modify food intake or food-associated drinking. No changes in the behavior were in the open field test were noted. The effect was not related to antidepressant-like effects |
| Dry extract (methanol, no further information), 0.3% hypericin, 3.8% hyperforin;<br><br>$CO_2$ extract, | Methanol extract: 62.5-500 mg/kg i.g.<br><br>$CO_2$ extract: 7.8-250 mg/kg i.g. | *In vivo*<br><br>msP rats with preference to alcohol<br><br>Forced swimming test | Perfumi *et al.* 2001 | Both extract reduced ethanol intake, the CO2 extract was about 8 times more potent. Food and water intake was not influenced |

| **Herbal preparation tested** | **Strength Dosage Route of administration** | **Experimental model** <br> ***In vivo/ In vitro*** | **Reference** <br> **Year of publication** | **Main non-clinical conclusions of the authors** |
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| 24.33% hyperforin | | | | |
| $CO_2$ extract, 24.33% hyperforin | 7, 31, 125 mg/kg i.g. | *In vivo* <br> msP rats with preference to alcohol <br> Tail flick test | Perfumi *et al.* 2003 | $CO_2$ extract reduced ethanol intake at 31 or 125 mg/kg, but not 7 mg/kg. <br> When naloxone 1 mg/kg was combined with the three doses of H. perforatum $CO_2$ extract, the attenuation of ethanol intake was more pronounced than that observed after the administration of the extract alone |
| $CO_2$ extract, 24.33% hyperforin | 7 and 125 mg/kg i.g <br> Acute or chornic (12 days) | *In vivo* <br> msP rats with preference to alcohol | Perfumi *et al.* 2005a | Chronic treatment markedly reduced ethanol intake at the dose of 125, but not at 7 mg/kg; the effect of 125 mg/kg was observed since the first day of treatment and remained constant across the 12 days. Treated rats promptly recovered baseline ethanol intake when treatment did not precede access to ethanol (on day 8) or after the end of treatment (day 13 and day 14), suggesting that *Hypericum* administrations did not induce conditioned aversion to alcohol |
| $CO_2$ extract, 24.33% hyperforin | 7, 31, 125 mg/kg i.g. | *In vivo* <br> msP rats with preference to alcohol | Perfumi *et al.* 2005b | Doses of 31 or 125 mg/kg but not 7 mg/kg, significantly reduced ethanol self-administration, while it did not modify saccharin self-administration. The same doses of the extract abolished the increased ethanol intake following ethanol deprivation. |
| Dry extract (methanol, no further details), | Methanolic extract: 15.6-1000 mg/kg | *In vivo* <br> C57BL/6J mice | Wright *et al.* 2003 | The dose of the hyperforin-rich extract required to significantly reduce 10% ethanol intake (5 mg/kg) was 125-fold less than that required for the crude extract |

| **Herbal preparation tested** | **Strength**<br>**Dosage**<br>**Route of administration** | **Experimental model**<br>***In vivo/***<br>***In vitro*** | **Reference**<br>**Year of publication** | **Main non-clinical conclusions of the authors** |
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| hyperforin not detectable<br>$CO_2$ extract, 45% hyperforin | CO2 extract: 1-10 mg/kg<br>oral | | | (625 mg/kg), and was comparable to the dose of fluoxetine (10 mg/kg) required to produce a similar effect |
| Dry extract (methanol 80% v/v, 4-7:1)<br>Dry extract (ethanol 50% m/m, 4-7:1), very low content of hyperforin | i.p.<br>Acute: 5-40 mg/kg, 3 times within 23 hours<br>Subacute: 10 mg/kg for 4 days | *In vivo*<br>Forced swimming test<br>Model for alcohol consumption | De Vry *et al.* 1999 | *Hypericum* extracts induced a significant reduction of immobility time.<br>Alcohol intake was significantly reduced dose-dependently |
| Dry extract (ethanol 50%, no further details) | 25-200 mg/kg BW, i.p. before ethanol withdrawal | *In vivo*<br>Withdrawal syndrome sin ethanol-dependent rats | Coskun *et al.* 2006 | *Hypericum* extract produced some dose dependent and significant inhibitory effects on locomotor hyperactivity at second and sixth hour of ethanol withdrawal. In addition, it significantly reduced the number of stereotyped behaviors at the same dose range. At doses of 50 and 100 mg/kg it produced some significant inhibitory effects on tremor and audiogenic seizures during withdrawal period |
| Dry extract (0.3% hypericin, no further details) | 100-600 mg/kg, oral | *In vivo*<br>Wistar rats<br>Ex vivo | Capasso *et al.* 2008 | Oral administration of SJW extract (100–600 mg kg−1) produced a dose-dependent decrease in gastric emptying.<br>*In vitro* studies showed that the extract was significantly |

| Herbal preparation tested | Strength<br>Dosage<br>Route of administration | Experimental model<br>***In vivo/***<br>***In vitro*** | Reference<br>Year of publication | Main non-clinical conclusions of the authors |
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| | Up to 1000 µg/m | Isolated stomach | | more active in inhibiting acetylcholine (or prostaglandin E2)-induced contractions than electrical field stimulation (EFS)-induced contractions. The effect of the extract on EFS-induced contractions was unaffected by drugs that inhibit intrinsic inhibitory nerves or by tachykinin antagonists, but it was reduced by the 5-hydroxytryptamine antagonist methysergide. The inhibitory effect of *Hypericum* extract on acetylcholine-induced contractions was reduced by the sarcoplasmic reticulum Ca2+-ATPase inhibitor cyclopiazonic acid, but not by the L-type Ca2+ channel blocker nifedipine or by methysergide. Among the chemical constituents of the extract tested, hyperforin and, to a lesser extent, the flavonoids kaempferol and quercitrin, inhibited acetylcholine-induced contractions |
| Dry extract (ethanol 80%, no further information) | 25-100 mg/kg BW, oral<br>3 days | *In vivo*<br>Hypthermic-restraint stress induced gastritis | Cayci & Dayioglu 2009 | Macroscopic analyses showed that treatment with the extract 25, 50, and 100 mg/kg/day significantly healed lesions compared to control groups by 65, 95, and 75% (p=0.001) |
| Dry extract (no further information) | 50-300 mg/kg BW, i.p.<br>3 days | *In vivo*<br>Rats with induced inflammatory bowel disease | Dost *et al.* 2009 | Colonic damage was significantly reduced by *Hypericum* extract. Macroscopic scoring of colonic damage was significantly reduced compared to untreated animals (P < 0.001). Blood catalase levels were reduced in the treatment group (150 mg/kg/day) compared with the untreated group (P < 0.01) |

| **Herbal preparation tested** | **Strength**<br>**Dosage**<br>**Route of administration** | **Experimental model**<br>***In vivo/***<br>***In vitro*** | **Reference**<br>**Year of publication** | **Main non-clinical conclusions of the authors** |
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| Extract (0.1% hypericin, no further details) | 150 – 450 mg/kg BW,<br>i.g. | *In vivo*<br>Rats with induced inflammatory bowel disease | Mozaffari *et al.* 2011 | A significant reduction in small bowel and colonic transit (450 mg/kg), TNF-α, myeloperoxidase (MPO), and lipid peroxidation and an increase in antioxidant power in all *Hypericum*-treated groups were seen as compared with the control group. Gastric emptying did not alter significantly when compared with the control group. Treatment with loperamide (10 mg/kg) significantly inhibited gastric emptying and small bowel and colonic transit, while flouxetine (10 mg/kg) decreased gastric emptying, TNF-α, MPO, and lipid peroxidation and increased the antioxidant power of the samples in comparison with the control group |
| Dry extract (ethanol 80%, no further information) | 25 µg/ml extract | *In vitro*<br>Murine 3T3-L1 preadipocytes<br>Fully developed 3T3-L1 cells | Amini *et al.* 2009 | *Hypericum* extract inhibited adipogenesis as judged by PPARγ and adiponectin levels. The extract inhibited insulin sensitive glucose uptake |
| Dry extract (ethanol 80%, no further information)<br>Hypericin<br>Hyperforin | 50 µg/ml<br>0.5 and 3 µM<br>0.2 and 0.47 µM | *In vitro*<br>Murine 3T3-L1 preadipocytes<br>Fully developed 3T3-L1 cells<br>Human adipocytes | Richard *et al.* 2012 | *Hypericum* extract attenuates insulin-sensitive glucose uptake in human adipocytes. Moreover, the extract inhibits IRS-1 tyrosine phosphorylation in both murine and human fat cells. The effects on adipogenesis, IRS-1 activation, and insulin-stimulated glucose uptake are not mediated by hypericin and/or hyperforin. |
| Dry extract (no further details) | Up to 50 µg/ml | *In vitro* | Hatano *et al.* 2014 | Oil Red O staining indicated that *Hypericum* extract promotes adipocyte differentiation, while immunoblots |

| Herbal preparation tested | Strength<br>Dosage<br>Route of administration | Experimental model<br><br>*In vivo/*<br>*In vitro* | Reference<br><br>Year of publication | Main non-clinical conclusions of the authors |
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| | | 3T3-L1 preadipocytes<br><br>Fully developed 3T3-L1 cells | | indicated that the extract increases the expression of peroxisome proliferator activated receptor γ (PPARγ), a nuclear receptor regulating adipocyte differentiation, and adiponectin, an anti-inflammatory adipokine. The anti-inflammatory activity of *Hypericum* was demonstrated by its inhibition of the activation of nuclear factor-κB (NF-κB), an inflammatory transcription factor. Stimulation of mature 3T3-L1 adipocytes by tumor necrosis factor-α (TNF-α) decreased the expression of the NF-κB inhibitor IκBα, and increased its phosphorylation. Treatment with *Hypericum* further decreased the TNF-α-induced perturbation in IκBα expression and phosphorylation, *Hypericum* decreased the TNF-α-induced increase in the mRNA levels of pro-inflammatory adipokines, interleukin-6 (IL-6), and monocyte chemoattractant protein-1 (MCP-1) |
| Dry extract (no further details) | Oral<br><br>200 mg/kg BW, 2 weeks | *In vivo* | Fuller *et al.* 2014 | Mice treated with *Hypericum* extract showed increased levels of adiponectin in white adipose tissue in a depot specific manner ($P < 0.01$). *Hypericum* extract also exerted an insulin-sensitizing effect as indicated by a significant increase in insulin-stimulated Akt serine phosphorylation in epididymal white adipose tissue ($P < 0.01$). Food intake, body weight, fasting blood glucose, and fasting insulin did not differ between the two groups |
| Dry extract (ethyl acetate, no further | Oral | *In vivo* | Arokiyaraj *et al.* | H. perforatum ethyl acetate extract showed dose dependent fall in fasting blood glucose (FBG). After 30 |

| **Herbal preparation tested** | **Strength**<br>**Dosage**<br>**Route of administration** | **Experimental model**<br>***In vivo/***<br>***In vitro*** | **Reference**<br>**Year of publication** | **Main non-clinical conclusions of the authors** |
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| information) | 50, 100 and 200 mg/kg BW | Streptozotocin induced diabetic rats | 2011 | min of extract administration, FBG was reduced significantly when compared with normal rats. H. perforatum ethyl acetate extract produced significant reduction in plasma glucose level, serum total cholesterol, triglycerides, glucose-6-phosphatase levels. Tissue glycogen content, HDL-cholesterol, glucose-6-phosphate dehydrogenase were significantly increased compared with diabetic control |
| Dry extract (ethanol 50%, no further details) | 125 mg and 250 mg/kg per day, i.p., 7 days | *In vivo*<br>Streptozotocin induced diabetic rats<br>Plus-maze<br>Activity cage<br>Modified forced swimming test<br>Active avoidance test | Can *et al.* 2011a | The reults show a diabetes mellitus (DM)-induced increase in anxiety and depression levels, decrease in spontaneous locomotor activities, and impairment of learning parameters in rats even in the early stages of the disease. Daily insulin replacement (2 IU/kg/day) could not restore these impaired parameters completely.<br>*Hypericum* extract provided significant improvement in all of the impaired parameters |
| Dry extract (ethanol 50%, no further details) | 125 mg and 250 mg/kg per day, i.p., 7 days | *In vivo*<br>Streptozotocin induced diabetic rats<br>Tail-pinch test<br>Tail-flick test | Can *et al.* 2011b | *Hypericum* extract induced significant decrease in high blood glucose levels of three weeks STZ-diabetic rats and improved their dysregulated metabolic parameters. In addition, the treatment caused restoration in the mechanical hyperalgesia of diabetic animals |

| Herbal preparation tested | Strength<br>Dosage<br>Route of administration | Experimental model<br><br>*In vivo/*<br>*In vitro* | Reference<br><br>Year of publication | Main non-clinical conclusions of the authors |
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| Dry extract (ethanol 50%, no further information), not less than 3% hyperforin, 0.3% hypericins | 100, 200 and 300 mg/kg BW, oral, 14 days | *In vivo*<br><br>Streptozotocin induced diabetic rats | Husain *et al.* 2009 | Daily oral administration of the Hypericum extract counteracted in a dose-dependent manner the alterations in blood glucose levels and lipid profile as well as liver glycogen content and body weight changes. In general, effects of the highest dose of the extract in this model were quite similar, but not identical, to those of a 10 mg/kg/day dose of glibenclamide. The effects of single oral doses of the extract in a rat oral glucose tolerance test conducted in fasted animals were also analogous to those of an antidiabetic drug therapeutic use |
| Fractions of an extract (ethanol 95%) | Undefined fraction, 50 and 200 mg/kg BW, 3 weeks | *In vivo*<br><br>High fat diet induced obese mice.<br><br>Ex vivo<br><br>Skeletal muscles | Tian *et al.* 2015 | The fraction 4 significantly improved the glucose and lipid metabolism in obese mice. *In vitro*, EHP inhibited the catalytic activity of recombinant human protein tyrosine phosphatase 1B (PTP1B) and reduced the protein and mRNA levels of PTP1B in the skeletal muscle. Moreover, expressions of genes related to fatty acid uptake and oxidation were changed in the skeletal muscle |
| Dry extract (ethanol 50%, no further details) | Rats<br><br>i.p. 0-400 mg extract/kg BW | Humoral antibody response<br><br>Blood leukocyte count<br><br>Body weight, spleen index | Aghili *et al.* 2014 | The IgG titer increased with higher doses of *Hypericum* extract. The extract increased number of lymphocytes at 200 mg but decreased at 400 mg, number of neutrophils decreased at 200 mg but increased at 400 mg, and number of monocytes increased at 100 mg and 200 mg but decreased at 400 mg ($p<0.01$). Increasing doses of the extract lowered BW ($p<0.01$). The extract |

| **Herbal preparation tested** | **Strength Dosage Route of administration** | **Experimental model** <br> ***In vivo/ In vitro*** | **Reference** <br> **Year of publication** | **Main non-clinical conclusions of the authors** |
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| | | | | increased spleen index at 100 mg and 200 mg but decreased at 400 mg (p>0.072) |
| Dry extract (ethanol 95%, no further details) | 110 mg/kg oral, 2 weeks | *In vivo* <br> Mice immunized with sheep red blood cells (SRBC) | Froushani *et al.* 2015 | The results indicate a significant increase in the level of anti-SRBC antibody and simultaneously a significant decrease in the level of cellular immunity, an enhancement in foot pad thickness, in treatment group compared to control group. The level of the respiratory burst in phagocytic cells and the level of lymphocyte proliferation in splenocytes were significantly decreased in the treatment group compared to control group. Moreover, extract caused a significant reduction in the production of pro-inflammatory IL-17 as well as IFN-γ, parallel to increasing the level of IL-6 |
| Dry extract (ethanol 95%, no further information) | 30 µg/ml <br><br> 110 mg/kg BW, oral, 5 days | *In vitro* <br> A549 human bronchial alveolar epithelial cells <br><br> *In vivo* <br> BALB/c mice infected with A/PR/8/34 H1N1 influenza virus | Huang *et al.* 2013 | In A549 cells, the extract significantly inhibited influenza virus induced monocyte chemotactic protein (MCP)-1 and interferon-γ induced protein 10 kD (IP-10), but dramatically increased interleukin-6 (IL-6). In mice inoculated intranasally with $10^{7.9}$ $EID_{50}$ of Influenza A/PR/8/34 H1N1 (high dose), daily oral treatment of H. perforatum extract increased lung viral titer, bronchoalveolar lavage (BAL) pro-inflammatory cytokine and chemokine levels, and the infiltration of pro-inflammatory cells in the lung 5 days post-inoculation, as compared to ethanol vehicle treated mice. Transcription of suppressor of cytokine signaling 3 (SOCS3) was increased by H. perforatum extract both in |

| **Herbal preparation tested** | **Strength**<br>**Dosage**<br>**Route of administration** | **Experimental model**<br>***In vivo/***<br>***In vitro*** | **Reference**<br>**Year of publication** | **Main non-clinical conclusions of the authors** |
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| | | | | A549 cells and BALB/c mice, which could have interrupted anti-viral immune response and thus led to the inefficient viral clearance and increased lung inflammation. H. perforatum treatment resulted in minor reduction in viral titer without affecting body weight when mice were inoculated with a lower dose (~$10^{5.0}$ $EID_{50}$) and H. perforatum was applied in the later phase of infection. Mice challenged intranasally with high dose of influenza virus ($10^{7.9}$ $EID_{50}$) suffered from a higher mortality rate when dosed with H. perforatum extract |
| Dry extract (ethanol, no further details) | 25-200 mg/kg orally | *In vivo*<br>Carrageenan-induced rat paw oedema | Savikin *et al.* 2007 | All extracts exhibited anti-inflammatory activity. The activity was independent of the hypericin content. |
| Hyperforin | *In vitro*:<br>0-2.5 µM<br>*In vivo*:<br>150 mg/kg hyperforin dicyclohexyl-ammonium salt | *In vitro*<br>IL-2/PHA-activated T cells<br>*In vivo*<br>Rats | Cabrelle *et al.* 2008 | Treatment with Hyperforin inhibited IFN-gamma production, with down-regulation of T-box (T-bet; marker of Th1 gene expression) and up-regulation of GATA-3 (marker gene of Th2) on IL-2/PHA-activated T cells. The chemokine receptor CXCR3 expression on activated T cells was strongly down-regulated.<br>Hyperforin attenuates the symptoms in an animal model of experimental allergic encephalomyelitis (EAE), a classic, Th1-mediated autoimmune disease of the CNS |
| Hyperforin | 4 mg/kg BW, i.p. | *In vivo* | Feißt *et al.* 2009 | Hyperforin significantly suppressed leukotriene B(4) formation in pleural exudates of carrageenan-treated |

| **Herbal preparation tested** | **Strength Dosage Route of administration** | **Experimental model**<br><br>***In vivo/ In vitro*** | **Reference**<br><br>**Year of publication** | **Main non-clinical conclusions of the authors** |
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| | | Carrageenan treated rats | | rats associated. Inhibition of 5-LO by hyperforin, but not by the iron-ligand type 5-LO inhibitor BWA4C or the nonredox-type inhibitor ZM230487, was abolished in the presence of phosphatidylcholine and strongly reduced by mutation (W13A-W75A-W102A) of the 5-LO C2-like domain. Moreover, hyperforin impaired the interaction of 5-LO with coactosin-like protein and abrogated 5-LO nuclear membrane translocation in ionomycin-stimulated neutrophils, processes that are typically mediated via the regulatory 5-LO C2-like domain |
| Dry extract (no further information) | 30-300 mg/kg BW, i.p., 30 minutes before paracetamol administration | *In vivo*<br><br>Paracetamol-induced lethality and liver damaga | Hohmann *et al.* 2015 | *Hypericum* extract dose-dependently reduced paracetamol-induced lethality. Paracetamol-induced increase in plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations, and hepatic myeloperoxidase activity, IL-1β, TNF-α, and IFN-γ concentrations as well as decreased reduced glutathione (GSH) concentrations and capacity to reduce 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate radical cation; ABTS˙(+) ) were inhibited by H. perforatum (300 mg/kg, i.p.) treatment |
| Dry extract (ethanol 95%, no further information) | Extract 58.9 µg/ml<br><br>Fractions 1.5-44.7 µg/ml | *In vitro*<br><br>RAW 264.7 mouse macrophages; C57/B6 mouse peritoneal macrophages | Huang *et al.* 2011 | Pseudohypericin, quercetin, amentoflavone and chlorogenic acid accounted for a significant part of the extract's inhibitory activity on PGE2, NO, tumor necrosis factor-α (TNF-α), and interleukin-1β (IL-1β) in RAW 264.7 as well as peritoneal macrophages. Pseudohypericin was the most important contributor of |

| **Herbal preparation tested** | **Strength**<br>**Dosage**<br>**Route of administration** | **Experimental model**<br>***In vivo/***<br>***In vitro*** | **Reference**<br>**Year of publication** | **Main non-clinical conclusions of the authors** |
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| | | | | the anti-inflammatory potential among these 4 compounds |
| Dry extract (ethanol 95%, no further information); 11 HPLC fractions | Extract 30 µg/ml<br>Pseudohypericin 0.008 µM<br>Quercetin 0.38 µM<br>Amentoflavone 0.03 µM<br>Chlorogenic acid 0.58 µM | *In vitro*<br>RAW 264.7 mouse macrophages | Huang *et al.* 2012 | siRNA was used to knockdown expression of SOCS3 in RAW 264.7 macrophages.<br>The SOCS3 knockdown significantly compromised the inhibition of PGE2 and nitric oxide (NO) by pseudohypericin, quercetin, amentoflavone and chlorogenic acid, but not by the extract. These 4 compounds, but not the extract, decreased interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), while both lowered interleukine-1β. SOCS3 knockdown further decreased IL-6 and TNF-α. Pseudohypericin was the major contributor to the PGE2 and NO inhibition in cells treated with the 4 compounds, and its activity was lost with the SOCS3 knockdown. Cyclooxygenase-2 (COX-2) and inducible NO synthase protein expression were not altered by the treatments, while COX-2 activity was decreased by the extract and the 4 compounds and increased by SOCS3 knockdown |
| Hyperforin | 1-30 µM | *In vitro*<br>Human lung carcinoma cells A549 | Koeberle *et al.* 2011 | Hyp significantly suppressed PGE2 formation in whole blood assays starting at 0.03–1 µM, whereas the concomitant generation of COX-derived 12(S)-hydroxy-5-cis-8,10-trans-heptadecatrienoic acid, thromboxane B2, and 6-keto PGF1α was not significantly suppressed up to 30 µM. In cell-free assays, Hyp efficiently blocked |

| Herbal preparation tested | Strength<br>Dosage<br>Route of administration | Experimental model<br>*In vivo/*<br>*In vitro* | Reference<br>Year of publication | Main non-clinical conclusions of the authors |
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| | 4 mg/kg BW, i.p. | *In vivo*<br>Carrageenan induced mouse paw edema<br>Carrageenan induced pleurisy in rats | | the conversion of PGH2 to PGE2 mediated by mPGES-1 ($IC_{50}$ = 1 μM), and isolated COX enzymes were not (COX-2) or hardly (COX-1) suppressed. Intraperitoneal (i.p.) administration of Hyp to rats impaired exudate volume and leukocyte numbers in carrageenan-induced pleurisy associated with reduced PGE2 levels, and Hyp (given i.p.) inhibited carrageenan-induced mouse paw edema formation ($ED_{50}$ = 1 mg $kg^{-1}$) being superior over indomethacin ($ED_{50}$ = 5 mg kg−1) |
| Dry extract (ethanol 50%, no further information)<br>Hyperforin | 75 µg/ml in culture medium | *In vitro*<br>LPS-induced NO production by microglia and macrophages<br>BV2 and N11 cells | Kraus *et al.* 2010 | The extract efficiently suppress lipopolysaccharide-induced NO release. Hyperforin was identified as the responsible compound, being effective at concentrations between 0.25 and 0.75 µM. The reduced NO production was mediated by diminished inducible nitric oxide synthase expression on the mRNA and protein level. At similar concentrations, hyperforin reduced zymosan phygocytosis to 20-40% and putatively acted by downregulating the CD206 macrophage mannose receptor and modulation of cell motility. The observed effects correlated with a suppression of the activated state of Nf-kappaB and phospho-CREB, while c-JUN, STAT1, and HIF-1alpha activity and cyclooxygenase-2 expression remained unaffected by hyperforin |
| Hyperoside | Up to 50 µM | *In vitro*<br>Primary human umbilical vein | Ku *et al.* 2014 | High glucose induced markedly increased vascular permeability, monocyte adhesion, expressions of cell adhesion molecules (CAMs), formation of reactive |

| **Herbal preparation tested** | **Strength**<br>**Dosage**<br>**Route of administration** | **Experimental model**<br>***In vivo/***<br>***In vitro*** | **Reference**<br>**Year of publication** | **Main non-clinical conclusions of the authors** |
|---|---|---|---|---|
| | <br>18.6 or 46.4 µg/mouse | endothelial cells<br>*In vivo*<br>High glucose induced vascular inflammation | | oxygen species (ROS), and activation of nuclear factor (NF)-κB. All of the above-mentioned vascular inflammatory effects were attenuated by pretreatment with hyperoside |
| CO2 extract containing 44.3% hyperforin<br>Hyperforin | Up to 10 µM | *In vitro*<br>DPPH test<br>Ex vivo<br>Test on radical formation in pig ear skin cells irradiated with solar simulated radiation | Meinke *et al.* 2012 | Hyperforin ($EC_{50}$ 0.7 µM corresponding to 0.42 µg/ml) was much more effective compared to Trolox ($EC_{50}$ 12 µg/ml) and N-acetylcysteine ($EC_{50}$ 847 µg/ml) without showing phototoxicity. The radical protection factor of a cream containing 1.5%w/w of a hyperforin-rich *Hypericum* extract was determined to be 200 × $10^{14}$ radicals/mg, indicating a high radical scavenging activity |
| Dry extract (0.34% hypericin, 4.1% hyperforin, 5% flavonoids, 10% tannins) | 2 mg/kg BW, oral, 8 days | *In vivo*<br>Experimentally induced periodontitis in rats | Paterniti *et al.* 2010 | The extract reduced significantly edema, inflammatory cell infiltration, alveolar bone loss, as well as other inflammation parameters |
| Extract (no further information) | Topical gel (10%)<br>300 mg/kg BW, oral | *In vivo*<br>Chemotherapy induced mucositis in hamsters | Tanideh *et al.* 2014 | Both of the H. perforatum extract treatment groups saw a significant relief in oral mucositis compared to the control and base gel groups; the systemic form was superior to the topical form |

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| Dry extract (no further details), containing 0.3% total hypericins | 0.1-100 µg/ml | *In vitro*<br>Reduction of DPPH radicals<br>Inhibition of lipid peroxidase formation | Benedi *et al.* 2004 | Inhibition of lipid peroxidation of rat brain cortex mitochondria; DPPH radical scavenging in dose dependent manner; attenuation of the increase of caspase-3 activity |
| Flavonoid-rich fraction of a commercial extract (no further details), 0.3% hypericin | 25-150 mg/kg/day<br>16 weeks, oral administration | *In vivo*<br>Wistar rats fed a cholesterol-rich diet | Zou *et al.* 2005 | The doses of 75 and 150 mg/kg/day significantly lowered the serum levels of total cholesterol, total triglycerides and low-density lipoprotein cholesterol, while the levels of high-density lipoprotein cholesterol were increased. The content of malondialdehyde decreased significantly in serum and liver. The activity of superoxide dismutase increased in serum and liver, the activity of catalase was elevated in the liver |
| Dry extract (ethanol 50%, no further information), not less than 3% hyperforin, 0.3% hypericins | 100 and 200 mg/kg BW, oral, 15 days | *In vivo*<br>Charles Foster rats<br>Fructose-induced hypertriglyceridemia and insulin resistance<br>High-fat-diet- induced obesity | Husain *et al.* 2011b | *Hypericum* significantly lowered total cholesterol and low-density cholesterol in normal rats. *Hypericum* significantly inhibited weight gain in high-fat-fed rats. In fructose-fed rats, *Hypericum* normalised the dyslipidemia induced by fructose feeding and improved the insulin sensitivity |
| Dry extract (ethanol 96%, no further details) | 150 mg extract / kg BW per day, oral | *In vivo*<br>Rabbits | Asgary *et al.* 2012 | *Hypericum perforatum* extract significantly decreased the levels of apolipoprotein B(apoB), apolipoprotein B/apolipoprotein A (apoB/apoA), triglyceride, cholesterol, low density lipoprotein cholesterol, oxidized |

| Herbal preparation tested | Strength<br>Dosage<br>Route of administration | Experimental model<br><br>*In vivo/*<br>*In vitro* | Reference<br><br>Year of publication | Main non-clinical conclusions of the authors |
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| | 60 days<br><br>Group I: standard diet<br><br>Group II: standard diet + *Hypericum* extract<br><br>Group III: standard diet + *Hypericum* extract + 1% cholesterol<br><br>Group IV: standard diet + 1% cholesterol<br><br>Group V: : standard diet + 1% cholesterol + 10 mg/kg lovastatin | | | LDL, malondialdehyde, and C-reactive protein (CRP) as well as atherosclerosis index, and increased high density lipoprotein and apoA in rabbits of Group III compared to the rabbits of Group IV. The effect of *Hypericum perforatum* extract in decreasing the level of some biochemical factors like apoB, apoB/apoA, and CRP was meaningfully more than that of lovastatin. Histopathological findings confirmed that hydroalcoholic extract of *Hypericum perforatum* restricted the atherosclerotic lesions |
| Hyperforin | 4 mg/kg i.p. | *In vivo*<br><br>Carrageenan-treated rats | Feißt *et al.* 2009 | Hyperforin significantly suppressed leukotriene B(4) formation in pleural exudates associated with anti-inflammatory effectiveness. Inhibition of 5-lipoxygenase (5-LO) by hyperforin, but not by the iron-ligand type 5-LO inhibitor BWA4C or the nonredox-type inhibitor ZM230487, was abolished in the presence of |

| **Herbal preparation tested** | **Strength Dosage Route of administration** | **Experimental model** *In vivo/ In vitro* | **Reference Year of publication** | **Main non-clinical conclusions of the authors** |
|---|---|---|---|---|
| | | | | phosphatidylcholine and strongly reduced by mutation (W13A-W75A-W102A) of the 5-LO C2-like domain. Moreover, hyperforin impaired the interaction of 5-LO with coactosin-like protein and abrogated 5-LO nuclear membrane translocation in ionomycin-stimulated neutrophils, processes that are typically mediated via the regulatory 5-LO C2-like domain |
| Dry extract (ethanol 70%, no further information) | 500 mg/kg BW, 6 weeks | *In vivo* Sham-operated and ovariectomsed (OVX) rats | You *et al.* 2014 | *Hypericum* showed estrogen-like effect on body weight gain, adipose tissue weight and food efficacy ratio in OVX rats. Hypericum prevented hypercholesterolemia induced by OVX more effectively than estradio. Estradiol increased uterus weight and epithelial proliferation rate in OVX rats, while Hypericum maintained them at the level of the sham-operated animals |
| Dry extract (water, no further information) | Exact concentrations not available | *In vivo* Morphine dependent rats | Feily & Abbasi 2009 | Clonidine was more effective than Hypericum at a dose of 0.4 mL/200 g and there was no significant statistical difference between the mean frequency of withdrawal signs of Hypericum at a dose of 0.8 mL/200 g compared with clonidine (0.2 mg/kg i.p.) but at a dose of 1.2 mL/200 g of the Hypericum extract was significantly stronger than clonidine in attenuation of the morphine withdrawal syndrome |
| Soft extract (ethanol 70%, no further | 20 mg/kg BW, oral; twice daily for 9 days; single | *In vivo* Induced opium dependence | Khan *et al.* 2014 | Hypericum reduced stereotype jumps and wet dog shake number in the chronic treatment compared to the saline control group ($F(2, 24) = 3.968$, $p < 0.05$) and |

| **Herbal preparation tested** | **Strength Dosage Route of administration** | **Experimental model** <br> ***In vivo/ In vitro*** | **Reference** <br> **Year of publication** | **Main non-clinical conclusions of the authors** |
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| information) | dose 1 h before induction of withdrawal symptoms | in rats | | ($F(2, 24) = 3.689$, $p < 0.05$), respectively. The plant extract in the acutely treated group reduced diarrhoea ($F(2, 24) = 4.850$, $p < 0.05$ vs. saline). It decreased rectal temperature by chronic treatment at 30 min ($F(2, 24) = 4.88$, $p < 0.05$), 60 min ($F(2, 240 = 5.364$, $p < 0.01$) and 120 min ($F(2, 24) = 4.907$, $p < 0.05$) |
| Dried aqueous extract (DER app. 4:1, no further information), dry extracts with ethanol 70% and ethanol 96% | 20 mg/kg BW, oral,2 x daily or single acute dose | *In vivo* <br> Withdrawal signs in heroin dependent rats | Subhan *et al.* 2009 | The aqueous extract attenuated abdominal constriction. Diarrhea was ameliorated by the ethanolic extracts |
| Dry extract (0.32% hypericin, no further information) <br> Hypericin, hyperforin, quercetin, amentoflavon, hyperoside | Gavage <br> Extract 5 mg/kg <br> Hypericin 0.016 mg/kg <br> Hyperforin 0.21 mg/kg <br> Quercetin 0.0415 mg/kg <br> Amentoflavon 0.0029 mg/kg | *In vivo* <br> Mice treated with morphine | Galeotti *et al.* 2014 | Co-administration of morphine and Hypericum extract or hypericin prevented phosphorylation in rodent periaqueductal grey matter inducing a potentiation of morphine analgesia in thermal pain |

| Herbal preparation tested | Strength<br>Dosage<br>Route of administration | Experimental model<br>*In vivo/*<br>*In vitro* | Reference<br>Year of publication | Main non-clinical conclusions of the authors |
|---|---|---|---|---|
| | Hyperoside 0.3175 mg/kg | | | |
| Extract prepared with ethanol 80%, fractionation with solvents of different polarity | 1 ml/kg BW | *In vivo*<br>Bioelectrical activity assay in rabbits | Ivetic *et al.* 2002 | Effects depend on the polarity of the extracts. The water fraction exerted the highest antiepileptic activity |
| Dry extract (DER 12:1, ethanol 80% V/V), 0.3% hypericin, 3% hyperforin, >20% flavonoids | 4-25 mg/kg, i.p. | *In vivo*<br>Passive avoidance conditioning test in mice | Khalifa 2001 | Acute administration of Hypericum extract before retrieval testing increased the step-down latency during the test session. The same doses of Hypericum extract, on the other hand, failed to reverse scopolamine-induced amnesia of a two-trial passive avoidance task. The involvement of serotonergic, adrenergic, and dopaminergic mechanisms in the facilitatory effect of Hypericum extract on retrieval memory was investigated. Pretreatment of the animals with (−)-pindolol (0.3, 1.0, and 3.0 mg/kg), spiperone (0.01, 0.03, and 0.1 mg/kg), phentolamine (1, 5, and 10 mg/kg), propranolol (5, 7.5, and 10 mg/kg), and sulpiride (5, 7.5, and 10 mg/kg) revealed the involvement of adrenergic and serotonergic 5-HT1A receptors in the facilitatory effect of Hypericum extract on retrieval memory |
| Dry extract (DER 3-7:1, methanol | 25-300 mg/kg extract | *In vivo*<br>Conditioned avoidance | Klusa *et al.* 2001 | 50 mg/kg/day of extract and 1.25 mg/kg/day of hyperforin improved the learning ability from day 2 until |

| **Herbal preparation tested** | **Strength<br>Dosage<br>Route of administration** | **Experimental model**<br><br>***In vivo/ In vitro*** | **Reference**<br><br>**Year of publication** | **Main non-clinical conclusions of the authors** |
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| 80% V/V); hyperforin | 1.25-2.5 mg/kg hyperforin<br><br>Oral administration. | response test in rats<br><br>Passive avoidance response test in mice<br><br>Scopolamine induced amnesia in mice<br><br>Behavioural despair test in rats | | day 7. The learned responses retained after 9 days without further treatment. A single dose of hyperforin (1.25 mg/kg) improved memory acquisition and completely reversed scopolamine-induced amnesia |
| Dry extract (ethanol 50%) | 100 and 200 mg/kg orally for 3 consecutive days | *In vivo*<br><br>Adult rats<br><br>Transfer latency in elevated plus-maze<br><br>Passive avoidance test<br><br>Active avoidance test<br><br>Scopolamine induced amnesia<br><br>Sodium nitrite induced amnesia | Kumar *et al.* 2000 | The extract exerted in the higher dose similar effects as compared to the known nootropic piracetam (500 mg/kg i.p.) |
| Dry extract (ethanol 50% by maceration) | Caffeine 4-16 mg/kg<br><br>Hypericum extract | *In vivo*<br><br>Effect of Hypericum on the caffeine-induced locomotor | Uzbay *et al.* 2007 | The highest dose of 48 mg/kg reduced significantly the locomotor activity. Lower doses did not change the activity. Doses between 6-24 mg/kg significantly blocked the caffeine (16 mg/kg) induced locomotor |

| **Herbal preparation tested** | **Strength Dosage Route of administration** | **Experimental model** <br> ***In vivo/ In vitro*** | **Reference** <br> **Year of publication** | **Main non-clinical conclusions of the authors** |
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| | 6-48 mg/kg | activity in mice | | activity |
| Hyperici herba, 0.3% hypericin | 350 mg/kg, 21 days, oral | *In vivo* <br> Water maze, elevated plus maze <br> Induced stress (corticosterone) | Trofimiuk *et al.* 2005 | H. perforatum prevented the deleterious effects of both chronic restraint stress and long-term corticosterone on learning and memory as measured in both, the object recognition and the water maze tests. It not only prevented stress- and corticosterone-induced memory impairments, but it significantly improved recognition memory ($p < 0.01$) in comparison to control |
| Hyperici herba, 0.3% hypericin | 350 mg/kg, 21 days, oral | *In vivo* <br> Chimney test <br> Passive avoidance test <br> Conditioned avoidance test <br> Induced stress (corticosterone) | Trofimiuk *et al.* 2006 | Hypericum significantly enhanced the recall of passive avoidance behavior, but had no effect on the acquisition of conditioned avoidance responses. The diminished recall in stressed rats was abolished by Hypericum |
| Hyperici herba, 0.2% hypericin | 350 mg/kg, 21 days, oral | *In vivo* <br> Water maze <br> Barnes maze <br> Induced stress (corticosterone) | Trofimiuk & Braszko 2008 | H. perforatum prevented the deleterious effects of both chronic restraint stress and prolonged corticosterone on working memory measured in both tests. The herb significantly improved hippocampus dependent spatial working memory in comparison with control ($p<0.01$) and alleviated some other negative effects of stress on cognitive functions |
| Dry extract (methanol 100%, | 300 mg/kg p.o., 7 | *In vivo* | Mohanasundari *et al.* | Hypericum in combination with bromocriptine: |

| **Herbal preparation tested** | **Strength**<br>**Dosage**<br>**Route of administration** | **Experimental model**<br>***In vivo/***<br>***In vitro*** | **Reference**<br>**Year of publication** | **Main non-clinical conclusions of the authors** |
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| 12:1) | days<br>Bromocriptine 10 mg/kg i.p. | Induced Parkinson's disease<br>Rotarod test (motor co-ordination)<br>Hang test (neuromuscular strength)<br>Forepaw stride length during walking | 2006 | significant improvement in all test systems.<br>Dopamine and 3,4-dihydroxyphenyl acetic acid levels were significantly improved. Significant reduction in lipid peroxidation |
| Dry extract (methanol 100%, 12:1) | 300 mg/kg p.o., 7 days | *In vivo*<br>Reaction of astrocytes in mice brain after i.p. administration of 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine | Mohanasundari *et al.* 2007 | Treatment with Hypericum inhibited monoamine oxidase-B activity and reduced astrocyte activation in striatal area |
| Dry extract (ethanol 70%, 0.37% hypericin, 3.1% hyperforin, 4.3% flavonoids) | 200 mg/kg BW, i.p., 2 weeks | *In vivo*<br>Induced Parkinson's disease<br>Rotational behaviour<br>Elevated narrow beam test<br>Oxidative stress assessment | Kiasalari *et al.* 2016 | The extract attenuated apomorphine-induced rotational behavior, decreased the latency to initiate and the total time on the narrow beam task, lowered striatal level of malondialdehyde and enhanced striatal catalase activity and reduced glutathione content, normalized striatal expression of glial fibrillary acidic protein, tumor necrosis factor α with no significant effect on mitogen-activated protein kinase, lowered nigral DNA fragmentation, and prevented damage of nigral dopaminergic neurons with a higher striatal tyrosine |

| **Herbal preparation tested** | **Strength Dosage Route of administration** | **Experimental model** ***In vivo/ In vitro*** | **Reference Year of publication** | **Main non-clinical conclusions of the authors** |
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| | | | | hydroxylase immunoreactivity |
| Dry extract (ethanol 80% v/v, DER 12:1) | 4.0, 8.0, 12.0, and 25.0 mg/kg i.p. | *In vivo* Passive avoidance test | El-Sherbiny *et al.* 2003 | The administration of 1.4 mg/kg of scopolamine impaired the retrieval memory of rats associated with elevated malondialdehyde and reduced glutathione level. Pretreatment of the animals with Hypericum extract (4, 8, and 12 mg/kg) resulted in an antioxidant effect through altering brain malondialdehyde, glutathione peroxidase, and/or glutathione level/activity |
| Hyperforin | 0.1-30 µmol/l | *In vitro* Brain membranes from young guinea pigs | Eckert & Müller 2001 | 0.3 µmol/l hyperforin significantly decreased the annular fluidity of lipids close to membrane proteins |
| Dry extract (petroleum ether, 1,2-dichloroethane, ethanol 50%) | 26.5 mg/kg | *In vivo* Mice | Girzu *et al.* 1997 | Marked sedation in mice Increase in sleep duration induced by pentobarbital |
| Dry extract (hydromethanolic), 0.3% hypericine | 1-300 µg/ml | Ex vivo Vas deferens (rat, human) preparations, contractility | Capasso *et al.* 2005 | Concentration dependent decrease of the amplitude of electrical field stimulation and agonist induced contractions with the same potency, suggesting direct inhibition of rat vas deferens smooth muscle |
| Dry extract (ethanol 50% V/V, DER app. 5:1) | 26.5 mg/kg, oral | *In vivo* Sedation measured with actimeter and test for sleep | Girzu *et al.* 1997 | Marked sedation induced by the total extract. Activity of fractions was less compared to entire extract |

