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Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Hypericum perforatum* L., herba

Final – Revision 1

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

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

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Hypericum perforatum* L., herba
Herbal preparation(s)	**Traditional use** a) Dry extract (DER 4-7:1), extraction solvent ethanol 38% (m/m) = 45% V/V b) Liquid extract (DER 1:4-20), extraction solvent vegetable oil c) Liquid extract (DER 1:13), extraction solvent maize oil or other suitable vegetable oil d) Tincture (ratio herbal substance: extraction solvent 1:5), extraction solvent ethanol 50-70% V/V e) Tincture (ratio herbal substance: extraction solvent 1:10), extraction solvent ethanol 45-50% V/V f) Liquid extract (DER 1:2-7), extraction solvent ethanol 50% V/V[1] g) Liquid extract from fresh herb (DER 1:1), extraction solvent ethanol 96% V/V h) Expressed juice from the fresh herb (DER 1:0.5-0.9) i) Stabilised expressed juice from fresh herb: The fresh herb is first stabilised over a boiling ethanol, then

[1] A narrow DER to be specified for an individual medicinal product.

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		pressed and adjusted with water to a DER of 1:1. j) Comminuted herbal substance k) Powdered herbal substance **Well-established use** a) Dry extract (DER 3-7:1), extraction solvent methanol 80% V/V 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** Comminuted herbal substance as herbal tea for oral use. Comminuted herbal substance for infusion preparation for cutaneous use. Herbal preparations a), k) in solid dosage forms for oral use. Herbal preparations b), c), d), e), f), g), h), i) in liquid dosage forms for oral use. Herbal preparations b), e), f) in liquid or semi-solid dosage forms for cutaneous use. **Well-established use** Herbal preparation in solid dosage forms for oral use.
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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). Pilepić *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 Pilepić *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% (specific extract '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.

Influence of the extraction solvent on the composition of the extract:

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.

Jürgenliemk & Nahrstedt (2003) investigated the dissolution profile of phenolic constituents from a herbal tea. In general, in a herbal tea the flavonoid glycosides were dissolved very well, followed by flavonoid aglycones. The content of hyperin and pseudohypericine was about 34% of the content in the drug substance. Hyperforin was only detectable in about 1% of the amount present in the herbal substance.

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 sunflower 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 the EU 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 the 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** **Posology** **Duration of use**	**Regulatory Status**
Comminuted herbal substance	Transient mental exhaustion	Herbal tea 2-3 times 1.7 - 1.75 g 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 2 times 1.5 g	TU 1996, 1998 CZ
Comminuted herbal substance	For the supportive treatment of nervous restlessness and sleep disorders	Herbal tea 1-2 teaspoons Hyperici herba	1986, DE, Standard Marketing Authorisation according to section 36 of

Active substance	Indication	Pharmaceutical form Posology Duration of use	Regulatory Status
		/150 ml boiling water 2 times daily	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 2 times 1.8 g	THMP 2004 SE
Comminuted herbal substance	Traditionally as a mean in symptoms of transcient mental exhaustion in adults	Herbal tea 2 times 2 g	PL 1992
Comminuted herbal substance	Traditionally in symptoms of transcient mental exhaustion in adults.	Herbal tea 2 times 2 g	PL 1992
Comminuted herbal substance	For topical use use in minor skin injuries in adults and adolescents.	For infusion preparation for cutaneous use 2 g per glass of boiling water	PL 1992
Comminuted herbal substance	Transient mental exhaustion	Coated tablets 3 times 300 mg 2 weeks	THMP 2011 AT
Comminuted herbal substance	Relief of symptoms of slightly low mood and mild anxiety	Capsules 3 times 300 mg	THMP UK
Comminuted herbal substance	For the improvement of condition in mental stress	Capsules, hard 2 times 500 mg	TU at least since 1976 DE
Dry extract, DER 4.6-	Relief of temporary Mental exhaustion	Single dose: 60-180 mg	At least 30 years (DE)

Active substance	Indication	Pharmaceutical form Posology Duration of use	Regulatory Status
6.5:1, extraction solvent ethanol 38% m/m (= 45% V/V)		Daily dose: 180-360 mg	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 times 2.5 ml	THMP UK
Liquid extract, DER 1:2, ethanol 50% V/V	Mild transient depressive conditions	3 times 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 times 10-20 ml, max. daily dose 30 ml	TU at least since 1976 DE
Tincture, DSR 1:5, extraction solvent ethanol 70% V/V	Traditionally as a mean in symptoms of transcient mental exhaustion in adults	5 ml diluted in water per day	PL 1990
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 2 times 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 3 times 300 mg	WEU 2002 BE
Dry extract, DER 5-8:1, ethanol 50% V/V	Mild to moderate depressive episodes	Film-coated tablets 1 times 612 mg	WEU 2005 AT
Dry extract, DER 5-8:1, ethanol 50% V/V	Mild to moderate depressive conditions	Coated tablets 1 times 612 mg	WEU 2010 SK

Active substance	Indication	Pharmaceutical form Posology Duration of use	Regulatory Status
Dry extract, DER 5-8:1, ethanol 50% V/V	Short term treatment of symptoms in mild depressive disorders	Film-coated tablets 1 times 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 1 times 612 mg	WEU 2008 LV
Dry extract, DER 5-8:1, ethanol 50% V/V	Mild temporary depressive disorders	Capsules, hard 2 times 306 mg	WEU at least since 1976 DE
Dry extract, DER 5-8:1, ethanol 50% V/V	Mild temporary depressive disorders	Film-coated tablets 1 times 612 mg	WEU at least since 1976 DE
Dry extract, DER 4–7:1, ethanol 57.9% V/V	Treatment of mild depression (depressed or labile mood, internal tension, feeling of tension, stress) and associated sleep disturbances	Film-coated tablets 1 times 500 mg	WEU 2003 HU
Dry extract, DER 4-6:1, ethanol 60% m/m	Low mood, psychovegetative disorders	Coated tablets 1 times 180 mg 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 1 times 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 1 times 250 mg	THMP UK
Dry extract, DER 3.5-6:1, ethanol 60% m/m	Mild temporary depressive disorders	Capsules, hard	WEU 1998 DE, 1999 DE

Active substance	Indication	Pharmaceutical form Posology Duration of use	Regulatory Status
		2-3 times 250 mg	
Dry extract, DER 3.5-6:1, ethanol 60% m/m	Low mood, psychovegetative disorders	Capsules, hard, film-coated tablets 1 times 425 - 600 mg 6 weeks	Authorization 9.2010 AT, 10.2000 AT, 9.2010 AT
Dry extract, DER 3.5-6:1, ethanol 60% m/m	Mild to moderate depressive episodes	Capsules, hard 1-2 times 425 mg Minimum 4 weeks	Authorization 8.2000 AT, 12.1999 AT
Dry extract, DER 3.5-6:1, ethanol 60% m/m	For the short term treatment of symptoms in mild depressive disorders	1-2 times 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 1 times 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 1 times 300 mg	THMP UK
Dry extract, DER 3.5-6:1, ethanol 60% m/m	Mild temporary depressive disorders	Capsules, hard 2 times 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 2 times 450 mg	WEU 1998 DE
Dry extract, DER 3.5-	Mild temporary depressive disorders	Film-coated tablets	WEU 2003 DE

Active substance	Indication	Pharmaceutical form Posology Duration of use	Regulatory Status
6:1, ethanol 60% m/m		2 times 325 mg	
Dry extract, DER 3.5-6:1, ethanol 60% m/m	Mild temporary depressive disorders	Coated tablets 3 times 200 mg	WEU at least since 1976 DE
Dry extract, DER 3.5-6:1, ethanol 60% m/m	Treatment of psychosomatic complaints, occurring as mild or moderate forms of depression, after exclusion of every severe pathology	Capsules, hard 2 times 425 mg	WEU 2007 BE
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 1-2 times 185 mg	WEU 10.1998 ES
Dry extract, DER 6-7:1, ethanol 60% m/m	Mild temporary depressive disorders	Capsules, hard; capsules, soft 2 times 185 mg 2-3 times 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 1 times 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 2 times 250 mg	WEU 6.1999 HR
Dry extract, DER 4.2-6.5:1, ethanol 60% V/V	Mild temporary depressive disorders	Coated tablets 3 times 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 3 times 150 mg	THMP UK

Active substance	Indication	Pharmaceutical form Posology Duration of use	Regulatory Status
Dry extract, DER 5-7:1, ethanol 60% V/V	Relief of symptoms of slightly low mood and mild anxiety	Tablets 1-2 times 170 mg	THMP UK
Dry extract, DER 5-7:1, ethanol 60% V/V	Relief of symptoms of slightly low mood and mild anxiety	Tablets 1-2 times 300 mg	THMP UK
Dry extract, DER 5-7:1, ethanol 60% V/V	Relief of symptoms of slightly low mood and mild anxiety	Tablets 1 times 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 1-2 times 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 1-2 times 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 1-2 times 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 2 times 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 2-3 times 100 mg	WEU 5.2000 ES
Dry extract, DER 5-7:1, ethanol 70% V/V	Mild temporary depressive disorders	Capsules, soft 2 times 270 mg	WEU at least since 1976 DE

Active substance	Indication	Pharmaceutical form Posology Duration of use	Regulatory Status
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 1 times 300 mg	WEU 5.1998 ES
Dry extract, DER 3-6:1, ethanol 80% V/V	Treatment of mild to moderate depressive episodes	Film-coated tablets 1 times 900 mg	WEU 7.2008 HR
Dry extract, DER 3-6:1, ethanol 80% V/V	Moderate temporary depressive disorders (depressive episodes)	Coated tablets 3 times 300 mg	WEU 1998 DE
Dry extract, DER 3-6:1, ethanol 80% V/V	Mild temporary depressive disorders (depressive episodes)	Coated tablets 3 times 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 1 times 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 1 times 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 3 times 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 2 times 450 mg 3 times 300 mg Minimum 4 weeks	Authorization 7.2002 AT, 2.1998 AT

Active substance	Indication	Pharmaceutical form Posology Duration of use	Regulatory Status
Dry extract, DER 3-6:1, methanol 80% V/V	Low mood	Film-coated tablets, coated tablets 1 times 450 mg 1-2 times 300 mg 6 weeks	Authorization 11.2004 AT, 7.1998 AT
Dry extract, DER 3-6:1, methanol 80% V/V	Mild to moderate depressive episodes (anxiety, restlessness, sleep disorders)	Coated tablets 3 times 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 3 times 300 mg	WEU 2006 BE
Dry extract, DER 3-6:1, methanol 80% V/V	Mild to moderate temporary depressive disorders	Coated tablets 3 times 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 1 times 450 mg	THMP UK
Dry extract, DER 3-6:1, methanol 80% V/V	Mild temporary depressive disorders	Film-coated Tablets 1-2 times 450 mg	WEU 2003 DE, 2004 DE
Dry extract, DER 3-6:1, methanol 80% V/V	Mild temporary depressive disorders	Coated Tablets 2 times 375 mg	WEU 2009 DE
Dry extract, DER 3-7:1, methanol 80% V/V	Herbal medicinal product used in slightly low mood, mild anxiety and temporary difficulties in falling asleep	Coated tablets 2 times 300 mg	WEU 1999 SE