| Herbal preparation tested | Strength<br>Dosage<br>Route of administration | Experimental model<br>***In vivo/***<br>***In vitro*** | Reference<br>Year of publication | Main non-clinical conclusions of the authors |
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| | | potentiation. Comparator diazepam 2mg/kg p.o. | | |
| Dry extract (ethanol 80% V/V, DER 12:1) | 500 mg/kg, acute<br>200 mg/kg for 3 days | *In vivo*<br>Passive avoidance test in rats<br>Proapoptotic and prepulse inhibition (PPI) of acoustic startle reflex experiment | Tadros *et al.* 2009b | Hypericum extract disrupted PPI, which is interpreted as indicator for limitations of Hypericum to manage cognitive disturbances in psychotic patients |
| Dry extract (methanol 80%, no further details) | 25-200 mg/kg BW i.p. | *In vivo*<br>Picrotoxin induced seizures | Etemad *et al.* 2011 | Latency of seizures, duration of seizures as well as death latency were significantly reduced |
| Dry extract (0.32% hypericines, no further information) | 5 mg/kg BW, oral, single dose | *In vivo*<br>Mouse model induced by nitric oxide (NO) donors administration | Galeotti & Ghelardini 2013a | The extract produced a prolonged relief from pain hypersensitivity. Similarly, preventive administration increased the latency to the induction of hyperalgesia and reduced the duration of the painful symptomatology. Among main components, hypericin showed a similar profile of activity, whereas flavonoids were devoid of any antihyperalgesic effect. The upregulation and increased phosphorylation of protein kinase γ and protein kinase ε isoforms within periaqueductal grey matter was prevented by Hypericum treatment |
| Dry extract (0.32% hypericines, | 5 mg/kg BW, oral, single dose | *In vivo*<br>Mouse model induced by | Galeotti & Ghelardini 2013b | Glyceryl trinitrate and sodium nitroprusside produced a delayed meningeal inflammation, as showed by the upregulation of interleukin (IL)-1β and inducible NO |

| Herbal preparation tested | Strength<br>Dosage<br>Route of administration | Experimental model<br>***In vivo/ In vitro*** | Reference<br>Year of publication | Main non-clinical conclusions of the authors |
|---|---|---|---|---|
| 4.28% rutin, 6.35% hyperoside) | | nitric oxide (NO) donors administration | | synthase (iNOS), and a prolonged cold allodynia and heat hyperalgesia with a time-course consistent with NO-induced migraine attacks. Hypericum extract counteracted the nociceptive behaviour and the overexpression of IL-1β and iNOS. To clarify the cellular pathways involved, the expression of protein kinase C (PKC) and downstream effectors was detected. NO donors increased expression and phosphorylation of PKCγ, PKCε and transcription factors, such as nuclear factor (NF)-κB, cyclic AMP response element binding protein (CREB), Signal Transducer and Activator of Transcription (STAT)-1. All these molecular events were prevented by Hypericum extract and hypericin |
| Dry extract (0.32% hypericines, 4.28% rutin, 6.35% hyperoside) | 1 and 5 mg/kg BW, oral<br>Hypericin 0.01 mg/kg, oral | *In vivo*<br>Mice<br>Nociceptive hypersensitivity induced by administration of the NO donors nitroglycerin (GTN) and sodium nitroprusside (SNP) was assessed by cold and hot plate tests. | Galeotti & Ghelardini 2013c | GTN and SNP produced a prolonged allodynia and hyperalgesia in mice. Hypericum extract or purified hypericin reversed the NO donor-induced nociceptive behavior whereas hyperforin and flavoinoids were ineffective. Investigating into the cellular pathways involved, an increased CREB and STAT1 phosphorylation, and activation of NF-κB were detected within PAG and thalamus following NO donors' administration. These cellular events were prevented by Hypericum extract or hypericin. Since hypericin showed PKC blocking properties, a role of PKC as an upstream modulator of these transcription factors was hypothesized. NO donors increased expression and |

| **Herbal preparation tested** | **Strength Dosage Route of administration** | **Experimental model** <br> ***In vivo/ In vitro*** | **Reference** <br> **Year of publication** | **Main non-clinical conclusions of the authors** |
|---|---|---|---|---|
| | | | | phosphorylation of protein kinase C (PKC) γ and ε isoforms, molecular events prevented by Hypericum extract or hypericin |
| Dry extract (0.32% hypericines, 4.28% rutin, 6.35% hyperoside) | 30-60 mg/kg, oral | *In vivo* <br> Chronic constriction injury model <br> Repeated administration of oxaliplatin | Galeotti *et al.* 2010 (BiochemPharmacol) | Hypericum extract reversed mechanical hyperalgesia with a prolonged effect, being effective up to 180 min after injection. Hyperforin and hypericin were responsible for the antihyperalgesic properties whereas flavonoids were ineffective. The effect of Hypericum on the protein kinase C (PKC) expression and activation was investigated in the periaqueductal grey (PAG) area by immunoblotting experiments. Mechanistic studies showed a robust over-expression and hyperphosphorylation of the PKCgamma (227.0+/-15.0% of control) and PKCepsilon (213.9+/-17.0) isoforms in the rat PAG area. A single oral administration of SJW produced a significant decrease of the PKCgamma (131.8+/-10.0) and PKCepsilon (105.2+/-12.0) phosphorylation in the PAG area due to the presence of hypericin. Furthermore, Hypericum showed a dual mechanism of action since hyperforin antinociception involves an opioid-dependent pathway |
| SHP1: Dry extract (0.3% hypericin, 6% hyperforin, 4% flavonoids) | 4 mg/kg BW, i.p. <br> Quercetin: 25 and 100 mg/kg, i.p. | *In vivo* <br> Neurodegeneration induced by rotenone | Gomez del Rio *et al.* 2013 | Pretreatment of the animals with SHP1 and SHP2 efficiently halted deleterious toxic effects of rotenone, revealing normalization of catalepsy in addition to amelioration of neurochemical parameters. Also, SHP1 reduced neuronal damage, diminishing substantia nigra |

| **Herbal preparation tested** | **Strength Dosage Route of administration** | **Experimental model** <br><br> ***In vivo/ In vitro*** | **Reference** <br><br> **Year of publication** | **Main non-clinical conclusions of the authors** |
|---|---|---|---|---|
| SHP2: Dry extract (Ze 117, free of hyperforin) <br><br> Quercetin | 45 days | | | dopaminergic cell death caused by the pesticide, indicating benefit of neuroprotective therapy. In general, the SHP1 was more active than SHP2. In addition, SHP1 inhibited the apoptotic cascade by decreasing Bax levels |
| Dry extract (boiling water, 0.3% hypericin, 3% hyperforin, >20% flavonoids) | 6, 12 and 25 mg/kg BW, oral, 25 days | *In vivo* <br><br> Streptozotocin induced diabetes | Hasanein & Shahidi 2011 | Diabetes caused impairment in acquisition and retrieval processes of passive avoidance learning and memory. Hypericum treatment (12 and 25 mg/kg) improved learning and memory in control rats and reversed learning and memory deficits in diabetic rats. A dose of 6 mg/kg did not affect cognitive function. Hypericum administration did not alter the body weight and plasma glucose levels |
| Dry extract (ethanol 50%, no further information), not less than 3% hyperforin, 0.3% hypericins | 100 and 200 mg/kg BW, oral, 14 days | *In vivo* <br><br> Strptozotocin induced diabetic rats <br><br> Open-field-exploration test (OFT) <br><br> Plus-maze test (EPM) <br><br> Forced swimming test (FST) | Husain *et al.* 2011a | Diabetic rats showed significant increase in anxiety in OFT and EPM compared to non diabetic normal control rats. Diabetic rats treated with Hypericum extract have shown significant anxiolytic activity in OFT and EPM test. In FST, immobility period of vehicle treated diabetic rats was significantly increased ($p < 0.05$) compared to normal control rats. Treatment with Hypericum extract significantly decreased ($p < 0.001$) immobility period compared to vehicle treated diabetic control rats. HpE treatment significantly reduced elevated blood glucose levels in diabetic rats |
| Dry extracts (water, ethanol | 400 mg/kg BW, 60 | *In vivo* | Hofrichter *et al.* | Extracts both attenuate Aβ-induced histopathology and alleviate memory impairments in APP-transgenic mice. |

| Herbal preparation tested | Strength<br>Dosage<br>Route of administration | Experimental model<br>*In vivo/*<br>*In vitro* | Reference<br>Year of publication | Main non-clinical conclusions of the authors |
|---|---|---|---|---|
| 60%, ethanol 80%) | days, oral | Mice expressing mutated human amyloid precursor protein (APP) and mutated presenilinel transgenes | 2013 | These effects are attained independently of hyperforin. The reduction of soluble Aβ42 species is the consequence of a highly increased export activity in the blood-brain barrier ABCC1transporter, which was found to play a fundamental role in Aβ excretion into the bloodstream |
| Dry extract (ethanol 70%), fractionated with water, n-butanol and ether | 0.1 g/kg BW, i.m., 31 days | *In vivo*<br>Rabbits<br>Epileptic focus induced by stimulation of hippocampus | Ivetic *et al.* 2011 | Animals treated with an ether extract of Hypericum required significantly weaker minimum current strengths for the development of epileptogenic focus, and displayed longer after-discharge (AD) times, while the number of electro-stimulations necessary for full kindling was less. In contrast, animals treated with water and n-butanol extracts required increased electro-stimulations for the development of epileptic discharge, and displayed shortened AD durations versus controls |
| Hyperforin | 10 mg/kg BW, i.p., 7 days | *In vivo*<br>Rats and mice<br>Foot shock-induced aggression<br>Isolation-induced aggression<br>Resident-intruder aggression<br>Water competition test | Kumar *et al.* 2009 | Hyperforin treatment significantly ($p < 0.001$) reduced various aggressive parameters viz. latency to first attack and number of fights in isolation induced aggression, resident intruder aggression and foot shock induced aggression tests. In water competition test, hyperforin treatment significantly ($p < 0.001$) reduced the duration of water consumption and frequency of water spout possession |

| Herbal preparation tested | Strength<br>Dosage<br>Route of administration | Experimental model<br>*In vivo/*<br>*In vitro* | Reference<br>Year of publication | Main non-clinical conclusions of the authors |
|---|---|---|---|---|
| Herbal substance (0.3% hypericin, up to 6% hyperforin, 2-4% flavonoids, 8-12% procyanidins) | 350 mg/kg BW, oral, 3 weeks | *In vivo*<br>Aged rats<br>Open field test<br>Plus maze test<br>Morris water maze test | Trofimiuk *et al.* 2010 | Hypericum significantly improved the processing of spatial information in the aged rats ($p < 0.001$). Also the herb increased the levels of phosphorylated cyclic adenosine 3', 5'-monophosphate response element binding protein pCREB in the aged rat's hippocampus ($p < 0.01$) as measured by western immunoblotting. Aging caused significant locomotor impairments as tested in the open field ($p < 0.001$) but not in the water maze test. However, these were unaffected by treatment with Hypericum |
| Herbal substance (0.2% hypericin, 2-4.5% hyperforin, 2-4% flavonoids, 8-12% procyanidins) | 350 mg/kg BW, oral, 3 weeks | *In vivo*<br>Chronic restraint stress in rats<br>Open field test<br>Barnes maze test (BM) | Trofimiuk *et al.* 2011 | Hypericum significantly improved processing of spatial information in the stressed and corticosterone-injected rats ($p < 0.001$). It statistically significantly ($p < 0.05$) increased levels of neuromoduline GAP-43 and synaptophysin, respectively in the hippocampi and prefrontal cortex as measured by western immunoblotting. Hypericum prevented the deleterious effects of both chronic restraint stress and prolonged corticosterone administration on working memory measured in the BM test. The herb significantly ($p < 0.01$) improved hippocampus-dependent spatial working memory in comparison with control and alleviated some other negative effects of stress on cognitive functions |
| Dry extract (0.3% hypericin, 3.2% | 100 – 1000 mg/kg | *In vivo* | Uchida *et al.* 2008 | Oral pretreatment with Hypericum attenuated significantly times of licking/biting both first and second |

| **Herbal preparation tested** | **Strength Dosage Route of administration** | **Experimental model** <br> ***In vivo/ In vitro*** | **Reference** <br> **Year of publication** | **Main non-clinical conclusions of the authors** |
|---|---|---|---|---|
| hyperforin) | BW, oral | Mice <br> Formalin test <br> Tail-flick test <br> Locomotor activity | | phases of formalin injection in mice in the dose-dependent manner. Formalin injection resulted in significant increase in the content of nitrites/nitrates (NO(x)) in mouse spinal cord. The rise of spinal NO(x) content by formalin was significantly attenuated by Hypericum. The pretreatment with Hypericum significantly potentiated an antinociceptive effect of morphine (0.3 mg/kg, s.c.), although concentrations of morphine in plasma and brain were not significantly changed |
| Dry extract (ethanol 60% m/m), 0.3% hypericin, 4.5% hyperforin, 50% flavonoids | 125-500 mg/kg, orally by gavage for 30 days | *In vivo* <br> Evaluation of signs of nicotine withdrawal | Mannucci *et al.* 2007 | Animals treated with Hypericum extract showed a significant reduction of total abstinence score. The cortical 5-HT content increased as well as the 5-HT1A receptors. |
| Dry extract (no further details), 50% flavonoids, 0.3% hypericin, 4.5% hyperforin | 125-500 mg/kg, oral, acute or for 7 or 14 days | *In vivo* <br> Nicotine dependent mice <br> 2 mg/kg,4 x daily i.p. for 2 weeks | Catania *et al.* 2003 | The locomotor activity reduction induced by nicotine withdrawal was abolished by Hypericum, which also significantly and dose-dependently reduced the total nicotine abstinence score when injected after nicotine withdrawal |
| Dry extract (no further details), 50% flavonoids, 0.3% hypericin, | 125-500 mg/kg, oral <br> For 30 days after nicotine | *In vivo* <br> Nicotine dependent mice <br> 2 mg/kg,4 x daily i.p. for 2 | Mannucci *et al.* 2007 | After nicotine withdrawal reduction of the 5-HT content while animals treated only with Hypericum extract showed a significant reduction of total abstinence score compared to controls. A selective 5-HT receptor |

| Herbal preparation tested | Strength<br>Dosage<br>Route of administration | Experimental model<br>*In vivo/*<br>*In vitro* | Reference<br>Year of publication | Main non-clinical conclusions of the authors |
|---|---|---|---|---|
| 4.5% hyperforin | withdrawal;<br>500 mg/kg, animals not treated with nicotine | weeks | | antagonist inhibited the reduction of total abstinence score induced by H. perforatum. Moreover, 5-HT1A expression has been evaluated 30 days after nicotine withdrawal. The results show a significant increase of cortical 5-HT content in nicotine dependent mice treated with H. perforatum, with a concomitant significant increase of 5-HT1A receptor |
| Dry extract (methanol, no further information), 0.34% hypericin, 4.1% hyperforin, 5% flavonoids, 10% tannins | 30 mg/kg, oral, 1 h before and 6 h after spinal cord injury | *In vivo*<br>Experimental spinal cord injury in mice | Genovese *et al.* 2006b | The degree of spinal cord inflammation and tissue injury (histological score), nitrotyrosine, poly(adenosine diphosphate-ribose), neutrophils infiltration, and the activation of signal transducer and activator transcription 3 was markedly reduced in spinal cord tissue obtained from H. perforatum extract treated mice. H. perforatum extract significantly ameliorated the recovery of limb function |
| Dry extract (0.1-0.3% hypericin, 6% flavonoids, 6% hyperforin, no further information) | 30 mg/kg BW, oral, 3 days | *In vivo*<br>Experimental spinal cord injury (SCI) in rats | Özdemir *et al.* 2016 | The SCI-induced TRPM2 and TRPV1 currents and cytosolic free Ca(2+) concentration were reduced by the extract. The SCI-induced decrease in glutathione peroxidase and cell viability values were ameliorated by Hypericum treatment, and the SCI-induced increase in apoptosis, caspase 3, caspase 9, cytosolic reactive oxygen species (ROS) production, and mitochondrial membrane depolarization values in dorsal root ganglion of SCI group were overcome by Hypericum treatment |
| Dry extract | 21 mg/day, oral | *In vivo* | Halicioglu *et al.* | Number of osteoclasts, capillaries, inflammatory cell |

| **Herbal preparation tested** | **Strength Dosage Route of administration** | **Experimental model** <br> ***In vivo/ In vitro*** | **Reference** <br> **Year of publication** | **Main non-clinical conclusions of the authors** |
|---|---|---|---|---|
| (water, no further information) | 17 days and 57 days | Bone formation in the expanded premaxillary suture in rats | 2015 | infiltration and new bone formation was higher in treated animals. |
| Dry extract (0.3% hypericin, no further information) <br> Hypericin | 15 mg/kg/day, oral, 5 days <br> 15-135 µg/kg/day, oral, 5 days | *In vivo* <br> Mouse model of oxygen-induced retinopathy | Higuchi *et al.* 2008 | Hypericin and Hypericum extract significantly inhibited the degree of retinal neovascularization, but did not affect the area of retinal vasoobliteration. Both had no effect on normal vascularization over the treatment time course. Treatment with Hypericum extract or hypericin reduced phosphorylation of extracellular signal-regulated kinase in the retina |
| Hydroalcoholic extract (no further information) | 300 and 500 mg/kg BW, oral, 28 days | *In vivo* <br> Ethylene glycol (EG) induced calcium oxalate deposits in rats | Khalili *et al.* 2012 | Urine level of free calcium in groups EG and EG + Hypericum (300 mg/kg) and phosphorous in EG + Hypericum (500 mg/kg) significantly decreased compared to controls ($P < .01$; $P < .05$; and $P < .05$, respectively). Treatment of the rats with high dose of Hypericum (500 mg/kg) markedly reduced decrementing effect of EG on serum level of free calcium ($P < .05$). Histological experiments showed that chronic administration of Hypericum could significantly reduce the size and number of calcium oxalate deposits in EG group |
| Hyperforin | 1-27 µM | *In vitro* <br> Neutrophils, monocytes, HUVE cells | Lorusso *et al.* 2009 | Treatment with non-cytotoxic concentrations of Hyperforin restrains, in a dose-dependent manner, the capacity of endothelial cells to migrate towards relevant chemotactic stimuli. Hyperforin inhibits the organisation |

| **Herbal preparation tested** | **Strength Dosage Route of administration** | **Experimental model** <br><br> ***In vivo/ In vitro*** | **Reference** <br><br> **Year of publication** | **Main non-clinical conclusions of the authors** |
|---|---|---|---|---|
| | 5.4 mg/kg BW i.p. | *In vivo*: <br><br> Matrigel sponge model of *in vivo* angiogenesis in mice <br><br> *In vivo* xenograft tumour growth | | of HUVE endothelial cells in capillary-like structures *in vitro*, and potently represses angiogenesis in vivo in the Matrigel sponge assay in response to diverse angiogenic agents. Immunofluorescent staining shows that in cytokine-activated endothelial HUVE cells Hyperforin prevents translocation to the nucleus of NF-kappaB, a transcription factor regulating numerous genes involved in cell growth, survival, angiogenesis and invasion. Under Hyperforin treatment *in vivo*, the growth of Kaposi's sarcoma - a highly angiogenic tumour - is strongly inhibited, with the resultant tumours remarkably reduced in size and in vascularisation as compared with controls. Hyperforin inhibits neutrophil and monocyte chemotaxis *in vitro* and angiogenesis *in vivo* induced by angiogenic chemokines (CXCL8 or CCL2) |
| Dry extract (0.1% hypericin, 3.8% hyperforin, no further information) | 125, 250 and 500 mg/kg BW, oral | *In vivo* <br><br> Rat model of binge eating (BE) | Micioni Di Bonaventura *et al.* 2012 | The doses of 250 and 500 mg/kg of Hypericum extract significantly reduced the BE episode, while 125 mg/kg was ineffective. The same doses did not affect intake of highly palatable food in the absence of BE. The dose of 250 mg/kg did not significantly modify stress-induced increase in serum corticosterone levels, suggesting that the effect on BE is not due to suppression of the stress response |
| Dry extract (no further | 0.05 mg/ml, 30 | Ex vivo | Tugrul *et al.* 2011 | Although there were significant reductions in the contractile responses to phenylephrine |

| Herbal preparation tested | Strength<br>Dosage<br>Route of administration | Experimental model<br>*In vivo/*<br>*In vitro* | Reference<br>Year of publication | Main non-clinical conclusions of the authors |
|---|---|---|---|---|
| information) | minutes incubation | Aortic rings<br>Contractile responses to phenylephrine, KCl, acetylcholine, sodium nitroprusside | | (1113.73 ± 164.11; 477.40 ± 39.94; $p < 0.05$) and potassium chloride (745.58 ± 66.73; 112.58 ± 26.58; $p < 0.05$), no differences in the relaxant responses to acetylcholine (94.61 ± 2.65; 87.79 ± 9.40) and sodium nitroprusside (108.82 ± 5.06; 106.43 ± 7.45) were observed. |

Hypericin is considered as a potential agent in the photodynamic therapy of cancer (Agostinis *et al.* 2002). However, since only isolated hypericin and not extracts have been tested, this approach is out of the scope of this assessment.

### 3.1.3. Safety pharmacology

No information available

### 3.1.4. Pharmacodynamic interactions

Jendzelovska *et al.* 2014

The effect of 24h hypericin pre-treatment on the cytotoxicity of cisplatin (CDDP) and mitoxantrone (MTX) in human cancer cell lines was investigated. CDDP-sensitive and -resistant ovarian adenocarcinoma cell lines A2780/A2780cis, together with HL-60 promyelocytic leukemia cells and ABCG2-over-expressing cBCRP subclone, were used. CDDP cytotoxicity was attenuated by hypericin pre-treatment (0.1 and 0.5 µM) in both A2780 and A2780cis cells and MTX cytotoxicity in HL-60 cells. In contrast, hypericin potentiated MTX-induced death in cBCRP cells. Hypericin did not restore cell proliferation in rescued cells. It increased the expression of MRP1 transporter in A2780 and A2780cis cells indicating the impact of hypericin on certain resistance mechanisms. The authors conclude that hypericin may affect the effectiveness of anti-cancer drugs.

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

Sattler *et al.,* 1997

Investigations indicate that a significant accumulation of hypericin in the cell membrane and the cell nucleus membrane of Caco-2-cells takes place. The authors conclude that hypericin is absorbed through the intestinal epithelium by passive transcellular diffusion and that increasing its solubility by cyclodextrin appears as a promising approach to increase its oral bioavailability for pharmaceutical formulations.

Gutmann *et al.,* 2002

The penetration of radioactive labelled amentoflavon was investigated in an *in vitro* model of the blood brain barrier (primary cell cultures of porcine brain capillary endothelial cells). The concentration dependent uptake in the range of 37 – 2000 nM indicates a passive diffusion. This finding was confirmed by transport experiments through the cell monolayer. Co-administration of a *Hypericum* extract (ethanol 50%, 4-7:1) increased amentoflavone transport significantly.

Cui *et al.,* 2004

Hyperforin was *in vitro* using liver microsomes from rats metabolised to 19-hydroxyhyperforin, 24-hydroxyhyperforin, 29-hydroxyhyperforin and 34-hydroxyhyperforin. Hydroxylation is therefore suggested as major biotransformation of hyperforin in the rat liver.

Shibayama *et al.,* 2004

The administration of 400 mg/kg/day of an extract (no further information) for 10 days to rats resulted in an increase of multidrug resistance protein 2 by 304%, of gluthathione-S-transferase by 252% and of CAP1A2 by 357% in the liver. The amounts of P-glycoprotein and multidrug resistance protein 1 remained unchanged. These increased levels lasted for 30 consecutive days. No increase of multidrug resistance protein 2 in the kidney was found.

Hu *et al.,* 2005

Rats received 400 mg/kg of a dry extract (methanol 80%, DER 3-6:1) by gavage for8 days. The gastrointestinal and haematological toxicities following injection of irinotecan were alleviated by hypericum. The pharmacokinetics of irinotecan and of the metabolite SN-38 in the rat were significantly altered.

Paulke *et al.,* 2008

After oral uptake the genuine flavonoids are deglycosylated in the small intestine, after absorption the quercetin aglycone is glucuronidated (yielding e.g. miquelianin). Further methylation is possibly leading to isorhamnetin and tamarixetin. These metabolites have the ability to penetrate the blood-brain-barrier. Moreover, a significant accumulation of these metabolites in the CNS tissue is observed. After a single dose of a Hypericum extract (1600 mg/kg rat) the quercetin plasma level increased rapidly and reached the maximum of about 700 ng/ml after 4 hours. After 24 hours, 50% of the $C_{max}$ was still measurable. In contrast the concentration level of isorhamnetin/tamarixetin increase much slower, the maximum was reached after 24 hours with a $C_{max}$ of 903 ng/ml. Repeated doses of 1600 mg/kg rat yielded a continuous increase in the plasma levels of quercetin and isorhamnetin for 5 days, after that time the concentration remained constant.

Ho *et al.,* 2009

A dry extract (no further details) was administered orally (doses 150 or 300 mg/day) to rats for 15 days. On day 16 indinavir was given. The plasma levels of indinavir were significantly lowered. In a perfusion study it could be demonstrated that both small intestine and the liver contributed to the reduction. The small intestine was the major site for metabolism of indinavir.

Nagai *et al.,* 2009

Among other herbal extracts a not further defined extract of Hypericum inhibited dose-dependently the sulfation of dopamine ($IC_{50}$ 114 µg/ml) and ritodrine ($IC_{50}$ 98 µg/ml) by SULT1A3, a cytosolic sulfotransferase.

Hokkanen *et al.,* 2011

The metabolism of hyperforin was characterized *in vitro* using human liver microsomes and recombinant heterologously expressed P450 enzymes. A total of 57 hyperforin metabolites were detected. Of those, six were identified as monohydroxylations, while the others were formed via two or more hydroxylation reactions, via dehydrogenation, or by combinations of these reactions. A combined approach of cDNA-expressed recombinant CYPs, CYP-selective chemical inhibitors and correlation with CYP-specific marker activities indicated a central role of the CYP2C and CYP3A families in the metabolism of hyperforin. In addition, hyperforin was found to inhibit CYP2D6 and CYP3A4 model activities potently.

Guo *et al.,* 2012

20 mg/kg hyperoside was given i.g. and i.p to rats. The concentration of hyperoside and its main metabolite 3´-O-methyl-hyperoside were measured using a microdialysis technique. Neither hyperoside nor its metabolite were detected in rat brain after i.g. administration but both compounds could be detected after i.p. administration. Maximum concentrations were 63.78 ng/mL (hyperoside) and 24.66 ng/mL (metabolite) after 20mg/kg i.p. administration.

Verjee *et al.,* 2015

The permeation characteristics of hypericin across Caco-2 monolayers were studied. Only negligible amounts were found in the basolateral compartment when hypericin was administered alone (5 µM). In the presence of 20 µM quercitrin the amount was increased to 4%. Hypericin was mainly accumulated in the cell membranes.

Hatanaka *et al.*, 2011

From a dry extract (ethanol [no further information], 0.3% hypericin, 3.2% hyperforin) several formulations were prepared, including cyclodextrin inclusion (SJW-CD), solid dispersion (SJW-SD), dry-emulsion (SJW-DE), and nano-emulsion (SJW-NE). After oral administration of the SJW-NE formulation (5.2 mg hyperforin/kg) in mice, higher hyperforin exposure in plasma (1188 ± 41 nM·h) and the brain (52.9 ± 1.6 pmol/g tissue·h) was observed with 2.8- and 1.3-fold increases of the area under the concentration curve (AUC) from 0 to 6 hours (0-6)) compared to those of the SJW extract (417 ± 41 nM·h in plasma and 41.6 ± 1.5 pmol/g tissue·h in the brain).

Wurglics *et al.,* 2006 (review)

The hyperforin plasma concentration in humans was investigated in a small number of studies. The results of these studies indicate a relevant plasma concentration, comparable with that used in *in vitro* tests. Furthermore, hyperforin is the only ingredient of *H. perforatum* that could be determined in the brain of rodents after oral administration of alcoholic extracts. The plasma concentrations of the hypericins were only one-tenth compared with hyperforin and until now the hypericins could not be found in the brain after oral administration of alcoholic *H. perforatum* extracts or pure hypericin.

Caccia & Gobbi 2009 (review)

Data so far suggest that the acylphloroglucinol hyperforin, the flavonol quercetin and its glycosylated forms and their metabolites, the biflavones amentoflavone and its I3,II8-analog biapigenin and the naphthodianthrones hypericin and pseudohypericin pass the blood-brain barrier poorly in animals. The brain concentrations of all these high-molecular weight, poorly water-soluble compounds after pharmacologically effective doses of the extracts are therefore far below those effective on neurotransmitter receptors and the mechanisms which are obviously important in the central effects of conventional, pharmacologically related drugs.

Butterweck *et al.,* 2003

Plasma levels of hypericin in rats in the presence and absence of procyanidin B2 or hyperoside were determined by reversed phase HPLC using fluorimetric detection. Both compounds increased the oral bioavailability of hypericin by ca. 58% (B2) and 34% (hyperoside). Procyanidin B2 and hyperoside had a different influence on the plasma kinetics of hypericin; median maximal plasma levels of hypericin were detected after 360 min ($C_{max}$ : 8.6 ng/ml) for B2, and after 150 min (Cmax : 8.8 ng/ml) for hyperoside. It can be speculated that, when administered together with these compounds, a significant accumulation of hypericin in rat plasma in the presence of both polyphenols might be responsible for the observed increased *in vivo* activity.

Juergenliemk *et al.,* 2003

In the Caco-2 cell line, miquelianin, a flavonoid contained in Hypericum perforatum, showed a higher uptake (1.93 ± 0.9 pmol × $min^{-1}$ × $cm^{-2}$) than hyperoside (0.55 ± 0.18 pmol × $min^{-1}$ × $cm^{-2}$) and quercitrin (0.22 ± 0.08 pmol × $min^{-1}$ × $cm^{-2}$). The permeability coefficient of miquelianin (*P* c = 0.4 ± 0.19 × 10 - 6 cm/sec) was in the range of orally available drugs assuming sufficient absorption from the small intestine. Uptake and permeability of the examined compounds was increased by the MRP-2 inhibitor MK-571 indicating a backwards transport by this membrane protein. Porcine cell cultures of brain capillary endothelial cells were used as a model of the blood-brain barrier (bbb) and epithelial cells of the plexus chorioidei as a model of the blood-CSF barrier (bcb). Results indicate no active transport in one direction. Although moderate, the permeability coefficients (bbb: *P* c = 1.34 ± 0.05 ×

10 - 6 cm/sec; bcb: *P* c = 2.0 ± 0.33 × 10 - 6 cm/sec) indicate the ability of miquelianin to cross both barriers to finally reach the CNS.

**Pharmacokinetic interactions**

Dürr *et al.,* 2000

Rats received for 14 days 1000 mg/kg Hypericum extract (methanol 80%, DER 4-7:1) by gavage. The treatment resulted in a 3.8-fold increase of intestinal P-glycoprotein/Mdr1 expression and in a 2.5-fold increase in hepatic CYP3A2 expression.

Cantoni *et al.,* 2003

Mice received for 3 days or 11 days 2 x daily 300 mg/kg a Hypericum hydroethanolic extract (4.5% hyperforin, no further details), and for another day once. Hyperforin was given at a dose of 18.1 mg/kg (similar amount compared to the extract) 2 x daily for 3 days or 11 days and on day 4 or 12 once. 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 too increased ERND activity within 4 days, much to the same extent as the extract (1.8 times the activity of controls), suggesting that it 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.

Bray *et al.,* 2002a

An extract (0.3% hypericins, 2.3% hyperforin) was administered in doses of 140 or 280 mg/kg/day orally to mice for up to 3 weeks. The catalytic activity of CYP1A remained unchanged, while after 3 weeks the activities of CYP2E1 and CYP3A were increased 2-fold.

Bray *et al.,* 2002b

The activities of CYP1A2, CYP2E1 and CYP3A in mice remained unchanged after oral administration of an extract (0.3% hypericins, 2.3% hyperforin, 435 mg/kg/day), hypericin (1 mg/kg/day) and hyperforin (10 mg/kg/day) for four days.

Chen *et al.,* 2004

Hyperforin is a high affinity ligand for pregnan x receptor (PXR) and activates the promoters of CYP3A4 and CYP2B6 through activation of PXR and PXR-specific cis-elements. The PXR-mediated activation of CYP2C9 by 0.2 nM hyperforin is consistent with the high affinity of this compound as a ligand for PXR.

Chaudhary & Willett 2006:

Seven flavonoids present in Hypericum perforatum and apigenin were screened for their inhibition of recombinant human CYP1B1 and CYP1A1. While seven flavonoids (myricetin, apigenin, kaempferol, quercetin, amentoflavone, quercitrin and rutin) were slightly more selective for CYP1B1 inhibition ($K_i$s 0.06–5.96 µM) compared to CYP1A1 ($K_i$s 0.20–1.6 µM) the difference in $K_i$s for the P450s were not significantly different. Rutin did not inhibit CYP1A1 at concentrations up to 10 µM. Kinetic analyses determined that apigenin and amentoflavone were competitive inhibitors of CYP1B1, while quercetin showed mixed type inhibition.

Dostalek *et al.,* 2005:

In the isolated perfused rat liver model the influence of a dry extract (methanol 80%, DER 3-6:1) on CYP2C6, CYP2D2 and CYP3A2 was investigated. Rats received 100 mg/kg extract i.p. once daily for 10 days. Hypericum administration resulted in a significant induction of CYP2D2 and CYP3A2 and in a significant inhibition of CYP2C6.

Komoroski *et al.,* 2005

Hyperforin (0.1, 0.5 or 1.5 μM/l) induced dose dependently the docetaxel metabolism in human hepatocytes.

Tukanovic *et al.,* 2009

An extract (methanol 80%, DER 3-7:1) was administered by gavage in the dose of 1 g/kg to rats for 14 days. The elimination of fexofenadine into the bile was enhanced.

Ott *et al.* 2010

In a blood-brain barrier model a Hypericum extract (not defined) and the isolated constituents hyperforin, hypericin and quercetin decreased P-glycoprotein transport activity in a dose-dependent and time-dependent manner. The extract and hyperforin directly inhibited P-glycoprotein activity, whereas hypericin and quercetin modulated transporter function through a mechanism involving protein kinase C.

Dostalek *et al.,* 2011

In the isolated perfused rat liver model the influence of a dry extract (methanol 80%, DER 3-6:1) on CYP 1A2 was investigated. Rats received 100 mg/kg extract i.p. once daily for 10 days. Phenacetin was used as marker substance for enzyme activity. The extract inhibited the enzyme activity significantly and also significantly more than the control inhibitor omeprazole (30 mg/kg).

Fukunaga & Orito 2012

300 mg dry extract (methanol 80%, DER 3-6:1) was administered orally to dogs for 14 days. The maximum whole-blood concentration and the AUC of ciclosporine given on day 7 and day 14 were significantly lowered.

Radwan *et al.,* 2012

Rats received daily 25 mg/kg a dry extract from fresh Hypericum (extraction solvent ethanol, DER 1:9) for 3 weeks. A significant change in the pharmacokinetic parameters of etoricoxib was observed, the steady state peak plasma concentration was reduced by 32%, the terminal half-life by 91%.

Yang *et al.,* 2012

Rats were orally given methotrexate alone or coadministered with 300 and 150 mg/kg of an extract (no further information, 0.3% hypericin). 300 mg/kg Hypericum extract increased significantly the AUC (163%) and $C_{max}$(60%), 150 mg/kg the AUC by 55%. The mortality of rats treated with Hypericum was higher than in the control group (methotrexate only).

Raskovic *et al.,* 2014

Oral pretreatment with an extract (ethanol 70%, no further information, 57.77 μg/ml hypericin, 155.38 μg/ml pseudohypericin; dosage 400 mg/kg, 4 times) potentiated in mice the effect of pentobarbital and impairment of motor coordination caused by diazepam and increased paracetamol plasma concentrations in comparison to the control group.

Silva *et al.,* 2016

A MTT proliferation assay was performed inWRL-68, HepG2 and HepaRG cells after exposition to different concentrations of H. perforatum extract, hypericin and hyperforin for 24 and 72 h. Then, a real-time PCR analysis was accomplished after incubating the cells with these products evaluating the relative CYP1A2 and CYP2D6 expression. A Hypericum extract (ethanol 70%, 0.3% hypericin), hypericin and hyperforin have relevant cytotoxicity at a 10 µM concentration. The extract led to a significant CYP1A2 and CYP2D6 induction in all cell lines. Hypericin seems to induce CYP1A2 in HepG2 cells and to inhibit its expression in HepaRG cells while hyperforin induced CYP1A2 in HepG2 and inWRL-68 cells. Additionally, hypericin and hyperforin induce CYP2D6 in HepG2 cells but inhibit its expression in HepaRG and in WRL-68 cells.

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

### 3.3.1. Single dose toxicity

Fox *et al.,* 2001

Intravenous application of hypericin was well tolerated by rhesus monkeys at a dose of 2 mg/kg, at 5 mg/kg transient severe photosensitivity rash occurred. The amount of hypericin administered daily in usual therapeutic dosages is not more than 3 mg for adults (= 0.04 mg/kg).

Leuschner 1996

Following a single oral administration of an extract (extraction solvent methanol 80%, DER 3-6:1) the no effect level was above 5000 mg/kg BW (no further details).

### 3.3.2. Repeat dose toxicity

Leuschner 1996

Studies on repeated dose toxicity in rats and dogs (extract extraction solvent methanol 80%, DER 3-6:1, 900 mg/kg and 2700 mg/kg extract per day, 26 weeks treatment) revealed only minor non-specific symptoms (weight loss, minor pathological changes in liver and kidney). All changes reverted to normal when treatment was stopped. Reproduction was not influenced. The dosages were approximately 70 and 200 times the mean therapeutic dosage.

### 3.3.3. Genotoxicity

Schimmer *et al.,* 1994

A tincture (ethanol 70%, 1:5) exhibited weak positive results in an AMES test (with and without metabolic activation, maximum dose 160 µl per plate) in the *S. typhimurium* strain TA98, while no effects were detected in TA100. The weak positive effects were assigned to quercetin, a constituent in the tincture.

Okpanyi *et al.,* 1990

An ethanolic extract (DER 1:5-7, 0.2-0.3% hypericin, 0.35 mg/g quercetin) was tested in several *in vivo* (mammalian spot test in mice, chromosome aberration test in Chinese hamsters) and *in vitro* test systems (HGPRT hypoxanthine-guanine-phosphoribosyl-transferase test, UDS unscheduled DNA synthesis test, cell transformation test). The authors could not detect any signs of a mutagenic potential of the extract.

Miadokova *et al.,* 2010

Hypericin was found to be not mutagenic in an AMES test (*S. typhimurium* TA97), with and without metabolic activation in the concentrations of 20-100 µg/plate. In a yeast assay (S. cerevisiae D7 strain) hypericin in concentrations of 1 x $10^{-5}$ and 1 x $10^{-6}$ M did not increase the frequency of mitotic crossovers or total aberrants, convertants and revertants at several loci. In a chromosome aberration assay (human HepG2 hepatoma cells, V79 Chinese hamster cells, human VH10 cells) hypericin in concentrations 100-1000 ng/ml did not alter the frequency of structural chromosome abberations.

Peron *et al.,* 2013

Hypericum powder (0.3% hypericin) was tested in a chromosomal aberration test using bone marrow cells from Wistar rats. For the acute treatment 0.3-30 mg/100 g BW i.p. or 3.0 and 30 mg/100 g BW by gavage were administered. In the subchronic test 0.3-30 mg/100 g BW were given by gavage for 8 days. No signs of mutagenicity or cytotoxicity could be observed.

### 3.3.4. Carcinogenicity

No data available.

### 3.3.5. Reproductive and developmental toxicity

Ondrizek *et al.,* 1999

Zona-free hamster oocytes were incubated for 1 hour in H. perforatum (no further details) or control medium before sperm-oocyte interaction. The DNA of herb-treated sperm was analyzed with denaturing gradient gel electrophoresis. Pre-treatment of oocytes with 0.6 mg/ml of Hypericum resulted in zero penetration. A lower concentration (0.06 mg/ml) had no effect. Exposure of sperm to Hypericum resulted in DNA denaturation. Sperm exposed to 0.6 mg/ml of St. John's wort showed mutation of the BRCA1 exon 11 gene. The data suggested in the view of the authors that St. John's wort at high concentrations damage reproductive cells. St. John's wort was mutagenic to sperm cells. Since the concentrations used were several orders of magnitude higher than considered as therapeutically relevant these effects should be considered with caution.

Gonzalez *et al.,* 1999

The administration of Hypericum (182 mg/kg/day, no further details) for 2 weeks before mating and throughout gestation did not have a major impact on selected cognitive tasks in mice offspring.

Rayburn *et al.,* 2000

The effect of antenatal exposure to Hypericum on neurobehaviour of developing mice was studied. The extract (no further details) was standardised to 0.3% hypericin. 0.75 mg extract was mixed with each gram of feed (= 180 mg/kg/day). In a randomized and placebo-controlled behavioural testing 45 mice received Hypericum extract equivalent to the dosage for humans over 2 weeks before conception and throughout gestation (placebo group: 42 mice). The antenatal exposure showed no long-term deficits on selected behavioural tasks by developing mice offspring.

Rayburn *et al.,* 2001a

In a following study with similar design as in Rayburn *et al.* (2000) no effect on long term growth and physical maturation of exposed mouse offspring was detectable.

Rayburn *et al.,* 2001b

Prenatal exposure to a therapeutic dose for Hypericum (same herbal preparation as in Rayburn *et al.* 2000, 180 mg/kg/day) did not have a major impact on certain cognitive tasks in mice offspring.

Cada *et al.*, 2001

Pregnant rats received an Hypericum plant powder in doses up to 4500 ppm in the diet from gestational day 3 until ending at offspring weaning on postnatal day 21. Behavioural (Morris water maze, elevated plus-maze) and physiological alterations were investigated. There were no effects detectable regarding maternal weight gain, duration of gestation and offspring body weight. There were no behavioural changed related to the treatment.

Chan *et al.*, 2001

Rat embryos were explanted at gestational day 9.5 and cultured *in vitro* for 2 days. Hypericin in concentrations up to 142 ng/ml was added. Embryos exposed to concentrations of 71 and 142 ng/ml had a significant lower total morphological score and number of somites compared with the control group. The authors are of the opinion that these teratogenic concentrations may be reached in humans after ingestion of 1800 mg Hypericum extract.

Gregoretti *et al.,* 2004

The purpose of the study was to investigate the effects of a treatment with Hypericum (methanolic extract, 0.3% total hypericins, no further details) administered prenatally and during breastfeeding (from 2 weeks before mating to 21 days after delivery) in Wistar rats. Two doses of the extract were chosen, 100 mg/kg per day, which, based on surface area, is comparable to the dose administered to humans, and 1000 mg/kg per day. A microscopic analysis of livers, kidneys, hearts, lungs, brains, and small bowels was performed. A severe damage was observed in the livers and kidneys of animals euthanized postnatally on days 0 and 21. The lesions were more severe with the higher dose and in animals that were breastfed for 21 days; however, an important renal and hepatic damage was evident also with the dose of 100 mg/kg per day. In addition, similar serious hepatic and renal lesions were evident also in animals that were exposed to Hypericum only during breastfeeding. In particular, a focal hepatic damage, with vacuolization, lobular fibrosis, and disorganization of hepatic arrays was evident; in the kidney, a reduction in glomerular size, disappearance of Bowman's space, and hyaline tubular degeneration were found. All important in-life data regarding dams and offspring did not show significant differences between the treatment groups. The results obtained in this study indicate that further, appropriate histological studies should be performed in other animal species to better evaluate the safety of Hypericum extracts taken during pregnancy and breastfeeding.

Borges *et al.,* 2005

The toxicity of an extract (methanol 80%, DER 3-7:1) was tested in pregnant female rats during the period of organogenesis (days 9-15 of pregnancy). No clinical signs of maternal toxicity after oral application of 36 mg/kg twice daily on days 9-15 of pregnancy were detectable. Based on body weight of foetuses and weight of the placenta the authors conclude that there was no embryo toxicity.

Garrovo *et al.,* 2006

A methanolic extract (no further details, content of total hypericins 0.3%, 100 mg/kg/day or 1000 mg/kg/day) was administered orally by gavage to Wistar rats for 2 weeks before mating and throughout gestation. The extract decreased in both concentrations significantly the transcripts of mdr1a, mdr1b, mrp1 and mrp2 genes in the liver of the foetuses and significantly increased mdr1a, mdr1b, mrp2and CYP3A22 genes in the livers of the mothers.

da Conceicao *et al.,* 2010

JEG-3 cells were used to investigate the influence of a Hypericum extract (10:1, 0.3% hypericin, no further information, 25-250 µg/ml) and of hypericin (7.5 and 75 ng/ml) on the *in vitro* placental $Ca^{2+}$ transport. All treatments resulted after 24-hours incubation in an increased intracellular $Ca^{2+}$

concentration, but not after 10 minutes time of incubation. The extract but not hypericin led to a significant decrease in translationally controlled tumor protein $Ca^{2+}$ handling protein. Hypericin increased the protein expression of the transient receptor potential vanilloid 6 $Ca^{2+}$ channel and 28-kDa calcium-binding protein, but decreased the protein expression of plasma membrane Ca2+ ATPase ¼.

Salje *et al.,* 2012

Rats orally treated with an extract (methanol 80%, DER 3-7:1, 1 g/kg) for nine days during late pregnancy induced P-glycoprotein expression in maternal jejunum and placenta significantly. In foetal organs the expression was substantially lower.

Dalmizrak *et al.,* 2012

The effect of hypericin on glutathione S-transerase-pi (GST-pi), one of the most important placental detoxification enzymes, purified from human placentas was investigated. Hypericin inhibited GST-pi competitively with respect to substrates of the enzyme.

Vieira *et al.,* 2013

Rats were treated by gavage with 100 mg/kg of an Hypericum extract (no details published) during pregnancy and lactation. In contrast to the control group (fluoxetine 7.5 mg/kg) the treatment with the Hypericum extract did not significantly influence in male pups the weight of the full seminal vesicle and in the number of spermatozoa.

Nakamura *et al.,* 2013

A modified embryonic stem cell (mES) test, which has been validated as an *in vitro* developmental toxicity protocol, mES cells, was used to assess embryotoxic potential of hyperforin. High concentrations of hyperforin (up to 10 µM) inhibited mouse ES cell population growth and induced apoptosis in fibroblasts. Under the cell culture conditions applied, ES cells mainly differentiated into cardiomyocytes, although various other cell types were also produced. In this condition, hyperforin affected ES cell differentiation into cardiomyocytes in a dose-dependent manner. Analysis of tissue-specific marker expression also revealed that hyperforin at high concentrations partially inhibited ES cell differentiation into mesodermal and endodermal lineages.

Campos *et al.,* 2017

The authors assessed the behaviours of adult male rats born from mothers treated with a Hypericum-extract (aqueous extract containing 0.3% hypericin, no further information). The mother animals received once daily 36 mg/kg, 72 mg/kg or 144 mg/kg by gavage. At 90 days of age the offspring undwerwent the following tests: rotarod test, pentobarbital-induced sleep time, elevatedplus maze, hole-board and forced swimming test. The results suggest changes in the performances in the several tests indicating that Hypericum may interfere with the behavioural development. Additionally the intensity of fluorescence was analysed in organs of the mothers and foetuses. The authors conclude from the observed fluorescence that the extract is present in all tissues analysed.

### 3.3.6. Local tolerance

Boiy *et al.,* 2008

The phototoxicity of hypericin (0.1-1%) and hypericin acetate (0.015-1.5%) was tested after topical application onto mouse ears. The application of hypericin resulted in limited phototoxicity probably due to confined penetration into the epidermal layers. In contrast hypericin acetate caused severe and prolonged responses after irraditation.

## 3.3.7. Other special studies

Vandenbogaerde *et al.,* 1997

The cytotoxicity of hypericin depends on the cell line. Photoactivated hypericin showed $IC_{50}$ values of 0.14 µM (A432 cells), 0.32 µM (HeLa cells) or 1.84 µM (MCF7 cells). Dark cytotoxicity was absent up to high concentrations of 25 µM.

Bernd *et al.,* 1999

In order to estimate the potential risk of phototoxic skin damage during antidepressive therapy, the authors investigated the phototoxic activity of hypericin extract using cultures of human keratinocytes and compared it with the effect of the well-known phototoxic agent psoralen. The absorbance spectrum of the Hypericum extract (extraction solvent methanol, 0.3% hypericins, no further data) revealed maxima in the whole UV range and in parts of the visible range. Human keratinocytes were cultivated in the presence of different Hypericum concentrations. The determination of the bromodeoxyuridine incorporation rate showed a concentration- and light-dependent decrease in DNA synthesis with high hypericin concentrations (≥ 50 µg/ml) combined with UVA or visible light radiation. In the case of UVB irradiation a clear phototoxic cell reaction was not detected. The authors found phototoxic effects even with 10 ng/ml psoralen using UVA with the same study design as in the case of the Hypericum extract. These results confirm the phototoxic activity of Hypericum extract on human keratinocytes. However, the blood levels that are to be expected during antidepressive therapy are presumably too low to induce phototoxic skin reactions.

Wilhelm *et al.,* 2001

The phototoxic potential of three H. perforatum extracts (no further details) from different sources as well as some of its main constituents was investigated. H. perforatum extracts demonstrated cytotoxicity and photocytotoxicity in a dose and UVA-dose dependent manner. Hypericin itself also evoked severe phototoxic effects and was thus identified as the main phototoxic constituent. Among the tested flavonoids quercitrin was found to be cytotoxic, while rutin unexpectedly demonstrated phototoxicity whereas quercitrin was effective to control the phototoxic activity of H. perforatum extracts.

Schempp *et al.,* 2002

The phototoxic and apoptosis-inducing capacity of hypericin and pseudohypericin were assessed in a cell culture model with human leukemic lymphoma cells (Jurkat). Both substances when photoactivated dose-dependently inhibited cell proliferation. Without photoactivation no effects were seen. The half-maximal inhibitory concentrations were 100 ng/ml for hypericin and 200 ng/ml for hyperforin. After photoactivation both substances increased DNA fragmentation.

Traynor *et al.,* 2005

HaCaT keratinocytes were used to investigate the photoclastogenic ability of hypericin on irradiation with UVA. The results show that although the combination of hypericin (0.1-1 µM) and UVA light (0.4 and 4 $J/cm^2$) increased the genotoxic burden, when all factors are taken into account, the risk of significant photogenotoxic damage incurred by the combination of H. extracts and UVA phototherapy may be low in the majority of individuals.

Schmitt *et al.,* 2006a

This study was conducted to determine whether the phototoxicity of hypericin in HaCaT keratinocytes could be attenuated by H. perforatum extracts and constituents. Two extracts, when supplemented with 20 µM hypericin: (1) an ethanol re-extraction of residue following a chloroform extraction (denoted ethanol(-chloroform)) (3.35 µM hypericin and 124.0 µM total flavonoids); and (2) a chloroform extract (hypericin and flavonoids not detected), showed 25% and 50% ($p<0.0001$) less phototoxicity than 20 µM hypericin alone. Two H. perforatum constituents, when supplemented with 20 µM hypericin: (1) 10 µM chlorogenic acid; and (2) 0.25 µM pyropheophorbide, exhibited 24% ($p<0.05$) and 40% ($p<0.05$) less phototoxicity than 20 µM hypericin alone. The peroxidation of arachidonic acid was assessed as a measure of oxidative damage by photo-activated hypericin, but this parameter of lipid peroxidation was not influenced by the extracts or constituents. However alpha-tocopherol, a known antioxidant also did not influence the amount of lipid peroxidation induced in this system. These observations indicate that hypericin combined with H. perforatum extracts or constituents may exert less phototoxicity than pure hypericin, but possibly not through a reduction in arachidonic acid peroxidation.

Schmitt *et al.,* 2006b

The cytotoxicity of *H. perforatum* extracts prepared in solvents ranging in polarity, fractions of one extract, and purified compounds in three cell lines was examined. All extracts exhibited significant cytotoxicity; those prepared in ethanol (no hyperforin, 3.6 microM hypericin, and 134.6 microM flavonoids) showed between 7.7 and 77.4% cell survival ($p < 0.0001$ and 0.01), whereas the chloroform and hexane extracts (hyperforin, hypericin, and flavonoids not detected) showed approximately 9.0 ($p < 0.0001$) and 4.0% ($p < 0.0001$) survival. Light-sensitive toxicity was observed primarily with the ethanol extracts sequentially extracted following removal of material extracted in either chloroform or hexane. The absence of light-sensitive toxicity with the H. perforatum extracts suggests that the hypericins were not playing a prominent role in the toxicity of the extracts.

Onoue at al., 2011

Out of several constituents of Hypericum only hypericin, pseudohypericin and hyperforin exhibited photosensitized peroxidation of linoleic acid, but did not show a photodynamic cleavage of plasmid DNA.

### 3.3.8. Conclusions

The few data available on acute and repeated-dose toxicity do not reveal signs of a risk to the patient. The weak positive outcome of tests on mutagenicity of ethanolic extracts can be explained with the presence of quercetin in the extracts. Numerous publications deal with the potential phototoxicity of hypericin and Hypericum extracts. Extracts exert less phototoxicity than pure hypericin. The data on reproductive toxicity are contradictory.

Despite an uncertainty in the extracts used in each study, several studies report in vitro and in vivo effects of Hypericum perforatum extracts and isolated compounds that could affect the development of fetuses from treated mothers. Therefore, the following text is included in section 4.6 of the WEU and TU monographs:

## *3.4.* ‘Safety during pregnancy and breast-feeding has not been established. Studies in animals have shown signs of reproductive toxicity (see section 5.3 ‘Preclinical safety data’). The use is not recommended during pregnancy and lactation. No fertility data available.*’Overall conclusions on non-clinical data*

There are numerous pharmacological findings published which propose a similar pharmacology to established synthetic antidepressant drugs. However, the discussion on the responsible compounds in the extract is still ongoing. Since several hydroethanolic and hydromethanolic extracts with different contents of hypericin and hyperforin have been tested positively, it could be concluded that the naphthodianthrones and the phloroglucine derivatives may contribute to the antidepressant activity. However, the clinical efficacy cannot attributed to certain constituents.

# 4. Clinical Data

## *4.1. Clinical pharmacology*

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

Johnson *et al.,* 1992

In a placebo controlled design the neurophysiological effects of an *Hypericum* extract (methanol 80%, 3-7:1) were compared with respect to the subjective state as well as to the performance. The authors suggest that *Hypericum* shows central effects (cognitive activation) similar to known antidepressants.

Sharpley *et al.,* 1998

Dry extract (DER 4-7:1, methanol 80% V/V), single oral dose of 900 mg (11 healthy subjects) or 1800 mg (10 healthy subjects). Both doses significantly increased the latency to rapid eye movement sleep without producing any other effect on the sleep architecture.

Böttcher *et al.,* 2000

An extract (ethanol 50% m/m, 4-7:1, nearly free of hyperforin), 250 mg twice daily, was administered for 43 days. The data of the electroencephalogram showed a marked increase in the amplitude of delta and theta waves as typical for antidepressants.

Schüle *et al.,* 2001

The plasma levels of cortisol, growth hormone and prolactin were determined in 12 healthy subjects after administration of 300 or 600 mg of *Hypericum* extract (ethanol 80% V/V, DER 3-7:1). *Hypericum* had no influence on the prolactin levels. After 300 mg *Hypericum* a small but significant elevation of growth hormone levels was observed. This posology had no influence on the cortisol levels. However, 600 mg *Hypericum* extract a clear-cut stimulation of cortisol was observed.

Carvalho *et al.,* 2009

The effect of antidepressants on 6-sulphatoxymelatonin (aMT6s), the main melatonin urinary metabolite, was examined in drug-free depressed patients - most of them antidepressant-naive. aMT6s was evaluated in 34 depressed patients, before and after 8 weeks of placebo (n = 12) or antidepressant (n = 22; fluoxetine, duloxetine or *Hypericum* perforatum [900 mg/day, no further information]). After treatment, aMT6s levels increased after antidepressants ($P < 0.01$), but not after placebo ($P > 0.05$). It is suggested that melatonin changes after antidepressants are more likely due to a pharmacological action of these drugs on melatonin secretion.

Molendijk *et al.,* 2011

Serum levels of the 'brain derived neurotrophic factor' (BDNF) were investigated in 962 depressed patients, 700 fully remitted persons (≥6 months) and 382 healthy controls. Serum BDNF levels were found to be low in antidepressant-free depressed patients relative to controls (P=0.007) and to depressed patients who were treated with an antidepressant (P=0.001). BDNF levels of fully remitted persons (whether unmedicated or treated with an antidepressant) were comparable to those of controls. Analyzing the sample of antidepressant-free depressed patients showed that BDNF levels were unrelated to the core clinical features of depression such as its severity or first versus a recurrent episode. The antidepressant associated upregulation of serum BDNF in depressed patients was confined to selective serotonin reuptake inhibitors (SSRIs) (P=0.003) and *Hypericum* (P=0.03).

Sacher *et al.,* 2011

23 participants (10 controls and 13 patients with major depressive disorder) were included. Depressed patients received either moclobemide (300 mg twice daily) or *Hypericum* (600 mg twice daily, no data regarding the type of the herbal preparation) for 6 weeks. Compared to the effects seen for the MAO-A inhibitor moclobemide (MAO-A density in the prefrontal anterior cingulate and anterior temporal cortices, putamen, thalamus, midbrain and hippocampus) *Hypericum* should not be classified as MAO-A inhibitor.

Arsic *et al.,* 2012

20 healthy volunteers received in a randomized, double-blind, placebo-controlled study creams containing 15% oil extracts of *Hypericum* (fresh material, olive oil, palm oil, sunflower oil, DER 1:5, extraction time 40 days). The investigated O/W creams demonstrated significant anti-inflammatory effects using a sodium lauryl sulphate test. Both skin parameters assessed in the study (electrical capacitance and erythema index), were restored to the baseline value after a seven-day treatment with the tested creams. Almost all investigated oil extracts and corresponding creams displayed the same antimicrobial activity against the most of the investigated microorganisms with obtained minimal inhibitory concentrations values of 1,280 µg/mL, 2,560 µg/mL or >2,560 µg/mL.

Arndt *et al.,* 2013

11 healthy volunteers received in a double-blind, placeb-controlled study a cream containing a hyperforin-rich extract (1.5% in base; no details to the extract). Electron paramagnetic resonance spectroscopy was applied to determine radical formation during VIS/NIR irradiation of the inner forearm. The results were compared to ex vivo investigations on excised porcine ear skin after a single application of the creams. The non-treated skin was measured as control. The absolute values and the kinetics are not comparable for ex vivo and *in vivo* radical formation. Whereas *in vivo*, the radical production decreases with time, it remains stable ex vivo over the investigated timescale. *In vivo* as well as ex vivo, the radical formation could be reduced by almost 80% when applying the hyperforin-rich cream onto the skin, whereas placebo resulted in about 60%. *In vivo*, a daylong protection effect could be validated after a 4-week application time of the cream indicating that a regular application is necessary to obtain the full effect.

Camfield *et al.,* 2013

The effects of a Hypericum extract (ethanol 50% m/m, 4-7:1, nearly free of hyperforin), Nicabate CQ Nicotine Replacement therapy (NRT) and combined NRT/*Hypericum* during conditions of smoking abstinence in 20 regular smokers aged between 18 and 60 years over a period of 10 weeks during smoking cessation. A Spatial Working Memory (SWM) task was completed at baseline, 4 weeks prior to quitting, as well as at the completion of the study, following the 10 weeks of treatment. Brain activity was recorded during the completion of the SWM task using Steady-State Probe Topography. Reaction time and accuracy on the SWM task were not found to be significantly different between treatment groups at retest.

Haag *et al.,* 2014

It was investigated whether topical treatment with a hyperforin-rich cream (1.5% extract in base; no details to the extract) increases the radical protection of the skin during VIS/NIR irradiation. Skin lipid profile was investigated applying HPTLC on skin lipid extracts. Furthermore, the absorption- and scattering coefficients, which influence radical formation, were determined. 11 volunteers were included in this study. After a single cream application, VIS/NIR-induced radical formation could be completely inhibited by both verum and placebo showing an immediate protection. After an application period of 4weeks, radical formation could be significantly reduced by 45% following placebo application and 78% after verum application showing a long-term protection. Skin lipids in both verum and placebo groups increased directly after a single cream application but only significantly for certain ceramides and squalene. After long-term cream application, concentration of cholesterol and the ceramides increased, but no significance was observed.

Naziroglu *et al.,* 2014a

The effect of a dry extract (3.6% hyperforin, no further details) on neutrophils taken from 9 patients diagnosed with Behcet's disease (BD) and 9 control subjects was investigated. The neutrophils from patients were divided into three subgroups and were incubated with the Hypericum extract, voltage-gated calcium channel (VGCC) blockers, (verapamil+dilitiazem) and non-specific TRPM2 channel blocker (2-aminoethyl diphenylborinate, 2-APB), respectively. The neutrophils were stimulated by fMLP as a $Ca^{2+}$-concentration agonist and oxidative stress former. Caspase-3, caspase-9, apoptosis, lipid peroxidation, and cytosolic-free $Ca^{2+}$ $[Ca^{2+}]_i$ values were high in the patient groups, although cell viability, glutathione (GSH), and glutathione peroxidase (GSH-Px) values were low in patient group. However, the $[Ca^{2+}]_i$, caspase-3, and caspase-9 values decreased markedly in patient+Hypericum group although GSH and GSH-Px values increased in the group. The $[Ca^{2+}]_i$ concentration was also decreased in the patient group by V+D, 2-APB, and HP incubations. In conclusion, we observed the importance of neutrophil $Ca^{2+}$ entry, apoptosis, and oxidative stress through gating VGCC and TRPM2 channels in the neutrophils in the pathogenesis and activation of the patients with BD.

Naziroglu *et al.,* 2014b

The effect of a dry extract (3.6% hyperforin, no further details) on neutrophils taken from 9 patients diagnosed with multiple sclerosis (MS) and 9 control subjects was investigated. Neutrophil and serum lipid peroxidation, neutrophil apoptosis and cytosolic-free $Ca^{2+}$ $[Ca^{2+}]_i$ values in patients with MS were higher than in control although their levels were decreased by Hypericum, the non-specific TRPM2 channel blocker 2-APB, and verapamil and diltiazem incubations. The modulator role of verapamil and diltiazem in MS and MS + Hypericum groups was higher than in the 2-APB group. Neutrophilic glutathione peroxidase (GSH-Px) and serum vitamin A and E concentrations were lower in the MS group than in control. However, the neutrophil GSH-Px activity was increased by HP incubation. The neutrophil reduced glutathione, serum vitamin C and β-carotene concentrations did not change in control and patients.

Siepmann *et al*., 2002

In a randomized, double-blind, cross over study 12 healthy male volunteers received capsules with 255-285 mg St John's wort extract (900 µg hypericin content), 25 mg amitriptyline and placebo three times daily for periods of 14 days each with at least 14 days in between. The doses of amitriptyline and St John's wort extract are comparable with respect to their antidepressant activity. Neither St John's wort extract nor amitriptyline had an influence on cognitive performance such as choice reaction, psychomotor coordination, short-term memory and responsiveness to distractive stimuli. Amitriptyline but not St John's wort extract decreased self rated activity ($P < 0.05$). Both drugs caused significant qEEG changes. St John's wort extract increased theta power density. Amitriptyline increased theta as well as fast alpha power density.

Siepmann *et al.,* 2004

A randomized, double-blind, crossover study was performed in healthy male volunteers aged 22 to 31 years (25 +/- 3 years; mean +/- SD) years by Siepmann *et al.* (2004). Subjects orally received capsules with 255 to 285 mg St. John's wort extract (900 µg hypericin content), 25 mg amitriptyline, and placebo 3 times daily for periods of 14 days each with at least 14 days between. Vasoconstrictor responses of cutaneous blood flow (VR) and skin conductance response (SR) following a single deep inspiration were employed as parameters of autonomic function. St. John's wort extract had no effect on VR and SR.

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

Staffeldt *et al.,* 1994

Pharmacokinetic parameters of hypericin and pseudohypericin were evaluated in 12 healthy volunteers after a single oral dose of a Hypericum dry extract (methanol 80%, 4-7:1) corresponding to 250, 750 and 1500 µg hypericin and 526, 1578 and 3156 µg pseudohypericin. $C_{max}$ levels were 1.5, 4.1 and 14.2 ng/ml for hypericin and 2.7, 1.7 and 30.6 ng/ml for pseudohypericin. The median elimination half-life for hypericin was 24.8-26.5 hours, for pseudohypericin 16.3 – 36.0 hours. The AUC for hypericin showed a non-linear increase with raising dose.

Brockmöller *et al.,* 1997

For a single dose period 13 volunteers received either placebo or 900, 1800 or 3600 mg of an Hypericum extract (methanol 80%, 4-7:1) containing 2.81, 5.62 and 11.25 mg of total hypericins. Maximum total hypericin plasma concentrations were observed 4 h after dosage (0.028, 0.061, 0.159 mg/l).

Schempp *et al.,* 1999

The authors describe the HPLC detection of hypericin and semiquantitative detection of pseudohypericin in human serum and skin blister fluid after an oral single dose (1 x 6 tablets) or after steady-state (3 x 1 tablet/day for 7 days) administration of the Hypericum extract LI 160 (methanol 80%, DER 3-6:1) in healthy volunteers (n = 12). Serum levels of hypericin and pseudohypericin were always significantly higher than skin levels (p </= 0.01). After oral single-dose administration of Hypericum extract the mean serum level of total hypericin (hypericin + pseudohypericin) was 43 ng/ml and the mean skin blister fluid level was 5.3 ng/ml. After steady-state administration the mean serum level of total hypericin was 12.5 ng/ml and the mean skin blister fluid level was 2.8 ng/ml. These skin levels are far below hypericin skin levels that are estimated to be phototoxic (>100 ng/ml).

Biber *et al*., 1998

The authors investigated the pharmacokinetics of hyperforin after oral administration of 300, 600 and 1200 mg of two different ethanolic extracts (5% and 0.5% hyperforin). Maximum plasma levels of hyperforin were reached after 2.8 to 3.6 hours. The 5% extract yielded a total $AUC_{0-\infty}$ of 1336, 2215 and 3378 h x ng/ml. The hyperforin pharmacokinetics were linear up to 600 mg of the extract, higher dosages resulted in a lower concentration than would be expected after linear extrapolation. The plasma concentrations of hyperforin were considerably lower after the administration of the extract containing 0.5% hyperforin. In a repeated dose study no accumulation of hyperforin could be detected, the steady state plasma concentration after 3 x 300 mg/day of the extract was approximately 100 ng/ml.

Schemp *et al.,* 1999 (abstract only)
After administration of a single dose of 1800 mg Hypericum extract (methanol 80%, 4-7:1, content of hypericin 0.1-0.3%) to 12 healthy volunteers the serum level of total hypericins was 43 ng/ml and the

mean skin blister fluid level was 5.3 nn/ml. After administration of 300 mg 3 x daily for 7 days the mean serum level was 12.5 ng/ml, in skin blister fluid 2.8 ng/ml. These skin levels are far below hypericin skin levels that were estimated to be phototoxic.

Böttcher *et al.,* 2000

With an extract (ethanol 50% m/m, 4-7:1, nearly free of hyperforin) maximum hypericin plasma concentration of 0.21 to 1.33 µg/L were achieved 6-12 hours after a single dose of 250 mg. The terminal half-life was 15.1 to 63.1 hours after a single dose. Steady state hypericin plasma concentrations following multiple bid doses of 250 mg extract were 2 to 3 µg/L. Steady state was reached before 14 days

Agrosi *et al.,* 2000

In an open single-dose study in 12 healthy volunteers the oral bioavailability of hyperforin and hypericin was measured. The study medication was 300 mg ethanolic dry extract (0.3% hypericin, 5% hyperforin, no further details) in an oily solution (soft capsule) or in dry state in hard capsules. Cmax for hyperforin was 168 ng/ml (soft capsule) and 84 ng/ml (hard capsule). Tmax for hyperforin was 2.50 h (soft capsule) and 3.08 h (hard capsule). Total AUC was measured as 1482 h*ng/ml (soft capsule) and 583 h*ng/ml (hard capsule). Hypericin was only detectable in half of the participants with a tendency towards higher individual absorption from soft capsules compared to the hard capsules.

Jacobson *et al.,* 2001

The pharmacokinetic parameters of hypericin were evaluated in 12 patients diagnosed with chronic hepatitis C virus infection. After a dose of 0.05 mg/kg the elimination half-life was 36.1 hours, after a dose of 0.1 mg/kg 33.8 hours. The AUC for these posologies were 1.5 and 3.1 µg/ml x hour, respectively.

Schulz *et al.,* 2005a

The pharmacokinetic parameters of an Hypericum extract (ethanol 80% V/V, 3-6:1) were investigated in 18 volunteers. Data were collected after single dose or multiple doses (612 mg extract per dose) over 14 days.

Single dose administration:

Hypericin: Cmax 3.8 ng/ml, elimination half-life 18.71 h, MRT 28.67 h

Pseudohypericin: Cmax 10.2 ng/ml, elimination half-life 17.19 h, MRT 20.21 h

Hyperforin: Cmax 122.0 ng/ml, elimination half-life 17.47 h, MRT 20.88 h

Quercetin: Cmax1 89.5 ng/ml, Cmax2 79.1 ng/ml, elimination half-life 2.60 h, MRT 4.68 h

Isorhamnetin: Cmax1 12.5 ng/ml, Cmax2 14.6 ng/ml, elimination half-life 5.61 h, MRT 10.29 h

Steady state: Similar results.

Schulz *et al.,* 2005b

The pharmacokinetic parameters of an Hypericum extract (ethanol 50% V/V, 5-8:1) were investigated in 18 volunteers. Data were collected after single dose or multiple doses (612 mg extract per dose) over 14 days.

Single dose administration:

Hypericin: Cmax 3.14 ng/ml, elimination half-life 23.76 h, MRT 34.74 h

Pseudohypericin: Cmax 8.5 ng/ml, elimination half-life 25.39 h, MRT 28.17 h

Hyperforin: Cmax 83.5 ng/ml, elimination half-life 19.64 h, MRT 21.77 h

Quercetin: Cmax1 47.7 ng/ml, Cmax2 43.8 ng/ml, elimination half-life 4.16 h, MRT 7.44 h

Isorhamnetin: Cmax1 7.6 ng/ml, Cmax2 9.0 ng/ml, elimination half-life 4.45 h, MRT 8.86 h

Steady state: Similar results.

## *4.2. Clinical efficacy*

### 4.2.1. Dose response studies

No data available

### 4.2.2. Clinical studies (case studies and clinical trials)

**<u>Clinical trials related to the indication 'depression'</u>**

**Clinical trials with the herbal preparation LI 160: extraction solvent methanol 80% V/V, DER 3-6:1, in some publications also 4-7:1**

Chemical characterisation:

Total hypericins: 0.12-0.28%

Hyperforin: app. 4.5% (Mueller *et al.* 2006)

Flavonoids: app. 8.3% (Mueller *et al.* 2006)

| **Study** | **Harrer *et al.,* 1994** | |
|---|---|---|
| **Indication** | Moderately severe depressive episodes, according to ICD-10, F 32.1 (HAMD 17-items >- 16) | |
| **Duration of use** | 4 weeks | |
| **Daily dosage** | 900 mg/d | |
| **Single dosage** | 300 mg | |
| **Relapse** | - | |
| **Study design** | *randomized* | yes |
| | *double blind* | yes |
| | *placebo-controlled* | no |
| | *reference-controlled* | Maprotiline (3 x 25 mg) |
| | *multicentre* | n=6 |
| | *number of patients* | 102: Hypericum 13 male, 38 female, mean age 43.8 years, 7 dropouts; maprotiline 16 male, 35 female, mean age 47.6 years, 9 dropouts |

| | | |
|---|---|---|
| | *Statistics* | Wilcoxon-Mann-Whitney U test; chi-squared test; significance level p = 0.05 |
| **Outcome** | HAMD reduction:<br><br>LI 160: 20.5 -> 12.2, no statistically significant difference compared to maprotiline.<br><br>Maprotiline: 21.5 -> 10.5<br><br>Responder rate: 61% in *Hypericum*, 67% in maprotiline<br><br>Onset of effects up to the second week of treatment. After 2 weeks of treatment more pronounced effect with maprotiline, after 4 weeks both groups similar. | |

| | | |
|---|---|---|
| **Study** | **Hänsgen *et al.,* 1994** | |
| **Indication** | Mild to moderate major depression, according to DSM-III-R (HAMD >- 16) | |
| **Duration of use** | 4 weeks (+2 weeks) | |
| **Daily dosage** | 900 mg/d | |
| **Single dosage** | 300 mg | |
| **Relapse** | - | |
| **Study design** | *randomized* | yes |
| | *double blind* | yes |
| | *placebo-controlled* | yes |
| | *reference-controlled* | no |
| | *multicentre* | n=11 |
| | *number of patients* | 67; Hypericum 14 male, 19 female, mean age 53.0 years, 1 dropout; placebo 11 male, 23 female, mean age 53.3 years, 4 dropouts |
| | *Statistics* | Per protocol, Wilcoxon-Mann-Whitney U test; chi-squared test |
| **Outcome** | HAMD reduction:<br><br>LI 160: 21.8 -> 9.2, statistically significant compared to placebo (p < 0.001).<br><br>Placebo: 20.4 -> 14.7<br><br>Responder rate: 81% in *Hypericum*, 26% in placebo<br><br>Further 2 weeks of verum-treatment in both groups: Similar improvement in the former placebo-group like in the first 2 weeks of treatment in the verum group. | |

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|---|---|
| **Study** | **Sommer & Harrer 1994** |

| | | |
|---|---|---|
| **Indication** | Depressive symptoms according ICD-09 300.4 (neurotic depression) and 309.0 (brief depressive reaction). | |
| **Duration of use** | 4 weeks | |
| **Daily dosage** | 900 mg/d | |
| **Single dosage** | 300 mg | |
| **Relapse** | - | |
| **Study design** | *randomized* | yes |
| | *double blind* | yes |
| | *placebo-controlled* | yes |
| | *reference-controlled* | no |
| | *multicentre* | n=3 |
| | *number of patients* | ITT 105 (no gender information), PP 42 (Hypericum) and 47 (placebo), mean age 45 years |
| | Statistics | Per protocol; Wilcoxon-Mann-Whitney U test; chi-squared test |
| Outcome | Only graphical presentation of data; significant improvement under *Hypericum* compared to placebo ($p < 0.05$ after 2 weeks, $p < 0.01$ after 4 weeks)<br>Responder rate: 67% in *Hypericum*, 28% in placebo | |

| | | |
|---|---|---|
| | **Hänsgen & Vesper 1996** | |
| | Mild to moderate major depression, according to DSM-III-R (HAMD >- 16) | |
| **se** | 4 weeks (+2 weeks) | |
| | 900 mg/d | |
| **e** | 300 mg | |
| | - | |
| | *randomized* | yes |
| | *double blind* | yes |
| | *placebo-controlled* | yes |
| | *reference-controlled* | no |
| | *multicentre* | n=17 |
| | *number of patients* | 101; Hypericum 20 male, 31 female, mean age 53.3 years, 2 dropouts; placebo 15 male, 35 female, mean age 50.9 years, 4 dropouts |
| | *Statistics* | Per protocol; Mann-Whitney U test; chi-squared |

| | | |
|---|---|---|
| | | test |
| | HAMD reduction:<br>LI 160: 21.0 -> 8.9, statistically significant compared to placebo ($p < 0.001$).<br>Placebo: 20.4 -> 14.4<br>Responder rate: 70% in *Hypericum*, 24% in placebo<br>Further 2 weeks of verum-treatment in both groups: Similar improvement in the former placebo-group like in the first 2 weeks of treatment in the verum group. | |

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|---|---|---|
| **Study** | **Vorbach *et al.,* 1994** | |
| **Indication** | Major depression according to DSM-III-R (single episode, recurrent episode, neurotic depression, adjustment disorder with depressed mood) | |
| **Duration of use** | 6 weeks | |
| **Daily dosage** | 900 mg | |
| **Single dosage** | 300 mg | |
| **Relapse** | - | |
| **Study design** | *randomized* | yes |
| | *double blind* | yes |
| | *placebo-controlled* | |
| | *reference-controlled* | 75 mg/d imipramine |
| | *multicentre* | n=20 |
| | *number of patients* | 135; Hypericum 34 male, 33 female, mean age 52.8 years, 1 dropout; imipramine 37 male, 31 female, mean age 54.0 years, 4 dropouts |
| | *Statistics* | Kruskal-Wallis test; chi-squared test; Fisher's exact test |
| **Outcome** | HAMD (17 items) reduction:<br>LI 160:from 20.2 to 8.8<br>Imipramine: from 19.4 to 10.7<br>Conclusion:<br>No significant difference between Hypericum and imipramine ($p = 0.05$). | |

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|---|---|
| **Study** | **Vorbach *et al.,* 1997** |
| **Indication** | Severe episode of a major depression according to ICD-10 F 33.2, recurrent, without psychotic symptoms |

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|---|---|---|
| **Duration of use** | 6 weeks | |
| **Daily dosage** | 1800 mg | |
| **Single dosage** | 600 mg | |
| **Relapse** | - | |
| **Study design** | *randomized* | yes |
| | *double blind* | yes |
| | *placebo-controlled* | |
| | *reference-controlled* | 150 mg/d imipramine |
| | *multicentre* | n=20 |
| | *number of patients* | 209; Hypericum 29 male, 78 female, mean age 48.8 years; imipramine 26 male, 76 female, mean age 50.1 years; no information regarding dropouts |
| | *Statistics* | PP and ITT |
| Outcome | HAMD (17 items) reduction:<br>LI 160: from 25.3±4.7 to 14.4±6.1<br>Imipramine: from 26.1±4.8 to 13.4±5.9<br>Conclusion:<br>Efficacy not statistically equivalent (p = 0.36), equivalence only in subgroups with more than 33% and 50% reduction of the HAMD total score. For LI 160 less adverse events reported. | |

| | | |
|---|---|---|
| **Study** | **Wheatley 1997** | |
| **Indication** | Mild to moderate major depression (HAMD-17 score: 17-24; according to DSM-IV) | |
| **Duration of use** | 6 weeks | |
| **Daily dosage** | 900 mg | |
| **Single dosage** | 300 mg | |
| **Relapse** | - | |
| **Study design** | *randomized* | yes |
| | *double blind* | yes |
| | *placebo-controlled* | no |
| | *reference-controlled* | 75 mg/d amitriptyline |
| | *multicentre* | n=19 |

| | | |
|---|---|---|
| | *number of patients* | 165; Hypericum 13 male, 70 female, mean age 42.0 years, 4 dropouts; amitriptyline 17 male, 56 female, mean age 38.0 years, 5 dropouts |
| | *Statistics* | ITT yes |
| **Outcome** | HAMD reduction:<br>LI 160: from 20 to 10<br>Amitriptyline: from 21 to 6<br>Conclusion:<br>No statistically significant difference between LI 160 and amitriptyline (p = .73); *Hypericum* is better tolerated. | |

| | | |
|---|---|---|
| **Reference** | **Brenner *et al.,* 2000** | |
| **Indication** | Mild to moderate depression (HAMD: ≥ 17, according to DSM IV) | |
| **Duration of use** | 7 weeks | |
| **Daily dosage** | 600 mg (1st week)<br>900 mg (6 weeks) | |
| **Single dosage** | 300 mg | |
| **Relapse** | - | |
| **Study design** | *Randomized* | yes |
| | *Double blind* | yes |
| | *Placebo-controlled* | no? |
| | *Reference-controlled* | 50 mg sertraline (1st week)<br>75 mg sertraline (6 weeks) |
| | *Multicentre* | no |
| | *Number of patients* | 30; Hypericum 5 male, 10 female, mean age 44.2 years, 2 dropouts; sertraline 6 male, 9 female, 46.9 years, no dropouts |
| | *Statistics* | ITT; ANCOVA, no further information |
| **Outcome** | HAMD reduction:<br>LI 160: -40 % ± 30 %; from 21.3 ± 3.2 to 12.7 ± 6.7)<br>Sertraline: -42 % ± 24 %, from 21.7 ± 2.7 to 12.5 ± 5.6)<br>Conclusion:<br>Significant improvement in both groups (p <0.01).LI 160 is as effective as sertraline in the treatment of mild to moderate depression (no significant statistical difference). | |

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| **Comment** | Small number of patients, relatively high drop out rate.<br>In the chapter 'Study medication' placebos are mentioned. However, there is no placebo group in the table of results. |

| | | |
|---|---|---|
| **Study** | **Montgomery *et al.,* 2000** | |
| **Indication** | Mild to moderate depression (DSM-IV) | |
| **Duration of use** | 12 weeks | |
| **Daily dosage** | 900 mg | |
| **Single dosage** | 300 mg | |
| **Relapse** | - | |
| **Study design** | *randomized* | yes |
| | *double blind* | yes |
| | *placebo-controlled* | yes |
| | *reference-controlled* | no |
| | *multicentre* | n=18; general practicioners and psychiatric outpatients clinics |
| | *number of patients* | 248, no gender information, no information on mean age and dropouts |
| | *Statistics* | ITT, per protocol |
| **Outcome** | Conclusion:<br>There is no statistically significant difference between placebo and LI 160. (HAMD-score after 6 weeks). Negative Outcome<br>Publication as abstract only. | |

| | | |
|---|---|---|
| **Study** | **Shelton *et al.,* 2001** | |
| **Indication** | Major depression (HAMD: ≥ 20 for more than 2 years, according to DSM-IV: major depression disorder, single episode or recurrent, without psychotic features) | |
| **Duration of use** | 8 weeks | |
| **Daily dosage** | 900 mg/d for 4 weeks, in case of not adequate response increase to 1200 mg/d | |
| **Single dosage** | 300 mg | |
| **Relapse** | - | |
| **Study design** | *randomized* | yes |
| | *double blind* | yes |

| | | |
|---|---|---|
| | *placebo-controlled* | yes |
| | *reference-controlled* | no |
| | *multicentre* | n=11 |
| | *number of patients* | 200; Hypericum 98 patients, 64.9% female, mean age 41.4 years, 3 dropouts; placebo 102 patients, 62.8% female, mean age 43.3 years, 2 dropouts |
| | *Statistics* | ITT: yes |
| **Outcome** | Response rate in the ITT-analysis:<br>LI 160: 26,5 % (for statistical significance 36,1 % is needed)<br>Placebo: 18,6 %<br>In the *Hypericum* group there was a significantly greater proportion of remissions.<br>Conclusion: LI160 is not effective in the treatment of major depression; good compliance; headache the only adverse effect. | |
| **Comment** | High number of patients with chronic major depression. | |

Wurglics *et al.* (2002) stated that although no significant difference between placebo and verum was detected, significant differences in the number of remissions and number of responders were observed. Patients included were severely depressed (duration of depression more than 10 years); the acute phase had a mean duration of 2 years. This is a clear difference to the clinical studies performed in Europe. The authors point out the extremely low responder rate under placebo in this study.

| | | |
|---|---|---|
| **Study** | **HDTSG 2002 (Hypericum depression trial study group)** | |
| **Indication** | Moderately severe major depressive disorder (according to DSM-IV; HAM-D ≥ 20; GAF ≤ 60) | |
| **Duration of use** | 8 weeks | |
| **Daily dosage** | 900-1800 mg (3 x 300- 3 x 600 mg) | |
| **Single dosage** | 300 – 600 mg | |
| **Relapse** | - | |
| **Study design** | *randomized* | yes |
| | *double blind* | yes |
| | *placebo-controlled* | yes |
| | *reference-controlled* | 50-150 mg sertraline |
| | *multicentre* | n=12 |

| | | |
|---|---|---|
| | *number of patients* | 340; Hypericum 40 male, 73 female, mean age 43.1 years, 31 dropouts; placebo 39 male, 77 female, mean age 40.1 years, 32 dropouts; sertraline 37 male, 74 female, mean age 43.9 years, 32 dropouts |
| | *Statistics* | Per protocol |
| **Outcome** | HAMD reduction/response rate:<br>Placebo: -9.20/-31,9 %<br>LI 160: -8,68/-23,9 %<br>Sertraline: -10.53/-24,8 %<br>Conclusion: No efficacy of LI 160 and of sertraline in the treatment of moderately severe major depression. Possible reason: Low assay sensitivity. | |
| **Comment** | No efficacy despite of increase of dosage during study | |

Grobler *et al.* 2014 performed a re-analysis of the data using different approaches regarding to the impact of missing data. No change of the outcome for Hypericum was found, while under some circumstances a significant difference between sertraline and placebo was calculated.

| | | |
|---|---|---|
| **Reference** | **Sarris *et al.,* 2012** | |
| **Indication** | Continuation of the study HDTSG 2002 | |
| **Duration of use** | Responders after 8 weeks treatment, follow up treatment until week 26 | |
| **Daily dosage** | 900 – 1500 mg | |
| **Single dosage** | 300 – 500 mg | |
| **Relapse** | Yes | |
| **Study design** | *Randomized* | yes |
| | *Double blind* | yes |
| | *Placebo-controlled* | yes |
| | *Reference-controlled* | 50 – 100 mg sertraline |
| | *Multicentre* | |
| | *Number of patients* | 124; Hypericum 35, placebo 40, sertraline 49 (according to reference overall 43 male, 77 female [not resulting in 124]), 82 remained until end of study (Hypericum 24, placebo 27, sertraline 31) |
| | *Statistics* | ITT yes |
| **Outcome** | No significant differences in reduction of HAMD score and relapse rates between treatment groups. | |