Active substance	**Indication**	**Pharmaceutical form** **Posology** **Duration of use**	**Regulatory Status**
Dry extract, DER 3-7:1, methanol 80% V/V	Mild temporary depressive disorders	Film-coated tablets 2 times 300 mg 1 times 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 1 times 600 mg	WEU 2005 DE
Dry extract, DER 3-7:1, methanol 80% V/V	Moderate temporary depressive disorders	Film-coated tablets 1 times 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 2-3 times 300 mg	WEU at least since 1976 DE
Dry extract from fresh herb, DER 3.1-4:1, ethanol 68% V/V 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 Relief of symptoms of slightly low mood and mild anxiety	Tablets 3 times 40-73 mg (corresponding to 0.33 mg total hypericines) Tablets 3 times 40-73 mg	WEU 2.2012 FI THMP UK
Expressed juice of fresh, flowering herb, DER 1:0.5-0.9	For the relief of temporary mental exhaustion	Expressed juice 2 times 10 ml	TU at least since 1976 DE
Liquid extract, DER 1:4-20, vegetable 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

Active substance	Indication	Pharmaceutical form Posology Duration of use	Regulatory Status
(*Hypericum* oil)			
Liquid extract (DER 1:13), extraction solvent maize oil or other suitable vegetable oil	For the relief of temporary mental exhaustion	Soft capsules 3 times 200 mg	TU at least since 1976 DE
Liquid extract from fresh herb (Syn. Succus Hyperici)	Traditionally used in indigestion in adults	100 ml of liquid contains 100 ml of stabilised juice of the fresh herb of *H. perforatum* L. Extraction solvent: ethanol 96% V/V, water. The fresh herb is first stabilised over a boiling ethanol, then pressed and adjusted with water to the same mass. Final product contains 25-35% of ethanol. 2.5 ml 3 times daily	PL 1987
Liquid extract from fresh herb (Syn. Intractum Hyperici)	Traditional herbal medicinal product used in transient states of nervous exhaustion in adults	100 ml of liquid contains 100 ml of ethanol extract of *H. perforatum* L., fresh herb (1:1), extraction solvent ethanol 96% V/V. Final product contains 52-62% of ethanol. 5 ml 4 times daily	PL 1987

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** **Posology** **Duration of use**	**Reference**
Comminuted herbal substance	Among others: neurasthenia, sleep disorders, nervous complaints, depression	Herbal tea, oral use	List & Hörhammer 1976
	Nervous restlessness, sleep disorders	30 ml of infusion (1:20)	
	Psychovegetative disorders, mood depression, anxiety, nervous restlessness	1.8 – 3.6 g, 2 times daily	Wichtl 1984
	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	1-2 g, 2 times daily	Hänsel *et al.,* 1993

Herbal preparation	Documented use / Traditional use	Pharmaceutical form Posology Duration of use	Reference
	Mild depression, support of emotional balance		Irion 1955
	Mild gastrointestinal discomfort	1 spoon, 3 times daily	
		2-4 g daily	ESCOP 2003, Barnes *et al.,* 2002
		2 g, 2 times daily	Ozarowski *et al.,* 1978
Liquid extract (DER 1:4-20), extraction solvent vegetable oil	Dyspeptic complaints Increase of bile flow Nervous stomach, gastritis	Oral use Daily dose corresponding to 2-4 g herbal substance (= 8-80 g *hypericum* oil)	Wichtl 1984 Hänsel *et al.,* 1993 Böhme *et al.,* 2006 Gerlach 2008
	Gout, rheumatic complaints, bruises, sprains	6-8 drops	Madaus 1938
Liquid extract (DER 1:4-20), extraction solvent vegetable oil	Injuries, myalgia, first grade burn wounds Rheumatic complaints Lumbago	Cutaneous use Apply undiluted	Madaus 1938 Auster 1958 Wichtl 1984 Hänsel *et al.,* 1993

Herbal preparation	Documented use / Traditional use	Pharmaceutical form Posology Duration of use	Reference
			Gerlach 2008
Tincture (DER 1:10), extraction solvent ethanol 45-50% V/V	Oral use Relief of temporary mental exhaustion Cutaneous use 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 2-4 ml, 3 times daily Apply undiluted to the affected area	Madaus 1983 Barnes *et al.,* 2002 Irion 1955, Barnes *et al.,* 2002, Gruenwald *et al.,* 2004
Tincture (DER 1:5), extraction solvent ethanol 50% -70% V/V	Oral use Relief of temporary mental exhaustion. Cutaneous use 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 times daily Apply undiluted to the affected area	Irion 1955, Hänsel *et al.,* 1993, Bradley 2006

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 such 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.

Because for the indication of a THMP terms like 'depression' or 'depressed mood' are not suitable, special attention is given 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 a period of 30 years.

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. TU a)	Oral use Relief of temporary mental exhaustion	Single dose: 60-180 mg 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	Cutaneous use 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 Wichtl 1984

Herbal preparation Pharmaceutical form	Indication	Posology, Strength	Period of medicinal use
oleum) TU b)			
Liquid extract (DER 1:13), extraction solvent maize oil TU c)	Oral use Relief of temporary mental exhaustion	3 times 200 mg	Since 1976 (DE)
Tincture (DER 1:10), extraction solvent ethanol 45-50% V/V TU e)	Oral use Relief of temporary mental exhaustion Cutaneous use: Symptomatic treatment of minor inflammations of the skin (such as sunburn) and as an aid in healing of minor wounds	2-4 ml, 3 times daily Apply undiluted to the affected area	According to references Madaus (1983), Irion (1955), Barnes et al. (2002) and Gruenwald et al. (2004)
Tincture (DER 1:5), extraction solvent ethanol 50% -70% V/V TU d)	Oral use: Relief of temporary mental exhaustion. Cutaneous use 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 times daily Apply undiluted to the affected area	According to reference Bradley (2006) Since 1990 (PL) Irion (1955)
Liquid extract DER 1:2, extraction solvent	Oral use Relief of temporary mental exhaustion	0.8-1.2 ml, 3 times daily	Since 1978 (DE)

Herbal preparation Pharmaceutical form	Indication	Posology, Strength	Period of medicinal use
ethanol 50% V/V Included in TU f)			
Liquid extract (DER 1:5-7), extraction solvent ethanol 50% V/V Included in TU f)	Oral use Relief of temporary mental exhaustion	1.3 ml, 3 times daily	Since 1976 (DE)
Liquid extract from fresh herb (DER 1:1), extraction solvent ethanol 96% V/V TU g)	Oral use Relief of temporary mental exhaustion	5 ml 4 times daily	Since 1987 (PL)
Expressed juice of fresh, flowering herb, DER 1:0.5-0.9 TU h)	Oral use Relief of temporary mental exhaustion	10-20 ml, 1-3 times daily (max. 30 ml daily)	Since 1976 (DE)
Stabilised expressed juice from fresh herb, DER 1:1 TU i)	Oral use Symptomatic relief of mild gastrointestinal discomfort	The fresh herb is first stabilised over a boiling ethanol, then pressed and adjusted with water to a DER of 1:1 2.5 ml 3 times daily	Since 1987 (PL)
Comminuted herbal	Oral use	1.5-2 g, 2-3 times daily	Irion (1955), List &

Herbal preparation Pharmaceutical form	Indication	Posology, Strength	Period of medicinal use
substance as herbal tea TU j)	Relief of temporary mental exhaustion Oral use Symptomatic relief of mild gastrointestinal discomfort Oral use For the supportive treatment of nervous restlessness and sleep disorders Cutaneous use Symptomatic treatment of minor inflammations of the skin (such as sunburn) and as an aid in healing of minor wounds	2 g, 2 times daily 1-2 teaspoons (= 2-3 g) / 150 ml boiling water, 2 times daily For infusion preparation for cutaneous use 2 g per glass of boiling water	Hörhammer (1976) Ozarowski et al., 1978 Since 1986 (DE) Since 1992 (PL)
Powdered herbal substance TU k)	Oral use Relief of temporary mental exhaustion	300 – 500 mg, 2-3 times daily (max. 1000 mg daily)	Since 1976 (DE)
Well-established use			
Dry extract (DER 3-7:1), extraction solvent methanol (80% V/V) 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 3-6:1),	Herbal medicinal product for the treatment of mild to moderate depressive episodes	Single = daily dose 900 mg	Several member states, at

Herbal preparation Pharmaceutical form	Indication	Posology, Strength	Period of medicinal use
extraction solvent ethanol (80% V/V) WEU b)	(according to ICD-10)		least since 1998
Dry extract (DER 2.5-8:1), extraction solvent ethanol (50-68% V/V) WEU c)	Herbal medicinal product for the short term treatment of symptoms in mild depressive disorders	600 or 612 mg 1 times daily Or 250 – 600 mg, 2-3 times daily 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** **Dosage** **Route of administration**	**Experimental model** ***In vivo/*** ***In vitro***	**Reference** **Year of publication**	**Main non-clinical conclusions of the authors**
Herbal substance	350 mg/kg per day for 3 weeks	*In vivo* (rats) Chronic restraint stress, prolonged corticosterone administration 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 8 weeks of treatment	*In vivo* 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* 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) 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

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**
Dry extract (DER 4-7:1, methanol 80% V/V)	240 mg/kg orally for 14 days	*In vitro* MAO-A and MAO-B assays Synaptosomal uptake assays *In vivo* 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 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* 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% hyperforin) CO_2-extract (30.14% hyperforin)	Methanol extract: 31.25 – 125 mg/kg CO_2 extract: 2.42-9.86 mg/kg Oral	*In vivo* Rats *In vivo* microdialysis in shell region of nucleus accumbens	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 of 5-HT neurons
Dry extract (DER 4-7:1, methanol 80% V/V), 1.5% hyperforin CO2 extract, 38.8%	240 mg/kg orally for 14 days	*In vitro* MAO-A and MAO-B assays Synaptosomal uptake	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

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**
hyperforin Pure hyperforin		assays *In vivo* Receptor binding test with rats		led to a significant down-regulation of ß-receptors
Dry extract (ethanol, no further details)	0.4-400 µg/ml	*In vitro* Effects of extracts on naloxone binding to the µ and κ-opioid receptors 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, methanol 80% V/V)	6.25 – 100 µg/ml 300 µl per plate	*In vitro* Human monocytic U-937 cells Corticosteroid receptor mRNA expression	Enning *et al.,* 2011	In a small concentration range the glucocorticoid receptor α mRNA was primarily and transiently up-regulated, after 16h 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%,	Equivalent to 5µM hypericin in the media	*In vitro* 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

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**
quercetin 0.13%, quercitrin 0.11%, biapigenin 0.048%				
Dry extract (Ze 117, DER 4-7:1, ethanol 50% m/m); hyperforin <0.02%		*In vitro* Rat brain slices 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 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* Rat cortical brain slices	Ruedeberg *et al.,* 2010	Dopamin uptake was inhibited in a dose dependent manner 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 Also in combination with a dry extract of	*In vitro* Serotinin re-uptake *In vivo*	Fiebich *et al.,* 2011	IC_{50} values for serotononin uptake: Hyperforin rich extract (2.7%): 13.0 µg/ml Hyperforin poor extract (1.1%): 88.2 µg/ml Combination with passionflower extract reduces the IC_{50} to 9.7