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|---|---|
| | Sertraline and Hypericum are regarded as therapeutically equivalent with a pronounced placebo effect impeding a significant result at week 26. |

| | | |
|---|---|---|
| **Reference** | **Bjerkenstedt *et al.,* 2005** | |
| **Indication** | Mild to moderate major depression (DSM-IV: 296.31, 296.32); minimum of a total score of 21 on the 21-item Hamilton Depression scale | |
| **Duration of use** | 4 weeks | |
| **Daily dosage** | 900 mg | |
| **Single dosage** | 300 mg | |
| **Relapse** | - | |
| **Study design** | *Randomized* | yes |
| | *Double blind* | yes |
| | *Placebo-controlled* | yes |
| | *Reference-controlled* | 20 mg/d fluoxetine |
| | *Multicentre* | n=15 |
| | *Number of patients* | 163 outpatients;Hypericum 43 female, 11 male, mean age 49.1 years, 6 drop outs, 10 protocol violationsfluoxetine 41 female, 13 male, mean age 50.4 years, 6 drop outs, 11 protocol violations<br>placebo 45 female, 10 male, mean age 51.4 years, 3 drop outs, 12 protocol violations<br>Age app. 50 ± 12 years |
| | *Statistics* | ITT yes<br>Standard descriptive statistics (mean, standard deviations, and frequencies).<br>Tests for treatment differences included χ2-test and Fisher's exact test for categorical and Student's t-test, ANOVA, Wilcoxon-Mann-Whitney-U test and Kruskal-Wallis test for continuous variables. All statistical tests were two-tailed with α set to 0.05. |
| **Outcome** | HAMD reduction:<br>LI 160: from 24.9±4.5 to 15.0±8.4 (-9.9±8.1)<br>Fluoxetine: from 23.8±3.7 to 14.9±8.4 (-8.9±8.0)<br>Placebo: from 25.2±4.6 to 15.5±6.7 (-9.7±7.0)<br>Conclusion: LI 160 and fluoxetine are not more effective in short-term treatment in mild to moderate depression than placebo.<br>*Hypericum* is better tolerated than fluoxetine | |

| | |
|---|---|
| **Comment** | Short time of treatment; high number of drop-outs in all groups (*Hypericum* start n=59, end n=38; fluoxetine start n=57, end n=37; placebo start n=58, end n=40) |

| | | |
|---|---|---|
| **Reference** | **Fava *et al.,* 2005** | |
| **Indication** | Major depressive disorder (HAMD-17 ≥ 16) | |
| **Duration of use** | 12 weeks | |
| **Daily dosage** | 900 mg | |
| **Single dosage** | 300 mg | |
| **Relapse** | - | |
| **Study design** | *Randomized* | yes |
| | *Double blind* | yes |
| | *Placebo-controlled* | yes |
| | *Reference-controlled* | 20 mg/d fluoxetine |
| | *Multicentre* | n=2 |
| | *Number of patients* | 135; Hypericum 45 patients, 53% women, mean age 37.4 years, 60% completed 12 weeks; fluoxetine 47 patients, 53% women, mean age 36.7 years, 51% completed 12 weeks; placebo 43 patients, 65% women, mean age 36.7 years, 49% completed 12 weeks<br>mean HAMD-17: 19.7 ± 3.2 |
| | *Statistics* | ITT yes<br>Significance was set at p <=0.05 |
| **Outcome** | HAMD reduction (ITT-analysis):<br>LI 160: -38 %, from 19.6± 3.5 to 10.2 ± 6.6<br>Fluoxetine: -30 %, from 19.6 ± 3.1 to 13.3 ± 7.3<br>Placebo: -21 %, from 19.9 ± 2.9 to 12.6 ± 6.4<br>Conclusion: LI 160 is significantly more effective than fluoxetine and superior to placebo. It is well tolerated and safe. | |
| **Comment** | Sample smaller than originally planned; the lack of efficacy of fluoxetine is explained by the fixed-dose approach. | |

| | |
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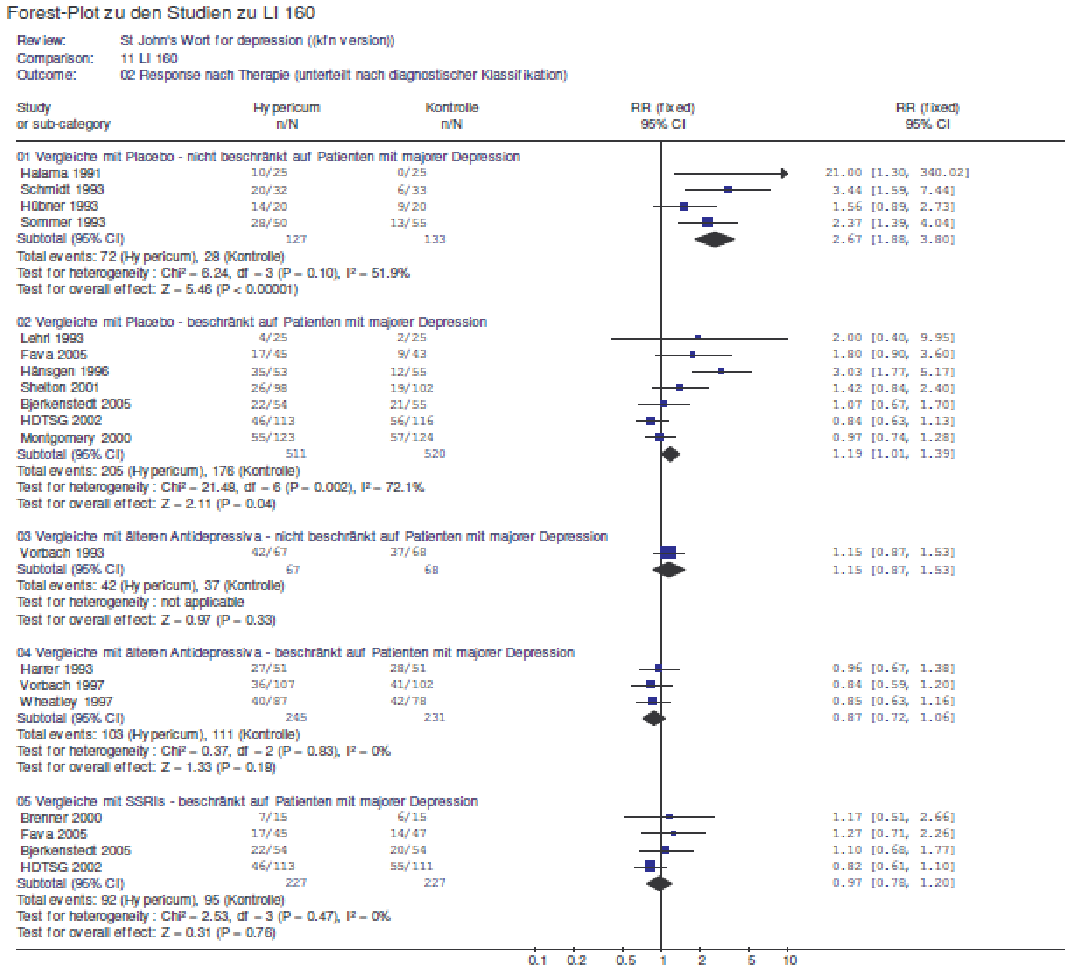
| **Reference** | **Mannel *et al.,* 2010** |

| | | |
|---|---|---|
| **Indication** | Depression with atypical features | |
| **Duration of use** | 8 weeks | |
| **Daily dosage** | 600 mg | |
| **Single dosage** | 300 mg | |
| **Relapse** | - | |
| **Study design** | *Randomized* | yes |
| | *Double blind* | yes |
| | *Placebo-controlled* | yes |
| | *Reference-controlled* | no |
| | *Multicentre* | n=19 |
| | *Number of patients* | 100 with mild severity of major depression<br>100 with moderate severity of major depression<br>18-70 years of age; Hypericum 19 male, 81 female, mean age 47.0 years; placebo 15 male, 85 female, mean age 46.6 years; in total 11 dropouts |
| | *Statistics* | ITT yes |
| **Outcome** | Significant absolute reduction of HAMD-D17<br>No significant benefit for the sum of the atypical vegetative items (p = 0.051)<br>Significant improvement of atypical vegetative items (hypersomnia, hyperphagia) in the group of moderately depressed patients. | |

Meta-analysis of clinical studies with LI 160 (Linde 2007):




Conclusion:

Reference controlled studies: All studies included patients with major depression; similar or insignificantly less efficacy compared to standard antidepressants; in some studies no difference between *Hypericum*, reference medication and placebo. Only one study (Vorbach *et al.* 1997) was designed for proof of equivalence.

Placebo controlled studies: Tendency that in older studies (published before 2000) better outcome for *Hypericum*; modern studies also with negative outcome.

Studies including patients with more severe depressive episodes (daily dosage up to 1800 mg extract) do not show sufficient efficacy.

**Clinical trials with the herbal preparation WS 5570: extraction solvent methanol 80% V/V, DER 3-7:1**

Chemical characterisation:

Total hypericins: 0.12-0.28%

Hyperforin: app. 3-6%

Flavonoids: ≥ 6.0%

| | | |
|---|---|---|
| **Study** | **Lecrubier *et al.*, 2002** | |
| **Indication** | Mild to moderate major depression (single or recurrent episode, DSM-IV code: 296.21, 296.22, 296.32, HAMD 17-item: 18-25) | |
| **Duration of use** | 6 weeks | |
| **Daily dosage** | 900 mg | |
| **Single dosage** | 300 mg | |
| **Relapse** | - | |
| **Study design** | *Randomized* | Yes |
| | *double blind* | Yes |
| | *placebo-controlled* | Yes |
| | *reference-controlled* | No |
| | *Multicentre* | n=26 |
| | *number of patients* | 375; Hypericum 44 male, 142 female, mean age 40.2 years, 18 dropouts; placebo 44 male, 145 female, mean age 41.2 years, 25 dropouts |
| | *Statistics* | ITT yes |
| **Outcome** | HAMD reduction/Responders<br>WS 5570: -9.9/52.7 %<br>Placebo: -8.1/42.3 % (The difference is significant, p =0.037))<br>Conclusion: WS 5570 is safe and more effective than placebo in the treatment of mild to moderate major depression | |

| | | |
|---|---|---|
| **Study** | **Szegedi *et al.*, 2005** | |
| **Indication** | Moderate to severe major depression (HAMD 17-item: ≥ 22; DSM-IV: 296.22, 296.23, 296.32, 296.33) | |
| **Duration of use** | 6 weeks | |
| **Daily dosage** | 900 mg-1800 mg; dose increase in week 2 in patients with HAMD improvement <20% | |
| **Single dosage** | 300 mg-600 mg | |
| **Relapse** | Responders (decrease in total HAMD score ≥ 50%) were invited to participate in a four month double blind maintenance phase | |
| **Study design** | *Randomized* | Yes |
| | *double blind* | Yes |
| | *placebo-controlled* | No |

| | | |
|---|---|---|
| | *reference-controlled* | 20 mg-40 mg/d paroxetine; dose increase in week 2 in patients with HAMD improvement <20% |
| | *Multicentre* | n=21 |
| | *number of patients* | 244; Hypericum 37 male, 85 female, mean age 49.0 years, 25 dropouts; paroxetine 39 male, 83 female, mean age 45.5 years, 31 dropouts |
| | *Statistics* | Test on non-inferiority, ITT yes |
| **Outcome** | HAMD recuction/ % responder:<br>WS 5570: -14.4 / 71%<br>Paroxetine: -11.4 / 60%<br>Conclusion: WS 5570 is as effective as paroxetin in the treatment of moderate to severe major depression | |

| | | |
|---|---|---|
| **Study** | **Anghelescu *et al.,* 2006** | |
| **Indication** | Moderate to severe depression according to DSM-IV criteria: 296.22, 296.23, 296.32 and 296.33 (HAMD 17-item: ≥ 22) | |
| **Duration of use** | 6 weeks of acute treatment | |
| **Daily dosage** | 900 mg or 1800 mg; dose increase in week 2 in patients with HAMD improvement <20% | |
| **Single dosage** | 300 mg or 600 mg | |
| **Relapse** | Patients with a HAM-D total score decrease of >=50% during the 6 weeks of acute treatment were asked to continue the treatment for another 16 weeks (n=133) | |
| **Study design** | *Randomized* | Yes |
| | *double blind* | Yes |
| | *placebo-controlled* | No |
| | *reference-controlled* | 20/40 mg/d paroxetine; dose increase in week 2 in patients with HAMD improvement <20% |
| | *Multicentre* | n=21 |
| | *number of patients* | 133; Hypericum 17 male, 54 female, mean age 48.6 years, continuation treatment completed by 38 patients; paroxetine 13 male, 49 female, mean age 46.9 years, continuation treatment completed by 36 patients |
| | *Statistics* | ITT: yes |
| **Outcome** | HAMD reduction:<br>WS 5570: .from 25.3+/-2.5 to 4.3+/-6.2 | |

| | |
|---|---|
| | Paroxetine: from 25.3+/-2.6 to 5.2+/-5.5<br><br>During maintenance treatment only 61.6% of the Hypericum group and 54.6% of the paroxetine group showed additional reduction of HAMD score. Remission occurred in 81.6% of the patients in the Hypericum group and in 71.4% in the paroxetine group. 3 Patients under Hypericum and 2 patients under paroxetine showed an increase in HAMD score of >5 during continuation treatment<br><br>Conclusion: WS 5570 and paroxetine are similarly effective in preventing relapse in a continuation treatment after recovery from an episode of moderate to severe depression |

| **Study** | **Kasper *et al.*, 2006** | |
|---|---|---|
| **Indication** | Mild or moderate major depressive episode (single or recurrent episode, DSM-IV criteria: 296.21, 296.22, 296.31, 296.32; HAMD 17-item: ≥ 18, "depressive mood" ≥ 2) | |
| **Duration of use** | 6 weeks | |
| **Daily dosage** | 600-1200 mg | |
| **Single dosage** | 600 mg | |
| **Relapse** | - | |
| **Study design** | *Randomized* | Yes |
| | *double blind* | Yes |
| | *placebo-controlled* | Yes |
| | *reference-controlled* | No |
| | *Multicentre* | n=16 |
| | *number of patients* | 324; Hypericum 600 mg 52 male, 67 female, mean age 46.3 years, 15 dropouts; Hypericum 1200 mg 42 male, mean age 46.1 years, 82 female, 20 dropouts; placebo 25 male, 56 female, mean age 46.9 years, 12 dropouts |
| | *Statistics* | ITT yes |
| **Outcome** | More patients in the WS 5570 1200 mg group met the criterion of remission (HAMD: ≤ 7 at treatment end)<br><br>HAMD reduction:<br><br>WS 5570 600 mg: -11.6 ± 6,4<br><br>WS 5570 1200 mg: -10.8 ± 7,3<br><br>Placebo: -6.0 ± 8,1<br><br>Conclusion: WS 5570 is safe and more effective than placebo (p <0.001) in treatment of mild to moderate depression. | |

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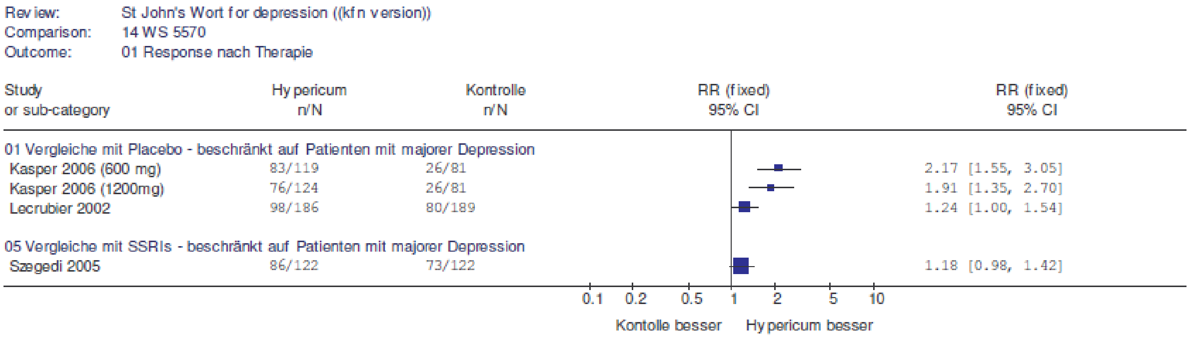
|---|---|
| **Continuation study** | Kasper *et al.* 2007<br><br>Those participants with a HAMD total score decrease ≥ 50% during acute treatment were eligible for 4 months of double blind continuation treatment. 69 patients 600 mg/day, 68 patients 1200 mg/day, 24 patients placebo. Additional slight decrease of HAMD in both active groups (0.8 and 0.4 points), deterioration in the placebo group (2.1 points). |

| | | |
|---|---|---|
| **Study** | Kasper *et al.*, 2008 | |
| **Indication** | Recurrent episode of moderate major depression; HAMD 17-item: ≥ 20, ≥ 3 previous episodes in 5 years (ICD-10 F33.0. F33.1, DSM-IV 296.3) | |
| **Duration of use** | 6 weeks single blind acute treatment, then 26 weeks double blind continuation treatment, then 52 weeks double blind maintenance treatment | |
| **Daily dosage** | 900 mg | |
| **Single dosage** | 300 mg | |
| **Relapse** | + | |
| **Study design** | *Randomized* | Yes |
| | *double blind* | Yes |
| | *placebo-controlled* | Yes |
| | *reference-controlled* | No |
| | *Multicentre* | Yes |
| | *number of patients* | 426; Hypericum 76 male, 206 female, mean age 47.5 years, 44 dropouts; placebo 35 male, 109 female, mean age 47.4 years, 19 dropouts |
| | *Statistics* | ITT yes |
| **Outcome** | Relapse rates: Hypericum 18.1%; placebo 25.7% (ITT p = 0.035)<br><br>Average time to relapse: Hypericum 177 days; placebo 163 days (ITT p = 0.035)<br><br>Conclusion: WS 5570 showed a beneficial effect in preventing relapse after recovery from acute depression. Tolerability in continuation and long-term maintenance treatment was on the placebo level. | |

Meta-analysis of clinical studies with WS 5570 (Linde 2007):

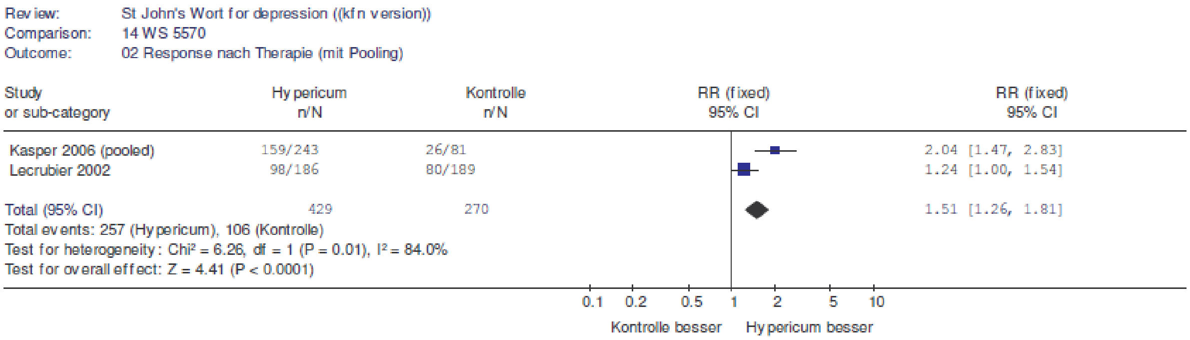
Forest-Plot zu den Studien mit WS 5570




Legende siehe Abbildung 1

Abbildung 2b

Forest-Plot zu den placebokontrollierten Studien mit WS 5570 mit Pooling




Conclusion: All studies are well designed. All studies report superiority compared to placebo or non-inferiority compared to standard medication.

Comparison with LI 160:
In contrast to the recent studies published for LI 160 all modern studies for WS 5570 demonstrated a positive outcome for *Hypericum*. Since the extracts LI 160 and WS 5570 are very similar in their key parameters, it seems to be justified to combine the results. It can be concluded that the efficacy of this type of extract in the treatment of mild to moderate severe depression is well documented.

**Clinical trials with the herbal preparation WS 5572: extraction solvent ethanol 60% V/V, DER 2.5-5:1**

Chemical characterisation:

Total hypericins: no information
Hyperforin: 5% (Laakmann *et al.* 1998, Lemmer *et al.* 1999, Rychlik *et al.* 2001); 1,5 % (Kalb *et al.* 2001)
Flavonoids: no information

| | |
|---|---|
| **Study** | Laakmann *et al.,* 1998a, Laakmann *et al.* 1998b |
| **Indication** | Mild or moderate depression according to DSM-IV criteria, HAMD ≥ 17 |
| **Duration of use** | 6 weeks |
| **Daily dosage** | 900 mg |
| **Single dosage** | 300 mg |

| | | |
|---|---|---|
| **Relapse** | - | |
| **Study design** | *Randomized* | Yes |
| | *double blind* | Yes |
| | *placebo-controlled* | Yes |
| | *reference-controlled* | 900 mg WS 5573 |
| | *Multicentre* | n=11 |
| | *number of patients* | 147; Hypericum WS 5572 9 male, 40 female, mean age 47.3 years; Hypericum WS 5573 7 male, 42 female, mean age 48.7 years; placebo 14 male, 35 female, mean age 51.0 years; in total 9 dropouts |
| | *Statistics* | ITT yes |
| **Outcome** | HAMD reduction / % responder:<br>WS 5572: 20.9 -> 10.7 / 49.0%<br>WS 5573: 20.3 -> 11.8 / 38.8%<br>Placebo: 21.2 -> 13.3 / 32.7%<br>Conclusion:<br>WS 5572 (5% hyperforin) was superior to placebo (p = 0.004)<br>WS 5573 (0.5% hyperforin) and placebo were descriptively comparable<br>The therapeutic effect depends on the content of hyperforin. | |
| **Comment** | WS 5572 and WS 5573 are produced with the identical manufacturing process (identical DER, extraction solvent), the only difference between the extracts relates to the content of hyperforin. The fingerprint chromatograms of the two extracts are identical except for hyperforin. It is not mentioned how the differences in the content of hyperforin are achieved.<br>The negative outcome for the extract with low content of hyperforin is in contrast to the positive findings with the extract ZE 117, which is said to be nearly free of hyperforin. | |

| | | |
|---|---|---|
| **Study** | **Kalb *et al.,* 2001** | |
| **Indication** | Mild to moderate major depressive disorder (according to DSM-IV criteria) (DSM-IV code: 296.21, 296.31, 296.22, 296.32, HAMD (17-items): ≥ 16) | |
| **Duration of use** | 6 weeks | |
| **Daily dosage** | 900 mg | |
| **Single dosage** | 300 mg | |
| **Relapse** | - | |
| **Study design** | *Randomized* | Yes |

| | | |
|---|---|---|
| | *double blind* | Yes |
| | *placebo-controlled* | Yes |
| | *reference-controlled* | No |
| | *Multicentre* | n=11 |
| | *number of patients* | 72; Hypericum 11 male, 26 female, mean age 48 years, 4 drop outs; placebo 13 male, 22 female, mean age 49 years, 4 drop outs |
| | *Statistics* | ITT yes |
| **Outcome** | HAMD reduction / % responder:<br><br>WS 5572: 19.7 -> 8.9 / 62.2%<br><br>Placebo: 20.1 -> 14.4 / 42.9%<br><br>Conclusion: Superior compared to placebo (day 28 p = 0.011, day 42 p < 0.001) | |
| **Comment** | Efficacy was already statistically significant at day 28<br><br>Adaptive 2-stage design | |

| **Study** | **Rychlik *et al.,* 2001** | |
|---|---|---|
| **Indication** | Mild to moderate depression (based on Clinical Global Impression CGI scale) | |
| **Duration of use** | 7 weeks | |
| **Daily dosage** | 600 mg/1200 mg | |
| **Single dosage** | 600 mg | |
| **Relapse** | - | |
| **Study design** | *Randomized* | No |
| | *double blind* | No |
| | *placebo-controlled* | No |
| | *reference-controlled* | comparison of 600 mg and 1200 mg daily |
| | *Multicentre* | n=446 |
| | *number of patients* | 2166; dose 600 mg 1385 patients (73.2% female), mea nage 49.56 years; dose 1200 mg 781 patients (72.3% female), mean age 50.76 years |
| | *Statistics* | - |
| **Outcome** | Evaluation of symptoms according to ICD-10 F32)<br><br>Responders:<br><br>600 mg: 83,7 % | |

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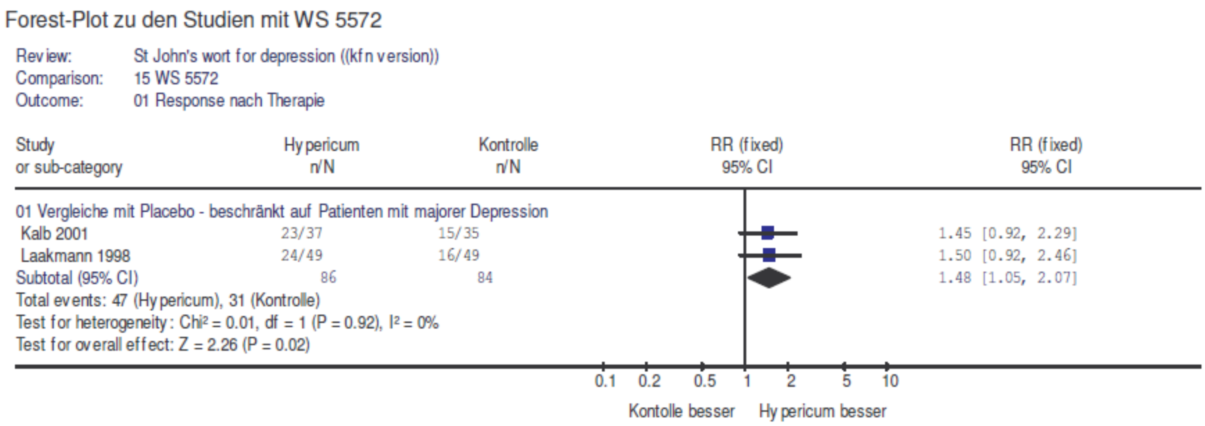
|---|---|
| | 1200 mg: 86,9 %<br><br>Conclusion: Good effectiveness and tolerability of WS 5572 |
| **Comment** | Observational study |

Meta-analysis of clinical studies with WS 5572 (Linde 2007):




Conclusion: WS 5572 is superior to placebo in the treatment of mild to moderate major depression.

**Clinical trials with the herbal preparation Ze 117: extraction solvent ethanol 50% m/m, DER 4-7:1**

Chemical characterisation:
- Total hypericins: 0.2%
- Hyperforin: nearly free
- Flavonoids: no information

| **Study** | **Schrader *et al.*, 1998** | |
|---|---|---|
| **Indication** | Mild to moderate depression (ICD-10; F 32-0 and F 32-1) | |
| **Duration of use** | 6 weeks | |
| **Daily dosage** | 500 mg (corresponding to 1 mg hypericin daily) | |
| **Single dosage** | 250 mg | |
| **Relapse** | - | |
| **Study design** | *randomized* | Yes |
| | *double blind* | Yes |
| | *placebo-controlled* | Yes |
| | *reference-controlled* | No |
| | *multicentre* | n=16 |
| | *number of patients* | 162; Hypericum 23 male, 58 female, median age 47 years, 14 drop outs; placebo 31 male, 50 female, median age 39 years, 9 drop outs |
| | *Statistics* | ITT yes |

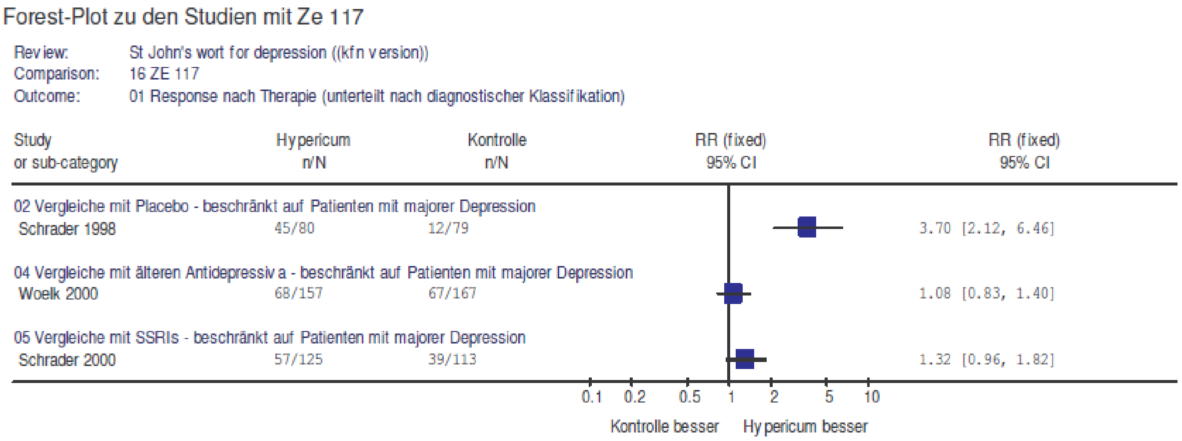
| | |
|---|---|
| **Outcome** | HAMD (21-item) reduction / % responder:<br>Ze 117: from 20.13 to 10.53 / 56%<br>Placebo: from 18.76 to 17.89 / 15%<br>Conclusion: ZE 117 is significantly superior (p < 0.001) compared to placebo and safe in treatment of mild to moderate depression |
| **Comment** | Contact of patients with investigators only at the beginning of the study and after 6 weeks in order to minimize the placebo effect. |

| | | |
|---|---|---|
| **Study** | **Schrader 2000 (Friede *et al.,* 2001)** | |
| **Indication** | Mild to moderate depression (ICD-10 F 32-0 and F 32-1, HAMD scale (21-item) 16-24) | |
| **Duration of use** | 6 weeks | |
| **Daily dosage** | 500 mg | |
| **Single dosage** | 250 mg | |
| **Relapse** | - | |
| **Study design** | *randomized* | Yes |
| | *double blind* | Yes |
| | *placebo-controlled* | No |
| | *reference-controlled* | 20 mg fluoxetine |
| | *multicentre* | n=7 |
| | *number of patients* | 240; Hypericum 36 male, 90 female, mean age 46 years; fluoxetine 47 male, 67 female, mean age 47 years; no drop outs |
| | *Statistics* | ITT yes |
| **Outcome** | HAMD reduction:<br>Ze 117: from 19.65 to 11.54/-7,25<br>Fluoxetine: from 19.50 to 12.20/-8,11<br>Conclusion:<br>Hypericum and fluoxetine are equipotent. The safety of Ze 117 was superior to that of fluoxetine. | |
| **Comment** | Contact of patients with investigators only at the beginning of the study and after 6 weeks in order to minimize the placebo effect.<br>Nearly identical data in the publication compared to Friede *et al.* 2001. However, no coincidence of the authors and the sponsor. | |

| | |
|---|---|
| **Study** | **Woelk 2000** |

| | | |
|---|---|---|
| **Indication** | Mild to moderate depression (ICD-10 codes F32.0, F33.0, F32.1, F 33.1; HAMD score (17-item) ≥ 18) | |
| **Duration of use** | 6 weeks | |
| **Daily dosage** | 500 mg | |
| **Single dosage** | 250 mg | |
| **Relapse** | - | |
| **Study design** | *randomized* | Yes |
| | *double blind* | Yes |
| | *placebo-controlled* | No |
| | *reference-controlled* | 150 mg/d imipramine |
| | *multicentre* | n=40 |
| | *number of patients* | 324; Hypericum 45 male, 112 female, mean age 46.5 years, 15 drop outs; imipramine 48 male, 119 female, mean age 45.4 years, 32 drop outs |
| | *Statistics* | ITT yes |
| **Outcome** | HAMD reduction:<br><br>Ze 117: from 22.4 to 12.0<br><br>Imipramine: from 22.1 to 12,75<br><br>Conclusion: Ze 117 and imipramine are therapeutically equivalent in the treatment of mild to moderate depression. In the treatment of depression with anxiety Hypericum has more benefit. There are fewer adverse events in the Ze 117 group. | |
| **Comment** | The dosage of imipramine is relatively high, which could be the reason for the high number of drop outs in the reference group. | |

Meta-analysis of clinical studies with ZE 117 (Linde 2007):




Conclusion: The superiority of the extract ZE 117 against placebo and the non-inferiority against imipramine and fluoxetine could be demonstrated. Compared with the results obtained with the extracts LI 160 and WS 5570 it can be concluded that hyperforin is not solely responsible for clinical efficacy. According to the manufacturer the extract is on the market in Germany at least since 1996. Therefore the minimum of 10 years of medicinal use is fulfilled.

**Clinical trials with the herbal preparation STW 3: extraction solvent ethanol 50% V/V, DER 5-8:1**

Chemical characterisation:
Total hypericins: 0.2%
Hyperforin: mean 2%
Flavonoids: mean 9%

| **Study** | **Gastpar *et al.,* 2005, Gastpar & Zeller 2005** | |
|---|---|---|
| **Indication** | Moderate depressive disorder (according to ICD-10 criteria: F32.1 or F33.1; HAMD 17-items: 20-24) | |
| **Duration of use** | 12 weeks | |
| **Daily dosage** | 612 mg | |
| **Single dosage** | 612 mg | |
| **Relapse** | after 12 weeks additional treatment for 12 weeks of n=161 | |
| **Study design** | *Randomized* | Yes |
| | *double blind* | Yes |
| | *placebo-controlled* | No |
| | *reference-controlled* | 50 mg sertraline |
| | *Multicentre* | n=18 |
| | *number of patients* | 241; Hypericum 123 (79.4% female), mean age 48.3 years, until week 12 17 dropouts, untl week 24 additional 16 dropouts; sertraline 118 (69.4% female), mean age 49.5 years, until week 12 19 dropouts, until week 24 additional 8 drop outs |
| | *Statistics* | Test on non-inferiority, ITT yes |
| **Outcome** | HAMD reduction: (week 12/week 24)<br>STW 3: from 22.0 to 8.3/5.7<br>Sertraline: from 22.1 to 8.1/7.1<br>Conclusion: STW 3 is therapeutically not inferior to sertraline ($p < 0.0001$) in moderate depression and it is well tolerated. | |
| **Comment** | Single daily dose | |

Conclusion: 1 study of adequate quality which demonstrates non-inferiority compared to sertraline (50 mg).

**Clinical trials with the herbal preparation STW3-VI: extraction solvent ethanol 80% V/V, DER 3-6:1**

Chemical characterisation:

Total hypericins: mean 0.2%
Hyperforin: mean 2%
Flavonoids: mean 9%

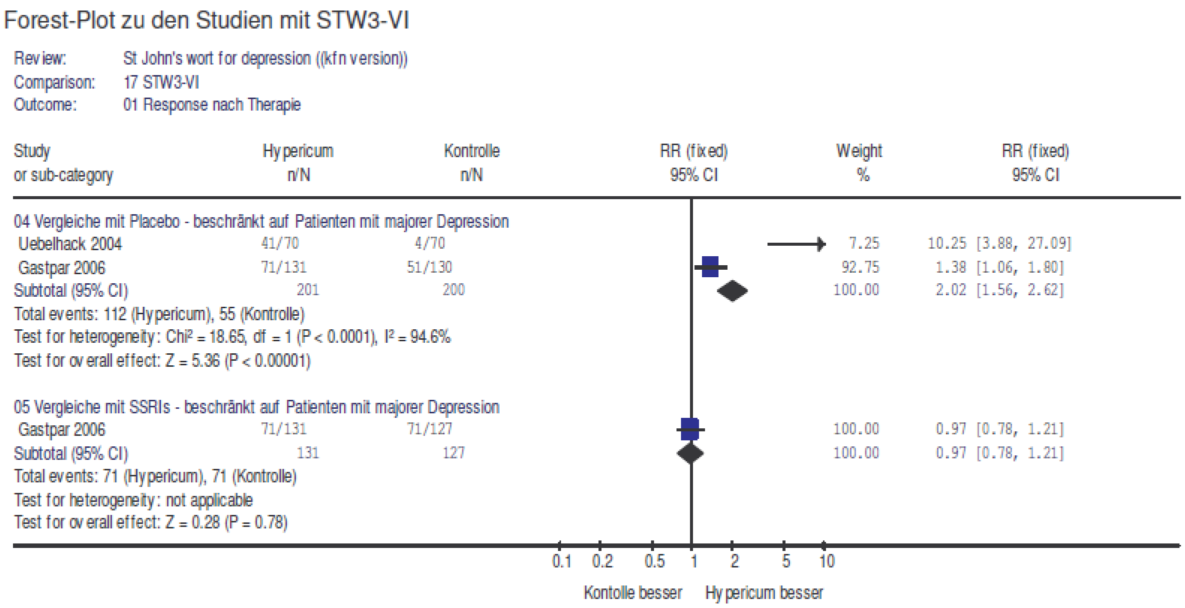
| | | |
|---|---|---|
| **Study** | **Uebelhack *et al.,* 2004** | |
| **Indication** | Moderate depressive disorders (ICD-10 F32.1, F33.1) and HAMD (17-items) score : 20-24 | |
| **Duration of use** | 6 weeks | |
| **Daily dosage** | 900 mg | |
| **Single dosage** | 900 mg | |
| **Relapse** | | |
| **Study design** | *Randomized* | Yes |
| | *double blind* | Yes |
| | *placebo-controlled* | Yes |
| | *reference-controlled* | No |
| | *Multicentre* | n=1 |
| | *number of patients* | 140; Hypericum 21 male, 49 female, mean age 46.4 years, 9 drop outs; placebo 25 male, 45 female, mean age 43.3 years, 10 drop outs |
| | *Statistics* | ITT yes |
| **Outcome** | HAMD reduction / % responder:<br>STW3-VI: 22.8 -> 11.8 / 58.6%<br>Placebo: 22.6 -> 19.2 / 5.7%<br>Conclusion: STW3-VI in a single daily dose is superior to placebo (p < 0.001). | |
| **Comment** | The low responder rate under placebo is explained by the authors with the inclusion of a high number of patients with moderate depression, while other studies included also a higher number of patients with mild depression. | |

| | | |
|---|---|---|
| **Study** | **Gastpar *et al.,* 2006** | |
| **Indication** | Moderate depression (HAMD 17-items score: 20-24, ICD-10. F32.1, F33.1, according to DSM-IV major depressive episode and recurrent major depression) | |
| **Duration of use** | 6 weeks | |
| **Daily dosage** | 900 mg | |
| **Single dosage** | 900 mg | |
| **Relapse** | - | |
| **Study design** | *Randomized* | Yes |
| | *double blind* | Yes |

| | | |
|---|---|---|
| | *placebo-controlled* | Yes |
| | *reference-controlled* | 20 mg citalopram |
| | *Multicentre* | n=21 |
| | *number of patients* | 388; Hypericum 131 (65.6% female), mean age 50.8 years, 30 drop outs; citalopram 127 (64.4% female), mean age 49.3 years, 23 drop outs; placebo 130 (73.1% female), mean age 49.4 years, 25 drop outs |
| | *Statistics* | ITT yes |
| **Outcome** | HAMD reduction / % responder:<br>Hypericum: 21.9 -> 10.3 / 54.2%<br>Citalopram: 21.8-> 10.3 / 55.9%<br>Placebo: 22.0 -> 13.0 / 39.2%<br>Conclusion:<br>The Hypericum group was statistically non-inferior to citalopram (p< 0.0001) and significantly superior to the placebo (p< 0.0001). | |
| **Comment** | Study of high methodological quality | |

In a retrospective study (Singer *et al.* 2008, Singer *et al.* 2011) 154 responders of the clinical study by Gastpar *et al.* (2006) in patients with moderate depression (HAMD score 20-24) which received STW 3-VI, citalopram or placebo for 6 weeks were observed for 5 years.
In 19.5% of the patients a relapse was observed with the highest ratio in the citalopram group (25.9%), followed by the placebo group (17.4%), the minimal relapse rate was in the Hypericum group (14.8%). The severity of the relapse was identical in all three groups.
Relapse + recurrence: Hypericum 44.4%, citalopram 48.1%, placebo 52.2%
Duration until relapse, recurrence: Hypericum 1833 days, citalopram 1755, placebo 802 days
The authors suggest that the prognosis under Hypericum is better compared to citalopram.

Meta-analysis of clinical studies with STW 3-VI (Linde 2007):




Conclusions: Superiority against placebo and non-inferiority against citalopram (20 mg) could be demonstrated.

**Clinical trials with the herbal preparation STEI 300: extraction solvent ethanol 60% m/m, DER 5-7:1**

Chemical characterisation:
Total hypericins: 0.2 – 0.3%
Hyperforin: 2-3%
Flavonoids: no information

| **Study** | **Philipp *et al.*,1999** | |
|---|---|---|
| **Indication** | Moderate depression according to ICD-10 (codes F32. 1 and F33.1) (HAMA score ≥ 18) | |
| **Duration of use** | 8 weeks | |
| **Daily dosage** | 1050 mg | |
| **Single dosage** | 350 mg | |
| **Relapse** | - | |
| **Study design** | *Randomized* | Yes |
| | *double blind* | Yes |
| | *placebo-controlled* | Yes |
| | *reference-controlled* | 100 mg/d imipramine (titrated within 4 days from 50 mg) |
| | *Multicentre* | n=18 |
| | *number of patients* | For safety evaluation 263: Hypericum 26 male, 80 female, mean age 47 years; imipramine 31 male, 79 female, mean age 48 years; placebo 9 male, 38 female, mean age 43 years; ITT population n=251, no further information) |
| | *Statistics* | ITT yes |
| **Outcome** | HAMD reduction / % responder:<br>STEI 300: 22.7-> 7.3 / 76%<br>Placebo: 22.7-> 10.6 / 63%<br>Imipramine: 22.2-> 8.0 / 66.7%<br>Conclusion: STEI 300 is as effective as imipramine and more effective than placebo in the treatment of moderate depression and it is safe. | |
| **Comment** | Study of high methodological quality | |

Conclusion: Superiority against placebo and non-inferiority against imipramine (100 mg) could be demonstrated.

**Clinical trials with the herbal preparation LoHyp-57: extraction solvent ethanol 60% V/V, DER 5-7:1**

Chemical characterisation:

Total hypericins: 0.2-0.3%

Hyperforin: 2-3%

Flavonoids: no information

Extract identical to STEI 300, but different dosage form and posology.

| **Study** | **Harrer *et al.,* 1999** | |
|---|---|---|
| **Indication** | Mild to moderate major depression according to ICD 10 (F32.0, F32.1) | |
| **Duration of use** | 6 weeks | |
| **Daily dosage** | 800 mg | |
| **Single dosage** | 400 mg | |
| **Relapse** | - | |
| **Study design** | *Randomized* | Yes |
| | *double blind* | Yes |
| | *placebo-controlled* | No |
| | *reference-controlled* | 20 mg fluoxetine (= 22.4 mg fluoxetine HCl) |
| | *Multicentre* | n=17 |
| | *number of patients* | 149; Hypericum 10 male, 60 female, mean age 68.4 years, 8 drop outs; fluoxetine 10 male, 69 female, mean age 69.1 years, 16 drop outs |
| | *Statistics* | ITT yes |
| **Outcome** | HAMD (17-items) reduction:<br>LoHyp 57: from 16.60 to 7.91 (mild: from 14.21 to 6.21; moderate: from 18.73 to 9.43)<br>Fluoxetine: from 17.18 to 8.11 (mild: from 15.21 to 7.46; moderate: from 19.10 to 8.75)<br>Responder rate:<br>LoHyp 57: 71.4% (mild subgroup: 81.8%; moderate subgroup: 62.2%)<br>Fluoxetine: 72.2% (mild subgroup: 76.9%; moderate subgroup: 67.5%)<br>Conclusion: LoHyp 57 is equivalent to fluoxetine ($p<0.05$) in the treatment of mild to moderate major depression particularly in elderly patients. | |
| **Comment** | 90% confidence interval, the patients were 60-80 years of age. | |

Conclusion: 800 mg of LoHyp-57 is equivalent to 20 mg fluoxetine in the treatment of mild to moderate major depression.

Table 7: Clinical studies on humans in mild to moderate depression, LI160 (DER 3-6:1, extraction solvent methanol 80% V/V)

| Reference | Study Design | Test Product(s): herbal preparation | Number of Subjects | Diagnosis | Outcomes | Statistical analysis | Comments on clinical relevance of results |
|---|---|---|---|---|---|---|---|
| Harrer *et al.* 1994 | Rand., 75 mg maprotiline | 3 x 300 mg<br>4 weeks | 102; Hypericum 13 male, 38 female; maprotiline 16 male, 35 female | ICD-10, F 32.1, HAMD ≥ 16 | HAMD reduction<br>After 4 weeks both groups similar | Wilcoxon-Mann-Whitney U test; chi-squared test | Short duration of study |
| Hänsgen *et al.* 1994 | Rand., placebo | 3 x 300 mg<br>4 weeks | 67; Hypericum 14 male, 19 female; placebo 11 male, 23 female | HAMD ≥ 16 | HAMD reduction<br>Hyp significantly superior | Per protocol<br>Wilcoxon-Mann-Whitney U test; chi-squared test | Short duration of study |
| Sommer & Harrer 1994 | Rand., placebo | 3 x 300 mg<br>4 weeks | 105 (no gender information) | ICD-09 300.4, 309.0 | HAMD reduction<br>Hyp significantly superior | Per protocol<br>Wilcoxon-Mann-Whitney U test; chi-squared test | Short duration of study; only graphical presentation of data |
| Hänsgen & Vesper 1996 | Rand., placebo | 3 x 300 mg<br>4 weeks | 101; Hypericum 20 male, 31 female; | HAMD ≥ 16 | HAMD reduction<br>Hyp significantly superior | Per protocol<br>Mann-Whitney U test; chi- | Short duration of study |

| Reference | Study Design | Test Product(s): herbal preparation | Number of Subjects | Diagnosis | Outcomes | Statistical analysis | Comments on clinical relevance of results |
|---|---|---|---|---|---|---|---|
| | | | placebo 15 male, 35 female | | | squared test | |
| Vorbach *et al.* 1997 | Rand., 150 mg imipramine | 3 x 600 mg<br>6 weeks | 209;<br>Hypericum 29 male, 78 female; imipramine 26 male, 76 female | ICD-10, F 33.2 | HAMD reduction<br>Equivalence of efficacy only in subgroups | PP and ITT yes | Major depression not included into the monograph |
| Wheatly 1997 | Rand., 75 mg amitriptyline | 3 x 300 mg<br>6 weeks | 156; Hypericum 13 male, 70 female; amitriptyline 17 male, 56 female | HAMD 17-24 | HAMD reduction<br>No difference between treatments | ITT yes | Relevant for monograph. Supports safety. |
| Brenner *et al.* 2000 | Rand., 75 mg sertraline | 3 x 300 mg<br>7 weeks | 30;<br>Hypericum 5 male, 10 female; sertraline 6 male, 9 female | HAMD ≥ 17 | HAMD reduction<br>Hyp as effective as sertraline | ITT yes | Small number of patients, relatively high drop out rate. |

| Reference | Study Design | Test Product(s): herbal preparation | Number of Subjects | Diagnosis | Outcomes | Statistical analysis | Comments on clinical relevance of results |
|---|---|---|---|---|---|---|---|
| Montgomery *et al.* 2000 | Rand., placebo | 3 x 300 mg<br>12 weeks | 248; no gender information | Mild to moderate depression | HAMD reduction<br>No difference between Hyp and placebo | ITT yes | Negative outcome, but in total the positive studies prevail. Publication as abstract only. |
| Shelton *et al.* 2001 | Rand., placebo | 3 x 300 mg<br>8 weeks | 200; Hypericum 64.9% female; placebo 62.8% female | HAMD ≥ 20 | Responder rate<br>Hyp not effective in major depressions | ITT yes | High number of patients with chronic major depression. Major depression not included into the monograph. |
| HDTSG 2002 | Rand., placebo, 50-150 mg sertraline | 3 x 300 – 3 x 600 mg<br>8 weeks | 340; Hypericum 40 male, 73 female; placebo 39 male, 77 female; sertraline 37 male, 74 female | Severe major depression, HAMD ≥ 20 | HAMD reduction<br>No efficacy of Hyp and sertraline | Per protocol | No efficacy despite of increase of dosage during study. Major depression not included into the monograph |
| Sarris *et al.* 2012 | Continuation of HDTSG 2002 | Repsonders treated until week 26 | 124; Hypericum 35, placebo 40, sertraline | Severe major depression, HAMD ≥ 20 | No significant differences in reduction of HAMD score and relapse | ITT yes | Prnonounced placebo effect |

| Reference | Study Design | Test Product(s): herbal preparation | Number of Subjects | Diagnosis | Outcomes | Statistical analysis | Comments on clinical relevance of results |
|---|---|---|---|---|---|---|---|
| | | | 49 (according to reference overall 43 male, 77 female [not resulting in 124]), 82 remained until end of study (Hypericum 24, placebo 27, sertraline 31) | | rates between treatment groups.<br>Sertraline and Hypericum are regarded as therapeutically equivalent. | | |
| Bjerkenstedt *et al.* 2005 | Rand., placebo, 20 mg fluoxetine | 3 x 300 mg<br>4 weeks | 163; Hypericum 43 female, 11 male; fluoxetine 41 female, 13 male;<br>placebo 45 female, 10 male | DSM-IV 296.31, 296.32 | HAMD reduction<br>No difference between study groups | ITT yes | Short duration of study, high number of drop-outs in all groups |
| Fava *et al.* | Rand., placebo, | 3 x 300 mg | 135; | HAMD ≥ 16 | HAMD reduction | ITT yes | Relevant for |

| Reference | Study Design | Test Product(s): herbal preparation | Number of Subjects | Diagnosis | Outcomes | Statistical analysis | Comments on clinical relevance of results |
|---|---|---|---|---|---|---|---|
| 2005 | 20 mg fluoxetine | 12 weeks | Hypericum 45 patients, 53% women; fluoxetine 47 patients, 53% women; placebo 43 patients, 65% women | | Hyp sign. superior to fluoxetine and placebo | | monograph |

Table 8: Clinical studies on humans in mild to moderate depression, WS 5570 (DER 3-7:1, extraction solvent methanol 80% V/V)

| Reference | Study Design | Test Product(s): herbal preparation | Number of Subjects | Diagnosis | Outcomes | Statistical analysis | Comments on clinical relevance of results |
|---|---|---|---|---|---|---|---|
| Lecrubier *et al.* 2002 | Rand., placebo | 3 x 300 mg<br>6 weeks | 375; Hypericum 44 male, 142 female; placebo 44 male, 145 female | DSM-IV 296.22, 296.23, 296.32, 296.33, HAMD 18-25 | HAMD reduction<br>Hyp more effective than placebo | ITT yes | Relevant for monograph |
| Szegedi *et al.* 2005 | Rand., 20/40 mg paroxetine | 3 x 300 mg<br>3 x 600 mg<br>6 weeks | 244; Hypericum 37 male, 85 female; paroxetine 39 | DSM-IV 296.22, 296.23, 296.32, 296.33, HAMD ≥ 22 | HAMD reduction<br>Hyp and paroxetine similar effective | ITT yes | Relevant for monograph |

| Reference | Study Design | Test Product(s): herbal preparation | Number of Subjects | Diagnosis | Outcomes | Statistical analysis | Comments on clinical relevance of results |
|---|---|---|---|---|---|---|---|
| | | | male, 83 female | | | | |
| Anghelescu *et al.* 2006 | Rand., 20/40 mg paroxetine | 3 x 300 mg<br>3 x 600 mg<br>6 weeks, responder for another 16 weeks | 133; Hypericum 17 male, 54 female; paroxetine 13 male, 49 female | DSM-IV 296.22, 296.23, 296.32, 296.33, HAMD ≥ 22 | HAMD reduction<br>Hyp and paroxetine similar effective | ITT yes | Relevant for monograph, relevant for relapse prevention |
| Kasper *et al.* 2006 | Rand., placebo | 1-2 x 600 mg<br>6 weeks | 324; Hypericum 600 mg 52 male, 67 female; Hypericum 1200 mg 42 male, 82 female; placebo 25 male, 56 female | DSM-IV 296.21, 296.22, 296.31, 296.32, HAMD ≥18 | HAMD reduction<br>Hyp more effective than placebo | ITT yes | Relevant for monograph |
| Kasper *et al.* 2008 | Rand., placebo | 3 x 300 mg<br>6 weeks<br>52 weeks maintenance treatment | 426; Hypericum 76 male, 206 female; placebo 35 male, 109 | ICD-10 F33.0, F33.1<br>HAMD ≥ 20 | HAMD reduction<br>Relapse rates<br>Hyp is superior in preventing relapse than placebo | ITT yes | Relevant for monograph, relevant for relapse prevention |

| Reference | Study Design | Test Product(s): herbal preparation | Number of Subjects | Diagnosis | Outcomes | Statistical analysis | Comments on clinical relevance of results |
| --- | --- | --- | --- | --- | --- | --- | --- |
| | | | female | | | | |

Table 9: Clinical studies on humans in mild to moderate depression, ZE 117 (DER 4-7:1, extraction solvent ethanol 50% V/V)

| Reference | Study Design | Test Product(s): herbal preparation | Number of Subjects | Diagnosis | Outcomes | Statistical analysis | Comments on clinical relevance of results |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Schrader 1998 | Rand., placebo | 2 x 250 mg<br>6 weeks | 162; Hypericum 23 male, 58 female; placebo 31 male, 50 female | ICD-10 F32.0, F32.1 | HAMD reduction<br>Hyp more effective than placebo | ITT yes | Relevant for monograph |
| Schrader 2000 | Rand., 20 mg fluoxetine | 2 x 250 mg<br>6 weeks | 240; Hypericum 36 male, 90 female; fluoxetine 47 male, 67 female | ICD-10 F32.0, F32.1, HAMD 16-24 | HAMD reduction<br>Hyp and fluoxetine similar effective | ITT yes | Relevant for monograph |
| Woelk 2000 | Rand., 150 mg imipramine | 2 x 250 mg<br>6 weeks | 324; Hypericum 45 male, 112 female; imipramine 48 male, 119 | ICD-10 F32.0, F32.1, F33.0, F33.1, HAMD ≥ 18 | HAMD reduction<br>Hyp and imipramine similar effective | ITT yes | Relevant for monograph.<br>The dosage of imipramine is relatively high, |

| Reference | Study Design | Test Product(s): herbal preparation | Number of Subjects | Diagnosis | Outcomes | Statistical analysis | Comments on clinical relevance of results |
|---|---|---|---|---|---|---|---|
| | | | female | | | | which could be the reason for the high number of drop outs in the reference group. |

Table 10: Clinical studies on humans in mild to moderate depression, STW 3 (DER 5-8:1, extraction solvent ethanol 50% V/V)

| Reference | Study Design | Test Product(s): herbal preparation | Number of Subjects | Diagnosis | Outcomes | Statistical analysis | Comments on clinical relevance of results |
|---|---|---|---|---|---|---|---|
| Gastpar *et al.* 2005 | Rand., 50 mg sertraline | 612 mg<br>12 weeks | 241; Hypericum 123 (79.4% female), sertraline 118 (69.4% female) | ICD-10 F32.1, F33.1, HAMD 20-24 | HAMD reduction<br>Hyp and sertraline similar effective | ITT yes | Relevant for monograph |

Table 11: Clinical studies on humans in mild to moderate depression, STEI 300 (DER 5-7:1, extraction solvent ethanol 60% V/V)

| Reference | Study Design | Test Product(s): herbal preparation | Number of Subjects | Diagnosis | Outcomes | Statistical analysis | Comments on clinical relevance of results |
|---|---|---|---|---|---|---|---|
| Philipp *et al.* 1999 | Rand., placebo, 100 mg | 3 x 350 mg<br>8 weeks | 263; Hypericum 26 male, 80 | ICD-10 F32.1, F33.1, HAMD ≥ 18 | HAMD reduction<br>Hyp and imipramine similar | ITT yes | Relevant for monograph |

| Reference | Study Design | Test Product(s): herbal preparation | Number of Subjects | Diagnosis | Outcomes | Statistical analysis | Comments on clinical relevance of results |
|---|---|---|---|---|---|---|---|
| | imipramine | | female; imipramine 31 male, 79 female; placebo 9 male, 38 female | | effective, superior to placebo | | |

Table 12: Clinical studies on humans in mild to moderate depression, LoHyp-57 (DER 5-7:1, extraction solvent ethanol 60% V/V)

| Reference | Study Design | Test Product(s): herbal preparation | Number of Subjects | Diagnosis | Outcomes | Statistical analysis | Comments on clinical relevance of results |
|---|---|---|---|---|---|---|---|
| Harrer *et al.* 1999 | Rand., 20 mg fluoxetine | 2 x 400 mg<br>6 weeks | 149; Hypericum 10 male, 60 female; fluoxetine 10 male, 69 female | ICD-10 F32.0, F32.1 | HAMD reduction<br>Hyp and fluoxetine similar effective | ITT yes | No confidence intervals were shown in the publication, therefore the non-inferiority has formally not been demonstrated. |

Table 13: Clinical studies on humans in mild to moderate depression, STW3-VI (DER 3-6:1, extraction solvent ethanol 80% V/V)

| Reference | Study Design | Test Product(s): herbal preparation | Number of Subjects | Diagnosis | Outcomes | Statistical analysis | Comments on clinical relevance of results |
|---|---|---|---|---|---|---|---|

| Reference | Study Design | Test Product(s): herbal preparation | Number of Subjects | Diagnosis | Outcomes | Statistical analysis | Comments on clinical relevance of results |
|---|---|---|---|---|---|---|---|
| Uebelhack *et al.* 2004 | Rand., placebo | 1 x 900 mg<br>6 weeks | 140; Hypericum 21 male, 49 female; placebo 25 male, 45 female | ICD-10 F32.1, F33.1, HAMD 20-24 | HAMD reduction<br>Hyp more effective than placebo | ITT yes | Relevant for monograph.<br>The low responder rate under placebo is explained by the authors with the inclusion of a high number of patients with moderate depression |
| Gastpar *et al.* 2006 | Rand., placebo, 20 mg citalopra m | 1 x 900 mg<br>6 weeks | 388; Hypericum 131 (65.6% female); citalopram 127 (64.4% female); placebo 130 (73.1% female) | ICD-10 F32.1, F33.1, HAMD 20-24 | HAMD reduction<br>Hyp and citalopram similar effective, superior to placebo | ITT yes | Relevant for monograph |
| Singer *et al.* 2011 | | 5 year follow up of study by Gastpar et al 2006 | 154 | | Relapse<br>Hyp delayed relapse better than citalopram | | Relevant for monograph, relevant for relapse prevention |

Table 14: Clinical studies on humans in mild to moderate depression, WS 5572 (DER 2.5-5:1, extraction solvent ethanol 60% V/V)

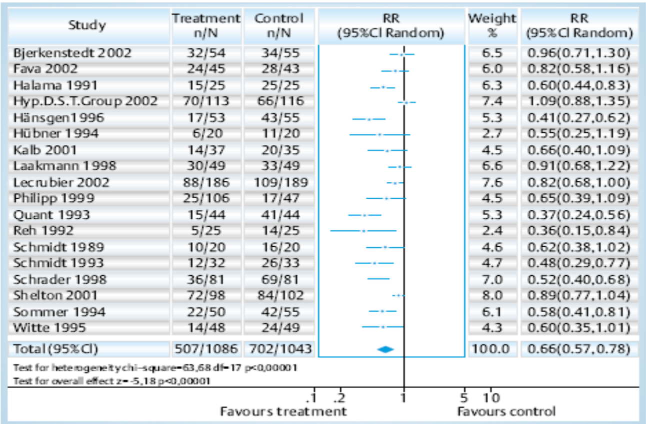
| Reference | Study Design | Test Product(s): herbal preparation | Number of Subjects | Diagnosis | Outcomes | Statistical analysis | Comments on clinical relevance of results |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Laakmann *et al.* 1998 | Rand., placebo, 900 mg extract low hyperforin | 3 x 300 mg<br>6 weeks | 147; Hypericum WS 5572 9 male, 40 female; Hypericum WS 5573 7 male, 42 female; placebo 14 male, 35 female | Mild to moderate depression, HAMD ≥ 17 | HAMD reduction<br>Hyp more effective than placebo; extract with low hyperforin similar to placebo | ITT yes | Relevant for monograph |
| Kalb *et al.* 2001 | Rand., placebo | 3 x 300 mg<br>6 weeks | 72; Hypericum 11 male, 26 female; placebo 13 male, 22 female | DSM-IV 296.21, 296.22, 296.31, 296.32, HAMD ≥ 16 | HAMD reduction<br>Hyp more effective than placebo | ITT yes | Relevant for monograph |

## Overall meta-analysis

Röder *et al.* (2004) published a meta-analysis of effectiveness and tolerability of treatment of mild to moderate depression with Hypericum extracts.

The results demonstrate a significant superiority of Hypericum extracts over placebo (mean response: Hypericum: 53.3 % and placebo: 32.7 %). Compared to standard antidepressives Hypericum is similarly effective for the treatment of depression (mean response: Hypericum: 53.2 %, synthetic antidepressives: 51.3 %). In the subgroup of mild to moderate depression Hypericum showed better results against the standard antidepressive group (mean response: 59.5 %/52.9 %) and a better side-effect profile. The fail-safe-N-test indicates that 423 studies with no effect would be needed to negate the presented result for placebo studies.

Relative risk of non-response after treatment with *Hypericum* or placebo (Linde 2007):



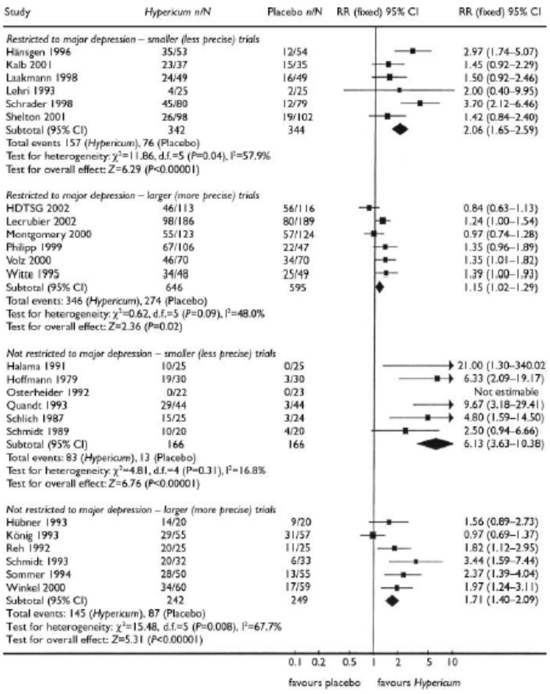
| Study | Treatment n/N | Control n/N | RR (95%CI Random) | Weight % | RR (95%CI Random) |
|---|---|---|---|---|---|
| Bjerkenstedt 2002 | 32/54 | 34/55 | | 6.5 | 0.96(0.71,1.30) |
| Fava 2002 | 24/45 | 28/43 | | 6.0 | 0.82(0.58,1.16) |
| Halama 1991 | 15/25 | 25/25 | | 6.3 | 0.60(0.44,0.83) |
| Hyp.D.S.T.Group 2002 | 70/113 | 66/116 | | 7.4 | 1.09(0.88,1.35) |
| Hänsgen1996 | 17/53 | 43/55 | | 5.3 | 0.41(0.27,0.62) |
| Hübner 1994 | 6/20 | 11/20 | | 2.7 | 0.55(0.25,1.19) |
| Kalb 2001 | 14/37 | 20/35 | | 4.5 | 0.66(0.40,1.09) |
| Laakmann 1998 | 30/49 | 33/49 | | 6.6 | 0.91(0.68,1.22) |
| Lecrubier 2002 | 88/186 | 109/189 | | 7.6 | 0.82(0.68,1.00) |
| Philipp 1999 | 25/106 | 17/47 | | 4.5 | 0.65(0.39,1.09) |
| Quant 1993 | 15/44 | 41/44 | | 5.3 | 0.37(0.24,0.56) |
| Reh 1992 | 5/25 | 14/25 | | 2.4 | 0.36(0.15,0.84) |
| Schmidt 1989 | 10/20 | 16/20 | | 4.6 | 0.62(0.38,1.02) |
| Schmidt 1993 | 12/32 | 26/33 | | 4.7 | 0.48(0.29,0.77) |
| Schrader 1998 | 36/81 | 69/81 | | 7.0 | 0.52(0.40,0.68) |
| Shelton 2001 | 72/98 | 84/102 | | 8.0 | 0.89(0.77,1.04) |
| Sommer 1994 | 22/50 | 42/55 | | 6.1 | 0.58(0.41,0.81) |
| Witte 1995 | 14/48 | 24/49 | | 4.3 | 0.60(0.35,1.01) |
| Total(95%CI) | 507/1086 | 702/1043 | | 100.0 | 0.66(0.57,0.78) |

Test for heterogeneity chi-square=63,68 df=17 p<0,00001
Test for overall effect z=-5,18 p<0,00001



Werneke *et al.* (2004) came to similar results. They found that the effect sizes in recent studies were smaller than those resulted from earlier studies.

Linde *et al.* (2005) concluded that the available data for major depression is confusing. While Hypericum has minimal beneficial effects over placebo, other trials suggest that Hypericum and standard antidepressants have equal efficacy (see figures below).



| Study | *Hypericum* n/N | Placebo n/N | RR (fixed) 95% CI |
|---|---|---|---|
| *Restricted to major depression – smaller (less precise) trials* | | | |
| Hänsgen 1996 | 35/53 | 12/54 | 2.97 (1.74–5.07) |
| Kalb 2001 | 23/37 | 15/35 | 1.45 (0.92–2.29) |
| Laakmann 1998 | 24/49 | 16/49 | 1.50 (0.92–2.46) |
| Lehri 1993 | 4/25 | 2/25 | 2.00 (0.40–9.95) |
| Schrader 1998 | 45/80 | 12/79 | 3.70 (2.12–6.46) |
| Shelton 2001 | 26/98 | 19/102 | 1.42 (0.84–2.40) |
| Subtotal (95% CI) | 342 | 344 | 2.06 (1.65–2.59) |
| Total events 157 (*Hypericum*), 76 (Placebo) | | | |
| Test for heterogeneity: $\chi^2$=11.86, d.f.=5 (*P*=0.04), I²=57.9% | | | |
| Test for overall effect: *Z*=6.29 (*P*<0.00001) | | | |
| *Restricted to major depression – larger (more precise) trials* | | | |
| HDTSG 2002 | 46/113 | 56/116 | 0.84 (0.63–1.13) |
| Lecrubier 2002 | 98/186 | 80/189 | 1.24 (1.00–1.54) |
| Montgomery 2000 | 55/123 | 57/124 | 0.97 (0.74–1.28) |
| Philipp 1999 | 67/106 | 22/47 | 1.35 (0.96–1.89) |
| Volz 2000 | 46/70 | 34/70 | 1.35 (1.01–1.82) |
| Witte 1995 | 34/48 | 25/49 | 1.39 (1.00–1.93) |
| Subtotal (95% CI) | 646 | 595 | 1.15 (1.02–1.29) |
| Total events: 346 (*Hypericum*), 274 (Placebo) | | | |
| Test for heterogeneity: $\chi^2$=0.62, d.f.=5 (*P*=0.09), I²=48.0% | | | |
| Test for overall effect: *Z*=2.36 (*P*=0.02) | | | |
| *Not restricted to major depression – smaller (less precise) trials* | | | |
| Halama 1991 | 10/25 | 0/25 | 21.00 (1.30–340.02 |
| Hoffmann 1979 | 19/30 | 3/30 | 6.33 (2.09–19.17) |
| Osterheider 1992 | 0/22 | 0/23 | Not estimable |
| Quandt 1993 | 29/44 | 3/44 | 9.67 (3.18–29.41) |
| Schlich 1987 | 15/25 | 3/24 | 4.80 (1.59–14.50) |
| Schmidt 1989 | 10/20 | 4/20 | 2.50 (0.94–6.66) |
| Subtotal (95% CI) | 166 | 166 | 6.13 (3.63–10.38) |
| Total events: 83 (*Hypericum*), 13 (Placebo) | | | |
| Test for heterogeneity: $\chi^2$=4.81, d.f.=4 (P=0.31), I²=16.8% | | | |
| Test for overall effect: *Z*=6.76 (*P*<0.00001) | | | |
| *Not restricted to major depression – larger (more precise) trials* | | | |
| Hübner 1993 | 14/20 | 9/20 | 1.56 (0.89–2.73) |
| König 1993 | 29/55 | 31/57 | 0.97 (0.69–1.37) |
| Reh 1992 | 20/25 | 11/25 | 1.82 (1.12–2.95) |
| Schmidt 1993 | 20/32 | 6/33 | 3.44 (1.59–7.44) |
| Sommer 1994 | 28/50 | 13/55 | 2.37 (1.39–4.04) |
| Winkel 2000 | 34/60 | 17/59 | 1.97 (1.24–3.11) |
| Subtotal (95% CI) | 242 | 249 | 1.71 (1.40–2.09) |
| Total events: 145 (*Hypericum*), 87 (Placebo) | | | |
| Test for heterogeneity: $\chi^2$=15.48, d.f.=5 (*P*=0.008), I²=67.7% | | | |
| Test for overall effect: *Z*=5.31 (*P*<0.00001) | | | |



**Fig. 3** Response to *Hypericum* extracts in depression. Results (fixed-effects model) from placebo-controlled trials stratified by type of depression (major and other) and study size (above and below median of variance). Studies identified by first author and year (HDTSG, Hypericum Depression Trial Study Group; *n*, number of responders; *N*, number of patients per group; RR, response rate ratio).

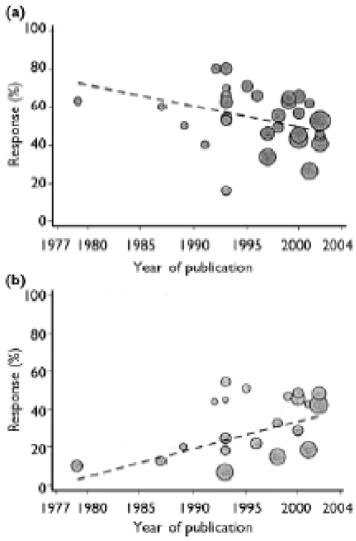
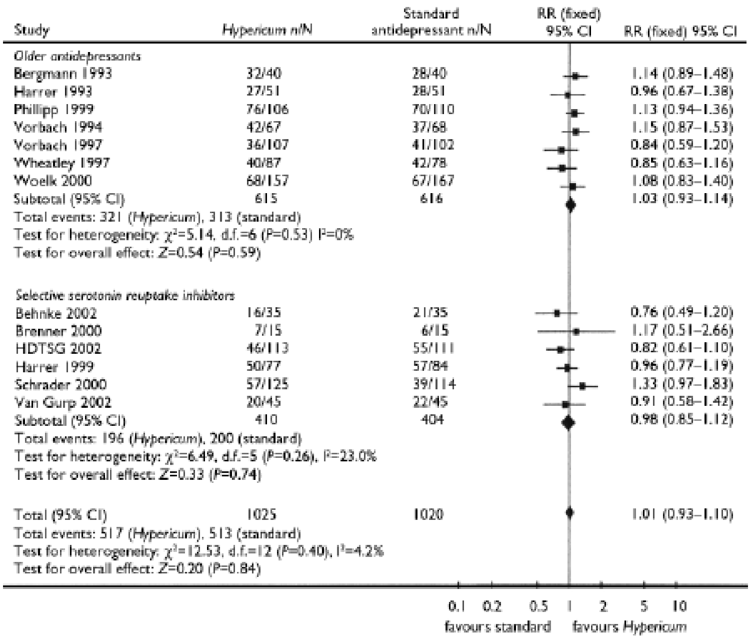



**Fig. 4** Response rates over time to (a) *Hypericum perforatum* extracts and (b) placebo, from 34 active and 22 placebo trial arms.



| Study | *Hypericum* n/N | Standard antidepressant n/N | RR (fixed) 95% CI |
|---|---|---|---|
| *Older antidepressants* | | | |
| Bergmann 1993 | 32/40 | 28/40 | 1.14 (0.89–1.48) |
| Harrer 1993 | 27/51 | 28/51 | 0.96 (0.67–1.38) |
| Phillipp 1999 | 76/106 | 70/110 | 1.13 (0.94–1.36) |
| Vorbach 1994 | 42/67 | 37/68 | 1.15 (0.87–1.53) |
| Vorbach 1997 | 36/107 | 41/102 | 0.84 (0.59–1.20) |
| Wheatley 1997 | 40/87 | 42/78 | 0.85 (0.63–1.16) |
| Woelk 2000 | 68/157 | 67/167 | 1.08 (0.83–1.40) |
| Subtotal (95% CI) | 615 | 616 | 1.03 (0.93–1.14) |
| Total events: 321 (*Hypericum*), 313 (standard) | | | |
| Test for heterogeneity: $\chi^2$=5.14, d.f.=6 (P=0.53) I²=0% | | | |
| Test for overall effect: *Z*=0.54 (P=0.59) | | | |
| *Selective serotonin reuptake inhibitors* | | | |
| Behnke 2002 | 16/35 | 21/35 | 0.76 (0.49–1.20) |
| Brenner 2000 | 7/15 | 6/15 | 1.17 (0.51–2.66) |
| HDTSG 2002 | 46/113 | 55/111 | 0.82 (0.61–1.10) |
| Harrer 1999 | 50/77 | 57/84 | 0.96 (0.77–1.19) |
| Schrader 2000 | 57/125 | 39/114 | 1.33 (0.97–1.83) |
| Van Gurp 2002 | 20/45 | 22/45 | 0.91 (0.58–1.42) |
| Subtotal (95% CI) | 410 | 404 | 0.98 (0.85–1.12) |
| Total events: 196 (*Hypericum*), 200 (standard) | | | |
| Test for heterogeneity: $\chi^2$=6.49, d.f.=5 (*P*=0.26), I²=23.0% | | | |
| Test for overall effect: *Z*=0.33 (*P*=0.74) | | | |
| Total (95% CI) | 1025 | 1020 | 1.01 (0.93–1.10) |
| Total events: 517 (*Hypericum*), 513 (standard) | | | |
| Test for heterogeneity: $\chi^2$=12.53, d.f.=12 (*P*=0.40), I²=4.2% | | | |
| Test for overall effect: *Z*=0.20 (P=0.84) | | | |



**Fig. 5** Response to *Hypericum perforatum* extracts in depression: results from controlled trials stratified by type of comparison drug. Studies identified by first author and year (HDTSG, Hypericum Depression Trial Study Group; *n*, number of responders; *N*, number of patients per group; RR, response rate ratio).

In a further Cochrane review Linde *et al.* (2008) assessed the outcome of studies in which exclusively patients with major depression were included. 29 studies in 5489 patients met the inclusion criteria; the duration of treatment was 4 to 12 weeks. Overall the Hypericum treatment was superior to placebo, similarly effective as standard antidepressants, and had fewer side effects than standard antidepressants. Studies from German speaking countries were more favourable to Hypericum compared to studies performed in other countries.

The cumulative evidence now suggests that Hypericum extracts have a modest effect over placebo in a similar range as standard antidepressants. An attempt of treating mild to moderate major depression with one of the Hypericum preparations positively tested in clinical trials is clearly justified.

However, the differences in the findings from different countries make clear-cut recommendations difficult.

**Assessor's conclusion:**

Overview of extracts with predominately positive study outcome (Superiority against placebo, equivalence to reference medication):

ICD-10 F32.0: mild depressive episode

ICD-10 F32.1: moderate depressive episode

ICD-10 F33.0: recurrent depressive disorder, current episode mild

ICD-10 F33.1: recurrent depressive disorder, current episode moderate

DSM-IV 296.21: major depressive disorder, single episode, mild

DSM-IV 296.22: major depressive disorder, single episode, moderate

DSM-IV 296.23: major depressive disorder, single episode, severe without psychotic features

DSM-IV 296.31: major depressive disorder, recurrent, mild

DSM-IV 296.32: major depressive disorder, recurrent, moderate

DSM-IV 296.33: major depressive disorder, recurrent, severe without psychotic features

| | single episode | | | | recurrent | | | | single | recurrent |
|---|---|---|---|---|---|---|---|---|---|---|
| | mild | | moderate | | mild | | moderate | | severe | severe |
| | ICD-10 F32.0 | DSM-IV 296.21 | ICD-10 F32.1 | DSM-IV 296.22 | ICD-10 F33.0 | DSM-IV 296.31 | ICD-10 F33.1 | DSM-IV 296.32 | DSM-IV 296.23 | DSM-IV 296.33 |
| LI 160 | | | **x** | | | **x** | | **x** | | |
| WS 5570 | | | | **x** | | | | **x** | **x** | **x** |
| STW3-VI | | | **x** | | | | **x** | | | |
| STEI 300 | | | **x** | | | | **x** | | | |
| LoHyp-57 | **x** | | **x** | | | | | | | |
| WS 5572 | | **x** | | **x** | | **x** | | **x** | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| STW3 | | | x | | | | x | | | |
| ZE 117 | x | | x | | x | | x | | | |

| | DER | extraction solvent | % hypericins | % hyperforin | % flavonoids | daily dosage | duration |
|---|---|---|---|---|---|---|---|
| LI 160 | 3-6:1 | methanol 80% v/v | 0.12-0.28 | app. 4.5% | app. 8.3% | 900 mg | 4-12 weeks |
| WS 5570 | 3-7:1 | methanol 80% v(v | 0.12-0.28 | 3-6% | ≥ 6.0% | 600-1800 mg | 6/26 weeks<br>relapse + |
| STW3-VI | 3-6:1 | ethanol 80% v/v | 0.26% (mean) | 4-5% | 7.17% (mean) | 900 mg | 6 weeks<br>relapse + |
| STEI 300 | 5-7:1 | ethanol 60% m/m | 0.2-0.3% | 2-3% | not specified | 1050 mg | 8 weeks |
| LoHyp-57 | 5-7:1 | ethanol 60% v/v | 0.2-0.3% | 2-3% | not specified | 800 mg | 6 weeks |
| WS 5572 | 2.5-5:1 | ethanol 60% v/v | not specified | 4-5% | not specified | 600-1200 mg | 6-7 weeks |
| STW3 | 5-8:1 | ethanol 50% v/v | 0.21% (mean) | 3.3% (mean) | 7.11% (mean) | 612 mg | 12 weeks |
| ZE 117 | 4-7:1 | ethanol 50% m/m | 0.2% | nearly free | not specified | 500 mg | 6 weeks |

Wording of the indication:

According to the 'Note for guidance on clinical investigation of medicinal products in the treatment of depression' several facts should be considered:

- Randomised double blind comparisons versus placebo are needed.
- Three-arm trials including both a placebo and an active control are recommended.
- Generally duration of about 6 weeks should be sufficient.
- For licensing it should be shown that a short-term effect can be maintained during the episode. For this a relapse prevention study is probably the best design.
- Recurrence prevention is not an obligatory part of a dossier.
- Demonstration of an acceptable benefit/risk in moderately ill patients will be considered sufficient for a registration package to get a license for 'Episodes of Major Depression".
- A 50% improvement on the usual rating scales is accepted as a clinically relevant response.

Data on relapse prevention are available from extract STW3-VI (DER 3-6:1, extraction solvent ethanol 80% v/v) and extract WS 5570 (DER 3-7:1, extraction solvent methanol 80% v/v). Extract LI 160 is very similar to WS 5570 with respect to DER, extraction solvent and content of major constituents.

Proposal of indication for these extracts:

Herbal medicinal product for the treatment of mild to moderate depressive episodes (according to ICD-10).

Overall the clinical evidence is also positive for the other extracts as reviewed by Linde *et al.* (2008). Due to the lack of data on relapse prevention the indication should be clearly different.

Proposal of indication for the remaining extracts:

Herbal medicinal product for the short term treatment of symptoms in mild depressive disorders.

**Further clinical trials related to the indication 'depression' (herbal preparation insufficiently characterised, insufficient information regarding the quality of the clinical trials):**

Extract 'Calmigen':

| | |
|---|---|
| **Extract** | No extract code |
| **Extraction solvent** | not specified |
| **DER** | not specified |
| **Total hypericins** | 0.3% |
| **Hyperforin** | not specified |

| | | |
|---|---|---|
| **Study** | **Behnke *et al.* 2002** | |
| **Herbal preparation** | | |
| **Indication** | Mild to moderate depression (ICD-10 F32), HAMD (17-items) score 16-24 | |
| **Duration of use** | 6 weeks | |
| **Daily dosage** | 300 mg | |
| **Single dosage** | 150 mg | |
| **Relapse** | - | |
| **Study design** | *Randomized* | yes |
| | *double blind* | yes |
| | *placebo-controlled* | no |
| | *reference-controlled* | 40 mg fluoxetin |
| | *Multicentre* | n=446 |
| | *number of patients* | 70 |
| | *Statistics* | ITT yes |
| **Outcome** | HAMD reduction / % responder;<br>Calmigen: 20.0 -> 10.0 / 55% | |

| | |
|---|---|
| | Fluoxetine: 20.7 -> 8.7 / 66% |
| **Comment** | Number of patients too small for a comparison of efficacy.<br>Not to be confused with Calmigen capsules (300 mg Hypericum extract, extraction solvent methanol 80%, 0.3% hypericin, authorized in DK) |

Dry extract (4-5:1, extraction solvent not specified):

| | |
|---|---|
| **Extract** | No extract code |
| **Extraction solvent** | not specified |
| **DER** | 4-5:1 (shoot tips) |
| **Total hypericins** | 0.5% |
| **Hyperforin** | not specified |

| | | |
|---|---|---|
| **Study** | **Lenoir *et al.* 1999** | |
| **Indication** | Mild to moderate depression (ICD-10) | |
| **Duration of use** | 6 weeks | |
| **Daily dosage** | corresponding to 0.17 mg, 0.33 mg or 1 mg hypericin | |
| **Single dosage** | | |
| **Relapse** | - | |
| **Study design** | *Randomized* | yes |
| | *double blind* | yes |
| | *placebo-controlled* | no |
| | *reference-controlled* | comparison of different dosages |
| | *Multicentre* | n=38 |
| | *number of patients* | 348 |
| | *Statistics* | ITT yes |
| **Outcome** | Reduction in HAMD score from initially 16-17 to 8-9 in all groups. Responder rate 62%-68%. The extract was effective at all three dosages. | |
| **Comment** | No placebo group | |

Extract HYP611:

| | |
|---|---|
| **Extract** | HYP611 |
| **Extraction solvent** | ethanol 60% |

| | |
|---|---|
| **DER** | 3.5-6:1 |
| **Total hypericins** | 0.18% (Wurglics *et al.* 2002) |
| **Hyperforin** | 2.22% (Wurglics *et al.* 2002) |

| | | |
|---|---|---|
| **Study** | Bracher 2001 | |
| **Indication** | Mild to moderate depression according to DSM IV | |
| **Duration of use** | 6 weeks | |
| **Daily dosage** | 650 mg extract | |
| **Single dosage** | 650 mg extract | |
| **Relapse** | - | |
| **Study design** | *Randomized* | yes |
| | *double blind* | yes |
| | *placebo-controlled* | no |
| | *reference-controlled* | comparison of different dosages |
| | *Multicentre* | n=? |
| | *number of patients* | 207 |
| | *Statistics* | - |
| **Outcome** | Reduction in Montgomery-Asberg depression rating scale in verum group 11.5 points, in placebo group 7.8 points; HAMD score decrease in verum group 8.6 points, in placebo group 6.3 points. | |
| **Comment** | Number of study centers not given. Publication in a not peer reviewed journal. However, Linde (2008) could retrieve detailed information about the study and included it in the latest Cochrane review. | |

A similar extract HYP 811 was tested in an observational post-marketing multicenter surveillance study with 607 patients over a period of 6 weeks (Mueller 1998).

Patients received 425 or 850 mg extract per day (no further information)

Indication: Depressive mood disorder.

Assessment of efficacy by using HAMD and van Zerssen Depression Scale (more suitable for emotional disturbances). The author found a clear reduction of symptoms.

<u>Liquid extract, DER, extraction solvent ethanol 50%</u>

| | |
|---|---|
| **Extract** | No extract code |
| **Extraction solvent** | ethanol 50% |

| | |
|---|---|
| **DER** | 1: 5-7 |
| **Total hypericins** | - |
| **Hyperforin** | - |

All studies performed with this type of extract (Harrer *et al.* 1991, Osterheider *et al.* 1992, Quandt *et al.* 1993, Schlich *et al.* 1987, Schmidt 1989) are not convincing from the current point of view. The methodology is inadequate, the number of included patients is small, and the drop-out-rate is considerably high. The studies do not fulfil the criteria for well-established use.

<u>Liquid extract (1:2, ethanol 50%):</u>

| | |
|---|---|
| **Extract** | No extract code |
| **Extraction solvent** | 50 % ethanol |
| **DER** | 1:2 |
| **Total hypericins** | 2 mg / ml |
| **Hyperforin** | not specified |

| | | |
|---|---|---|
| **Study** | **Hoffmann & *Kühl* 1979** | |
| **Indication** | Mild to severe forms of depression | |
| **Duration of use** | 6 weeks | |
| **Daily dosage** | 90 drops (= 3.6 ml = 7.2 mg hypericin) | |
| **Single dosage** | 30 drops | |
| **Relapse** | - | |
| **Study design** | *Randomized* | Yes |
| | *double blind* | Yes |
| | *placebo-controlled* | Yes |
| | *reference-controlled* | - |
| | *Multicentre* | - |
| | *number of patients* | 60 |
| | *Statistics* | - |
| **Outcome** | Not standardised symptom score with 47 items; improvement under Hypericum after 6 weeks 61.4%, in the placebo group 16.8%.<br>Responder: | |

| | |
|---|---|
| | Hypericum: 80%<br><br>Placebo: 33% |
| **Comment** | Lack of statistical evaluation; inadequate study design |

Dry extract (DER 2-5.5:1, ethanol 60%):

Daily dosage: 213-252 mg extract;

| | |
|---|---|
| **Extract** | No extract code |
| **Extraction solvent** | 60 % ethanol |
| **DER** | 2-5,5:1 |
| **Total hypericins** | 0.1% |
| **Hyperforin** | not specified |
| **Flavonoids** | not specified |

| | | |
|---|---|---|
| **Study** | **Bergmann *et al.* 1993** | |
| **Indication** | Mild to moderate depression (ICD-10 F32.0, F32.1, F33.0, F33.1) | |
| **Duration of use** | 6 weeks | |
| **Daily dosage** | 213-252 mg extract preparation (including excipients, = 3 x 60 mg native extract corresponding to 0.75 mg hypericin) | |
| **Single dosage** | | |
| **Relapse** | - | |
| **Study design** | *Randomized* | Yes |
| | *placebo-controlled* | No |
| | *reference-controlled* | 30 mg amitriptyline/day |
| | *Multicentre* | n=1 |
| | *number of patients* | 80 |
| | *Statistics* | Per protocol |
| **Outcome** | HAMD reduction / % responder:<br><br>Esbericum: from 15.82 to 6.43 / 84.2%<br><br>Amitriptyline: from 15.26 to 6.65 / 73.7%<br><br><br>From the fact that the low dosages of amitriptyline and of Hypericum were effective it can be assumed that only patients with mild symptoms were included. This is also reflected by the relatively low starting values of the HAMD scale. | |

| | |
|---|---|
| **Comment** | Low dosage of amitriptyline |

Conclusion: In the context with the results of the other extracts this study contributes to the overall evidence on the use of Hypericum extracts for the improvement of depressive symptoms.