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
	Passiflorae herba (DER 6.25-7.1:1, ethanol 60% m/m)	Sprague-Dawley rats Forced swimming test Open field test		and 14.0 µg (ml respectively). *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
Dry extract (ethanol 80%, no further information) Hypericin Hyperforin	Extract: 50 µg/ml Hyperforin 10 µM Hypericin 1 µM	*In vitro* Live cell calcium imaging 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* Male and female mice Male rats Induced sleeping time Forced swimming test 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. 500 mg/kg *Hypericum* extract causes a significant decrease of immobility time in the tail suspension test. 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	*In vivo*	Butterweck *et al.,* 2000	Fractions containing mainly hyperoside, isoquercitrin, miquellianin, quercitrin and astilbin were active in the forced

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
	Acute treatment, 12 days treatment	Forced swimming test Open field test		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
Dry extract (DER 4-7:1, methanol 80% V/V) Hypericin	Extract: 500 mg/kg p.o. Hypericin: 0.2 mg/kg p.o. 2 weeks, 8 weeks	*In vivo* 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) Rutin	Oral administration by gavage	*In vivo* Forced swimming test with male rats 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* Receptor binding assay in	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

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**
		rat brain		
Dry extracts (DER 3-7:1, methanol 80% V/V)	150 – 500 mg/kg, oral, in 3 portions Elevated T-maze: up to 300 mg/kg oral, 7 days	*In vivo* Forced swimming test Elevated T-maze Open field test	Beijamini & Andreatini 2003	All doses significantly reduced the immobility time. 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 Acute treatment 62.5- 250 mg/kg, oral 14 days	*In vivo* Elevated T-maze Light/dark transition 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 CO_2 extract (26.2% hyperforin)	Oral Methanolic extract: 500 mg/kg Hypericin 0.2 mg/kg Hyperoside 0.6 mg/kg	*In vivo* Radioligand receptor binding studies 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

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**
Hypericin Hyperoside Hyperforin-trimethoxybenzoate	CO2 extract: 27 mg/kg Hyperforin-trimethoxybenzoate 8 mg/kg			weeks, 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) Dry extracts (DER 4-7:1, ethanol 50% m/m)	i.p. Acute administration: Imipramine 0-30 mg/kg Fluoxetine 0-30 mg/kg Extracts 0-40 mg/kg Subacute administration: Up to 10 mg/kg	*In vivo* Rat forced swimming model cAA rat model of alcoholism	De Vry *et al.,* 1999	Rat forced swimming model Minimal effective dose: imipramine 30 mg/kg; fluoxetine 10 mg/kg; *Hypericum* extracts 20 mg/kg. The anti-immobility effects were more pronounced after subacute treatment. cAA model of alcoholism For all substances a dose dependent reduced alcohol intake was observed. 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 6% flavonoids Dry extracts (DER 4-7:1, ethanol 50%	Oral Acute administration Comparison with fluoxetine	*In vivo* Male Sprague-Dawley rats Levels of neurotransmitters in different regions of the brain	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 extract rich in flavonoids was able to increase the noradrenaline content

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**
m/m) containing 50% flavonoids				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) Liquid extract (DER 1:2, ethanol 50% [no further information])	Dry extract 15-600 mg/kg, p.o. Liquid extract 0.5-4.0 ml, i.v.	*In vivo* Male cats 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) Dry extract (DER 2.5-5:1, ethanol 60% V/V)	30 – 300 mg/kg, 7 days, oral	*In vivo* Forced swimming test in rats	Nöldner & Schötz 2002	Ethanolic extract: dose dependent reduction of immobility time. Methanolic extract: inactive; after addition of rutin comparable effect to the ethanolic extract
Dry extract (DER 2.5-5:1, ethanol 60% V/V)	Oral administration 9 days 12.5-800 mg/kg extract 3-30 mg/kg rutin 3-10 mg/kg isorhamnetin	*In vivo* 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. 48 mg/kg rutin showed the same activity as 30 mg/kg imipramine. 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%	Oral by feeding needle 125 – 1000 mg/kg	*In vivo* 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

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
hyperforin, 9.4% flavonoids				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* Male Sprague-Dawley rats Restrained stress conditions 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 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. Fluoxetine 10 mg/kg p.o.	*In vivo*, rats 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) Hypericin	Extract: 500 mg/kg p.o. Hypericin: 1.25 mg/kg p.o. Hyperforin: 2.14 mg/kg	*In vivo* (rats, mice) 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

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
Hyperforin	p.o.			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
Aqueous extract (no further information); extract free of hyperforin, total hypericins 0.16% m/m	5-100 mg/kg	*In vivo* Male Charles River mice 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* Male CD1 mice 7 weeks corticosterone administration (5 mg/kg per 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	10 µg/ml	*In vitro*	Gobbi *et al.,* 2001	The methanolic extract (4.5% hyperforin) interacted with a

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**
extract (no details), CO_2 extract, hypericin, hyperforin, biapigenin		Binding assay		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 further details)	0.1-1000 µg/ml	*In vitro* Human µ- and k-opioid receptors	Simmen *et al.,* 1998	*Hypericum* extract inhibited the binding of naloxone to the µ- and k-opioid receptor (IC_{50} values 25 and 90 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. 0.5% extract: 3.12-12.5 mg/kg i.p.	*In vivo* 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 62.5-500 mg/kg	*In vivo* Male rats Brain content of tryptophan, 5-hydroxytryptamine, 5-hydroxyindoleacetic acid, norepinephrine, dopamine 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	Oral administration	*In vivo* Adult male rats and mice	Bhattacharya *et al.* (1998)	The antidepressant activities of 50, 150 and 300 mg/kg per day ethanolic extract were similar to those of 5, 15 and 30 mg/kg per day of the CO_2 extract. The ethanol extract potentiated

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**
hypericins, 38.8% hyperforin)		Behavioral despair test in rats Muricidal behaviour in rats 5-hydroxytryptophan-induced head twitches in mice L-DOPA-induced behaviour in mice Apomorphine-induced stereotypy in rats Post-swim grooming response in mice Elevated plus-maze in mice		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 extract treated groups
Amentoflavone	Concentrations between 10^{-1} and 10^{3} nM	*In vitro* 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* 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

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
				respectively. The effect size was comparable to 2.0 mg/kg BW of bupropion. The effect was antagonised by the dopamine antagonist sulpiride (100 mg/kg BW i.p.)
Hypericin	1.56 mg/kg Comparator venlafaxine 7.81 mg/kg	*In vivo* 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* 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* 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** **Dosage** **Route of administration**	**Experimental model** ***In vivo/*** ***In vitro***	**Reference** **Year of publication**	**Main non-clinical conclusions of the authors**
Hyperosid	1 µM hyperforin; 1 µM hyperosid	*In vitro* 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. 20-40 mg/kg p.o.	*In vivo*, mice, rats Open field test Pentobarbital sleeping time Hot-plate test Acetic-acid induced writhing test Forced swimming test	Schulte Haas *et al.,* 2011	20-40 mg/kg i.p.: exploratory behaviour in the open-field test reduced 20 mg/kg i.p.: pentobartbital sleeping time increased, but not sleeping latency No activity in the hot-plate test and acetic acid-induced writhing test Antidepressant-like effect in forced swimming test (10-20 mg/kg i.p. in mice; 1.8 mg/kg per day p.o. in rats)
Dry extract (ethanol), 4.5% hyperforin CO_2 extract, 38.8% hyperforin	Oral administration for 3 consecutive days Ethanolic extract 50-300 mg/kg CO_2 extract 5-30 mg/kg	*In vitro* *In vivo* Synaptosomal preparations of rat striatum MAO-A and MAO-B in the whole mouse brain Behavioural despair in rats Learned helplessness in	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 The effects in the *in vivo* test systems correlated with the content of hyperforin in the extracts

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
		rats		
Hypericin, dry extract (no further details)	5 µg/ml	*In vitro* Receptor binding studies	Cott 1997	Hypericin affinity only to NMDA receptor 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* Receptor binding studies	Raffa 1998	30 receptors or reuptake sites Modest affinity (49% inhibition) for muscarinic cholinergic receptors and similar affinity (48% inhibition) for σ receptors
Hypericin	1-100 µM	*In vitro* 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* 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 decay kinetics
Hyperforin	10 mg/kg, i.p.	*In vivo* Push-pull superfusion	Kaehler *et al.,* 1999	Hyperforin enhanced the extracellular levels of dopamine, noradrenaline, serotonin and glutamate. The levels of the serotonin metabolite 5-hydroxyindolacetic acid, of GABA,

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
		technique Rat locus caeruleus		taurine, aspartate, serine and arginine were not influenced
Hyperforin	2 µM	*In vitro* Synaptosomal preparations from the frontal cortex of female mice Blood from human volunteers	Singer *et al.,* 1999	Hyperforin inhibits serotonin uptake by elevating free intracellular Na^+
Hyperforin		*In vitro* *In vivo* Choline (CH) uptake Rat brain synaptosomes 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. 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
Hyperforin	*In vivo* 4 mg/kg i.p.	*In vitro* Primary cultures of cortical neurons	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

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
		In vivo Male C57Bl6/J mice, 4 weeks treatment		p-CREB. *In vivo*, hyperforin augmented the expression of TrkB in the corte 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 10 mg/kg i.p.	*In vitro* *In vivo* Models of N-methyl-D-aspartate receptor antagonism and neuroprotection	Kumar *et al.,* 2006	*In vitro* Inhibition of N-methyl-D-aspartate (NMDA) induced calcium influx into cortical neurons. Inhibition of NMDA-receptor mediated release of choline from phospholipids *In vivo* Inactive in models of brain edema formation, middle cerebral artery occlusion, water intoxication
Hyperforin	0.3-10 µM	*In vitro* 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 the neurotrophin brain-derived neurotrophic factor (BDNF) in hippocampal pyramidal neurons
Hyperforin	1-10 µM	*In vitro* HEK-293 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

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
		Transfected HEK-293 cells expressing TRPC6 cDNA Primary mouse cortical microglia cells		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 1 µM hyperoside 6 days	*In vitro* Rat C6 glioblastoma cells	Jakobs *et al.,* 2013	Reduced ß2-adrenergic receptor density in plasma membranes Reduced downstream signalling
Hyperforin, adhyperforin	10^{-4} – 10^{2} M	*In vitro* Synaptosomal uptake assay in rat brain tissue 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* Forced swimming test Tail suspension assay Open field test *In vitro*	Tian *et al*., 2014	Adhyperforin reduced the immobility time of mice in the 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