<u>Dry extract (no information on DER and extraction solvent)</u>

| | |
|---|---|
| **Extract** | PM235 |
| **Extraction solvent** | No information |
| **DER** | No information |
| **Total hypericins** | 0.12% / tablet or 0.18% / tablet |
| **Hyperforin** | not specified |
| **Flavonoids** | not specified |

| | | |
|---|---|---|
| **Study** | **Randlov *et al.* 2006** | |
| **Indication** | Mild to moderate depression (ICD-10 F32.0, F32.1, F33.0, F33.1). Also patients with dysthymia (F34.1) were included. | |
| **Duration of use** | 6 weeks | |
| **Daily dosage** | 810 mg | |
| **Single dosage** | 270 mg | |
| **Relapse** | - | |
| **Study design** | *Randomized* | Yes |
| | *placebo-controlled* | No |
| | *reference-controlled* | - |
| | *Multicentre* | n=1 |
| | *number of patients* | 150 |
| | *Statistics* | Per protocol |
| **Outcome** | Large discrepancy in response between dysthymic and non-dysthymic patients. HAMD improvement not statistically significant for Hypericum. Non-dysthymic patients improved more frequent. After pooling of both Hypericum-treated groups a clinical significant effect in minor depressed and non-dysthymic patients (HAMD ≤ 17) could be concluded. | |
| **Comment** | Insufficient characterisation of the herbal preparation. | |

Dry extract (no information on DER and extraction solvent)

| | |
|---|---|
| **Extract** | - |
| **Extraction solvent** | No information |
| **DER** | No information |
| **Total hypericins** | not specified |
| **Hyperforin** | not specified |
| **Flavonoids** | not specified |

| | | |
|---|---|---|
| **Study** | Rapaport *et al.* 2011 | |
| **Indication** | Mild depression (HAMD 10-17) | |
| **Duration of use** | 12 weeks | |
| **Daily dosage** | 810 mg | |
| **Single dosage** | 270 mg | |
| **Relapse** | - | |
| **Study design** | *Randomized* | Yes |
| | *placebo-controlled* | No |
| | *reference-controlled* | Citalopram 20 mg |
| | *Multicentre* | n=1 |
| | *number of patients* | 100 |
| | *Statistics* | ITT |
| **Outcome** | 19 drop outs prior to randomization.<br>29 patients Hypericum group, 27 citalopram, 25 placebo.<br>Drop outs: citalopram 4 (side effects), Hypericum 1, placebo 2<br>Effects in all 3 groups were similar. Citalopram and Hypericum not superior to placebo. | |
| **Comment** | Insufficient characterisation of the herbal preparation. | |

**Other clinical trials, oral use:**

**Seasonal affective disorders**

Martinez *et al.* (1994) compared light therapy (2 hours daily) and Hypericum (3 x 300 mg dry extract, methanol 80%, 4-7:1) in 20 patients. After 4 weeks a significant reduction in the HAMD scale was observed in both treatment groups but no significant difference between the treatment groups.

**Somatoform disorders**

Volz *et al.* (2002) conducted a multicentre, randomised, placebo controlled, 6-week trial comparing the efficacy of LI 160 (600 mg/day) and placebo in 151 out-patients suffering from somatisation disorder (ICD-10: F45.0), undifferentiated somatoform disorder (F45.1), or somatoform autonomic dysfunctions (F45.3). The primary outcome measure was the decrease of the Hamilton Anxiety Scale, subfactor somatic anxiety (HAMA-SOM), during the trial period. The Hypericum extract was of superior effectiveness concerning the primary outcome criterion HAMA-SOM [decrease from 15.39 (SD 2.68) to 6.64 (4.32) in the Hypericum group and from 15.55 (2.94) to 11.97 (5.58) in the placebo group (statistically significant difference, P=0.001)]. This was corroborated by the result of a statistically significant superior efficacy in the outcome criteria additionally used such as Clinical Global Impression, HAMA-total score, HAMA, subscore psychic anxiety, Hamilton Depression Scale, Self-Report Symptom Inventory 90 items - revised (SCL-90-R), and SCL-90-R, subscore somatic anxiety. The efficacy of LI 160 was preserved after splitting the population in those with and those without mild depressive symptoms [corrected]. Tolerability of LI 160 was excellent. The efficacy was independent of an existing depressive mood.

In a prospective, randomized, placebo-controlled double-blind parallel group study, 184 outpatients with somatisation disorder (ICD-10 F45.0), undifferentiated somatoform disorder (F45.1), and somatoform autonomic dysfunction (F45.3), but not major depression, received either 300 mg of Hypericum extract LI 160 twice daily or matching placebo for 6 weeks (Müller *et al.* 2004). Six outcome measures were evaluated as a combined measure by means of the Wei Lachin test: Somatoform Disorders Screening Instrument--7 days (SOMS-7), somatic subscore of the HAMA, somatic subscore of the SCL-90-R, subscores "improvement" and "efficacy" of the CGI, and the global judgment of efficacy by the patient. In the intention to treat population (N=173), for each of the six primary efficacy measures as well as for the combined test, statistically significant medium to large-sized superiority of Hypericum extract treatment over placebo was demonstrated (p <.0001). Of the Hypericum extract patients, 45.4% were classified as responders compared with 20.9% with placebo (p =.0006). Tolerability of Hypericum extract treatment was equivalent to placebo.

**Fatigue**

Ina pilot study Stevinson *et al.* (1998) investigated the effect of Hypericum (dry extract, methanol 80%, DER 3-6:1) in 20 patients suffering from fatigue, tiredness or exhaustion without any overt medical reason. The patients received 3 x 300 mg extract for 6 weeks. Compared to baseline values, perceived fatigue was significantly lower after 2 weeks of treatment and reduced significantly further after 6 weeks.

**Schizophrenia**

Hypericum extract LI 160 has demonstrated a ketamine-antagonising effect. Therefore Murck *et al.* (2006) examined whether LI 160 reverses changes of a low dose ketamine on auditory evoked potentials (AEP) in healthy subjects. The authors performed a double-blind randomized treatment with either 2 x daily 750 mg LI 160 or placebo given for one week, using a crossover design, in 16 healthy subjects. A test-battery including AEPs, the oculodynamic test (ODT) and a cognitive test were performed before and after an infusion with 4 mg of S-ketamine over a period of 1 hour. S-ketamine led to a significant decrease in the N100-P200 peak to peak (ptp) amplitude after the placebo treatment, whereas ptp was significantly increased by S-ketamine infusion in the LI 160 treated subjects. The ODT and the cognitive testing revealed no significant effect of ketamine-infusion and therefore no interaction between treatment groups. Provided that ketamine mimics cognitive deficits in schizophrenia, LI 160 might be effective to treat these symptoms.

**Obsessive-compulsive disorder**

Taylor *et al.* (2000) treated 12 subjects with the diagnosis of obsessive-compulsive disorder for 12 weeks with 2 x daily 450 mg Hypericum extract (0.3% hypericin). Evaluation of the response was based on the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS). After 1 week of treatment a significant change in the BOCS was observed which increase towards the end of the trial.

Kobak *et al.* (2005) investigated the effect of Hypericum (600-18000 mg dry extract per day for 12 weeks, extraction solvent methanol 80%, DER 3-6:1) in 60 patients with obsessive-compulsive disorder. Primary endpoint was the change in the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS). The mean change in this scale with Hypericum was not significantly different than the mean change found with placebo.

**Social phobia**

In this pilot study by Kobak *et al.* (2005a) no significant difference between Hypericum treatment (2-6 x daily 300 mg dry extract; extraction solvent methanol 80%, DER 3-6:1) and placebo after 12 weeks measured by the Liebowitz Social Anxiety Scale in 40 patients suffering from social phobia.

**Attention-deficit hyperactivity disorder**

Niederhofer (2010) treated 3 adolescents diagnosed with ADHD for 4 weeks with Hypericum (30 mg per day, no further information). Patient's mean scores improved for Conners' hyperactivity, inattention and immaturity factors.

**Autistic disorders**

3 outpatients meeting ICD-10 criteria for autistic disorders received 20 mg Hypericum daily (no further information) for 4 weeks (Niederhofer 2009). Only slight improvements on the Aberrant Behavior Checklist, irritability, stereotypy and inappropriate speech were observed. Clinician ratings did not improve significantly.

**Generalised anxiety disorders**

Davidson & Connor (2001) present 3 case reports of successful treatment of patients with generalised anxiety disorders with Hypericum supplementation.

*Assessor's comment:*

*As the herbal preparations are insufficiently characterised no conclusion can be drawn.*

**Restless leg syndrome**

In an open-label pilot trial Pereira *et al.* (2013) treated 21 patients with Willis-Ekbom's disease (formerly known as restless leg syndrome) with a Hypericum extract (300 mg daily, no further information) for 3 months. In 17 patients the severity of symptoms were reduced.

**Premenstrual syndrome**

19 women with premenstrual syndrome who were in otherwise good physical and mental health and not taking other treatments for premenstrual syndrome were investigated in a prospective, open, uncontrolled, observational pilot study (Stevinson & Ernst 2000). The participants took Hypericum tablets for two complete menstrual cycles (1 x 300 mg Hypericum extract per day standardised to 900 µg hypericin). Symptoms were rated daily throughout the trial using a validated measure. The Hospital Anxiety and Depression scale and modified Social Adjustment Scale were administered at baseline and after one and two cycles of treatment. There were significant reductions in all outcome measures. The degree of improvement in overall premenstrual syndrome scores between baseline and the end of the trial was 51%, with over two-thirds of the sample demonstrating at least a 50% decrease in symptom severity. Tolerance and compliance with the treatment were encouraging.

Hicks *et al.* (2004) performed a randomized, double-blinded, placebo-controlled trial with two parallel treatment groups. After a no-treatment baseline cycle, volunteers were randomized to either Hypericum extract or placebo for a further two menstrual cycles. 169 normally menstruating women who experienced recurrent premenstrual symptoms were recruited onto the study. 125 completed the protocol and were included in the analysis. Study medication: 600 mg of Hypericum extract (standardized to contain 1800 µg of hypericin) or placebo (containing lactose and cellulose). A menstrual diary was used to assess changes in premenstrual symptoms. The anxiety-related subgroup of symptoms of this instrument was used as the primary outcome measure. After averaging the effects of treatment over both treatment cycles it was found that there was a trend for Hypericum extract to be superior to placebo. However, this finding was statistically not significant.

Canning *et al.* 2010 investigated the efficacy of the herbal preparation LI 160 (DER 3-6:1, extraction solvent methanol 80% V/V) in a ten-cycle, randomised, double-blind, crossover, placebo-controlled study in 36 women diagnosed with mild premenstrual syndrome. After three screening cycles and a two-cycle placebo run-in phase the patients received either 900 mg/day Hypericum or placebo for two cycles. After a placebo-treated washout cycle, the women crossed over to receive either placebo or Hypericum for additional two cycles. The treatment was statistically superior to placebo in improving physical and behavioural symptoms of PMS. No difference was found for mood- and pain-related PMS symptoms. No changes on the plasma hormone and cytokine levels were found.

Ghazanfarpour *et al.* 2011 report from a prospective, randomised, double-blind, placebo controlled trial including 170 women with premenstrual syndrome. The participants received either 2 tablets containing Hypericum (no information regarding the type of the herbal preparation; 680 µg hypericin per tablet) or placebo for 8 weeks. Hypericum significantly lowered PMS scores. Details on the validity of the scores are missing.

Ryoo *et al.* (2010) investigated the effect of Hypericum extract (2 x 300 mg; 0.3% hypericin, 3% hyperforin, no further information) on mood symptoms in women with premenstrual syndrome. 30 women were observed for 3 menstrual cycles. No difference to placebo was found in a pain visual analogue scale, in the Beck depression inventory and the premenstrual assessment form. However, significant improvements were found regarding emotional lability, hostility/anger and impulsivity.

**Menopausal symptoms**

In a drug-monitoring study Grube *et al.* (1999) investigated 12 weeks of treatment with St. John's Wort, one tablet three times daily (900 mg Hypericum extract LI 160), in 111 women from a general medical practice. The patients who were between 43 and 65 years old had climacteric symptoms characteristic of the pre- and postmenopausal state. Treatment outcome was evaluated by the Menopause Rating Scale, a self-designed questionnaire for assessing sexuality, and the Clinical Global Impression scale. The incidence and severity of typical psychological, psychosomatic, and vasomotor symptoms were recorded at baseline and after 5, 8, and 12 weeks of treatment. Substantial improvement in psychological and psychosomatic symptoms was observed. Climacteric complaints diminished or disappeared completely in the majority of women (76.4% by patient evaluation and 79.2% by physician evaluation). Sexual well-being also improved after treatment with St. John's Wort extract.

Abdali *et al.* (2010) investigated the effect of a Hypericum extract (0.2 mg/ml hypericin, no further details) in 100 women suffering from menopausal problems. After 8 weeks the authors found a statistically significant improvement in the Hypericum group.

*Assessor’s comment:*

*As essential data regarding characterisation of the herbal preparation and posology are missing the outcome of this trial cannot be assessed.*

Additionally some clinical trials with fixed combinations are published (e.g., combination Hypericum + Cimicifuga racemosa Uebelhack *et al.* 2006, Chung *et al.* 2007). Although the authors report a positive outcome with regard to climacteric complaints, such publications are considered only marginally because the amount of the contribution of each combination partner to the overall efficacy cannot be estimated.

Al-Akoum *et al.* (2009) investigated an extract (extraction solvent ethanol 50%, 0.3% hypericin) in breast cancer survivors in a randomised pilot trial. 47 women received 900 mg extract daily. After 12 weeks of treatment no significant difference between treatment and placebo was found regarding the frequency of daily hot flushes. However, an improvement in sleep disorders and the menopause-specific quality of life could be demonstrated.

Fahami et al (2010) compared the efficacy of a Hypericum preparation with that of a passion flower preparation in 59 women with menopausal symptoms. Although the authors found improvements in both groups the results remain unclear due to a missing characterisation of the herbal preparations and due to the missing comparison with a placebo group.

Laakmann *et al.* (2012) conclude in their review that monotherapy with Hypericum is not superior to placebo. Liu *et al.* (2014) come in their meta-analysis to a more favourable result. However, this may be due to the unclear distinction between single and combination studies.

**Support in smoking cessation**

Barnes *et al.* (2006) performed a randomised, open, uncontrolled pilot study with LI 160 (DER 3-6:1, extraction solvent methanol 80% V/V). 28 smokers (10 or more cigarettes per day for more than 1 year) received 300 or 600 mg extract for 3 months. Additionally all participants received motivational support. The study did not provide convincing evidence that Hypericum is likely to be effective as an aid in smoking cessation.

In a clinical trial Parson *et al.* (2009) smokers received 3 x daily 300 mg Hypericum extract (methanol 80%, DER 3-6:1) or placebo and additionally chromium or placebo. Treatment started 2 weeks prior to quit day and continued for 14 weeks. Smoking abstinence was observed until 6 months. Hypericum treatment turned out to be ineffective for smoking cessation when compared with placebo.

Sood *et al.* (2010) included 118 smokers (app. 20 cigarettes/day) in a randomised, placebo-controlled 3-arm study. The participants received a Hypericum extract (no further details) at doses of 300 mg or 600 mg, 3 x daily for 12 weeks. No difference in the abstinence rate between the 2 Hypericum groups and placebo were observed. Hypericum did not attenuate withdrawal symptoms among abstinente subjects.

In the review of Kitikannakorn *et al.* (2013) the authors conclude that there is only limited evidence for the effectiveness of Hypericum preparations used for smoking cessation.

In the Cochrane Review on the use of antidepressants in smoking cessation by Hughes *et al.* (2014) the clinical trials mentioned above were included. In the opinion of the authors there is no evidence that Hypericum aids long-term smoking cessation.

**Irritable bowel syndrome**

Saito *et al.* (2009) investigated the effect of Hypericum (2 x daily 450 mg for 12 weeks, no further information) in 70 patients with irritable bowel syndrome. Primary end point was self-reported overall bowel symptom score. Hypericum turned out to be less effective than placebo.

Wan & Chen (2010) investigated the administration of Hypericum (3 x 300 mg extract for 8 weeks, no further information) in 30 patients with irritable bowel syndrome. Beside some parameters related to responses of the autonomic nervous system also gastrointestinal symptoms improved significantly.

**Burning mouth syndrome**

Sardella *et al.* (2008) treated for 12 weeks 39 patients with burning mouth syndrome with a Hypericum extract (3 x 300 mg extract, 0.31% hypericin, 3.0% hyperforin, no further information). The intensity of burning was evaluated using a visual-analogue scale. The treatment did not improve the symptoms compared to placebo. Only the number of sites with burning sensation was significantly reduced.

**Other clinical trials, cutaneous use:**

Meinke *et al.* 2012

In 22 healthy volunteers (4 male, 18 female; aged 19-59 years) duplicate panels of test areas on the back were occlusively treated with vehicle, a cream containing 1.5% of a CO2 extract (44.3% hyperforin) or were left untreated. After 30 minutes one panel of the test areas was irriadiated with 1.5 minimal erythema doses of UVB, and the other panel was left unirradiated. The erythema of all test areas was measured photometrically 48 h after irradiation. Hypericum cream significantly reduced UVB-induced erythema as opposed to the vehicle. Occlusive application of the cream on non-irradiated test sites did not cause any skin irritation.

Schempp *et al.* (2000a) investigated the effects of Hypericum oil (hypericin 110 mg/ml) and Hypericum ointment (hypericin 30 mg/ml) on skin sensitivity to solar simulated radiation. Sixteen volunteers of the skin types II and III were tested on their volar forearms with solar simulated radiation for photosensitizing effects of Hypericum oil (n=8) and Hypericum ointment (n=8). The minimal erythema dose (MED) was determined by visual assessment, and skin erythema was evaluated photometrically. With the visual erythema score, no change of the MED could be detected after application of either Hypericum oil or Hypericum ointment (P>0.05). With the more sensitive photometric measurement, an increase of the erythema-index after treatment with the Hypericum oil could be detected (P< or =0.01). The results do not provide evidence for a severe phototoxic potential of Hypericum oil and Hypericum ointment, detectable by the clinically relevant visual erythema score. However, the trend towards increased photosensitivity detected with the more sensitive photometric measurement could become relevant in fair-skinned individuals, in diseased skin or after extended solar irradiation.

*Assessor's comment:*

*The mentioned content of hypericin is in contrast to investigations from Maisenbacher et al (1992), these authors found only artefacts of hypericin. From traditional use of Hypericum oil it is known that the exposure to sunlight of treated parts of the skin would lead to skin irritations. In traditional medicine it is recommended to protect treated skin from sunlight.*

In a half-side comparison study Schempp *et al.* (2003a) assessed the efficacy of a cream containing Hypericum: Extract (supercritical CO2) standardised to 1.5% hyperforin (verum) in comparison to the corresponding vehicle (placebo) for the treatment of subacute atopic dermatitis. The study design was a prospective randomised placebo-controlled double-blind monocentric study. In twenty one patients suffering from mild to moderate atopic dermatitis (mean SCORAD 44.5) the treatment with verum or placebo was randomly allocated to the left or right side of the body, respectively. The patients were treated twice daily over a period of four weeks. Eighteen patients completed the study. The severity of the skin lesions on the left and right side was determined by means of a modified SCORAD-index (primary endpoint). The intensity of the eczematous lesions improved on both sides of treatment.

However, the Hypericum-cream was significantly superior to the vehicle at all clinical visits (days 7, 14, 28) ($p < 0.05$). Skin colonisation with Staphylococcus aureus was reduced by both verum and placebo, showing a trend to better antibacterial activity of the Hypericum cream ($p = 0.064$). Skin tolerance and cosmetic acceptability was good or excellent with both the Hypericum cream and the vehicle (secondary endpoints).

Kacerovska *et al.* 2008 investigated the efficacy of topical Hypericum extract (chromatographically purified dry extract, extraction solvent ethanol 96%; 1.5-2.5 mg/ml hypericines). The extract was applied under occlusion in patients with actinic keratosis, basal cell carcinoma and morbus Bowen (in total 34 patients). After 2 hours the application sites were irradiated with 75 J/cm2 of red light. The treatment was performed weekly for 6 weeks. Complete clinical response was seen in 50% of patients with actinic keratosis, in 28% of patients with superficial basal cell carcinoma and in 40% of patients with morbus Bowen. Only partial remission was seen in patients with nodular basal cell carcinoma. All patients complained of burning ans pain sensation during irradiation.

Najafizadeh *et al.* (2012) treated 10 patients with plaque-type psoriasis with a Hypericum ointment. The ointment contained 5% of a Hypericum extract (no further details). The ointment was applied to one side of the patient's body and the placebo (vehicle) to the other side. Hypericum treatment significantly lowered the Modified Psoriasis Area Severity index with the factors erythema, scaling and thickness.

Samadi *et al.* (2010) investigated the effect of an oily Hypericum extract (extraction solvent grapeseed oil, no further information) on cesarean wound healing and hypertrophic scar. 144 women were included, in the verum group the ointment (20% oily extract) was applied 3 x daily for 16 days. At day 10 after cesarean section significant differences in would healing and at day 40 in scar formation compared to placebo and control group were detected. Additionally the participants reported lower pain and pruritus.

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

Hübner & Kirste (2001) investigated a Hypericum extract LI 160 (DER 3-6:1, methanol 80% V/V) in children under 12 years with symptoms of depression and psychovegetative disturbances.

Study design: Multi-center, post-marketing surveillance study; n=101 children under 12 years, dosage: 300 to 1800 mg per day.

Based on the data available for analysis, the number of physicians rating effectiveness as 'good' or 'excellent' was 72% after 2 weeks, 97% after 4 weeks and 100% after 6 weeks. The ratings by parents were very similar. There was, however, an increasing amount of missing data at each assessment point with the final evaluation including only 76% of the initial sample. Tolerability was good and no adverse events were reported.

Findling *et al.* (2003) conducted an open-label prospective outpatient pilot study in juvenile depression. Children and adolescents 6 to 16 years of age meeting DSM-IV criteria for major depressive disorder received in the prospective, open-label, outpatient study a dosage: 3 x 150-300 mg. The extract is not further specified. 33 children with a mean age of 10.5 (2.9) years were enrolled. After 4 weeks of St. John's wort therapy, 22 youths had their dose increased to 900 mg/day. Twenty-five of the patients met response criteria after 8 weeks of treatment. Overall, St. John's wort was well tolerated. The authors conclude that Hypericum may be an effective treatment for youths diagnosed with major depressive disorder.

*Assessor's comment:*

*The Hypericum extract is characterized improperly.*

In an 8 week open-label study on efficacy and safety of 3 x 300 mg Hypericum extract (0.3% hypericin, 3% hyperforin, no further information) 26 adolescents were enrolled (Simeon *et al.* 2005). In 7 adolescents the symptoms persisted or worsened, 8 of the 26 were noncompliant. Therefore only 11 participants finished the study. In 9 of the 11 patients a significant clinical improvement was observed.

Fegert *et al.* (2006) analysed the prescription data of antidepressants in Germany in the years 2000-2003. Approximately 280.000 persons under the age of 20 were accessed. Hypericum and tricyclic antidepressants accounted for more than 80% of antidepressant use. In all of the 4 years Hypericum was the preferably prescribed antidepressant in this age group:

2000: Hypericum 55.59%, Imipramine 16.25%
2001: Hypericum 52.24%, Imipramine 14.93%
2002: Hypericum 50.58%, Imipramine 11.85%
2003: Hypericum 40.36%, Omipramol 10.72%

In a randomized, double-blind, placebo controlled study (Weber *et al.* 2008) 54 children aged 6 to 17 years received 300 mg Hypericum extract (containing 0.3% Hypericin) 3 times daily for 8 weeks. All participants met the diagnostic criteria for ADHD. Hypericum did not improve the symptoms.

***Assessor's conclusion on the use in the paediatric population:***

*Although there are no controlled studies with children and adolescents published it can be concluded that there is a widespread documented use of Hypericum extracts among adolescents. However, there are no data available on the efficacy and safety in this population. Therefore the oral use in children and adolescents below 18 years of age is not recommended.*

## *4.4. Overall conclusions on clinical pharmacology and efficacy*

From the assessment of the controlled clinical trials it can be concluded that for dry extracts with a DER 3-7:1 and extraction solvent methanol 80% V/V as well as with DER 3-6:1 and extraction solvent ethanol 80% V/V the indication 'Herbal medicinal product for the treatment of mild to moderate depressive episodes (according to ICD-10)' can be proposed. Dry extracts with a DER 2.5-8:1 and extraction solvent ethanol 50-68% V/V are recommende for the short term treatment of symptoms in mild depressive disorders.

The evidence of clinical efficacy for all other herbal preparations is insufficient. Therefore only traditional medicinal use can be proposed, provided that all requirements according to Dir. 2001/83 as amended are fulfilled.

This conclusion is in line with the conclusions of Ravindran *et al.* (2009) in the Canadian 'Clinical guideline for the management of major depressive disoriders' that for Hypericum preparations there is a level 1 evidence for the treatment of mild to moderate major depressive disorders.

# 5. Clinical Safety/Pharmacovigilance

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

Table 15: Clinical safety data from clinical trials

| Type | Study | Test Product(s) | Number of subjects | Type of subjects | Adverse reactions | Comments |
|---|---|---|---|---|---|---|
| Harrer *et al.* 1994 | Controlled study<br>4 weeks | LI 160<br>3 x 300 mg | 51 Hypericum<br>51 Maprotiline | Moderately severe depressive episodes, according to ICD-10, F 32.1 (HAMD 17-items >- 16) | Hypericum: 25% of patients<br>Maprotilin: 35%<br>Hypericum: gastrointestinal complaints, dizziness, confusion | Side effects considered in the monograph |
| Hänsgen *et al.* 1994 | Controlled study<br>4 weeks | LI 160<br>3 x 300 mg | 33 Hypericum<br>34 Placebo | Mild to moderate major depression, according to DSM-III-R (HAMD >- 16) | 1 patient: sleep disturbances | Side effects considered in the monograph |
| Sommer & Harrer 1994 | Controlled study<br>4 weeks | LI 160<br>3 x 300 mg | 42 Hypericum<br>47 Placebo | Depressive symptoms according ICD-09 300.4 (neurotic depression) and 309.0 (brief depressive reaction) | 2 patients: skin reddening, itching , tiredness | Side effects considered in the monograph |
| Vorbach *et al.* 1994 | Controlled study<br>6 weeks | LI 160<br>3 x 300 mg | 67 Hypericum<br>68 Imipramine | Major depression according to DSM-III-R (single episode, recurrent episode, neurotic depression, adjustment disorder with depressed mood) | 11.9%: most frequent dry mouth, dizziness. | Dry mouth not considered in the monograph |
| Hänsgen & Vesper 1996 | Controlled study | LI 160 | 51 Hypericum | Mild to moderate major depression, according to DSM- | 1 patient: sleep disturbances | Side effects considered in the |

| Type | Study | Test Product(s) | Number of subjects | Type of subjects | Adverse reactions | Comments |
|---|---|---|---|---|---|---|
| | 4 weeks | 3 x 300 mg | 50 Placebo | III-R (HAMD >- 16) | | monograph |
| Wheatley 1997 | Controlled study<br>6 weeks | LI 160<br>3 x 300 mg | 83 Hypericum<br>73 Amitriptyline | Mild to moderate major depression (HAMD-17 score: 17-24; according to DSM-IV) | 37 % of the patients<br>Dry mouth (5%)<br>Drowsiness (1%)<br>Sleepiness (2%)<br>Dizziness (1%)<br>Lethargy (1%)<br>Nausea/Vomiting (7%)<br>Headache (7%)<br>Constipation (5%)<br>Pruritus (2%) | Gastrointestinal and nervous symptoms considered in the monograph. |
| Vorbach *et al.* 1997 | Controlled study<br>6 weeks | LI 160<br>3 x 600 mg | 107 Hypericum<br>102 Imipramine | Severe episode of a major depression according to ICD-10 F 33.2, recurrent, without psychotic symptoms | 23% of the patients<br>n=37<br>Dry mouth (3)<br>Gastric symptoms (5)<br>tiredness/sedation (5)<br>Restlessness (6)<br>Tremor (2) | Gastrointestinal and nervous symptoms considered in the monograph. |
| Czekalla *et* | Randomized, placebo- | LI 160 | 84 patients in Hypericum | Patients suffering from | Imipramine: significant increase in first degree AV- | Authors conclude that Hypericum |

| Type | Study | Test Product(s) | Number of subjects | Type of subjects | Adverse reactions | Comments |
|---|---|---|---|---|---|---|
| *al.* 1997 | controlled, multicentre safety study<br>6 weeks | Hypericum extract (DER 4-7:1, methanol 80% V/V): 1800 mg daily, 6 weeks<br>Imipramine: 150 mg daily, 6 weeks | group; 76 patients in imipramine group<br>Mean age 48-49 years | depression | blocks and abnormalities of repolarization.<br>Hypericum: significant decrease of such findings | extract is safer with regard to the cardiac function than tricyclic antidepressants. |
| Grube *et al.* 1997 | Non interventional study<br>5 weeks | LI 160<br>Hypericum extract (DER 4-7:1, methanol 80% V/V): 3 x daily 300 mg | 118 patients | Patients suffering from transient and mild depressive mood disorders | Adverse reactions reported from 7 patients (GI disorders, nervousness) | |
| Brockmöller *et* al*.* 1997 | Placebo-controlled<br>Single dose | LI 160<br>Hypericum extract (DER 4-7:1, methanol 80% V/V)<br>900, 1800 or | 13 volunteers | Healthy volunteers | No dose-related trend in light sensitivity. Sensitivity | Common therapeutic doses do not influence photosensitivity |

| Type | Study | Test Product(s) | Number of subjects | Type of subjects | Adverse reactions | Comments |
|---|---|---|---|---|---|---|
| | | 3600 mg; single administration<br><br>1800 mg daily, 15 days | <br><br>50 volunteers | | to UV-A increased only at highest dose<br><br>Slight increase in sensibility. | |
| Holsboer-Trachsler & Vanoni 1999 | Non interventiona l study<br><br>6 weeks | LI 160<br><br>Hypericum extract (DER 4-7:1, methanol 80% V/V): 3 x daily 300 mg | 647 patients | Patients suffering from mild to moderate depressive mood disorders | Adverse reactions in 17%<br><br>Diarrhoea, Nausea (10%)<br><br>Photodermatosis (3%)<br><br>Headache, tiredness (7%) | Gastrointestinal and nervous symptoms considered in the monograph. |
| Brenner *et al.* 2000 | Controlled study<br><br>7 weeks | LI 160<br><br>3 x 300 mg | 15 Hypericum<br><br>15 Sertraline | Mild to moderate depression (HAMD: ≥ 17, according to DSM IV) | 2 patients: headache, dizziness | Side effects considered in the monograph |
| Montgomery *et al.* 2000 | Controlled study<br><br>12 weeks | LI 160<br><br>3 x 300 mg | 124 Hypericum<br><br>124 Placebo | Mild to moderate depression (DSM-IV) | No information | |
| Shelton *et al.* 2001 | Controlled study<br><br>8 weeks | LI 160<br><br>3 x 300 mg, if no response 4 | 98 Hypericum<br><br>102 Placebo | Major depression (HAMD: ≥ 20 for more than 2 years, according to DSM-IV: major depression disorder, single episode or | Headache (41%)<br><br>Abdominal pain (≥ 10%) | Side effects considered in the monograph |

| Type | Study | Test Product(s) | Number of subjects | Type of subjects | Adverse reactions | Comments |
|---|---|---|---|---|---|---|
| | | x 300 mg | | recurrent, without psychotic features) | | |
| Hypericum depression trial study group 2002 | Controlled study<br>8 weeks | LI 160<br>3 x 300 mg to 5 x 300 mg | 113 Hypericum<br>111 Sertraline | Moderately severe major depressive disorder (according to DSM-IV; HAM-D ≥ 20; GAF ≤ 60) | Diarrhoea (21%)<br>Nausea (19%)<br>Anorgasmia (25%)<br>Forgetfulness (25%)<br>Frequent urination (27%)<br>Sweating (18%)<br>Swelling (19%) | Gastrointestinal and nervous symptoms considered in the monograph. |
| Schempp *et al.* 2003b | Controlled safety study<br>7 days | LI 160<br>Dry extract (methanol 80%, DER 3-6:1)<br>In the single-dose study the volunteers received 6 or 12 coated tablets (5400 or 10800 mg hypericin). In the steady-state study | 72 | Healthy volunteers, skin types II and III | No | After both single-dose and steady-state administration, no significant influence on the erythema-index or melanin-index could be detected, with the exception of a marginal influence on UVB induced pigmentation (p = 0.0471) in the single-dose study. |

| Type | Study | Test Product(s) | Number of subjects | Type of subjects | Adverse reactions | Comments |
|---|---|---|---|---|---|---|
| | | the volunteers (n = 24) received an initial dose of 6 tablets (5400 mg hypericin), and subsequently 3 x 1 tablets (2700 mg hypericin) per day for 7 days. | | | | The results do not provide evidence for a phototoxic potential of the Hypericum extract. |
| Sarris *et al.* 2012 | Continuation of the study above<br>26 weeks | LI 160<br>3 x 300 mg to 5 x 300 mg | 35 Hypericum<br>49 Sertraline<br>40 Placebo | Moderately severe major depressive disorder (according to DSM-IV; HAM-D ≥ 20; GAF ≤ 60) | No information | |
| Bjerkenstedt *et al.* 2005 | Controlled study<br>4 weeks | LI 160<br>3 x 300 mg | 54 Hypericum<br>54 Fluoxetine<br>55 Placebo | Mild to moderate major depression (DSM-IV: 296.31, 296.32); minimum of a total score of 21 on the 21-item Hamilton Depression scale | Adverse events: 38<br>Patients with adverse events: 35.1%<br>Adverse events possibly related to study medication: 24<br>Body as a whole (13) | Gastrointestinal and nervous symptoms considered in the monograph. |

| Type | Study | Test Product(s) | Number of subjects | Type of subjects | Adverse reactions | Comments |
|---|---|---|---|---|---|---|
| | | | | | Gastro-intestinal system disorders (6)<br>Autonomic nervous system disorders (10)<br>Central & peripheral nervous system disorders (10)<br>Skin and appendages disorders (9)<br>Psychiatric disorders (2)<br>Others (5) | |
| Fava *et al.* 2005 | Controlled study<br>12 weeks | LI 160<br>3 x 300 mg | 45 Hypericum<br>47 Fluoxetine<br>43 Placebo | Major depressive disorder (HAMD-17 ≥ 16) | Most common adverse events.<br>Headache (42%)<br>Dry mouth (22%)<br>Nausea (20%)<br>Gastrointestinal upset (20%)<br>Sleepiness (18%) | Gastrointestinal and nervous symptoms considered in the monograph. |
| Mannel *et al.* 2010 | Controlled study<br>8 weeks | LI 160<br>3 x 300 mg | 100 Hypericum<br>100 Placebo | Depression with atypical features | 13 patients<br>No systematic adverse drug reaction | |

| Type | Study | Test Product(s) | Number of subjects | Type of subjects | Adverse reactions | Comments |
|---|---|---|---|---|---|---|
| Lecrubier *et al.* 2002 | Controlled study<br>6 weeks | WS 5570<br>Hypericum extract (DER 3-7:1, methanol 80% V/V)<br>3 x 300 mg | 186 Hypericum<br>189 Placebo | Mild to moderate major depression (single or recurrent episode, DSM-IV code: 296.21, 296.22, 296.32, HAMD 17-item: 18-25) | N=21<br>Nausea (4.8%)<br>Headache (1.6%)<br>Dizziness (2.2%)<br>Abdominal pain (1.1%)<br>Insomnia (1.6%) | Gastrointestinal and nervous symptoms considered in the monograph. |
| Szegedi *et al.* 2005 | Controlled study<br>6 weeks | WS 5570<br>900 mg or 1800 mg daily | 125 Hypericum<br>126 Paroxetine | Moderate to severe major depression (HAMD 17-item: ≥ 22; DSM-IV: 296.22, 296.23, 296.32, 296.33) | Adverse events per day<br>WS 5570 900 mg (0.029)<br>WS 5570 1800 mg (0.039)<br>Upper abdominal pain (9.6%)<br>Diarrhoea (9.6%)<br>Dry mouth (12.8%)<br>Nausea (7.2%)<br>Fatigue (11.2%)<br>Dizziness (7.2%)<br>Headache (10.4%)<br>Sleep disorder (4%)<br>Increased sweating (7.2%)<br>Highest incidence: | Gastrointestinal and nervous symptoms considered in the monograph. |

| Type | Study | Test Product(s) | Number of subjects | Type of subjects | Adverse reactions | Comments |
|---|---|---|---|---|---|---|
| | | | | | Gastrointestinal disorders (59 events in 42 patients)<br>Nervous system disorders ( 35 events in 29 patients)<br>2 serious adverse events (psychic decompensation attributable to social problems, hypertensive crisis), both not caused by Hypericum | |
| Anghelescu *et al.* 2006 | Controlled study<br>6 weeks | WS 5570<br>900 mg or 1800 mg daily | 71 Hypericum<br>62 Paroxetine | Moderate to severe depression according to DSM-IV criteria: 296.22, 296.23, 296.32 and 296.33 (HAMD 17-item: ≥ 22) | 26.8% of 71<br>no "typical adverse events (except: 1 allergic reaction to sunlight → early study termination)<br>0.006 AE/d | |
| Kasper *et al.* 2006 | Controlled study<br>6 weeks | WS 5570<br>600 mg or 1200 mg | 119 Hypericum 600 mg<br>124 Hypericum 1200 mg<br>81 Placebo | Mild or moderate major depressive episode (single or recurrent episode, DSM-IV criteria: 296.21, 296.22, 296.31, 296.32; HAMD 17-item: ≥ 18, "depressive mood" ≥ 2) | All adverse events: 49 (39.8%)<br>Serious events 1 (tendon rupture attributable to accidental injury)<br>Ear and labyrinth disorders 3 (2.4%)<br>Gastrointestinal disorders 24 | Gastrointestinal and nervous symptoms considered in the monograph. |

| Type | Study | Test Product(s) | Number of subjects | Type of subjects | Adverse reactions | Comments |
|---|---|---|---|---|---|---|
| | | | | | (19.5%)<br><br>General disorders and administration site conditions 2 (1.6%)<br><br>Infection and infestations 7 (5.7%)<br><br>Injury, poisoning and procedural complications 1 (0.8%)<br><br>Investigations 1 (0.8%)<br><br>Metabolism and nutrition disorders 1 (0.8%)<br><br>Musculoskeletal and connective tissue disorder 1 (0.8%)<br><br>Nervous system disorder 6 (4.9%)<br><br>Psychiatric disorders 2 (1.6%)<br><br>Renal and urinary disorders 1 (0.8%)<br><br>Reproductive system and breast disorders 1 (0.8 %) | |

| Type | Study | Test Product(s) | Number of subjects | Type of subjects | Adverse reactions | Comments |
|---|---|---|---|---|---|---|
| | | | | | Respiratory, thoracic and mediastinal disorders 4 (3.3%)<br><br>Skin and subcutaneous disorders4 (3.3%)<br><br>Vascular disorders 1 (0.8%) | |
| Kasper *et al.* 2008 | Continuation of study above<br><br>12 months | WS 5570<br><br>3 x 300 mg | 282 Hypericum<br><br>144 Placebo | Recurrent episode of moderate major depression; HAMD 17-item: ≥ 20, ≥ 3 previous episodes in 5 years (ICD-10 F33.0. F33.1, DSM-IV 296.3) | 18 adverse events with possible causal relationship to study medication<br><br>Highest incidences for infections, musculoskeletal disorders, gastrointestinal disorders | Gastrointestinal and nervous symptoms considered in the monograph. |
| Kasper *et al.* 2010 | Re-analysis of 4 clinical trials | WS 5570: DER 3-7:1, extraction solvent methanol 80% V/V | 1264 patients Hypericum; 126 patients paroxetine; 271 patients placebo | Acute major depression | No significant effects on sedation, no significant anticholinergic reactions, gastrointestinal disturbances, no sexual dysfunction.<br><br>Highest number reported for diarrhea and nausea (1.7% of patients). | Percentage of patients with adverse events similar for Hypericum or placebo, but significantly lower compared to paroxetine. |
| Musselmann *et al.* 2011 | Open non-interventional study | WS 5570: DER 3-7:1, extraction | 1300 patients | Mild to moderate derpession | Incidence of adverse reactions 0.46%. No serious ADRs. In total 9 different | No influence on monograph |

| Type | Study | Test Product(s) | Number of subjects | Type of subjects | Adverse reactions | Comments |
|---|---|---|---|---|---|---|
| | 8-10 weeks | solvent methanol 80% V/V<br>1 x 600 mg per day<br>8-10 weeks | | | ADRs were reported, each ADR occurred once. | |
| Laakmann *et al.* 1998a, Laakmann *et al.* 1998b | Controlled study<br>6 weeks | WS 5572 (hyperforin 5%), WS 5573 (hyperforin 0.5%)<br>3 x 300 mg | 49 Hypericum WS 5572<br>49 Hypericum WS 5573<br>49 Placebo | Mild or moderate depression according to DSM-IV criteria, HAMD ≥ 17 | WS 5573 (28.6% of 49 patients)<br>WS 5572 (28.6% of 49 patients)<br>Bronchitis (3/1)<br>Influenza-like symptoms (2/0)<br>Cough (2/0)<br>Infection (1/0) | No influence on monograph |
| Lemmer *et al.* 1999 | Open non-interventional study<br>Ca. 6 weeks | WS 5572 (hyperforin 5%), WS 5573 (hyperforin 0.5%)<br>3 x 300 mg | 6154 | Mild to moderate derpession | 0.7% adverse events, only 0.1% were considered causally related to medication. Break through bleeding in 0.05% of women below 50 years of age, which is considered as the usual frequency in the untreated population. | No influence on monograph |

| Type | Study | Test Product(s) | Number of subjects | Type of subjects | Adverse reactions | Comments |
|---|---|---|---|---|---|---|
| | | | | | No cases of interactions were observed. | |
| Kalb *et al.* 2001 | Controlled study<br>6 weeks | WS 5572<br>3 x 300 mg | 37 Hypericum<br>35 Placebo | Mild to moderate major depressive disorder (according to DSM-IV criteria) (DSM-IV code: 296.21, 296.31, 296.22, 296.32, HAMD (17-items): ≥ 16) | Sinusitis<br>Bronchitis<br>Common cold | No influence on monograph |
| Rychlik *et al.* 2001 | Observationa l study<br>7 weeks | WS 5572<br>3 x 300 mg or 4 x 300 mg | 2166 | Mild to moderate depression (based on Clinical Global Impression CGI scale) | 17 patients<br>n=21 (13 with relation to Hypericum)<br>AEs frequency < 1%<br>Skin irritation, pruritus<br>Allergic exanthema<br>Nervousness, restlessness<br>Gastrointestinal disorders (4)<br>Diarrhea<br>Insomnia | Gastrointestinal and nervous symptoms considered in the monograph. |
| Schrader *et al.* 1998 | Controlled study<br>6 weeks | Ze 117<br>2 x 250 mg | 81 Hypericum<br>81 Placebo | Mild to moderate depression (ICD-10; F 32-0 and F 32-1) | 6 patients (abdominal pain, diarrhoea, melancholia, acute deterioration, dry mouth) | Gastrointestinal and nervous symptoms considered in the monograph. |