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
		Synaptosomes from frontal cortex		serotonin and norepinephrine transporters
Amentoflavon	10^{-10} – 10^{-3} M	*In vitro* 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** ***In vivo/ In vitro***	**Reference** **Year of publication**	**Main non-clinical conclusions of the authors**
Laboratory extracts prepared with methanol/acetone 1:1 *Hypericum perforatum* subsp. *perforatum* and subsp. veronense	Up to 10 µg/ml	*In vitro* 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* Transiently transfected K562 cells	Orhan *et al.,* 2014	Some of the tested oil macerates reduced TNFα-induced NF-κB activation in a concentration dependent manner.
Oil macerate (1:10) from dried *Hypericum perforatum* 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 Artificial wounds	Prisăcaru *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. Cutaneous application: in ointment base (no data o concentration)	*In vivo* Linear incision wound model (rats) Circular incision wound model (rats) Excision wound model (rats) Acetic acid-induced increase in capillary	Süntar *et al.,* 2010	Statistically significant faster wound healing compared to placebo. Bioassay-guided fractionation led to the suggestion that the flavonoids play a major role in wound healing. The ethanolic extract exhibited a dose-dependent anti-inflammatory activity

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
		permeability (mice)		
Oil extract (1:5), sunflower oil Oil extract (1:10), sunflower oil Oil extract (first extraction solvent ethanol 96%, second extraction solvent sunflower oil) Quercetin and I3,II8-biapigenin	1.25 ml/kg p.o.	*In vivo* Rat paw edema 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* Human epidermal cell suspensions 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%) Lipophilic extract (CO2) Ethylacetic fraction of an ethanolic extract (ethanol 70%) hypericin, adhyperforin, amentoflavone, hyperoside, isoquercitrin, hyperforin dicyclohexylammonium (DHCA) salt		*In vivo* Croton oil induced ear oedema in mice	Sosa *et al.,* 2007	Dose-dependent oedema reduction. 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

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
and dicyclohexylamine				hyperoside were less active (ID50 about 1 µmol cm-2)
Dry extract (ethanol 50%,	1-100 mg/ml	*In vitro* 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 ***In vivo/*** ***In vitro***	Reference Year of publication	Main non-clinical conclusions of the authors
Fractionated ethanolic extract (80%) and isolated constituents	Results calculated on the actual amount of a constituent in the fractions	*In vitro* Amyloid-β-peptide induced cell death in rat cultured hippocampal neurons. Lipid peroxidation in rat cortical synaptosomes	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 phenols, mainly chlorogenic acid-type phenolics (21%, 77% and 98%, respectively) 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). Total extract as well as fractions containing hypericin and flavonoids inhibited Amyloid-β-peptide induced decrease in cell volume, chromatin condensation and nuclear fragmentation

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**
Hyperforin	Rats injected with amyloid-β-fibrils alone or together with 6 µM hyperforin in the hippocampus	*In vivo* Circular water maze 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* 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 information)	1250 mg/kg 60 or 120 days	*In vivo* 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 expression
Dry extract (acidified methanol, no further information) Hyperforin Hypericin Pseudihypericin	Up to 4.66 mg/ml culture	*In vitro* 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

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**
				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* 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* HeLa 37 cells for HIV studies Equine dermal cells for EIAV studies	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 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 times daily, 5 days, oral	*In vivo* 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-α in lung tissue. In contrast it enhanced the lung and serum levels of IL-10 and INF-γ

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**
Hypericin	2, 10 and 50 µl/ml	*In vitro* 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 ≥ 50 µl/mL
Hyperforin Aristoforin	Up to 50 µM 2 mM daily for 2 weeks, peritumoral injections	*In vitro* Primary human lymphatic endothelial cells (LEC) Human umbilical vein endothelial cells (HUVEC) *In vivo* Tumor induced lymphangiogenesis in rats	Rothley *et al.,* 2009	At concentrations less than 10 µM, hyperforin and 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* Chronic lymphoid leukemia cells (CLL) Acute myeloid leukeima cells (AML)	Billard *et al.,* 2013	Review article 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

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
				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 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* 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 zymograph
Hyperforin	18 µM, up to 12 µg/ml	*Ex vivo* Human chronic lymphocytic leukemia cells (CLL) 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

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
				interaction of Noxa with Mcl-1 and the dissociation of Mcl-1/Bak complex, revealing that upregulated Noxa 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 the	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** **Dosage** **Route of administration**	**Experimental model** ***In vivo/*** ***In vitro***	**Reference** **Year of publication**	**Main non-clinical conclusions of the authors**
		nuclear enzyme PAR synthetase		
Dry extract (methanol, no further details) Hypericin	1h exposure 0.5-5.0 µg/ml hypericin as isolated substance or corresponding amounts of the extract	*In vitro* Cytotoxic properties 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 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* P12 cells	Lu *et al.,* 2004 (abstract only)	Protective effect against trauma of P12 cells induced by H2O2 Reactive oxygen species levels decreased significantly The extract blocked DNA fragmentation
Hypericin	0.1-10 µM	*In vitro* Cerebellar granule cells	Kaltschmidt *et al.,* 2002	Hypericin induced short-time activation of NF-kB. Cell death was induced at 10 µM 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* Hepatic ischaemia / reperfusion model in rats	Bayramoglu *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 tissue homogenates compared to ischaemia/reperfusion-induced rats without treatment-control

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				group ($p < 0.05$)
Dry hydroethanolic extract (no further information) Hyperforin	Extract: 25 µg/ml Hyperforin: 1-5 µM	*In vitro* Rat insulinoma cells INS-1E Pancreatic islands of rats 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) Hyperforin	Extract: 200 µg/ml Hyperforin: 2 µM	*In vitro* Pancreatic islands of rats 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 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

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				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* 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 Aristoforin	0.01-10 mM	*In vitro* Plasmid DNA 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 Kaempferol Biapigenin	10 µM	*In vitro* 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 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	Up to 50 µg/ml	*In vitro*	Zou *et al.,* 2010	Following a 4 h exposure of PC12 cells to H2O2, a significant

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extract		PC12 cells		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 Dry extract (CO_2), 24.33% hyperforin	Ethanolic extract 62.5-500 mg/kg i.g. CO_2 extract 7.8-250 mg/kg i.g.	*In vivo* msP rats Intake of ethanol 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 80% V/V)	Oral Acute: Fawn-hooded (FH) rats: 100-800 mg/kg High-alcohol drinking (HAD) rats: 100-600 mg/kg Chronic: FH rats 400 mg/kg,	*In vivo* Rats accustomed to ethanol	Rezvani *et al.,* 1999	*Hypericum* significantly reduced alcohol intake in both types of rats. FH rats did not develop tolerance to the effects of *Hypericum* in chronic treatment

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**
	15 days			
Dry extract (no further details), 0.3% hypericin	125-500 mg/kg, intragastral, acute	*In vivo* msP rats with preference to alcohol Forced swimming test 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 per 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; CO_2 extract, 24.33% hyperforin	Methanol extract: 62.5-500 mg/kg i.g. CO_2 extract: 7.8-250 mg/kg i.g.	*In vivo* msP rats with preference to alcohol 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
CO_2 extract, 24.33% hyperforin	7, 31, 125 mg/kg i.g.	*In vivo* msP rats with preference to alcohol Tail flick test	Perfumi *et al.,* 2003	CO_2 extract reduced ethanol intake at 31 or 125 mg/kg, but not 7 mg/kg. 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 Acute or chornic (12	*In vivo* msP rats with preference to	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

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	days)	alcohol		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* 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), hyperforin not detectable CO_2 extract, 45% hyperforin	Methanolic extract: 15.6-1000 mg/kg CO2 extract: 1-10 mg/kg oral	*In vivo* 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 (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) Dry extract (ethanol 50% m/m, 4-7:1), very low content of hyperforin	i.p. Acute: 5-40 mg/kg, 3 times within 23h Subacute: 10 mg/kg for 4 days	*In vivo* Forced swimming test Model for alcohol consumption	De Vry *et al.,* 1999	*Hypericum* extracts induced a significant reduction of immobility time Alcohol intake was significantly reduced dose-dependently

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Dry extract (ethanol 50%, no further details)	25-200 mg/kg BW, i.p. before ethanol withdrawal	*In vivo* 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 Up to 1000 µg/m	*In vivo* Wistar rats *Ex vivo* Isolated stomach	Capasso *et al.,* 2008	Oral administration of SJW extract (100–600 mg kg−1) produced a dose-dependent decrease in gastric emptying *In vitro* studies showed that the extract was significantly 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	25-100 mg/kg BW, oral	*In vivo* Hypthermic-restraint stress	Cayci & Dayioglu 2009	Macroscopic analyses showed that treatment with the extract 25, 50, and 100 mg/kg per day significantly healed lesions

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information)	3 days	induced gastritis		compared to control groups by 65, 95, and 75% (p=0.001)
Dry extract (no further information)	50-300 mg/kg BW, i.p. 3 days	*In vivo* 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 per day) compared with the untreated group ($P < 0.01$)
Extract (0.1% hypericin, no further details)	150 – 450 mg/kg BW, i.g.	*In vivo* 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* Murine 3T3-L1 preadipocytes 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	50 µg/ml	*In vitro* Murine 3T3-L1 preadipocytes	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.

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information) Hypericin Hyperforin	 0.5 and 3 µM 0.2 and 0.47 µM	Fully developed 3T3-L1 cells Human adipocytes		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* 3T3-L1 preadipocytes Fully developed 3T3-L1 cells	Hatano *et al.,* 2014	Oil Red O staining indicated that *Hypericum* extract promotes adipocyte differentiation, while immunoblots 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 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,

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				fasting blood glucose, and fasting insulin did not differ between the two groups
Dry extract (ethyl acetate, no further information)	Oral 50, 100 and 200 mg/kg BW	*In vivo* Streptozotocin induced diabetic rats	Arokiyaraj *et al.,* 2011	*H. perforatum* ethyl acetate extract showed dose dependent fall in fasting blood glucose (FBG). After 30 minutes 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* Streptozotocin induced diabetic rats Plus-maze Activity cage Modified forced swimming test 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 per day) could not restore these impaired parameters completely. *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* Streptozotocin induced diabetic rats	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

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		Tail-pinch test Tail-flick test		diabetic animals
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* Streptozotocin induced diabetic rats	Husain *et al.,* 2009	Daily oral administration of the *Hypericu*m 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 per 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* High fat diet induced obese mice. *Ex vivo* 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 i.p. 0-400 mg extract/kg BW	Humoral antibody response Blood leukocyte count 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 increased spleen index at 100 mg and 200 mg but

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				decreased at 400 mg (p>0.072)
Dry extract (ethanol 95%, no further details)	110 mg/kg oral, 2 weeks	*In vivo* 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 110 mg/kg BW, oral, 5 days	*In vitro* A549 human bronchial alveolar epithelial cells *In vivo* 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 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

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				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* 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* 0-2.5 µM *In vivo* 150 mg/kg hyperforin dicyclohexyl-ammonium salt	*In vitro* IL-2/PHA-activated T cells *In vivo* 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 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* Carrageenan treated rats	Feisst *et al.,* 2009	Hyperforin significantly suppressed leukotriene B(4) formation in pleural exudates of carrageenan-treated 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

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				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* 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; $ABTS^{\cdot}(+)$) were inhibited by *H. perforatum* (300 mg/kg, i.p.) treatment
Dry extract (ethanol 95%, no further information)	Extract 58.9 µg/ml Fractions 1.5-44.7 µg/ml	*In vitro* 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 the anti-inflammatory potential among these 4 compounds
Dry extract (ethanol 95%, no further information); 11 HPLC fractions	Extract 30 µg/ml Pseudohypericin 0.008 µM Quercetin 0.38 µM Amentoflavone 0.03	*In vitro* RAW 264.7 mouse macrophages	Huang *et al.,* 2012	siRNA was used to knockdown expression of SOCS3 in RAW 264.7 macrophages. 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-

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	µM Chlorogenic acid 0.58 µM			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 4 mg/kg BW, i.p.	*In vitro* Human lung carcinoma cells A549 *In vivo* Carrageenan induced mouse paw edema Carrageenan induced pleurisy in rats	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 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	75 µg/ml in culture medium	*In vitro* LPS-induced NO production by	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

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
information) Hyperforin		microglia and macrophages BV2 and N11 cells		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 18.6 or 46.4 µg/mouse	*In vitro* Primary human umbilical vein endothelial cells *In vivo* High glucose induced vascular inflammation	Ku *et al.,* 2014	High glucose induced markedly increased vascular permeability, monocyte adhesion, expressions of cell adhesion molecules (CAMs), formation of reactive 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 Hyperforin	Up to 10 µM	*In vitro* DPPH test *Ex vivo* Test on radical formation in pig	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

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**
		ear skin cells irradiated with solar simulated radiation		radical scavenging activity
Dry extract (0.34% hypericin, 4.1% hyperforin, 5% flavonoids, 10% tannins)	2 mg/kg BW, oral, 8 days	*In vivo* 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%) 300 mg/kg BW, oral	*In vivo* 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
Dry extract (no further details), containing 0.3% total hypericins	0.1-100 µg/ml	*In vitro* Reduction of DPPH radicals 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 per day 16 weeks, oral administration	*In vivo* Wistar rats fed a cholesterol-rich diet	Zou *et al.,* 2005	The doses of 75 and 150 mg/kg per 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	100 and 200 mg/kg	*In vivo*	Husain *et al.,* 2011b	*Hypericum* significantly lowered total cholesterol and low-

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50%, no further information), not less than 3% hyperforin, 0.3% hypericins	BW, oral, 15 days	Charles Foster rats Fructose-induced hypertriglyceridemia and insulin resistance High-fat-diet- induced obesity		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 60 days Group I: standard diet Group II: standard diet + *Hypericum* extract Group III: standard diet + *Hypericum* extract + 1% cholesterol Group IV: standard diet + 1% cholesterol Group V: : standard diet + 1%	*In vivo* 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 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

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**
	cholesterol + 10 mg/kg lovastatin			
Hyperforin	4 mg/kg i.p.	*In vivo* 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 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

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
				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 information)	20 mg/kg BW, oral; twice daily for 9 days; single dose 1 h before induction of withdrawal symptoms	*In vivo* Induced opium dependence in rats	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 ($F(2, 24) = 3.689$, $p < 0.05$), 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 minutes ($F(2, 24) = 4.88$, $p < 0.05$), 60 minutes ($F(2, 240 = 5.364$, $p < 0.01$) and 120 minutes ($F(2, 24) = 4.907$, $p < 0.05$)
Dried aqueous extract (DER app. 4:1, no further information), dry extracts with ethanol 70% and ethanol 96%	20 mg/kg BW, oral,2 times daily or single acute dose	*In vivo* 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) Hypericin, hyperforin, quercetin,	Gavage Extract 5 mg/kg Hypericin 0.016 mg/kg Hyperforin 0.21	*In vivo* 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** **Dosage** **Route of administration**	**Experimental model** ***In vivo*/** ***In vitro***	**Reference** **Year of publication**	**Main non-clinical conclusions of the authors**
amentoflavon, hyperoside	mg/kg Quercetin 0.0415 mg/kg Amentoflavon 0.0029 mg/kg Hyperoside 0.3175 mg/kg			
Extract prepared with ethanol 80%, fractionation with solvents of different polarity	1 ml/kg BW	*In vivo* 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* 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-

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**
				HT1A receptors in the facilitatory effect of *Hypericum* extract on retrieval memory
Dry extract (DER 3-7:1, methanol 80% V/V); hyperforin	25-300 mg/kg extract 1.25-2.5 mg/kg hyperforin Oral administration.	*In vivo* Conditioned avoidance response test in rats Passive avoidance response test in mice Scopolamine induced amnesia in mice Behavioural despair test in rats	Klusa *et al.,* 2001	50 mg/kg per day of extract and 1.25 mg/kg per day of hyperforin improved the learning ability from day 2 until 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* Adult rats Transfer latency in elevated plus-maze Passive avoidance test Active avoidance test Scopolamine induced amnesia 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 *Hypericum* extract 6-	*In vivo* Effect of *Hypericum* on the	Uzbay *et al.,* 2007	The highest dose of 48 mg/kg reduced significantly the locomotor activity. Lower doses did not change the activity.

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	48 mg/kg	caffeine-induced locomotor activity in mice		Doses between 6-24 mg/kg significantly blocked the caffeine (16 mg/kg) induced locomotor activity
Hyperici herba, 0.3% hypericin	350 mg/kg, 21 days, oral	*In vivo* Water maze, elevated plus maze 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* Chimney test Passive avoidance test Conditioned avoidance test 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* Water maze Barnes maze 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 days	*In vivo* Induced Parkinson's disease	Mohanasundari *et al.,* 2006	*Hypericum* in combination with bromocriptine: significant improvement in all test systems

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12:1)	Bromocriptine 10 mg/kg i.p.	Rotarod test (motor co-ordination) Hang test (neuromuscular strength) Forepaw stride length during walking		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* 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* Induced Parkinson's disease Rotational behaviour Elevated narrow beam test 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 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

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				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 potentiation. Comparator diazepam 2mg/kg p.o.	Girzu *et al.,* 1997	Marked sedation induced by the total extract. Activity of fractions was less compared to entire extract
Dry extract (ethanol 80% V/V, DER 12:1)	500 mg/kg, acute 200 mg/kg for 3	*In vivo* Passive avoidance test in rats	Tadros *et al.,* 2009b	*Hypericum* extract disrupted PPI, which is interpreted as indicator for limitations of *Hypericum* to manage cognitive disturbances in psychotic patients

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
	days	Proapoptotic and prepulse inhibition (PPI) of acoustic startle reflex experiment		
Dry extract (methanol 80%, no further details)	25-200 mg/kg BW i.p.	*In vivo* 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* 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, 4.28% rutin, 6.35% hyperoside)	5 mg/kg BW, oral, single dose	*In vivo* Mouse model induced by nitric oxide (NO) donors administration	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 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

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
				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 Hypericin 0.01 mg/kg, oral	*In vivo* Mice 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 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* Chronic constriction injury model Repeated administration of oxaliplatin	Galeotti *et al.,* 2010 (BiochemPharmacol)	*Hypericum* extract reversed mechanical hyperalgesia with a prolonged effect, being effective up to 180 minutes 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

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
				(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) SHP2: Dry extract (Ze 117, free of hyperforin) Quercetin	4 mg/kg BW, i.p. Quercetin: 25 and 100 mg/kg, i.p. 45 days	*In vivo* 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 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* 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	100 and 200 mg/kg	*In vivo*	Husain *et al.,* 2011a	Diabetic rats showed significant increase in anxiety in OFT and

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**
50%, no further information), not less than 3% hyperforin, 0.3% hypericins	BW, oral, 14 days	Strptozotocin induced diabetic rats Open-field-exploration test (OFT) Plus-maze test (EPM) Forced swimming test (FST)		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 60%, ethanol 80%)	400 mg/kg BW, 60 days, oral	*In vivo* Mice expressing mutated human amyloid precursor protein (APP) and mutated presenilinel transgenes	Hofrichter *et al.,* 2013	Extracts both attenuate Aβ-induced histopathology and alleviate memory impairments in APP-transgenic mice. 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* Rabbits 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	*In vivo*	Kumar *et al.,* 2009	Hyperforin treatment significantly ($p < 0.001$) reduced various

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	days	Rats and mice Foot shock-induced aggression Isolation-induced aggression Resident-intruder aggression Water competition test		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 substance (0.3% hypericin, up to 6% hyperforin, 2-4% flavonoids, 8-12% procyanidins)	350 mg/kg BW, oral, 3 weeks	*In vivo* Aged rats Open field test Plus maze test 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* Chronic restraint stress in rats Open field test 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

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**
				comparison with control and alleviated some other negative effects of stress on cognitive functions
Dry extract (0.3% hypericin, 3.2% hyperforin)	100 – 1000 mg/kg BW, oral	*In vivo* Mice Formalin test Tail-flick test Locomotor activity	Uchida *et al.,* 2008	Oral pretreatment with *Hypericum* attenuated significantly times of licking/biting both first and second 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* 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* Nicotine dependent mice 2 mg/kg,4 times 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%	125-500 mg/kg, oral For 30 days after	*In vivo* Nicotine dependent mice	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

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**
hypericin, 4.5% hyperforin	nicotine withdrawal; 500 mg/kg, animals not treated with nicotine	2 mg/kg,4 times daily i.p. for 2 weeks		controls. A selective 5-HT receptor 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* 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* 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 (water, no further information)	21 mg per day, oral 17 days and 57 days	*In vivo* Bone formation in the expanded	Halicioglu *et al.,* 2015	Number of osteoclasts, capillaries, inflammatory cell infiltration and new bone formation was higher in treated animals

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**
		premaxillary suture in rats		
Dry extract (0.3% hypericin, no further information) Hypericin	15 mg/kg/day, oral, 5 days 15-135 µg/kg/day, oral, 5 days	*In vivo* 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* 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 5.4 mg/kg BW i.p.	*In vitro* Neutrophils, monocytes, HUVE cells *In vivo* Matrigel sponge model of *in vivo* angiogenesis in mice	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 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,

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
		In vivo xenograft tumour growth		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* 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 information)	0.05 mg/ml, 30 minutes incubation	*Ex vivo* Aortic rings Contractile responses to phenylephrine, KCl, acetylcholine, sodium nitroprusside	Tugrul *et al.,* 2011	Although there were significant reductions in the contractile responses to phenylephrine (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

Jendželovská *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 per 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 4h. After 24h, 50% of the C_{max} was still measurable. In contrast the concentration level of isorhamnetin/tamarixetin increase much slower, the maximum was reached after 24h 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 per 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 6h (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 minutes (C_{max} : 8.6 ng/ml) for B2, and after 150 minutes (C_{max} : 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 times 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 times 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 per 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 per day), hypericin (1 mg/kg per day) and hyperforin (10 mg/kg per 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_is 0.06–5.96 μM) compared to CYP1A1 (K_is 0.20–1.6 μM) the difference in K_is 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.

Turkanovic *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).

Rašković *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 1h 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 per 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 per 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 per 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 per day or 1000 mg/kg per 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 Conceição *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 24h 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 Ca^{2+} 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.