| Type | Study | Test Product(s) | Number of subjects | Type of subjects | Adverse reactions | Comments |
|---|---|---|---|---|---|---|
| Schrader *et al.* 2000 | Controlled study<br><br>6 weeks | Ze 117<br><br>2 x 250 mg | 126 Hypericum<br><br>114 Fluoxetine | Mild to moderate depression (ICD-10 F 32-0 and F 32-1, HAMD scale (21-item) 16-24) | n=6 of 81 (7.4%)<br>Abdominal pain (2) Moderate<br>Diarrhoea (1) Moderate<br>Melancholia (1) Moderate<br>Acute deterioration (1) Moderate<br>Dry mouth (1) Mild | Gastrointestinal and nervous symptoms considered in the monograph. |
| Woelk 2000 | Controlled study<br><br>6 weeks | Ze 117<br><br>2 x 250 mg | 157 Hypericum<br><br>157 Imipramine | Mild to moderate depression (ICD-10 codes F32.0, F33.0, F32.1, F 33.1; HAMD score (17-item) ≥ 18) | 62 of 157 (39%)<br><br>Dry mouth (13)<br><br>Headache (3)<br><br>Sweating (2)<br><br>Asthenia (2)<br><br>Nausea (1) | Gastrointestinal and nervous symptoms considered in the monograph. |
| Brattström *et al.* 2009 | Open trial<br><br>Up to 1 year | Ze 117<br><br>Dry extract (DER 4-7:1, ethanol 50% V/V, <1% hyperforin) | 440 | ICD-10 codes F32.0, F33.0, F32.1, F 33.1; HAMD score (17-item) ≥ 16 | 49% of the patients reported 504 adverse events, were 30 of which were possibly or probably related to the study medication. Most adverse events were related to hypersensitivity of gastro-intestinal complaints | Gastrointestinal and nervous symptoms considered in the monograph. |
| Gastpar *et* | Controlled | STW3 | 123 Hypericum | Moderate depressive disorder (according to ICD-10 criteria: | 9.8% related to study | Gastrointestinal and nervous |

| Type | Study | Test Product(s) | Number of subjects | Type of subjects | Adverse reactions | Comments |
| --- | --- | --- | --- | --- | --- | --- |
| *al.* 2005 | study<br>6 weeks | 612 mg daily | 118 Sertraline | F32.1 or F33.1; HAMD 17-items: 20-24) | medication<br>Diarrhoea (1)<br>Serious adverse events (3): shoulder blade after falling down the stairs, somatic disorder, cerebral haemorrhage | symptoms considered in the monograph. |
| Uebelhack *et al.* 2004 | Controlled study<br>6 weeks | STW3-VI<br>1 x 900 mg | 70 Hypericum<br>70 Placebo | Moderate depressive disorders (ICD-10 F32.1, F33.1) and HAMD (17-items) score: 20-24 | 16 AE in Hypericum group (mainly gastrointestinal disorders) | Gastrointestinal and nervous symptoms considered in the monograph. |
| Demling *et al.* 2004 | Non-interventional study<br>12 weeks | STW3-VI<br>1 x 900 mg | 4188 patients | Moderate depressive disorders ICD-10<br>F 32.0: 34.8%<br>F 33.0: 16.1%<br>F 32.1: 32.7%<br>F 33.1: 10.9%<br>F 34.1: 5.9% | 6.1% of the patients terminated early. In 0.6% AE were registered. No serious AE related to the study medicine. | No influence on monograph |
| Rudolf & Zeller 2004 | Non-interventional study<br>12 weeks | Hypericum extract (DER 5-8:1, ethanol 50% V/V) | 4337 patients | Depressive outpatients | Adverse reactions in 0.09% (= 4 patients), no further details.<br>No reports for drug | No influence on monograph |

| Type | Study | Test Product(s) | Number of subjects | Type of subjects | Adverse reactions | Comments |
|---|---|---|---|---|---|---|
| | | 600 mg daily | | | interactions. | |
| Gastpar *et al.* 2006 | Controlled study<br>6 weeks | Dry extract (DER 3-6:1, ethanol 80%, STW3-VI)<br>1 x 900 mg | 131 Hypericum<br>127 Citalopram130 Placebo | Moderate depression (HAMD 17-items score: 20-24, ICD-10. F32.1, F33.1, according to DSM-IV major depressive episode and recurrent major depression) | 17.2%<br>Total AEs. 58<br>Related: 10<br>Gastrointestinal disorders (6)<br>Ear and labyrinth disorders (1)<br>Skin and subcutaneous tissue disorders (1) | Gastrointestinal and nervous symptoms considered in the monograph. |
| Schulz *et al.* 2006a | Open trial<br>Determination of the minimal erythema dose<br>2 weeks | Dry extract (DER 3-6:1, ethanol 80%, STW3-VI)<br>Dry extract (DER 5-8:1, ethanol 50%, STW3)<br>1 tablet per day (no further information) | 20 | healthy volunteers | No significant changes in the minimal erythema dose | No influence on monograph |
| Kresimon *et* | Non-interventiona | STW3-VI | 281 Hypericum | Moderate depression | 97% of the Hypericum patients rated the | No influence on |

| Type | Study | Test Product(s) | Number of subjects | Type of subjects | Adverse reactions | Comments |
|---|---|---|---|---|---|---|
| *al.* 2012 | l study<br>6 months | Hypericum extract (DER 5-8:1, ethanol 80% V/V)<br>1 x 900 mg daily | 128 other SSRIs | | tolerability with very good or good compared to 86.4% in the SSRI group. 2.3% of the adverse events were rated as potentially caused by Hypericum | monograph |
| Philipp *et al.* 1999 | Controlled study<br>8 weeks | STEI 300<br>3 x 350 mg | 100 Hypericum<br>105 Imipramine<br>46 Placebo | Moderate depression according to ICD-10 (codes F32. 1 and F33.1) (HAMA score ≥ 18) | 0.5 events per patient (22%)<br>most frequently reported adverse event: Nausea | Gastrointestinal and nervous symptoms considered in the monograph. |
| Schempp *et al.* 2000a | Randomised, prospective safety study<br>24 hours | Hypericum oil containing 110 µg/ml hypericin<br>Ethanolic extract (no further details) in cream, 30 µg/ml hypericin | 16 healthy volunteers (both sexes, 18-59 years of age, skin types II and III) | Healthy volunteers<br>Skin types II and III | No change of minimal erythema dose (visual erythema score).<br>Increase detectable when measured photometrically. | No evidence for severe phototoxic potential of Hypericum oil and H. ointment. The slight trend towards increased photosensitivity could become relevant in fair-skinned individuals. Considered in monograph. |
| Melzer *et al.* | Open non-interventiona | Dry extract, DER 3.5-6:1, | 1778 patients included, 1541 | Depressive episodes F32.0-F33.9 | Incidence of adverse reactions 3.54%. No serious | Gastrointestinal and nervous |

| Type | Study | Test Product(s) | Number of subjects | Type of subjects | Adverse reactions | Comments |
|---|---|---|---|---|---|---|
| 2010 | l study<br>12 weeks | extraction solvent ethanol 60% (m/m)<br>Mean daily dose app. 750-822.5 mg<br>12 weeks | finished the study | in 83.3% of the patients | ADRs. 65% of the patients rated the tolerance as 'very good'.<br>Only ADR reported by >1% of the patients were gastrointestinal troubles (1.12%) and tiredness (1.07%). Further more frequent ADRs were photosensibilisation (0.62%) and restlessness (0.56%). All other ADRs below 0.2% | symptoms considered in the monograph. |
| Köppel *et al.* 2008 | Open trial<br>Determination of the minimal erythema dose<br>2 weeks | Dry extract (ethanol 60% V/V, DER 3.5-6:1)<br>180 mg, 2 x daily | 20 | healthy volunteers | No significant changes in the minimal erythema dose | No influence on monograph |
| Reuter *et al.* 2008 | Controlled study<br>24 hours | Bath oil containing a Hypericum extract (sypercritical $CO_2$, 1.5% hyperforin) | 18 | healthy volunteers | The test oil was applied under occlusion. Skin erythema and transepidermal water loss were not different to the water control. | No influence on monograph |

According to the review by Greeson *et al.* (2001) the overall incidence of ADR is in the range of 2%. The most commonly reported side effects were gastrointestinal irritations (0.6%), allergic reactions (0.5%), fatigue (0.4%) and restlessness (0.3%). In comparison, the overall ADR incidence for SSRIs is between 20% and 50%, including more serious side effects.

### 5.2. Patient exposure

Patients included in clinical trials:

LI 160: 2002 patients

WS 5570: 3471 patients

WS 5572: 8406 patients

Ze 117: 804 patients

STW 3: 123 patients

STW 3-VI: 9027 patients

STEI 300: 100 patients

No information on actual patient exposure of marketed products is publicly available.

### 5.3. Adverse events, serious adverse events and deaths

Agollo *et al.* (2014) report a case of suspected hepatotoxicity related to the use of Hypericum. Neither data on the herbal preparation nor the posology are provided. The patient took additionally copaiba (Copaifera sp.), levothyroxine, omega 3 fatty acids, glucosamine and chondroitin. The causal relationship of this case report with the ingestion of Hypericum remains doubtful.

Booth & McGwin (2009) investigated the relationship between self-reported use of Hypericum products and cataractogenesis. The data were obtained from a National Health Interview Survey in the USA with 30,981 responders. People reporting having cataracts were approximately 60% more likely to report the use of Hypericum products in the last 12 months.

Bove 1998 reported a case of acute neuropathy in sun-exposed areas of the body after 4 weeks of intake of 500 mg/day of ground Hyperici herba. After withdrawal of Hypericum the symptoms started to improve after 3 weeks and disappeared gradually over the next 2 months.

Gahr *et al.* (2015) evaluated the potential risk of bleeding associated with selective and non-selective serotonergic antidepressants on the basis of data in pharmacovigilance databases. The authors conclude that serotonin reuptake inhibition is not associated with an increased risk of bleeding. The detected increased risk of bleeding with Hypericum may be due to pharmacokinetic drug interactions.

Jones *et al.* (2014) report a case of suspected syndrome of inappropriate secretion of antidiuretic hormone (SIADH) associated with the use of Hypericum. The patient was brought to emergency room after being found wandering outdoors. Serum sodium was reduced, while the concentration in the urine was increased. The patient reported to ingest daily 600 – 900 mg of Hypericum (no further details). After stopping Hypericum ingestion the sodium levels returned to normal.

Lampri *et al.* (2014) (= Ioachim *et al.* 2009) describe a case with an unusual hepatocellular carcinoma with syncytial giant cells in a patient with a 6-year history of alcoholic cirrhosis. The authors suggest that the 6-month history of Hypericum self-medication (no further details) may have prompted this unusual manifestation.

Lane-Brown (2000) presents 3 cases of phototoxicity related to the use of Hypericum. A person treated cutaneous lupus erythematosus additionally to conventional treatment also with Hypericum, orally and topically. Sun-exposed parts reacted with an erythematobullous dermatosis. Another patient started psoriasis treatment using phototherapy. The patient developed within 30 minutes of a 70 mJ dose of UVB a follicular erythema. He stated that he took 6 pills of Hypericum (no further details) daily. In the third case a women developed bullae on the frontal and maxillary areas after a day at the beach. The areas which were sun-exposed have been treated for 3 weeks with a Hypericum cream (no further details).

Holme & Roberts (2000) describe a case where depressed patient under dosulepin therapy started with additional intake of Hypericum (333 mg capsules, no further information). The patient developed on day 4 of Hypericum intake erythroderma, also on non-light exposed areas.

Schreiber *et al.* (2010) present a case of radiation induced optic neuropathy. A patient with glioblastoma multiforme received concomitant radiochemotherapy with temozolomide. Due to a depressive episode she took also Hypericum (900 mg per day, no further information). 5 months after cessation of cancer therapy the patient developed bilateral amaurosis due to radiation induced optic neuropathy. The authors assume that the comedication with Hypericum may have contributed to this effect.

Parker *et al.* (2001) report a case of a patient who developed a serotonin syndrome when taking clonazepam and Hypericum (no further information) together. In another case the authors make Hypericum (no further information) responsible for hair loss of a patient on olanzapine.

O'Breasail & Argouarch (1998) published two cases where Hypericum (no details of the product) may be linked to the development of hypomania.

Nierenberg *et al*. (1999) report a case of a man who had a history of mania, which precipitated after starting taking Hypericum (900 mg daily, extract containing 0.2% hypericin).

Moses & Mallinger (2000) published 3 cases with possible mania induction due to intake of Hypericum (insufficient information on posology, no information regarding type of the herbal preparation). All patients had psychiatric disorders which were treated conventionally. After starting taking Hypericum the signs of mania developed which could be resolved by lowering the Hypericum dose.

Patel *et al.* (2002) report a case of a hypertensive crisis of a man starting Hypericum administration (no further information). No other reason for the crisis could be revealed.

Piccolo *et al.* (2009) report a case of drug induced acute hepatitis during treatment with pegylated interferon $\alpha$ in a patient with chronic hepatitis C infection. Although the authors admit that interferon $\alpha$ may also hepatitis it is assumed that the concomitant use of Hypericum (2 capsules daily for 6 weeks, no further information) at least worsened the acute hepatitis.

Yildrim & Canan (2013) report a panic attack supposedly caused by the intake of a glass of Hypericum extract (no further details).

Irefin & Sprung (2000) present a case of a woman who became hypotensive during general anaesthesia. The anaesthetics given were the same like in a surgery 2 years before where no adverse events have been observed. The only difference was that the patient reported to take Hypericum for 6 months (no details regarding the type of the herbal preparation and the posology).

In the course of a phase I clinical trial (in order to investigate the interaction with rifampicin) Hohmann *et al.* (2016) administered Hypericum extract (DER 3-6:1, extraction solvent methanol 80% V/V) to healthy volunteers. After increase of the dose to 3 times daily 600 mg 6 female participiants developed ambient temperature-dependent allodynia and paresthesia in sun-exposed areas. None of the male participants showed any of these effects. The authors conclude that there is an increased risk to develop neuropathy during long-term treatment with high doses of Hypericum.

A review of adverse effects caused by Hypericum was published by Hammerness *et al.* (2003).

Stevinson & Ernst (2004) reviewed systematically the clinical evidence associating Hypericum extract with psychotic events. Seventeen case reports associated the use of Hypericum extract with psychotic events. In 12 instances, the diagnosis was mania or hypomania. Causality is in most cases possible. In no case a positive rechallenge has been reported. These case reports raise the possibility that Hypericum extract may trigger episodes of mania in vulnerable patients.

## *5.4. Laboratory findings*

Ferko & Levine (2001) tried to assess the association between Hypericum intake and elevated thyroid-stimulating hormone levels in a retrospective case-control study. Although the authors a probable association suggest the results are of highly preliminary quality. In total only 6 participants out of 74 reported the intake of Hypericum, no information is provided regarding the kind of herbal preparation, posology and actual duration of use.

## *5.5. Safety in special populations and situations*

### 5.5.1. Use in children and adolescents

Zhou *et al.* (2009) treated in an open trial 77 adolescents diagnosed with depression for 8 weeks with Hypericum (900 mg /day, no further information). The authors found significant improvements in HAMD and HAMA scores.

Hoffmann *et al.* (2012) analysed the health insurance data from Germany regarding the treatment of adolescents with depression. Among the 4295 patients matching the inclusion criteria received 8.5% Hypericum containing medicinal products. As only cases where the purchase of the product was based on a prescription were included the number the use of Hypericum may be underestimated.

Popper (2013) concludes that for treatment of mood disorders in youth Hypericum is a promising alternative. Adverse effects and potential for drug interactions are estimated as comparable to synthetic antidepressants.

### 5.5.2. Contraindications

Hypericum dry extract induces the activity of CYP3A4, CYP2C9, CYP2C19 and P-glycoprotein. The concomitant use of Hypericum with drugs which metabolism may be influenced by Hypericum and which are used in serious conditions is contraindicated. Such drugs are for example of ciclosporine, tacrolimus for systemic use, amprenavir, indinavir and other protease inhibitors, irinotecan and warfarin. The monograph does not contain a complete list of such drugs as the content of the list would have been adapted continuously on the basis of relevant publications.

Pregnancy is not considered as a contraindication as the available data are not convincing enough.

### 5.5.3. Special Warnings and precautions for use

During the treatment intense UV-exposure should be avoided.

### 5.5.4. Drug interactions and other forms of interaction

Using well-established probe drugs, a great number of clinical trials have consistently shown that St. John's wort induced P-glycoprotein as well as CYP3A4, CYP2E1 and CYP2C19, with no effect on CYP1A2, CYP2D6 or CYP2C9. Induction of CYP enzymes and P-glycoprotein is caused by hyperforin via activation of the pregnane X receptor (Mueller *et al.* 2006, Pal & Mitra 2006, Izzo & Ernst 2009, Izzo 2012). Well-documented and clinically relevant interactions include: (1) reduced blood ciclosporine concentration associated in some cases to rejection episodes; (2) reduced efficacy of the oral pill, resulting in unwanted pregnancy; (3) reduced plasma concentration of antiretroviral (e.g. indinavir, nevirapine) and anticancer drugs (e.g. imatinib, irinotecan).

Table 16: Data on interactions from clinical trials and case reports

| Reference | Study Design | Test Product(s): herbal preparation, pharmaceutical form;<br>Dosage | Probe drug | Number Subjects | Outcome |
|---|---|---|---|---|---|
| Bolley *et al.* 2002 | Case report | 3 x 300 mg, DER 3-7:1, extraction solvent methanol 80% V/V | Tacrolimus | 1 renal transplant patient | Hypericum intake led to a significant drop in the tacrolimus trough levels which returned to normal values after stopping the administration of Hypericum. |
| Mai *et al.* 2003 | Controlled study<br>2 weeks | 600 mg Hypericum extract (methanol 80%, DER 3-6:1) | Tacrolimus<br>Mycophenolat mofetil | 10 stable renal transplant patients | The AUC of tacrolimus decreased significantly. To maintain the therapeutic tacrolimus concentrations a dose adjustment from 4.5 mg/day to 8 mg/day was necessary. The pharmacokinetics of mycophenolate mofetil remained unchanged. |
| Hebert *et al.* 2004 | Controlled study<br>18 days | 3 x 300 mg dry extract, methanol 80%, DER 3-6:1 | Tacrolimus | 10 healthy volunteers | The co-administration resulted in a significant decrease of the AUC of tacrolimus and in an increase of clearance and volume of distribution at steady state. |
| Breidenbach *et al.* 2000a, Breidenbach *et al.* 2000b | Case reports | 3 x 300 mg (no further information) | Ciclosporin | 30 patients after kidney transplantation | Intake of Hypericum caused a significant drop in ciclosporine levels. With discontinuation of Hypericum the blood levels of ciclosporine increased markedly within several days. |
| Karliova *et al.* 2000 | Case report | 2 x 900 mg per day, no further information | Ciclosporin | Patient with liver allograft | In this case a patient developed a severe acute rejection of a liver allograft 14 months after transplantation. 2 weeks before he had started taking. The ciclosporine dose had to be doubled. After stopping intake of Hypericum the blood levels of ciclosporine |

| Reference | Study Design | Test Product(s): herbal preparation, pharmaceutical form;<br><br>Dosage | Probe drug | Number Subjects | Outcome |
|---|---|---|---|---|---|
| | | | | | returned to normal immediately. |
| Barone *et al.* 2000 | Case report | 1-2 x daily 300 mg (0.3% hypericin, no further information) | Ciclosporin | Patient 54 months after kidney and pancreas transplantation | After 30 days of supplementation the ciclosporine trough concentration dropped significantly, after additional 3 weeks first signs of organ rejection appeared. Although the supplementation was stopped and ciclosporine levels returned to therapeutic levels a chronic rejection developed. |
| Ruschitzka *et al.* 2000 | Case report | 3 x 300 mg extract (methanol 80%, 3-6:1) | Ciclosporin | 2 heart transplant patients | under standard maintaining therapy (ciclosporine, azathioprine, corticosteroids) developed heart transplant rejection after commencing intake of 3 x 300 mg Hypericum extract (methanol 80%, 4-7:1) due to decreased ciclosporine plasma concentrations. |
| Barone *et al.* 2001 | Case report | extract containing 0.3% hypericin, no further details; 600 to 900 mg per day | Ciclosporin | 2 transplant patients | One patient who already took Hypericum for 6 months stopped intake resulting in an increase of the blood levels of ciclosporine. The other patient started taking Hypericum during the administration of ciclosporine resulting in a continuous drop of the blood levels of ciclosporine. After stopping taking Hypericum the blood levels returned to normal values. |
| Beer & Ostermann 2001 | Case report | 3 x 300 mg, DER 3-7:1, methanol 80% V/V | Ciclosporin | 1 patient after kidney transplantation | The ciclosporine blood level dropped significantly when the patient started taking a Hypericum. After stopping the Hypericum medication the blood levels returned to therapeutic values. |
| Alscher 2003 | Case report | Herbal tea mixture containing Hypericum (no | Ciclosporin | 1 patient after kidney transplantation | A significant drop in the blood levels of ciclosporine was reported. The patient had started to drink regularly a herbal tea mixture, which contained also Hypericum. The blood levels returned to usual |

| Reference | Study Design | Test Product(s): herbal preparation, pharmaceutical form;<br><br>Dosage | Probe drug | Number Subjects | Outcome |
|---|---|---|---|---|---|
| | | further information) | | | levels after stopping the intake of the herbal tea. |
| Bauer *et al.* 2003 | Controlled study<br><br>2 weeks | 600 mg of extract (DER 3-6:1, extraction solvent methanol 80% V/V) | Ciclosporin | 11 renal transplant patients | The first dose correction of ciclosporine was necessary 3 days after start of the trial. The dose-corrected AUC, Cmax and Ctrough values decreased by 46%, 42% and 41%, respectively. |
| Mai *et al.* 2004: | Controlled study<br><br>2 weeks | 3 x 300 mg, methanol 80% V/V, 3-6:1, 2.3% hyperforin<br><br>Low hyperforin extract: removal of hyperforin, 0.03% hyperforin | Ciclosporin | 10 renal transplant patients | The low hyperforin extract did not alter significantly the pharmacokinetics of ciclosporine, while the high hyperforin extract reduced the plasma levels of ciclosporine significantly. |
| de Maat *et al.* 2001 | Case report | No information | Nevirapine | 5 HIV patients | Hypericum intake resulted in an increase in the oral clearance of nevirapine by 35%. |
| Jackson *et al.* 2014 | Controlled study<br><br>2 weeks | 600 mg once daily, no further information | Boceprevir | 17 healthy volunteers | Hypericum did not show any influence on the plasma concentration. |
| Hafner *et al.* 2010 | Controlled study<br><br>2 weeks | 3 x daily 300 mg; extraction solvent methanol 80%, DER | Ritonavir (CYP3A inhibitor) | 12 healthy volunteers | Probe drug midazolam.<br><br>Combined administration of inducer and inhibitor resulted in a predominance of enzyme inhibition: co-administration of Hypericum |

| Reference | Study Design | Test Product(s): herbal preparation, pharmaceutical form;<br><br>Dosage | Probe drug | Number Subjects | Outcome |
|---|---|---|---|---|---|
| | | 3-6:1 | | | and ritonavir with intravenous administration of midazolam resulted in an increase in the area under the plasma concentration-time curve (AUC)(0-8 h) of midazolam to 180% of baseline value, whereas with orally administered midazolam, the AUC(0-6 h) increased to 412% of baseline value (P < 0.05 for each). After cessation of the co-administered drugs, the AUC(0-6 h) of orally administered midazolam decreased to 6% of the level observed during combined administration, and the AUC(0-8 h) of intravenously administered midazolam decreased to 33% of the values observed during combined administration (P < 0.001 for each). |
| Piscitelli *et al.* 2000 | Controlled study<br><br>2 weeks | 3 x 300 mg Hypericum extract (0.3% hypericin, no further information) | Indinavir | 8 healthy volunteers | The AUC of indinavir was reduced by 57%, the trough values were reduced by 81%. |
| L'homme *et al.* 2006 | Controlled study<br><br>2 weeks | Herbal tea, 2 x daily | Nevirapine | 36 healthy volunteers | No changes in the half-life of nevirapine. |
| Goey *et al.* 2014 | Controlled study<br><br>2 weeks | 3 x 300 mg, no information regarding type of herbal preparation; 0.36-0.84 mg hypericin and 9-19 | Docetaxel | 10 cancer patients | The AUC of docetaxel significantly decreased, while the clearance significantly increased. The maximum plasma concentration and elimination half-life were non-significantly decreased. |

| Reference | Study Design | Test Product(s): herbal preparation, pharmaceutical form;<br><br>Dosage | Probe drug | Number Subjects | Outcome |
|---|---|---|---|---|---|
| | | mg hyperforin per tablet | | | |
| Frye *et al.* 2004 | Controlled study<br><br>2 weeks | 3 x daily 300 mg (methanol 80%, DER 3-6:1 | Imatinib | 12 healthy volunteers | Hypericum increased the imatinib clearance by 43%. AUC, half-life and maximum concentration were decreased significantly. |
| Smith *et al.* 2004, Smith 2004 | Controlled study<br><br>2 weeks | 3 x daily 300 mg for 2 weeks, no further information | Imatinib | 10 healthy volunteers | Hypericum reduced in 10 healthy volunteers the AUC, Cmax andhalf-life of imatinib. |
| Mathijssen *et al.* 2002 | Controlled study<br><br>18 days | 900 mg Hypericum extract (no further information) | Irinotecan | 5 cancer patients | Plasma levels of the active metabolite SN-38 decreased dramatically. |
| Hall *et al.* 2003 | Controlled study<br><br>3 cycles | Hypericum extract (food supplement, 900 mg per day) | Ethinylestradiol<br><br>Norethindrone<br><br>Midazolam | 12 women | Concomitant use resulted in a significant increase of oral clearance of norethindrone (8.2 ± 2.7 L/h to 9.5 ± 3.4 L/h, P = 0.42) and a significant reduction in the half-life of ethinylestradiol (23.4 ± 19.5 hours to 12.2 ± 7.1 hours, P = 0.23). The oral clearance of midazolam was significantly increased, the systemic clearance remained unchanged. Serum concentrations of follicle-stimulating hormone, luteinizing hormone and progesterone were not significantly affected. Breakthrough bleeding occurred in 2 of 12 women in the control phase compared to 7 of 12 women in the Hypericum phase. No ovulation was found. Therefore an increase of breakthrough bleeding is not necessarily associated with a loss of |

| Reference | Study Design | Test Product(s): herbal preparation, pharmaceutical form;<br><br>Dosage | Probe drug | Number Subjects | Outcome |
|---|---|---|---|---|---|
| | | | | | contraceptive efficacy. The authors interpret the changes in pharmacokinetics of norethindrone and ethinylestradiol as 'modest'. |
| Pfrunder et al 2003 | Controlled study<br><br>2 cycles | 2 x 300 mg or 3 x 300 mg Hypericum extract daily (LI 160: DER 3-6:1, methanol 80% V/V) | Ethinylestradiol<br><br>Desogestrel | 18 women | No change in follicle maturation, serum estradiol or progesterone concentrations were found. Significantly more subjects reported intracyclic bleeding, the AUC and Cmax of ethinyl estradiol remained unchanged, whereas the AUC and Cmax of 3-ketodesogestrel decreased significantly. There was no evidence of ovulation, but intracyclic bleeding episodes may adversely affect compliance to oral contraceptives. The decrease in serum 3-ketodesogestrel may enhance the risk of unintended pregnancies. |
| Schwarz et. al. 2003 | Case report | up to 1700 mg of a Hypericum extract (ethanol 60% m/m, DER 3.5-6:1). | Ethinylestradiol<br><br>Dienogesterol | 1 woman | Unwanted pregnancy |
| Will-Shahab *et al.* 2009 | Controlled study<br><br>2 weeks | 2 x 250 mg, ethanol 50% V/V, DER 4-7:1, < 1mg hyperforin per daily dose | Ethinylestradiol<br><br>Desogestrel | 16 healthy female volunteers | Hypericum intake resulted in a small loss of bioavailability but remained within the limits for bioequivalence. |
| Fogle *et al.* 2006 | Controlled study<br><br>2 cycles | 3 x daily 300 mg extract (standardised to 0.3% hypericin and 3.7% hyperforin) | Ethinylestradiol<br><br>Norethindrone | 15 healthy women | No significant changes in the androgene levels and in the level of sex hormone-binding globuline (SHBG) were observed. |

| Reference | Study Design | Test Product(s): herbal preparation, pharmaceutical form; Dosage | Probe drug | Number Subjects | Outcome |
|---|---|---|---|---|---|
| Murphy *et al.* 2005 | Controlled study<br>2 cycles | 3 x 300 mg, alcoholic extract, 0.3% hypericin, 3.7% hyperforin | Ethinylestradiol<br>Norethindrone | 16 healthy women | Significant 13-15% reduction in the dose exposure. Breakthrough bleeding increased, and there was evidence of follicle growth and probable ovulation. |
| Trana *et al.* 2013 | Open study<br>2 weeks | 3 x 300 mg Hypericum (no further information) | Clopidogrel | 15 Hypericum<br>8 Placebo | Patients did not respond to clopidogrel. Due to the induction of CYP3A4 platelet inhibition could be improved by Hypericum. |
| Lau *et al.* 2011 | Controlled study<br>2 weeks | 3 x daily 300 mg dry extract containing 1.7% hyperforin; extraction solvent methanol 80%, DER 3-6:1 | Clopidogrel | 10 hyporesponders to clipodgrel | The co-medication increased the platelet inhibition in hyporesponders. |
| Maurer *et al.* 1999 | Controlled study<br>11 days | 3 x 300 mg dry extract, methanol 80%, DER 4-7:1 | Phenprocoumon | 10 healthy volunteers | Hypericum administration resulted in a significant decrease of the AUC of phenprocoumon. |
| Jiang *et al.* 2004 | Controlled study<br>2 weeks | each tablet equivalent to 1 g flowering herb; dry extract containing 0.825 mg hypericin and 12.5 mg | Warfarin | 12 healthy volunteers | Hypericum significantly induced the apparent clearance: the apparent clearance from S-warfarin changed from 198 ml/min to 270 ml/min, form R-warfarin it changed from 110 ml/min to 142 ml/min. |

| Reference | Study Design | Test Product(s): herbal preparation, pharmaceutical form;<br><br>Dosage | Probe drug | Number Subjects | Outcome |
|---|---|---|---|---|---|
| | | hyperforin per tablet; 3 x daily 1 tablet | | | |
| Lei *et al.* 2010 | Controlled study<br><br>2 weeks | 325 mg, 3 x daily, no further details | Bupropion | 18 healthy volunteers | Bupropion is metabolized by CYP2B6. Hypericum caused a decrease of the AUC and an increase in the oral clearance. |
| Kawaguchi *et al.* 2004 | Controlled study<br><br>2 weeks | 3 x 300 mg Hypericum (0.3% hypericin; no further information) | Quazepam | 13 healthy volunteers | Hypericum significantly decreased Cmax and AUC of quazepam. However, the pharmacodynamics profile of quazepam remained unchanged. |
| Johne et al. 2002b | Controlled study<br><br>2 weeks | 3 x 300 mg dry extract, methanol 80%, DER 3-6:1 | Amitriptylin | 12 patients requiring amitriptyline treatment | Co-medication resulted in a significant decrease in AUC. |
| Barbenel *et al.* 2000 | Case report | No information | Sertraline | Patient with bilateral orchiectomy | The patient continued to take a Hypericum supplementation (no information regarding type of herbal preparation and posology). The patient developed a manic episode. |
| Bonetto *et al.* 2007 | Case report | No information | Fluoxetine | Patient on fluoxetine and eletriptan | After one month of Hypericum intake the patient developed a serotonin syndrome with the symptoms: Epileptic fit with clonic convulsions, mental slowness, tremor of fingers, slightly elevated temperature, diffuse myalgia. Highly elevated levels of creatine kinase and D-dimer were found. The medication was stopped. After 10 days the blood examination was returned to normal values. |

| Reference | Study Design | Test Product(s): herbal preparation, pharmaceutical form;<br><br>Dosage | Probe drug | Number Subjects | Outcome |
|---|---|---|---|---|---|
| Evans (2008) | Reply to the case report | | | | In the opinion of Evans the authors did not rule out other possible reasons for the presented symptoms. In the opinion of Evans the symptoms would fit much better to an infectious aetiology than to a serotonin syndrome. The author revised all cases reported from the FDA on serotonin syndrome. Out of the alleged 29 cases only 7 cases met the Sternbach criteria for serotonin syndrome, but no case met the Hunter criteria. In the opinion of Evans it is premature to give a warning when more than 1 million patients have been exposed to the drug combinations (triptans and SSRI) with only 7 cases meeting just one set of criteria.<br><br><u>*Assessor's comment:*</u><br><br>*Bonetto is of the opinion that Hypericum is an inhibitor of CYP3A4 like fluoxetine. This wrong fact is used by Bonetto to make the serotonin syndrome more plausible. In fact Hypericum should counteract the inhibition of CYP3A4 by fluoxetine.* |
| Lantz *et al.* 1999 | Case report | 900 mg, no further information | Sertraline<br><br>Nefazodone | 5 depressant patients | The patients developed a serotonine syndrome after combining prescribed antidepressants with Hypericum. |
| Waksman *et al.* 2000 | Case report | 600 mg / day, no further information | Paroxetine | 1 depressant patient | The patient took Hypericum, discontinued 3 days prior to presentation and paroxetine 20 mg on the day of presentation. Symptoms: restlessness, uncontrollable movements of all four extremities. These symptoms were classified as serotonin syndrome. |
| Gordon | Case | 600 mg powder / | Paroxetine | 1 depressant | A female patient (50 years old) stopped taking paroxetine 40 mg and |

| Reference | Study Design | Test Product(s): herbal preparation, pharmaceutical form;<br>Dosage | Probe drug | Number Subjects | Outcome |
|---|---|---|---|---|---|
| 1998 | report | day, no further information | | patient | started taking Hypericum. After 10 days she took 20 mg paroxetine additionally. She was found to be incoherent, groggy, slow-moving and almost unable to get out of bed. Next day the signs returned to normal. |
| Dannawi 2002 | Case report | Extract equivalent to 2000 mg herbal substance and 1 mg hypericin | Buspirone | Patient with anxiety disorders | In order to treat symptoms of depression the patient started taking Hypericum, 250 mg tyrosine and 25 mg magnesium. After a first improvement of the depression symptoms of a serotonin syndrome appeared, which disappeared within 1 week after stopping the supplementation. |
| Eich-Höchlie *et al.* 2003 | Open study<br>Up to 47 days | LI 160 (3 x 300 mg / day | Methadone | 4 patients under maintenance treatment | Methadone concentration was reduced to 19-60% of the original concentration. 2 patients reported symptoms that suggested a withdrawal syndrome |
| Peltoniemi *et al.* 2012 | Controlled study<br>2 weeks | 3 x daily 300 mg dry extract (methanol 80%, DER 3-6:1) | Ketamine | 12 healthy volunteers | Hypericum decreased the AUC by 58% and Cmax by 66%. The decrease was not associated with significant changes in the analgesic or behavioral effects of ketamine. |
| Galeotti *et al.* 2014 | Controlled study<br>Single dose | 300 mg standardised to 0.3% hypericin | Morphine | 8 healthy volunteers | The score of pain assessment was decreased by 40% when morphine was co-administered with Hypericum. The Hypericum dose was largely below the doses used to obtain antidepressant effects. |
| Mueller *et* | Controll | 500 mg of powdered | Midazolam | 20 healthy | The pharmacokinetic parameters of the probe drug midazolam did |

| Reference | Study Design | Test Product(s): herbal preparation, pharmaceutical form;<br><br>Dosage | Probe drug | Number Subjects | Outcome |
|---|---|---|---|---|---|
| *al.* 2009 | ed study<br><br>2 weeks | Hyperici herba with a content of 0.06 mg total hyperforin per capsule | | volunteers | not change significantly. |
| Imai *et al.* 2008: | Controll ed study<br><br>2 weeks | 3 x 300 mg (no further information) | Midazolam | 12 healthy volunteers | The elevated clearance returned to the control level 7 days after completion of the Hypericum intake. |
| Hojo *et al.* 2011 | Controll ed study<br><br>2 weeks | 3 x 300 mg dry extract, methanol 80%, DER 3-6:1 | Zolpidem | 14 healthy volunteers | The pharmacokinetic paramaters of zolpidem changed significantly after intake of Hypericum: AUC and Cmax decreased significantly while oral clearance increased significantly. The authors propose an individualised approach of this combination as in 3 participants in contrary to the other participants the AUC slightly increased. |
| Xu et al 2008 | Controll ed study<br><br>2 weeks | LI 160 (3 x 300 mg / day | Gliclazide | 21 healthy subjects with different CYP2C9 genotypes | Hypericum administration significantly increased the apparent clearance of gliclazide which is independent of the CYP2C9 genotype. |
| Stage et. al. 2014 | Controll ed study<br><br>3 weeks | 2 x daily 240-294 mg dry extract corresponding to 900 µg total hypericin | Metformin | 20 healthy volunteers | Hypericum decreased the renal clearance of metformin but did not affect any other pharmacokinetic parameter. A 2 hours glucose tolerance test revealed that Hypericum decreases the AUC of glucose. This effect was caused by a significant increase in the acute insulin response. |
| Fan *et al.* | Controll | 3 x 325 mg (no | Repaglinide | 15 healthy | Volunteers with specific solute carrier organic anion transporter |

| Reference | Study Design | Test Product(s): herbal preparation, pharmaceutical form;<br><br>Dosage | Probe drug | Number Subjects | Outcome |
|---|---|---|---|---|---|
| 2011 | ed study<br><br>2 weeks | further information) | | volunteers | family member 1B1 (SLCO1B1) genotypes.<br><br>Hypericum did not influence the pharmacokinetic parameters of a single dose of repaglinide. Also no changes in the pharmacological effects of repaglinide were observed. |
| Sugimoto *et al.* 2001 | Controlled study<br><br>2 weeks | 3 x 300 mg Hypericum extract (0.3% hypericin, no further information) | Simvastatin<br><br>Pravastatin | 16 healthy volunteers | Hypericum significantly lowered the plasma concentration of simvastatin hydroxyl acid, the active metabolite of simvastatin. Hypericum did not alter the plasma concentrations of pravastatin. |
| Eggertsen et al 2007 | Controlled study<br><br>4 weeks | 3 x 300 mg (DER 4-7:1, methanol 80% V/V | Simvastatin | 24 patients with hypercholesterolemia | The co-medication with Hypericum led to increased serum levels of LDL cholesterol and total cholesterol. |
| Gordon *et al.* 2009 | Case report | Hypericum (300 mg, no further information), rosemary (80 mg, no further information) and spirulina (40 mg, no further information) | Rosuvastatin | | Intake of the supplements caused an increase of total cholesterol and LDL cholesterol. After stopping the intake of the supplements the cholesterol levels returned to those prior to the intake of the supplement. The authors suggest that this interaction may be caused by to the upregulation of CYP2C9 and CYP2C19 due to the intake of Hypericum. |
| Andren *et al.* 2007 | Controlled study | 3 x 300 mg (DER 4-7:1, methanol 80% V/V | Atorvastatin | 16 patients with hypercholesterolemia | The co-medication with Hypericum led to increased serum levels of LDL cholesterol and total cholesterol. No change was observed regarding HDL cholesterol and triglycerides. |

| Reference | Study Design | Test Product(s): herbal preparation, pharmaceutical form;<br><br>Dosage | Probe drug | Number Subjects | Outcome |
|---|---|---|---|---|---|
| | 4 weeks | | | | |
| Wang *et al.* 2009 | Controlled study<br><br>2 weeks | 3 x 300 mg, 0.3% hypericin, 5% hyperforin, no further information | Nifedipine | 10 healthy volunteers | The authors investigated the relationship between the two most frequent haplotypes (H1 and H2) of the human pregnane X receptor and basal as well as Hypericum-induced CYP3A4 activity. H1/H1 of the human pregnane X receptor gene had weaker basal transcriptional activity but greater inducible transcriptional activity to CYP3A4 than H1/H2 and H2/H2. |
| Tannergren *et al.* 2004 | Controlled study<br><br>2 weeks | 3 x 300 mg dry extract, methanol 80% V/V, DER 4-7:1 | Verapamil | 8 healthy volunteers | Hypericum significantly decreased the bioavailability of R- and S-verapamil in healthy volunteers. This effect is caused by induction of first-pass CYP3A4 metabolism. |
| Schwarz *et al.* 2007 | Controlled study<br><br>12 days | 3 x 300 mg dry extract for 12 days, methanol 80%, DER 3-6:1 | Talinolol | 9 healthy volunteers | Hypericum increased P-glycoprotein levels in the duodenal mucosa. Hypericum reduced the oral bioavailability of talinolol by 25%, the AUC by 31% and increased oral clearance by 93%. |
| Markert *et al.* 2014 | Controlled study<br><br>10 days | 3 x daily 300 mg extract (methanol 80%, DER 3-6:1) | Bosentan | 9 healthy extensive metabolisers of CYP2C9 and 4 poor metabolisers | Midazolam was used as probe drug in order to quantify changes in CYP3A4 activity. Hypericum extract increased CYP3A4 activity, but had no consistent effect on bosentan clearance. |
| Markert *et al.* 2015 | Controlled study | 3 x daily 300 mg extract (methanol | Ambrisentan | 20 healthy volunteers (10 | Ambrisentan is metabolized by CYP2C19. Midazolam was used as probe drug in order to quantify changes in CYP3A4 activity. At |

| Reference | Study Design | Test Product(s): herbal preparation, pharmaceutical form;<br><br>Dosage | Probe drug | Number Subjects | Outcome |
|---|---|---|---|---|---|
| | 10 days | 80%, DER 3-6:1) | | CYP2C19 extensive, 4 poor and 6 ultrarapid metabolisers) | steady-state, ambrisentan exposure was similar in extensive and ultrarapid metabolisers but 43% larger in poor metabolisers. In all volunteers Hypericum reduced ambrisentan exposure (17-26%). The extent of the interaction did not correlate with the changes in CYP3A4 activity. The authors conclude that the extent of this interaction is small and thus likely without clinical relevance. |
| Portoles *et al.* 2006 | Controlled study<br><br>2 weeks | 3 x 300 mg extract (methanol 80%, DER 3-6:1) | Ivabradine | 12 healthy volunteers | AUC and Cmax were significantly decreased. |
| Xie *et al.* 2005 | Controlled study<br><br>10 days | 3 x 300 mg, no further information | Fexofenadine<br><br>Midazolam | 30 healthy subjects of different ethnics (Caucasian, African, Americans, Hispanics, Chinese, Indians and Malays) | In all ethnic groups the clearance of the P-glycoprotein substrate fexofenadine and the CYP3A4 substrate midazolam was significantly increased. The extent of induction was comparable among the evaluated ethnic groups. |
| Wang *et al.* 2002 | Controlled study<br><br>2 weeks | 3 x 300 mg, 0.3% hypericin, no further information | Fexofenadine | 12 healthy volunteers | After the single dose the Cmax of fexofenadine increased while the oral clearance decreased significantly. Long term administration resulted in a 35% decrease of Cmax and a 47% increase in oral clearance. |