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. However, also studies are published where no signs of toxicity have been detected. Therefore, the following text is included in section 4.6 of the WEU and TU monographs: *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.*

In section 5.3 the following statement is proposed: *Several studies on extracts of and isolated compounds from Hypericum perforatum report in vitro and in vivo effects that could affect the development of fetuses from treated mothers.*

3.4. 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 be 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 per 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.

Arsić *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 day-long 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.

Naziroğlu *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.

Naziroğlu *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 24.8-26.5h, for pseudohypericin 16.3 – 36.0h. 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 times 6 tablets) or after steady-state (3 times 1 tablet per 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 3.6h. 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 times 300 mg per 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 times 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 6-12h after a single dose of 250 mg. The terminal half-life was 15.1 to 63.1h 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. C_{max} for hyperforin was 168 ng/ml (soft capsule) and 84 ng/ml (hard capsule). T_{max} 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 h, after a dose of 0.1 mg/kg 33.8h. 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: C_{max} 3.8 ng/ml, elimination half-life 18.71 h, MRT 28.67 h

Pseudohypericin: C_{max} 10.2 ng/ml, elimination half-life 17.19 h, MRT 20.21 h

Hyperforin: C_{max} 122.0 ng/ml, elimination half-life 17.47 h, MRT 20.88 h

Quercetin: $C_{max}1$ 89.5 ng/ml, $C_{max}2$ 79.1 ng/ml, elimination half-life 2.60 h, MRT 4.68 h

Isorhamnetin: $C_{max}1$ 12.5 ng/ml, $C_{max}2$ 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: C_{max} 3.14 ng/ml, elimination half-life 23.76 h, MRT 34.74 h

Pseudohypericin: C_{max} 8.5 ng/ml, elimination half-life 25.39 h, MRT 28.17 h

Hyperforin: C_{max} 83.5 ng/ml, elimination half-life 19.64 h, MRT 21.77 h

Quercetin: $C_{max}1$ 47.7 ng/ml, $C_{max}2$ 43.8 ng/ml, elimination half-life 4.16 h, MRT 7.44 h

Isorhamnetin: $C_{max}1$ 7.6 ng/ml, $C_{max}2$ 9.0 ng/ml, elimination half-life 4.45 h, MRT 8.86 h

Steady state: Similar results.

Assessor's comment:

The following information is included in section 5.2 of the monograph:
"The absorption of hypericin is delayed and starts about 2h after administration. The time to maximum plasma concentration (T_{max}) of hypericin is 4-12h and the elimination half-life ($T_{½}$) is about 19-36h.

Maximum hyperforin levels (T_{max}) are reached about 3-4 h after administration and the elimination half-life ($T_{½}$) is about 15-63h. Hyperforin can cross the blood-brain-barrier."

4.2. Clinical efficacy

4.2.1. Dose response studies

No data available

4.2.2. Clinical studies (case studies and clinical trials)

Clinical trials related to the indication 'depression'

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 times 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: LI 160: 20.5 -> 12.2, no statistically significant difference compared to maprotiline. Maprotiline: 21.5 -> 10.5 Responder rate: 61% in *Hypericum*, 67% in maprotiline 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: LI 160: 21.8 -> 9.2, statistically significant compared to placebo ($p < 0.001$). Placebo: 20.4 -> 14.7 Responder rate: 81% in *Hypericum*, 26% in placebo 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.	

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) Responder rate: 67% in *Hypericum*, 28% in placebo	

Study	**Hänsgen & Vesper 1996**
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=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
Outcome	HAMD reduction: LI 160: 21.0 -> 8.9, statistically significant compared to placebo ($p < 0.001$). Placebo: 20.4 -> 14.4 Responder rate: 70% in *Hypericum*, 24% in placebo 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.	

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: LI 160:from 20.2 to 8.8 Imipramine: from 19.4 to 10.7 Conclusion: No significant difference between *Hypericum* and imipramine (p = 0.05).	

Study	**Vorbach *et al.,* 1997**	
Indication	Severe episode of a major depression according to ICD-10 F 33.2, recurrent, without psychotic symptoms	
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: LI 160: from 25.3±4.7 to 14.4±6.1 Imipramine: from 26.1±4.8 to 13.4±5.9 Conclusion: 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: LI 160: from 20 to 10 Amitriptyline: from 21 to 6 Conclusion: 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) 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) 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: LI 160: -40 % ± 30 %; from 21.3 ± 3.2 to 12.7 ± 6.7) Sertraline: -42 % ± 24 %, from 21.7 ± 2.7 to 12.5 ± 5.6) Conclusion: 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).	
Comment	Small number of patients, relatively high drop out rate. 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: There is no statistically significant difference between placebo and LI 160. (HAMD-score after 6 weeks). Negative Outcome. 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: LI 160: 26,5 % (for statistical significance 36,1 % is needed) Placebo: 18,6 % In the *Hypericum* group there was a significantly greater proportion of remissions. 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 times 300- 3 times 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: Placebo: -9.20/-31,9 % LI 160: -8,68/-23,9 % Sertraline: -10.53/-24,8 % 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. 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 placebo 45 female, 10 male, mean age 51.4 years, 3 drop outs, 12 protocol violations Age app. 50 ± 12 years
	Statistics	ITT yes Standard descriptive statistics (mean, standard deviations, and frequencies). Tests for treatment differences included $\chi 2$-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

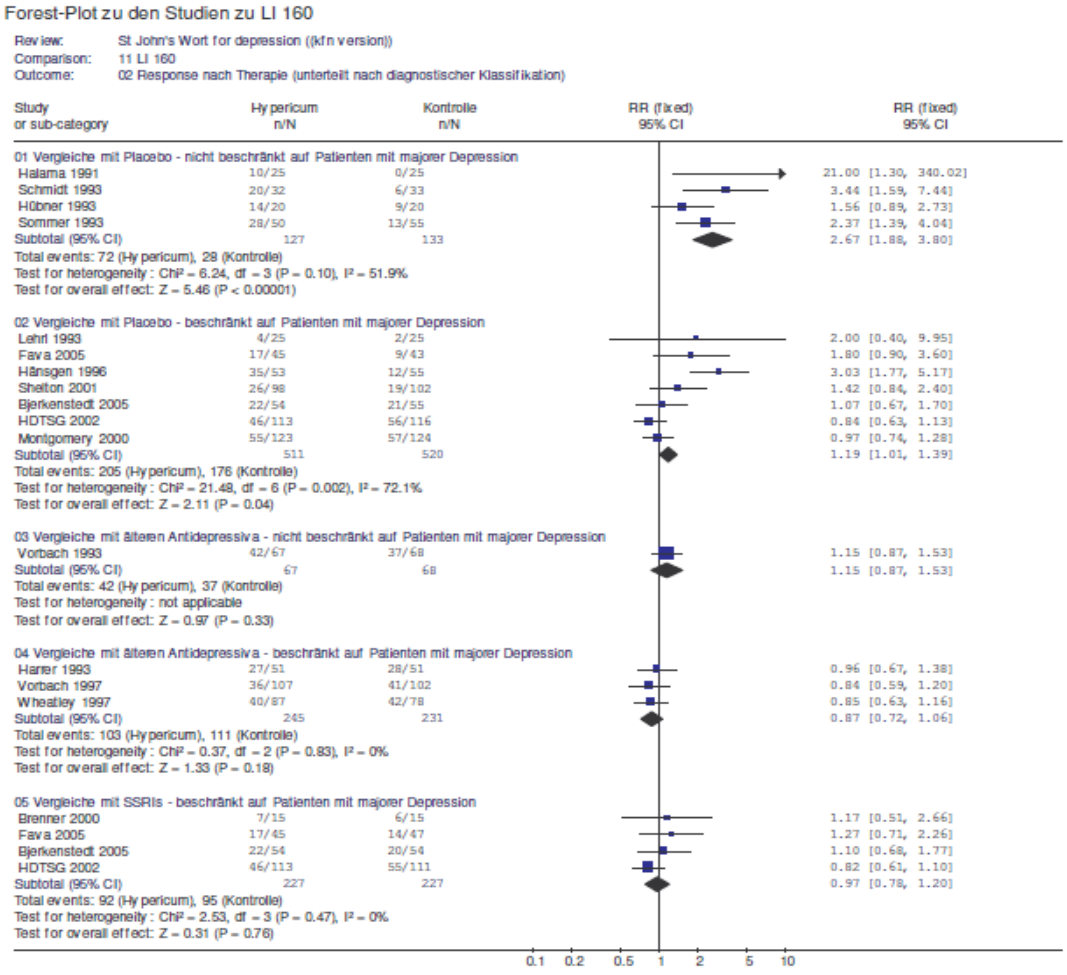
		statistical tests were two-tailed with α set to 0.05.
Outcome	HAMD reduction: LI 160: from 24.9±4.5 to 15.0±8.4 (-9.9±8.1) Fluoxetine: from 23.8±3.7 to 14.9±8.4 (-8.9±8.0) Placebo: from 25.2±4.6 to 15.5±6.7 (-9.7±7.0) Conclusion: LI 160 and fluoxetine are not more effective in short-term treatment in mild to moderate depression than placebo. *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 mean HAMD-17: 19.7 ± 3.2
	Statistics	ITT yes Significance was set at p <=0.05
Outcome	HAMD reduction (ITT-analysis): LI 160: -38 %, from 19.6± 3.5 to 10.2 ± 6.6	

	Fluoxetine: -30 %, from 19.6 ± 3.1 to 13.3 ± 7.3 Placebo: -21 %, from 19.9 ± 2.9 to 12.6 ± 6.4 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.

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 100 with moderate severity of major depression 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 No significant benefit for the sum of the atypical vegetative items (p = 0.051) 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 WS 5570: -9.9/52.7 % Placebo: -8.1/42.3 % (The difference is significant, p =0.037)) 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: WS 5570: -14.4 / 71% Paroxetine: -11.4 / 60% 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: WS 5570: .from 25.3+/-2.5 to 4.3+/-6.2	

	Paroxetine: from 25.3+/-2.6 to 5.2+/-5.5 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 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

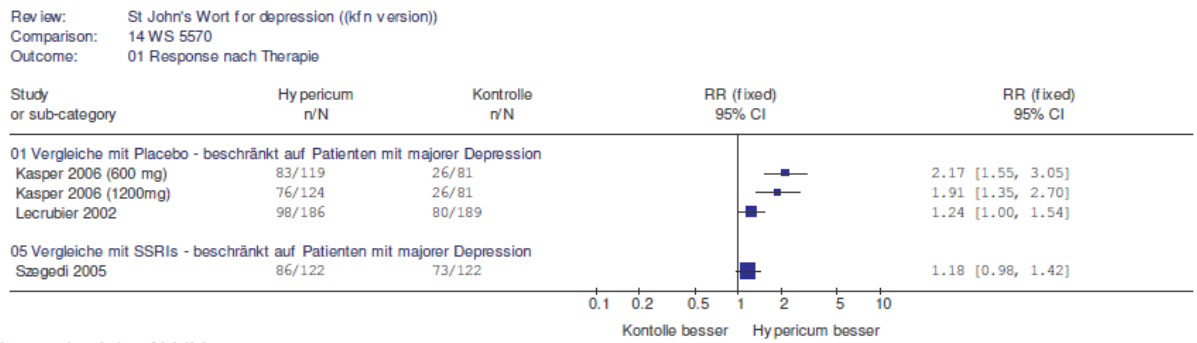
Study	**Kasper *et al.*, 2006**	
Indication	Mild or moderate major depressive episode (single or recurrent episode, DSM-IV criteria: 296.21, 296.22, 296.31, 296.32; HAMD 17-item: ≥ 18, "depressive mood" ≥ 2)	
Duration of use	6 weeks	
Daily dosage	600-1200 mg	
Single dosage	600 mg	
Relapse	-	
Study design	*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) HAMD reduction: WS 5570 600 mg: -11.6 ± 6,4 WS 5570 1200 mg: -10.8 ± 7,3 Placebo: -6.0 ± 8,1 Conclusion: WS 5570 is safe and more effective than placebo (p <0.001) in treatment of mild to moderate depression.	

Continuation study	Kasper *et al.,* 2007 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 per day, 68 patients 1200 mg per 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) Average time to relapse: *Hypericum* 177 days; placebo 163 days (ITT p = 0.035) 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):

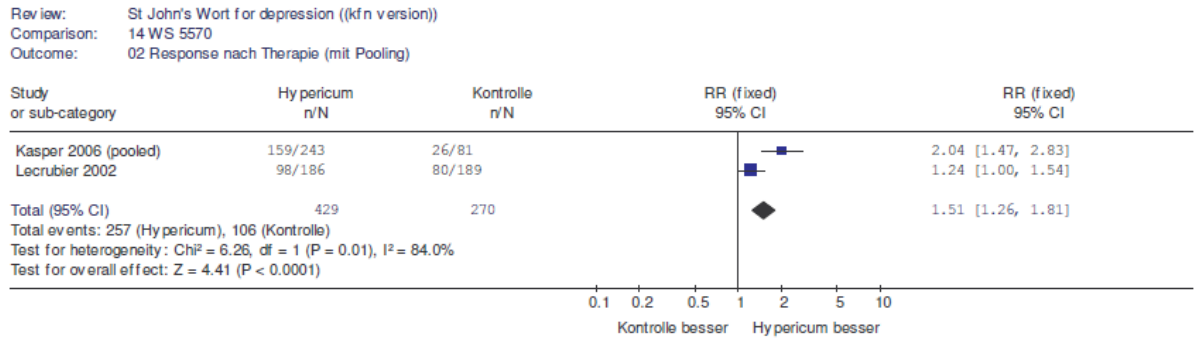
Forest-Plot zu den Studien mit WS 5570



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: WS 5572: 20.9 -> 10.7 / 49.0% WS 5573: 20.3 -> 11.8 / 38.8% Placebo: 21.2 -> 13.3 / 32.7% Conclusion: WS 5572 (5% hyperforin) was superior to placebo (p = 0.004) WS 5573 (0.5% hyperforin) and placebo were descriptively comparable 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. 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.	

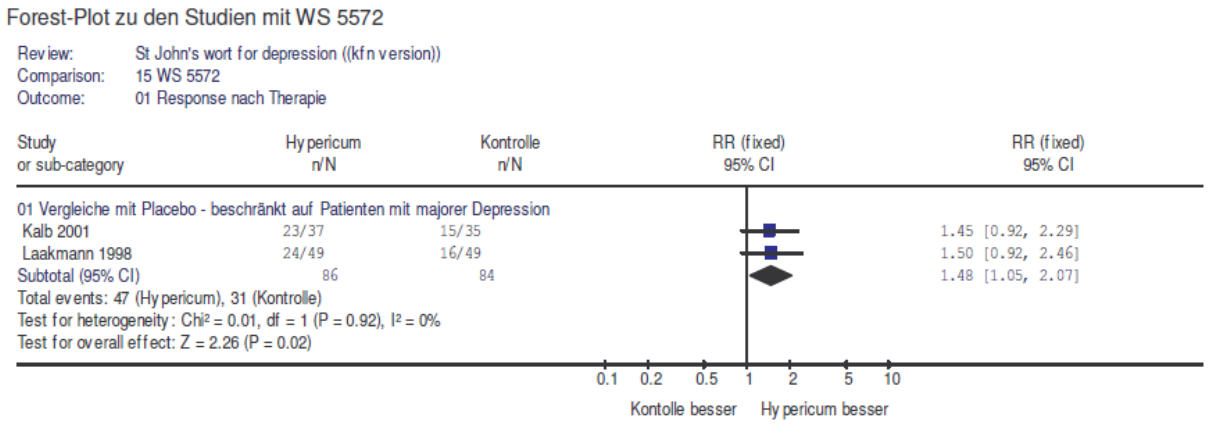
Study	**Kalb *et al.,* 2001**	
Indication	Mild to moderate major depressive disorder (according to DSM-IV criteria) (DSM-IV code: 296.21, 296.31, 296.22, 296.32, HAMD (17-items): ≥ 16)	
Duration of use	6 weeks	
Daily dosage	900 mg	
Single dosage	300 mg	
Relapse	-	
Study design	*Randomized*	Yes

	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: WS 5572: 19.7 -> 8.9 / 62.2% Placebo: 20.1 -> 14.4 / 42.9% Conclusion: Superior compared to placebo (day 28 p = 0.011, day 42 p < 0.001)	
Comment	Efficacy was already statistically significant at day 28 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) Responders: 600 mg: 83,7 %	

	1200 mg: 86,9 % 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

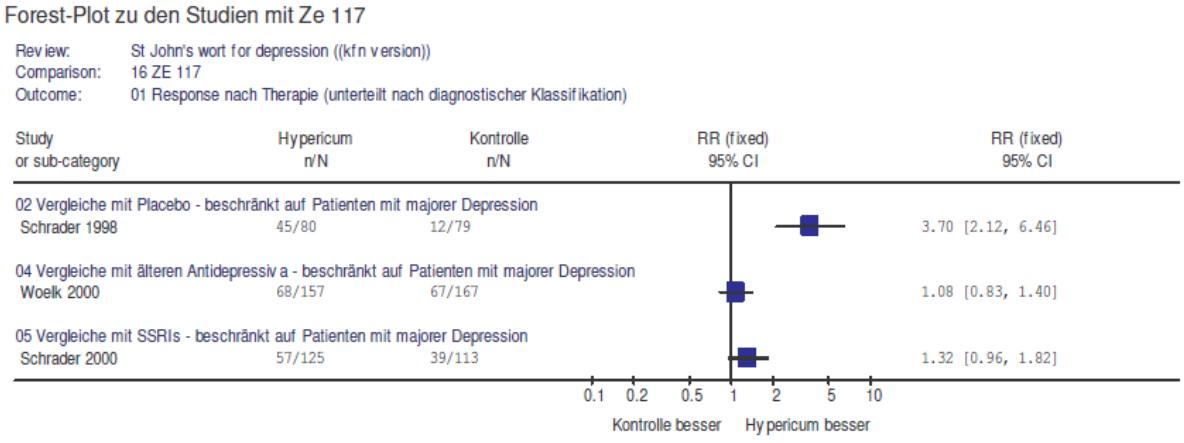
Outcome	HAMD (21-item) reduction / % responder: Ze 117: from 20.13 to 10.53 / 56% Placebo: from 18.76 to 17.89 / 15% Conclusion: ZE 117 is significantly superior ($p < 0.001$) compared to placebo and safe in treatment of mild to moderate depression
Comment	Contact of patients with investigators only 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: Ze 117: from 19.65 to 11.54/-7,25 Fluoxetine: from 19.50 to 12.20/-8,11 Conclusion: *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. 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: Ze 117: from 22.4 to 12.0 Imipramine: from 22.1 to 12,75 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, □ntil 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) STW 3: from 22.0 to 8.3/5.7 Sertraline: from 22.1 to 8.1/7.1 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:

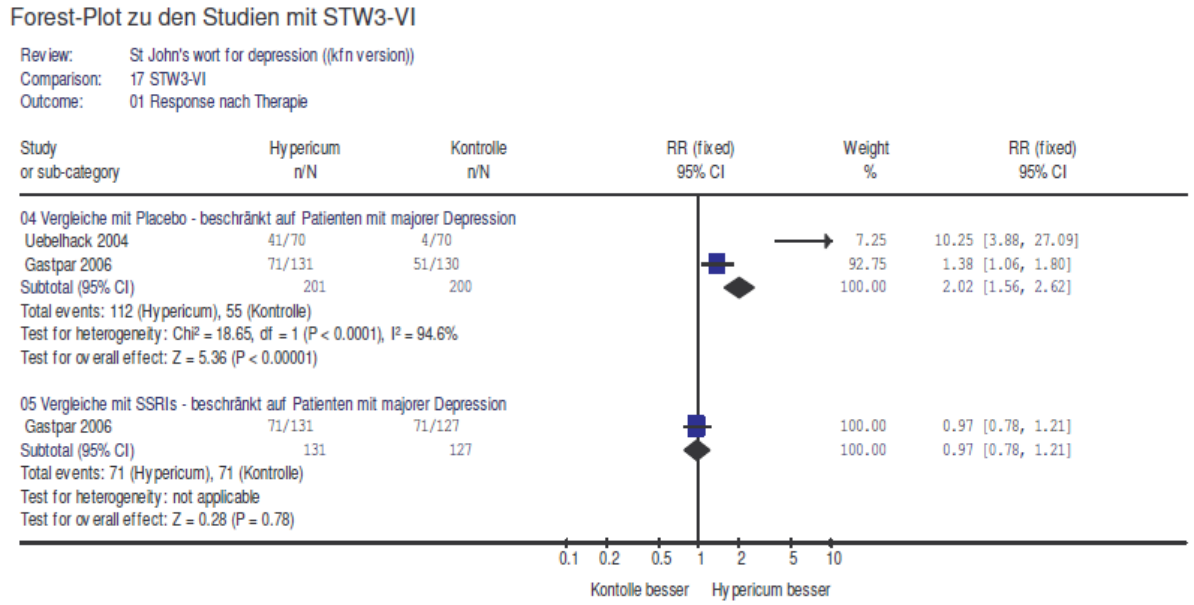
Total hypericins: mean 0.2%
Hyperforin: mean 2%
Flavonoids: mean 9%