| Reference | Study Design | Test Product(s): herbal preparation, pharmaceutical form;<br><br>Dosage | Probe drug | Number Subjects | Outcome |
|---|---|---|---|---|---|
| Rengelshausen *et al.* 2005 | Controlled study<br><br>15 days | LI 160 (3 x 300 mg / day | Voriconazole | 16 healthy volunteers | Day 1: Short-term clinically irrelevant increase in voriconazole parameters. This is limited to the absorption phase of voriconazole.<br><br>Day 15: Extensive reduction of voriconazole exposure |
| Burstein *et al.* 2000 | Controlled study<br><br>3 weeks | 3 x 300 mg standardised to 0.3% hypericin | Carbamazepin | 8 healthy volunteers | The intake of this Hypericum preparation did not change the pharmacokinetic parameters of carbamazepine. |
| Wang et *al.* 2004a | Controlled study<br><br>2 weeks | 3 x 300 mg, 0.3% hypericin, no further information | Mephenytoin<br><br>Caffein | 6 extensive metabolisers of CYP2C19 and 6 poor metabolisers | Hypericum treatment significantly increased CYP2C19 activity in wild-type metabolisers, whereas no alteration was observed for poor metabolisers. Hypericum administration did not alter CYP1A2 activity. |
| Van Strater & Bogers 2012 | Case report | 3 x 300 mg extract, 0.36-0.84 mg hypericin, min. 9 mg hyperforin | Clozapine | 1 patient with schizophrenia stable on a fixed dose of clozapine | The decrease of the plasma concentrations of clozapine resulted in disorganization and tension. After stopping the intake of Hypericum the plasma levels returned towards normal values and the psychiatric condition improved |
| Nieminen *et al.* 2010 | Controlled study<br><br>15 days | 3 x daily 300 mg dry extract (methanol 80%, DER 3-6:1) | Oxycodone | 12 healthy volunteers | The AUC of oxycodone was 50% decreased and the elimination half-life shortened. The self-reported drug effect of oxycodone decreased significantly. |
| Bell *et al.* | Controll | 3 x 300 mg standardised to 0.3% | Ibuprofen | 8 healthy | Hypericum had no apparent impact on the pharmacokinetic parameters of ibuprofen. It is concluded that Hypericum does not |

| Reference | Study Design | Test Product(s): herbal preparation, pharmaceutical form;<br><br>Dosage | Probe drug | Number Subjects | Outcome |
|---|---|---|---|---|---|
| 2007a | ed study<br><br>3 weeks | hypericin | | volunteers | influence CYP2C9. |
| Wang *et al.* 2004b: | Controll ed study<br><br>2 weeks | 300 mg Hypericum (0.3% total hypericin, minimum 4% hyperforin, no further details) | Omeprazol (CYP2C19) | 12 healthy volunteers | After 2 weeks of Hypericum administration the peak plasma concentration decreases significantly by 37.5%-50%, the AUC decreased by 37.9 – 43.9% (depending on CYP2C19 genotype). The peak plasma concentration and the AUC of omeprazole sulfone increased by 160% and by 136%. |
| Wenk *et al.* 2004: | Controll ed study<br><br>2 weeks | 3 x 300 mg, methanol 80% V/V, 3-6:1 | Cortisol<br><br>Dextrometorph an | 16 healthy volunteers | The ratios of the treatment to baseline values for CYP3A4 using cortisol as the probe were 1.5 for males, and 1.9 for females. The corresponding ratios using dextromethorphan as the probe for CYP2D6 were 0.9 for males and 1.9 for females. For CYP1A2, a significant increase in the metabolic ratios was found only for females (ratio of values 1.2). No influence of Hypericum extract on CYP2D6, NAT2, and XO activities was observed. |
| Bauer *et al.* 2002 | Controll ed study<br><br>2 weeks | 1800 mg of Hypericum extract (DER 3-6:1, extraction solvent methanol 80% V/V | - | 48 healthy volunteers | The treatment caused a significant increase of the urinary excretion of 6ß-hydroxycortisol. The excretion of free cortisol and of D-glucaric acid was not affected. The changes are interpreted as signal for induced CYP3A activity. |
| Bell *et al.* 2007b | Controll ed study<br><br>4 weeks | 3 x 300 mg standardised to 0.3% hypericin | Prednisone<br><br>Prednisolone | 8 healthy volunteers | Although the test substances are metabolised by CAP3A4 no influence on the pharmacokinetic parameters was observed. |

| Reference | Study Design | Test Product(s): herbal preparation, pharmaceutical form;<br><br>Dosage | Probe drug | Number Subjects | Outcome |
|---|---|---|---|---|---|
| Lundahl *et al.* 2009 | Controlled study<br><br>2 weeks | 2 x daily 300 mg dry extract, extraction solvent methanol 80% V/V, DER 4-7:1, 4% hyperforin | Finasteride | 12 healthy volunteers | Hypericum medication caused a significant reduction of Cmax, AUC and elimination half-life. |
| Ladner *et al.* 2001 | Case report | No further information on product | Aminolaevulinic acid | 1 patient | The authors describe a phototoxic reaction in a patient treated with d-aminolaevulinic acid-induced protoporphyrine IX for photodiagnosis of breast tumours during a clinical trial. The patient also took a Hypericum product. |
| Andelic 2003 | Case report | Hypericum tea, 2 litres per day | Digoxin | 80 years old man | The patient developed heart arrhythmia after stopping the intake of Hypericum. The author concludes that discontinuation of a Hypericum medication should only be done under supervision of a doctor. |
| Johne *et al.* 1999 | Controlled study<br><br>10 days | 900 mg extract (methanol 80%, DER 3-6:1) | Digoxin | 13 healthy volunteers Hypericum<br><br>12 Placebo | Hypericum treatment resulted in a decrease of digoxin AUC by 25%. After multiple dosing a reduction in trough concentration (33%) and Cmax (26%) was observed, the effects were time-dependent. |
| Dürr *et al.* 2000 | Controlled study<br><br>2 weeks | 3 x 300 mg extract (methanol 80%, DER 3-6:1 | Digoxin | 8 healthy volunteers | The treatment resulted in a 18% decrease of digoxin exposure, in a 1.4 fold increased expression of duodenal P-glycoprotein/MDR1, in a 1.5 fold increased expression of duodenal CYP3A4, and in a 1.4 fold increase in the functional activity of hepatic CYP3A4. |
| Mueller et | Controll | Dry extract | Digoxin | 96 healthy | Co-medication with 2 g powder without hyperforin, tea, juice, oil |

| Reference | Study Design | Test Product(s): herbal preparation, pharmaceutical form;<br><br>Dosage | Probe drug | Number Subjects | Outcome |
|---|---|---|---|---|---|
| *al.* 2004a | ed study<br><br>2 weeks | (methanol 80%, 3-6:1, LI 160): 28.9 mg hyperforin / day<br><br>Hyperforin-containing powder: 21.1 mg hyperforin / day<br><br>Hypericum oil: 3 x 2 capsules containing 200 mg oil extract each. 0.13 mg hyperforin / day.<br><br>Tea (prepared from 1.75 g): 2 x 1 cup. 0.04 mg hyperforin / day.<br><br>Hyperforin-reduced powder: 2 g per day: 0.3 mg hyperforin /day<br><br>Fresh plant juice: 2 x 10 ml. 3.56 mg | | volunteers | extract, hyperforin-free extract (Ze 117), 1g or 0.5 g hyperforin-containing powder: No significant interaction.<br><br>Co-medication with LI 160: reduction of AUC -24.8%, Cmax -37%, Ctrough -19%.<br><br>Co-medication with 4 g hyperforin-containing powder: Similar interaction compared to LI 106. |

| Reference | Study Design | Test Product(s): herbal preparation, pharmaceutical form;<br><br>Dosage | Probe drug | Number Subjects | Outcome |
|---|---|---|---|---|---|
| | | hyperforin / day<br><br>Dry extract (ethanol 50%, 4-7:1, Ze117): 0.38 mg hyperforin / day | | | |
| Nebel *et al.* 1999 | Case report | 300 mg/day, no further information | Theophylline | | The patient was established to 2 x 300 mg theophylline daily. After some time the dosage was increased to 800 mg bid because the plasma concentrations were lower than desired. She took several other drugs, but the only change was the new addition of Hypericum extract (300 mg/day). After stopping Hypericum the plasma concentration of theophylline increased about 100% after 7 days. |
| Morimoto *et al.* (2004) | Controlled study<br><br>2 weeks | 3 x 300 mg standardised to 0.3% hypericin | Theophylline | 12 healthy volunteers | The treatment of Hypericum produced no significant difference compared to the control group.<br><br>*Assessor's comment:*<br><br>*Theophylline is metabolized via CYP1A2, which is not influenced by Hypericum (Wang et al 2001). There is no rationale for a pharmacokinetic interaction between Hypericum and theophylline.* |
| Wang *et al.* 2001: | Controlled study<br><br>2 weeks | 3 x 300 mg per day, 900 µg hypericin per capsule, no further details | Tolbutamide<br><br>Caffeine<br><br>Dextrometorphan | 12 healthy volunteers | No change in CYP2C9, CYP1A2, CYP2D6 were detected. In contrast a significant and selective induction of CYP3A4 activity in the intestinal wall was observed. |

| Reference | Study Design | Test Product(s): herbal preparation, pharmaceutical form;<br><br>Dosage | Probe drug | Number Subjects | Outcome |
|---|---|---|---|---|---|
| | | | Midazolam | | |
| Gurley *et al.* 2005 | Controlled study<br><br>28 days | 3 x daily 300 mg (no details regarding herbal preparation, 0.3% hypericin; app. 5.5 mg hyperforin per day | Midazolam<br><br>Caffeine | 12 elderly volunteers | Hypericum induced the activity of CYP3A4 and CYP2E1. |
| Dresser *et al.* 2003: | Controlled study<br><br>12 days | 3 x 300 mg (methanol 80% V/V, 3-6:1) | Midazolam<br><br>Fexofenadine<br><br>Ciclosporin | 21 healthy volunteers | Midazolam was administered orally and intravenously in order to assess CYP3A activity, fexofenadine after oral dose for a measure of MDR1 (= P-glycoprotein) function; the oral plasma concentration profile of ciclosporine was considered to reflect both CYP3A and MDR1 activities. The clearance of all drugs was significantly enhanced (1.5-2.7-fold). |
| Johne *et al.* 2004: | Controlled study<br><br>18 days | 3 x 300 mg (methanol 80% V/V, 3-6:1) | Cimetidine<br><br>Carbamazepin<br><br>Placebo | 33 healthy volunteers | Between-group comparisons showed no statistically significant differences in AUC(0-24), C(max), and t(max) values for hypericin and pseudohypericin. Within-group comparisons, however, revealed a statistically significant increase in hypericin AUC(0-24) from a median of 119 (range 82-163 mg h/l) to 149 mg h/l (61-202 mg h/l) with cimetidine co-medication and a decrease in pseudohypericin AUC(0-24) from a median of 51.0 (16.4-102.9 mg h/l) to 36.4 mg h/l (14.0-102.0 mg h/l) with carbamazepine co-medication compared to the baseline pharmacokinetics in each group. Hypericin and pseudohypericin pharmacokinetics were only marginally influenced by co-medication with the enzyme inhibitors and inducers |

| Reference | Study Design | Test Product(s): herbal preparation, pharmaceutical form;<br><br>Dosage | Probe drug | Number Subjects | Outcome |
|---|---|---|---|---|---|
| | | | | | cimetidine and carbamazepine. |
| Arold *et al.* 2005: | Controlled study<br><br>11 days | 240 mg extract (ethanol 60% V/V, 3.5-6:1) containing 3.5 mg hyperforin | Alparzolam (CYP3A4)<br><br>Caffeine (CYP1A2)<br><br>Tolbutamide (CYP2C9)<br><br>Digoxin (marker for p-glycoprotein) | 28 healthy volunteers | No significant changes in the primary kinetic parameters of all of the probe drugs were observed. |
| Etogo-Asse *et al.* 2008 | Case report | Herbal tea from 2 g daily | Hydroxychloroquine sulfate<br><br>Tibolone | | A 57 year old women developed a mixed-type liver injury with prolonged cholestasis and features of the vanishing bile duct syndrome. She started taking an infusion of 2 g Hyperici herba daily. App. 2 months later fatigue, reduced appetite, dark urine jaundice and pruritus occurred. Tibolone and Hypericum administration was stopped. During the treatment with ursodesoxycholic acid the clinical and biochemical features slowly improved. |
| Hennessy *et al.* 2002 | Controlled study<br><br>16 days | 3 x daily 600 mg Hypericum (0.15% hypericin, no further information) | - | 15 healthy volunteers | P-glycoprotein expression increased 4.2 fold from baseline. |
| Zahner et | Controll | 1 x daily 500 mg dry | Caffeine | 20 healthy | Probe cocktail at days 1, 8 and 17, administration of Hypericum |

| Reference | Study Design | Test Product(s): herbal preparation, pharmaceutical form; <br><br> Dosage | Probe drug | Number Subjects | Outcome |
| --- | --- | --- | --- | --- | --- |
| al. 2019 | ed study <br><br> 16 days | extract ZE 117 (extraction solvent ethanol 50% m/m, DER 4-7:1), hyperforin content 0.96 mg per tablet | (CYP1A2), <br><br> bupropion (CYP2B6), flurbiprofen (CYP2C9), omeprazole (CYP2C19), dextromethorphan (CYP2D6), midazolam (CYP3A4), fexofenadine (P-gp). | volunteers | extract daily from day 8 to day 17. <br><br> No significant change, all probe drugs remained in the predefined bioequivalence range of 80-125%. |

Whitten *et al.* 2006 (review):

The authors reviewed prospective clinical trials for effects on CYP3A. Thirty-one studies met the eligibility criteria. More than two-thirds of the studies employed a before-and-after design, less than one-third of the studies used a crossover design, and only three studies were double-blind and placebo controlled. In 12 studies the SJW extract had been assayed, and 14 studies stated the specific SJW extract used. Results from 26 studies, including all of the 19 studies that used high-dose hyperforin extracts (>10 mg $day^{-1}$), had outcomes consistent with CYP3A induction. The three studies using low-dose hyperforin extracts (<4 mg $day^{-1}$) demonstrated no significant effect on CYP3A. In one of these studies an extract (ethanol 50% m/m, 4-7:1, nearly free of hyperforin) was given to 16 females in a dose of 500 mg daily for 14 days. The women started 3 months prior to the study taking 20 µg ethinyl estradiol and 150 µg desogestrel daily. Pharmacokinetic testing on days 7 and 22 after the treatment with Hypericum extract revealed no significant differences in ethinyl estradiol or 3-ketodesgestrel (active metabolite). It is not known whether a longer treatment with this extract would induce CYP3A4.

Volz & Zeller 2000

The authors report data from an observational trial (11.296 patients receiving STW3-VI, Laif 600, DER 5-8:1, per tablet 2 mg hypericin, 30 mg flavonoids, 11 mg hyperforin; 612 mg extract per tablet). The mean period was 65 days. No reports of interactions were received although 10% of the patients used ß-blockers, 10% ACE-inhibitors, 6% diuretics, 7.8% thyroid hormones, 4.2% oral contraceptives and 3.8% estrogens.

Several meta-analyses are published on the pharmacokinetic interactions of Hypericum with the cytochrome-P450 enzyme complex and P-glycoprotein (Pal & Mitra 2006, Madabushi *et al.* 2006, Whitten *et al.* 2006, Mills *et al.* 2004, Henderson *et al.* 2002, Izzo & Ernst 2009, Borelli & Izzo 2009, Russo *et al.* 2014).

*Assessor's comments on drug interactions:*

*Due to the content of hyperforin the plasma levels of numerous drug substances may be reduced resulting in the risk of therapeutic failure. Additionally side effects of antidepressants with similar pharmacodynamic activity may be increased when administered together with Hypericum.*

*Based on the published clinical trials and case reports as well as based on the product informations from authorised medicinal products the following list of drugs substances with potential interactions is established and evaluated with regard of relevance for product informations of Hypericum medicinal products.*

Table 17: Drug substances which are reported to be interacted by Hypericum

| **Drug substance** | **Source, kind of evidence** | **Conclusion** |
|---|---|---|
| Abacavir | Not metabolised with P450, no interactions to be expectd | Considered not relevant |
| Acenocoumarol | SmPC; induction of CYP2C9 may reduce plasma levels | Relevant |
| Agomelatine | Metabolism via CYP1A2 and CYP2C9/CYP2C19; in SmPC concomitant use of potent CYP1A2 inhibitors is contraindicated | Hyperforin is a CYP1A2 inducer, therefore considered not relevant |

| | | |
|---|---|---|
| Alprazolam | SmPC; induction of CYP3A4 may reduce plasma levels; clinical relevance not clear | Relevant |
| Ambrisentan | Clinical trial; no interaction with CYP3A4 | Considered not relevant |
| Amitriptyline | Clinical trial; induction of P450 enzymes may reduce plasma levels | Relevant |
| Amlodipine | SmPC, induction of CYP3A4 may reduce plasma levels | Relevant |
| Amprenavir | Marketing authorisation withdrawn | Considered not relevant |
| Atorvastatin | SmPC; induction of CYP3A4 may reduce plasma levels | Relevant |
| Bosentan | Clinical trial; induction of CYP3A4 did not effect bosentan clearance | Considered not relevant |
| Bupropion | Clinical trial; induction of CYP2B6 may reduce plasma levels | Relevant |
| Buspiron | Induction of CYP3A1 may reduce plasma levels | Relevant |
| Carbamazapine | Clinical trial; metabolism via CYP3A1, 1A2, 2C9; no influence on metabolism detected | Considered not relevant |
| Carvedilol | SmPC; induction of P450 enzymes may influence plasma levels | Relevant |
| Chlorzoxazone | No up-to-date information available | Considered not relevant |
| Ciclosporine (oral administration) | Case reports, clinical trials; inducers of CYP3A4 and p-glycoprotein are expected to decrease ciclosporin levels; contraindication | Relevant |
| Citalopram, Escilatopram | SmPC; possible pharmacodynamics interactions | Relevant |
| Clomipramine | SmPC; induction of CYP3A4, CYP2C19, CYP2D6 and CYP1A2 may reduce plasma levels | Relevant |
| Clopidogrel | SmPC; induction of CYP2C19 may increase plasma levels of active metabolites; Hypericum not mentioned in SmPC | Considered not relevant |
| Clozapine | Case report; not mentioned in SmPC | Relevant |
| Cortisol | Clinical trial; no interactions with Hypericum mentioned in SmPC | Considered not relevant |
| Debrisoquine | No up-to-date information available | Considered not relevant |

| | | |
|---|---|---|
| Desogestrel | Clinical trial; induction of CYP3A4 may reduce plasma levels | Relevant |
| Diazepam | Not mentioned in SmPC; induction of CYP3A4 may reduce plasma levels | Relevant |
| Dienogestrel | Case report, SmPC; induction of CYP3A4 may reduce plasma levels | Relevant |
| Digoxine | Case reports, clinical trials; inducers of p-glycoprotein are expected to decrease digoxin levels | Relevant |
| Diltiazem | Not mentioned in SmPC; induction of CYP3A4 may reduce plasma levels | Relevant |
| Docetaxel | Clinical trial; induction of CYP3A4 may reduce plasma levels | Relevant |
| Doxepin | Not mentioned in SmPC; no relevant pharmacokinetic or pharmacodynamics interactions to be expected | Considered not relevant |
| Duloxetine | SmPC; possible pharmacodynamics interactions | Relevant |
| Dutasteride | Not mentioned in SmPC; no publications | Considered not relevant |
| Efavirenz | SmPC; induction of CYP3A4 and CYP2B6 may reduce plasma levels; contraindication | Relevant |
| Erythromycine | SmPC; induction of CYP3A4 may reduce plasma levels | Relevant |
| Esomeprazol | SmPC; induction of CYP3A4 and CYP2C19 may reduce plasma levels | Relevant |
| Eszopiclone | SmPC; induction of CYP3A4 may reduce plasma levels | Relevant |
| Ethinylestradiol | Clinical trials; induction of CYP3A4 may reduce plasma levels | Relevant |
| Etravirine | SmPC; induction of CYP3A4, CYP2C9 and CYP2C19 may reduce plasma levels | Relevant |
| Everolimus | SmPC; induction of CYP3A4 and CYP2D6 may reduce plasma levels | Relevant |
| Fentanyl | SmPC; induction of CYP3A4 may reduce plasma levels | Relevant |
| Fexofenadine | Clinical trial; not in SmPC | Considered not relevant |
| Finasteride | Clinical trial; induction of CYP3A4 may reduce plasma levels | Relevant |

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|---|---|---|
| Fluoxetine | Case report, SmPC; mild forms of pharmacodynamics interactions may be possible (serotonine syndrome) | Relevant |
| Fluvoxamine | SmPC; possible pharmacodynamics interactions | Relevant |
| Fosamprenavir | SmPC contraindication; induction of CYP3A4 may reduce plasma levels | Relevant |
| Gliclazide | Clinical trial, SmPC; induction of CYP2C9 and CYP2C19 may reduce plasma levels; regular control of blood sugar levels | Relevant |
| Imatinib | Clinical trials; induction of CYP3A4 may reduce plasma levels | Relevant |
| Indinavir | Clinical trial, case reports; induction of CYP3A4 may reduce plasma levels | Relevant |
| Irinotecan | Case report; induction of CYP3A4 may reduce plasma levels | Relevant |
| Itraconazole | Clinical trial; induction of CYP3A4 may reduce plasma levels | Relevant |
| Ivabradine | Clinical trial; induction of CYP3A4 may reduce plasma levels | Relevant |
| Ketamine | Clinical trial;not mentioned in SmPC, increased dosage might be necessary | Indirectly considered as a warning regarding to elective surgery is taken up into the monograph |
| Lansoprazol | SmPC; induction of CYP3A4 and CYP2C19 may reduce plasma levels | Relevant |
| Macitentan | SmPC; induction of CYP3A4 may reduce plasma levels | Relevant |
| Mephenytoin | Clinical trial, SmPC; induction of CYP3A4 and CYP2C9 may reduce plasma levels | Relevant |
| Metformin | Clinical trial; not mentioned in SmPC | Considered not relevant |
| Methadone | Clinical trial, SmPC; induction of CYP3A4 may reduce plasma levels | Relevant |
| Metoprolol | Not in SmPC; induction of CYP2D6 may reduce plasma levels | Considered not relevant |
| Mianserine | Induction of CYP3A4 may reduce plasma levels, no cases reported | Considered not relevant |
| Midazolam | Clinical trials; induction of CYP3A4 may reduce plasma levels | Relevant |

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|---|---|---|
| Mirtazapine | SmPC; possible pharmacodynamics interactions | Relevant |
| Moclobemide | SmPC; possible pharmacodynamics interactions | Relevant |
| Nefazodone | Case report; obsolete | Considered not relevant |
| Nelfinavir | No product on the market | Considered not relevant |
| Nevirapine | Case report, SmPC; induction of CYP3A4 and CYP2B6 may reduce plasma levels | Relevant |
| Nicardipine | Hypericum not explicitely mentioned in SmPC, but induction of CYP3A4 may reduce plasma levels | Relevant |
| Nifedipine | Hypericum not explicitely mentioned in SmPC, but induction of CYP3A4 may reduce plasma levels | Relevant |
| Norethindrone | Clinical trial; induction of CYP3A4 may reduce plasma levels | Relevant |
| Omeprazol | Clinical trial, SmPC; induction of CYP3A4 and CYP2C19 may reduce plasma levels | Relevant |
| Oxacarbazepine | Hypericum not explicitely mentioned in SmPC, but induction of CYP3A4 may reduce plasma levels | Relevant |
| Oxycodon | Clinical trial, SmPC; induction of CYP3A4 may reduce plasma levels | Relevant |
| Paclitaxel | Hypericum not explicitely mentioned in SmPC, but induction of CYP3A4 may reduce plasma levels | Relevant |
| Pantoprazol | SmPC; induction of CYP3A4 and CYP2C19 may reduce plasma levels | Relevant |
| Paroxetine | Case reports, SmPC; possible pharmacodynamics interactions | Relevant |
| Phenobarbital | SmPC; induction of CYP2C9 may reduce plasma levels | Relevant |
| Phenprocoumon | Clinical trial; induction of CYP3A4 and CYP2C9 may reduce plasma levels | Relevant |
| Phenytoine | Clinical trial, SmPC; induction of CYP3A4 and CYP2C9 may reduce plasma levels | Relevant |
| Propofol | No published information regarding pharmacokinetic or pharmacodynamics interactions; Hypericum not mentioned in | Indirectly considered as a warning regarding to elective surgery is taken up into the |

| | SmPC | monograph |
|---|---|---|
| Propranolol | No published information regarding pharmacokinetic or pharmacodynamics interactions; Hypericum not mentioned in SmPC | Considered not relevant |
| Quazepam | Clinical trial; obsolete | Considered not relevant |
| Quetiapine | Hypericum not explicitely mentioned in SmPC, but induction of CYP3A4 may reduce plasma levels | Relevant |
| Rabeprazol | Hypericum not explicitely mentioned in SmPC, but induction of CYP3A4 and CYP2C19 may reduce plasma levels | Relevant |
| Rilpivirine | SmPC; induction of CYP3A4 may reduce plasma levels | Relevant |
| Risperidone | Hypericum not explicitely mentioned in SmPC, but induction of CYP3A4 may reduce plasma levels | Relevant |
| Rosuvastatin | Case report; according to SmPC no interaction with CYP 450 isoenzymes | Considered not relevant |
| Saquinavir | According to SmPC interactions with Hypericum not studied; but induction of CYP3A4 may reduce plasma levels | Relevant |
| Sertraline | Case reports; possible pharmacodynamics interactions | Relevant |
| Simvastatine | Clinical trial, case reports; Hypericum not explicitely mentioned in SmPC, but induction of CYP3A4 may reduce plasma levels | Relevant |
| Sirolimus | SmPC; induction of CYP3A4 may reduce plasma levels | Relevant |
| Sitaxentan | Product withdrawn | Considered not relevant |
| Tacrolimus (oral) | Clinical trial, case reports; induction of CYP3A4 may reduce plasma levels | Relevant |
| Tacrolimus (cutaneous) | No interaction to be expected | Considered not relevant |
| Talinolol | No product on the market?? | Considered not relevant |
| Telaprevir | No product on the market | Considered not relevant |
| Temazepam | Hypericum not explicitely mentioned in SmPC, but induction of CYP3A4 may reduce plasma levels | Relevant |

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|---|---|---|
| Theophylline | Theophylline metabolised by CYP1A2 which is not influenced by Hypericum | Considered not relevant |
| Ticlopidine | Hypericum not mentioned in SmPC | Considered not relevant |
| Tolbutamide | No interactions observed in clinical trials | Considered not relevant |
| Tramadol | Possible pharmacodynamics interactions | Relevant |
| Trazodon | Possible pharmacodynamics interactions | Relevant |
| Triazolam | Hypericum not explicitely mentioned in SmPC, but induction of CYP3A4 may reduce plasma levels | Relevant |
| Venlafaxine | Possible pharmacodynamics interactions | Relevant |
| Verapamil | Clinical trial, SmPC; induction of CYP3A4, CYP1A2, CYP2C8, CYP2C9 and CYP2C19 may reduce plasma levels | Relevant |
| Voriconazol | Clinical trial; SmPC contraindication; induction of CYP3A4 may reduce plasma levels | Relevant |
| Warfarine | Clinical trials; SmPC contraindication | Relevant |
| Zaleplon | Central marketing authorisation withdrawn | Considered not relevant |
| Ziprasidone | Not mentioned in SmPC, but risk for serotonine syndrome, possible pharmacodynamics interaction | Relevant |
| Zolpidem | Clinical trial; induction of CYP3A4 may reduce plasma levels | Relevant |

The HMPC agreed that the content of the monograph concerning drug interactions should be kept in a more general style as each new detected interaction would require a revision of the monograph. Moreover a more general style including examples would encourage a prescriber / pharmacist to search for current status of knowledge on interactions at the time of use of a Hypericum product.

At the time of the establishment of this assessment report interactions of Hypericum products with the following drug substances are considered relevant:

List of drug substances which may be pharmacokinetically interacted by Hypericum:

Concommitant use contraindicated:

Warfarin

Immunosuppressants such as ciclosporine, tacrolimus (oral administration), sirolimus, everolimus

Anti-HIV drugs belonging to nucleoside reverse transcriptase inhibitors (NNRTIs)(e.g. efavirenz, etravirine, nevirapine) and protease inhibitors (e.g. indinavir, fosamprenavir, rilpivirine, saquinavir)

Some cytostatic drugs (e.g. imatinib, irinotecan)

Concommitant use to be monitored:

Special care should be taken in case of concomitant use of all drug substances the metabolism of which is influenced by CYP3A4, CYP2C9, CYP2C19 or P-glycoprotein because a reduction of plasma concentrations is possible. These include:

- Anti-androgenics: Finasteride

- Anti-arrhythmic agents: Digoxin

- Antibiotics: Erythromycin

- Anti-coagulants (coumarin type): Acenocoumarol. phenprocoumon (for warfarin see contraindications).

- Anticonvulsants: Mephenytoin, oxcarbazepine, phenobarbital, phenytoin

- Antidepressants (anxiolytic, NDRI, SARI, SNRI, SSRI, tricyclic, with potential pharmacopkinetic interactions): Buspirone, bupropion, amitriptyline, clomipramine

- Antidiabetics: Gliclazide

- Antifungals: Itraconazole, voriconazole

- Antipsychotics: Clozapine, quetiapine, risperidone, ziprasidone

- Benzodiazepines: Alprazolam, diazepam, midazolam, temazepam, triazolam

- Beta blockers: Carvedilol

- Calcium channel blockers: Amlodipine, diltiazem, nicardipine, nifedipine, verapamil

- Contraceptives: Desogestrel, dienogestrel, ethinylestradiol, norethindrone

- Cytostatics: Docetaxel, paclitaxel

- Endothelin receptor antagonists: macitentan

- $I_f$ channel inhibitors: Ivabradine

- NMDA receptor antagonists: Ketamine

- Nonbenzodiazepines: Eszopiclone, zolpidem

- Opioids: Fentanyl, methadone, oxycodone, tramadol

- Proton-pump inhibitors: Esomeprazole, lansoprazole, omeprazole, pantoprazole, rabeprazole

- Statins: Atorvastatin, simvastatin

List of drug substances which may be pharmacodynamically interacted by Hypericum:

Citalopram, escilatopram, fluoxetine, fluvoxamine, mirtazapine, moclobemide, paroxetine, sertraline, trazodone, venlafaxine: Hypericum dry extract may contribute to serotonergic effects when combined with antidepressants with increased incidence of adverse reactions.

There is evidence that Hypericum preparations containing low amounts of hyperforin do not induce enzyme activity under certain circumstances (oral dose 1 x daily 500 mg, less than 1 mg hyperforin per day, duration of intake 1 week). As the duration of use required for achievement of an antidepressant activity is significantly longer the possible omission of contraindications and warnings in the product information should be assessed within procedures for marketing authorisation for a concrete product case by case.

For the monograph the following more general wording is proposed:

Pharmacokinetic interactions:

Hypericum dry extract induces the activity of CYP3A4, CYP2C9, CYP2C19 and P-glycoprotein. The concomitant use of ciclosporine, tacrolimus for systemic use, amprenavir, indinavir and other protease inhibitors, irinotecan and warfarin is contraindicated (see section 4.3. 'Contraindications').

Special care should be taken in case of concomitant use of all drug substances the metabolism of which is influenced by CYP3A4, CYP2C9, CYP2C19 or P-glycoprotein (e.g., amitriptyline, fexofenadine, benzodiazepines, methadone, simvastatin, digoxin, finasteride), because a reduction of plasma concentrations is possible.

The reduction of plasma concentrations of hormonal contraceptives may lead to increased intermenstrual bleeding and reduced safety in birth control. Women using oral contraceptives should take additional contraceptive measures.

Prior to elective surgery possible interactions with products used during general and regional anaesthesia should be identified. If necessary the herbal medicinal product should be discontinued.

The elevated enzyme activity returns within 1 week after cessation to normal level.

Pharmacodynamic interactions:

Hypericum dry extract may contribute to serotonergic effects when combined with antidepressants such as serotonin reuptake inhibitors (e.g. sertraline, paroxetine, nefazodone), buspirone or with triptans.

Patients taking other medicines on prescription should consult a doctor or pharmacist before taking Hypericum.

### 5.5.5. Fertility, pregnancy and lactation

Klier *et al.* (2002) report from a mother with post-natal depression. She took 3 x 300 mg Hypericum extract (LI 160). Four breast milk samples were analysed. Only hyperforin is excreted into breast milk at a low level, hyperforin and hypericin were below the detection limit in the infant's plasma. No side effects were seen in mother or infant.

Five mothers who were taking 300 mg of Hypericum extract (LI 160) 3 times daily and their breastfed infants were assessed by Klier at al (2006). Thirty-six breast milk samples (foremilk and hindmilk collected during an 18-hour period) and 5 mothers' and 2 infants' plasma samples were analyzed for hyperforin levels. Hyperforin is excreted into breast milk at low levels. However, the compound was at the limit of quantification in the 2 infants' plasma samples (0.1 ng/ml). Milk/plasma ratios ranged from 0.04 to 0.13. The relative infant doses of 0.9% to 2.5% indicate that infant exposure to hyperforin through milk is comparable to levels reported in most studies assessing anti-depressants or neuroleptics. No side effects were seen in the mothers or infants. The authors conclude that these results add to the evidence of the relative safety of St. John's wort while breast-feeding which was found in previous observational studies.

Lee *et al.* (2003) conducted a prospective, observational cohort study. 33 breastfeeding women received Hypericum (704.9 ± 463.6 mg/day, no further characterization) compared with 101 disease matched and 33 age and parity-matched nondisease controls. No statistically significant differences in milk production, maternal adverse events and infant weight over the first year of life were observed.

Moretti et al (2009, 2010) compared in a prospective study pregnant women taking Hypericum (n=54), with pregnant women receiving synthetic drugs for treatment of depression (n=54) and healthy pregnant women (n=54). In the Hypericum group most women (n=49) took tablets (mean daily amount of extract 615 mg). The other women used herbal tea, tincture or granules. The results indicate that the rates of malformations were similar across the groups. This outcome was also not different to the risk expected for the general population. Also live birth and prematurity rates did not differ among the groups.

In a review Dugoua *et al.* (2006) searched 7 electronic databases and compiled data according to the grade of evidence found. The authors found very weak scientific evidence based on a case report that St Johns wort is of minimal risk when taken during pregnancy. There is *in vitro* evidence from animal studies that St John's wort during pregnancy does not affect cognitive development nor cause long-term behavioural defects, but may lower offspring birth weight. There is weak scientific evidence that the use of St. John's wort during lactation does not affect maternal milk production nor affect infant weight, but, in a few cases, may cause colic, drowsiness or lethargy. There is weak scientific evidence that St John's wort induces CYP450 enzymes, which may lower serum medication levels below therapeutic range; this may be of concern when administering medications during pregnancy and lactation. Caution is warranted with the use of St John's wort during pregnancy until further high quality human research is conducted in order to determine its safety. The use of St John's wort during lactation appears to be of minimal risk, but may cause side effects. Caution is warranted when using medications along with St John's wort.

Grush *et al.* (1998) report of two pregnant women taking Hypericum extract (not more characterized, 900 mg/day). No signs of toxicity or other harmful effects are reported.

Kolding *et al.* (2015) (erratum Kolding *et al.* 2016) investigated the safety of Hypericum during pregnancy. The authors used the data from the Danish National Birth Cohort. Among more than 90,000 pregnancies only 38 women reported the use of Hypericum. Preterm birth, head circumference, length and birth weight did not differ across the groups. Although the prevalence of malformations was slightly higher in the Hypericum group than in control group the authors conclude that this difference is based only on 3 cases and was not of a specific pattern.

### 5.5.6. Overdose

Karalapillai & Bellomo (2007) reported a case of overdose in suicidal intention of a 16-year-old girl. It has been reported that the girl had taken up to 15 tablets per day for 2 weeks and 50 tablets just before hospitalisation. Seizures and confusion were diagnosed, after 6 days the EEG was normal, no further seizures occurred in the following 6 months. The published data on the composition of the tablets are not clear (‘300 µg tablets’).

*Assessor’s comment:*

*In a personal communication the author confirmed that there is a typing error in the publication. The product contained 300 mg extract per tablet. These symptoms occurred therefore after ingestion of*

*4500 mg extract per day over a period of 2 weeks (approximately the 5-fold therapeutic dose) and an additional dose of 15000 mg extract (approximately the 17-fold therapeutic dose).*

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

Friede *et al.* (1998) studied potential sedative effects of Hypericum in a placebo-controlled clinical study in cross-over design on 19 healthy persons over a period of 15 days. Study medication: Ze117, 250 mg per coated tablet 2 times daily. Ze 117 was shown to have no sedative effect in mental performance and reaction time tests under controlled conditions, so no impairment of the ability to drive vehicles or operate machinery is anticipated. In addition, cognition was not further impaired when Hypericum was administered concomitantly with alcohol (0.5‰ blood alcohol level).

In a double-blind randomized three-way cross-over trial with 18 volunteers Herberg (1994) studied the influence of Hypericum (1 capsule contains Hypericum extract with 0.25 mg total hypericines, 3 capsules daily, 10 days) combined with alcohol (blood alcohol level of 0.57‰) on mental capability. Tests studied optical orientation, permanent concentration, acoustic reaction time, coordination of motor functions. The authors did not find differences between placebo and verum.

Schmidt (1991) found in a placebo-controlled study (verum 17 paients, placebo 15 patients) that four week treatment with Hypericum extract (methanol 80%, DER 3-6:1, 900 mg daily) did not impair coordination, concentration and attention of patients.

Schmidt *et al.* (1993) investigated in a placebo-controlled double-blind study in 32 healthy volunteers the influence of alcohol intake and Hypericum extract on mental performance.

Group 1: 3 x 300 mg LI160 per day for 7 days; then 7 days placebo

Group 2: first placebo, then active treatment

After consumption of alcohol (blood alcohol concentration between 0.045 and 0.08%) psychometric tests were performed on day 7 and on day 14. Interactions between Hypericum and alcohol on the level of psychomotor and mental performance can be ruled out. The authors suggest that Hypericum can be used safely when driving or using machines.

*Assessor's comment:*

*The conclusions are only partly correct. The data suggest that additionally to alcohol there is no impairment on mental performance. Only the studies of Friede et al (1998) and Schmidt (1991) studied the influence of Hypericum alone.Considering the small number of treated persons and the findings on sedation in animals of Girzu et al (1997) it can be concluded that adequate studies in order to clarify this aspect are missing.*

### 5.5.8. Safety in other special situations

Beattie *et al.* (2005)

The effect of 10 days administration of 1020 mg of a Hypericum extract (equivalent to 3 mg hypericin) on erythemal response during high-dose UV A1 therapy was investigated in 11 adult volunteers. The visual erythemal peak was lower after Hypericum administration. Median intensity of postirradiation erythema increased at all time-points. However, the maximum slope of the dose-response curve was not increased.

Johne *et al.* (2002a) investigated the influence of impaired liver function on the pharmacokinetic data of major constituents of Hypericum. 8 Patients with mild and 8 with moderate liver cirrhosis received a single dose of the extract LI 160 (900 mg) and for 12 days 3 x 300 mg of this extract. The data were compared to 8 healthy volunteers. The authors conclude that moderate liver cirrhosis may increase plasma levels of hypericin, pseudohypericin and hyperforin.

### *5.6. Overall conclusions on clinical safety*

The adverse events observed in clinical trials which are most probably linked to the study medication are in general mild, the frequency is considerably lower in comparison to synthetic antidepressants. The induction of CYP3A4, CYP2C9, CYP2C19 and P-glycoprotein is well documented; the amount is directly correlated with the content of hyperforin in the herbal preparation. Pharmacokinetic interactions are documented for several drug substances metabolised via the mentioned enzymes having with a narrow therapeutic range. Hypericum extracts should not be used concomitantly with these substances or the therapeutic activity / plasma concentration is to be monitored. Provided that the product-specific risks are communicated in the product information properly, the risk / benefit assessment favours the benefits of the Hypericum dry extracts.

The induction of the mentioned enzymes is reversible within approximately 1 week after stopping ingestion of Hypericum extracts.

Adequate studies with extracts with low hyperforin content are available which could justify exemptions in the wording of contraindications, special warnings and in the interactions section of the SmPC.

No influence on the mental performance at least in case of low-hyperforin extracts was observed. Hypericum extracts do not additionally impair mental capability in persons after alcohol intake.

The only risk of the cutaneous application of Hypericum oil seems to be the phototoxicity when treated skin is exposed to intense sunlight. A special warning should inform the patient.

## 6. Overall conclusions (benefit-risk assessment)

Dry extracts of *H. perforatum* demonstrated superiority over placebo and non inferiority against standard medication in mild to moderate major depression in several controlled clinical trials. Therefore these types of extracts are proposed for 'well-established use'. The herbal tea and other mostly liquid extracts orally applied have a long tradition in folk medicine for the treatment of low mood, anxiety, to 'strenghten the nerves'. The cutaneous application of oil preparations is also plausible based on long-standing medicinal use and experience.

**Benefit – Risk – Assessment**

<u>Well-established use:</u>

Numerous clinical trials have shown the efficacy of defined herbal preparations containing Hypericum in the respective indications. A comparison with therapeutic alternatives reveals that for many herbal preparations containing Hypericum a non-inferiority compared to synthetic antidepressants could be demonstrated.

Although there are no controlled studies with children and adolescents published it can be concluded that there is a widespread documented use of Hypericum extracts among adolescents. However, there are no data available on the efficacy in this population. Therefore the oral use in children and adolescents below 18 years of age is not recommended for well-established use.

Hyperforin, which may be present in the herbal preparations, is responsible for interactions with other drug substances which are metabolized by certain CYP450 isoenzymes. Nevertheless, the rate and severity of adverse effects was clearly lower for Hypericum than for synthetic antidepressants (Schulz 2006c). Therefore the well-established use can be recommended for the herbal preparations, indications and posologies given in the monograph.

Herbal preparations have to be quantified regarding to hypericines as specified in the Pharm. Eur. monograph on St. John's Wort dry extract, quantified.

<u>Traditional use:</u>

At least in the alpine regions of Central Europe *H. perforatum* is the most important and most frequently used herb in traditional medicine. The efficacy in the proposed indications is plausible due to the longstanding medicinal use and experience of defined herbal preparations.

*Oral administration:*

Possible risks with the oral administration of preparations of Hypericum are related with pharmacokinetic interactions which are caused by the constituent hyperforin. The extent of the induction of the metabolic enzymes is dose-dependent and time-dependent. The oral use for the traditional preparations is limited with 2 weeks. This duration of use may be sufficient for the induction of the activity of the CYP-enzymes in the case of high-hyperforin preparations. In cases where the daily intake of hyperforin is higher than 1 mg the full information regarding enzyme induction, related contraindications and warnings related to interactions should be included in the product information. The content of hyperforin of the herbal preparation should be specified in the dossier.

As there are no adequate safety data for the use in the paediatric population available the use of traditional herbal medicinal products is restricted to adults.

*Cutaneous administration:*

The use of liquid Hypericum preparations in the mentioned indication is documented for a long period. Beside the risk of increased photosensitivity of the treated skin areas no concerns are known. The risk is at an acceptable level for traditional herbal medicinal products. Due to the lower risk assosciated with the cutaneous use traditional herbal medicinal products may be used in adults and adolescents.

# Annex

## *List of references*