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: STW3-VI: 22.8 -> 11.8 / 58.6% Placebo: 22.6 -> 19.2 / 5.7% 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: *Hypericum*: 21.9 -> 10.3 / 54.2% Citalopram: 21.8-> 10.3 / 55.9% Placebo: 22.0 -> 13.0 / 39.2% Conclusion: 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: STEI 300: 22.7-> 7.3 / 76% Placebo: 22.7-> 10.6 / 63% Imipramine: 22.2-> 8.0 / 66.7% 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: LoHyp 57: from 16.60 to 7.91 (mild: from 14.21 to 6.21; moderate: from 18.73 to 9.43) Fluoxetine: from 17.18 to 8.11 (mild: from 15.21 to 7.46; moderate: from 19.10 to 8.75) Responder rate: LoHyp 57: 71.4% (mild subgroup: 81.8%; moderate subgroup: 62.2%) Fluoxetine: 72.2% (mild subgroup: 76.9%; moderate subgroup: 67.5%) 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 times 300 mg 4 weeks	102; *Hypericum* 13 male, 38 female; maprotiline 16 male, 35 female	ICD-10, F 32.1, HAMD ≥ 16	HAMD reduction 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 times 300 mg 4 weeks	67; *Hypericum* 14 male, 19 female; placebo 11 male, 23 female	HAMD ≥ 16	HAMD reduction Hyp significantly superior	Per protocol Wilcoxon-Mann-Whitney U test; chi-squared test	Short duration of study
Sommer & Harrer 1994	Rand., placebo	3 times 300 mg 4 weeks	105 (no gender information)	ICD-09 300.4, 309.0	HAMD reduction Hyp significantly superior	Per protocol Wilcoxon-Mann-Whitney U test; chi-squared test	Short duration of study; only graphical presentation of data
Hänsgen & Vesper 1996	Rand., placebo	3 times 300 mg 4 weeks	101; *Hypericum* 20 male, 31 female; placebo 15 male, 35	HAMD ≥ 16	HAMD reduction Hyp significantly superior	Per protocol Mann-Whitney U test; chi-squared test	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
			female				
Vorbach *et al.,* 1997	Rand., 150 mg imipramine	3 times 600 mg 6 weeks	209; *Hypericum* 29 male, 78 female; imipramine 26 male, 76 female	ICD-10, F 33.2	HAMD reduction Equivalence of efficacy only in subgroups	PP and ITT yes	Major depression not included into the monograph
Wheatly 1997	Rand., 75 mg amitriptyline	3 times 300 mg 6 weeks	156; *Hypericum* 13 male, 70 female; amitriptyline 17 male, 56 female	HAMD 17-24	HAMD reduction No difference between treatments	ITT yes	Relevant for monograph. Supports safety
Brenner *et al.,* 2000	Rand., 75 mg sertraline	3 times 300 mg 7 weeks	30; *Hypericum* 5 male, 10 female; sertraline 6 male, 9 female	HAMD ≥ 17	HAMD reduction Hyp as effective as sertraline	ITT yes	Small number of patients, relatively high drop out rate
Montgomery *et al.,* 2000	Rand., placebo	3 times 300 mg 12 weeks	248; no gender information	Mild to moderate depression	HAMD reduction No difference between Hyp and placebo	ITT yes	Negative outcome, but in total the positive studies prevail. Publication

Reference	Study Design	Test Product(s): herbal preparation	Number of Subjects	Diagnosis	Outcomes	Statistical analysis	Comments on clinical relevance of results
							as abstract only
Shelton *et al.,* 2001	Rand., placebo	3 times 300 mg 8 weeks	200; *Hypericum* 64.9% female; placebo 62.8% female	HAMD ≥ 20	Responder rate 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 times 300 – 3 times 600 mg 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 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 49 (according to reference overall 43 male, 77 female [not	Severe major depression, HAMD ≥ 20	No significant differences in reduction of HAMD score and relapse rates between treatment groups. Sertraline and *Hypericum* are regarded as therapeutically	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
			resulting in 124]), 82 remained until end of study (*Hypericum* 24, placebo 27, sertraline 31)		equivalent.		
Bjerkenstedt *et al.,* 2005	Rand., placebo, 20 mg fluoxetine	3 times 300 mg 4 weeks	163; *Hypericum* 43 female, 11 male; fluoxetine 41 female, 13 male; placebo 45 female, 10 male	DSM-IV 296.31, 296.32	HAMD reduction No difference between study groups	ITT yes	Short duration of study, high number of drop-outs in all groups
Fava *et al.,* 2005	Rand., placebo, 20 mg fluoxetine	3 times 300 mg 12 weeks	135; *Hypericum* 45 patients, 53% women; fluoxetine 47 patients, 53% women; placebo 43	HAMD ≥ 16	HAMD reduction Hyp sign. superior to fluoxetine and placebo	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
			patients, 65% women				

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 times 300 mg 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 Hyp more effective than placebo	ITT yes	Relevant for monograph
Szegedi *et al.,* 2005	Rand., 20/40 mg paroxetine	3 times 300 mg 3 times 600 mg 6 weeks	244; *Hypericum* 37 male, 85 female; paroxetine 39 male, 83 female	DSM-IV 296.22, 296.23, 296.32, 296.33, HAMD ≥ 22	HAMD reduction Hyp and paroxetine similar effective	ITT yes	Relevant for monograph
Anghelescu *et al.,* 2006	Rand., 20/40 mg paroxetine	3 times 300 mg 3 times 600 mg 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 Hyp and paroxetine similar effective	ITT yes	Relevant for monograph, relevant for relapse prevention
Kasper *et al.,* 2006	Rand., placebo	1-2 times 600 mg 6 weeks	324; *Hypericum* 600 mg 52 male, 67 female;	DSM-IV 296.21, 296.22, 296.31, 296.32, HAMD ≥18	HAMD reduction Hyp more 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
			Hypericum 1200 mg 42 male, 82 female; placebo 25 male, 56 female		than placebo		
Kasper *et al.,* 2008	Rand., placebo	3 times 300 mg 6 weeks 52 weeks maintenance treatment	426; *Hypericum* 76 male, 206 female; placebo 35 male, 109 female	ICD-10 F33.0, F33.1 HAMD ≥ 20	HAMD reduction Relapse rates Hyp is superior in preventing relapse than placebo	ITT yes	Relevant for monograph, relevant for relapse prevention

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 times 250 mg 6 weeks	162; *Hypericum* 23 male, 58 female; placebo 31 male, 50 female	ICD-10 F32.0, F32.1	HAMD reduction Hyp more effective than placebo	ITT yes	Relevant for monograph
Schrader 2000	Rand., 20 mg fluoxetine	2 times 250 mg 6 weeks	240; *Hypericum* 36 male, 90 female; fluoxetine 47	ICD-10 F32.0, F32.1, HAMD 16-24	HAMD reduction Hyp and fluoxetine 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, 67 female				
Woelk 2000	Rand., 150 mg imipramine	2 times 250 mg 6 weeks	324; *Hypericum* 45 male, 112 female; imipramine 48 male, 119 female	ICD-10 F32.0, F32.1, F33.0, F33.1, HAMD ≥ 18	HAMD reduction Hyp and imipramine similar effective	ITT yes	Relevant for monograph The dosage of imipramine is relatively high, 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 12 weeks	241; *Hypericum* 123 (79.4% female), sertraline 118 (69.4% female)	ICD-10 F32.1, F33.1, HAMD 20-24	HAMD reduction 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 imipramine	3 times 350 mg 8 weeks	263; *Hypericum* 26 male, 80 female; imipramine 31 male, 79 female; placebo 9 male, 38 female	ICD-10 F32.1, F33.1, HAMD ≥ 18	HAMD reduction Hyp and imipramine similar effective, superior to placebo	ITT yes	Relevant for monograph

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 times 400 mg 6 weeks	149; *Hypericum* 10 male, 60 female; fluoxetine 10 male, 69 female	ICD-10 F32.0, F32.1	HAMD reduction 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
Uebelhack *et al.,*	Rand.,	1 times 900 mg	140;	ICD-10 F32.1,	HAMD reduction	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
2004	placebo	6 weeks	*Hypericum* 21 male, 49 female; placebo 25 male, 45 female	F33.1, HAMD 20-24	Hyp more effective than placebo		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 citalopram	1 times 900 mg 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 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 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)

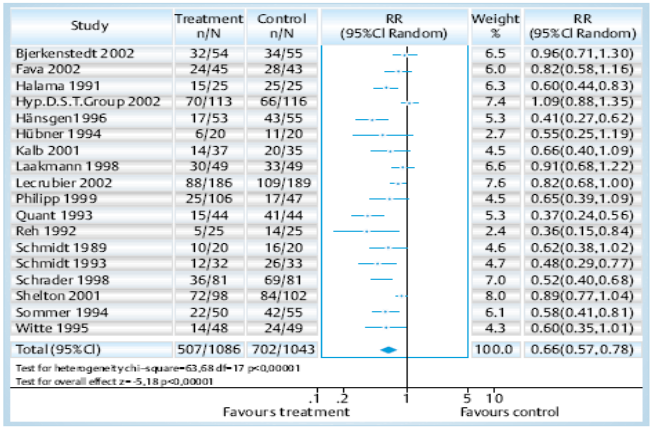
Reference	Study Design	Test Product(s): herbal preparation	Number of Subjects	Diagnosis	Outcomes	Statistical analysis	Comments on clinical relevance of results
Laakmann *et al.,* 1998	Rand., placebo, 900 mg extract low hyperforin	3 times 300 mg 6 weeks	147; *Hypericum* WS 5572 9 male, 40 female; *Hypericum*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



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).

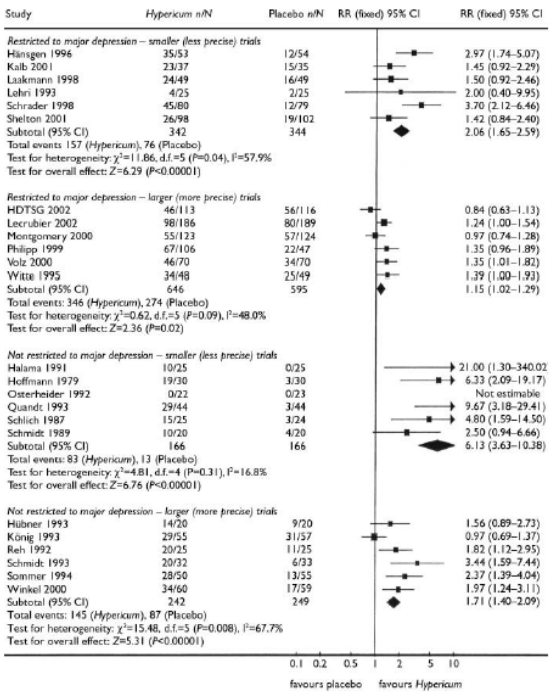


Fig. 3 Response to *Hypericum* extracts in depression. Results (fixed-effects model) from placebo-controlled trials stratified by type of depression (major and other) and study size (above and below median of variance). Studies identified by first author and year (HDTSG, Hypericum Depression Trial Study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

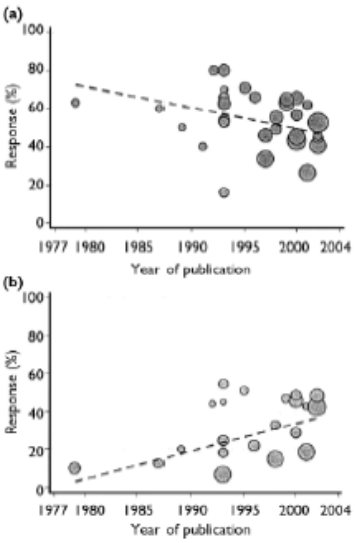


Fig. 4 Response rates over time to (a) *Hypericum perforatum* extracts and (b) placebo, from 34 active and 22 placebo trial arms.

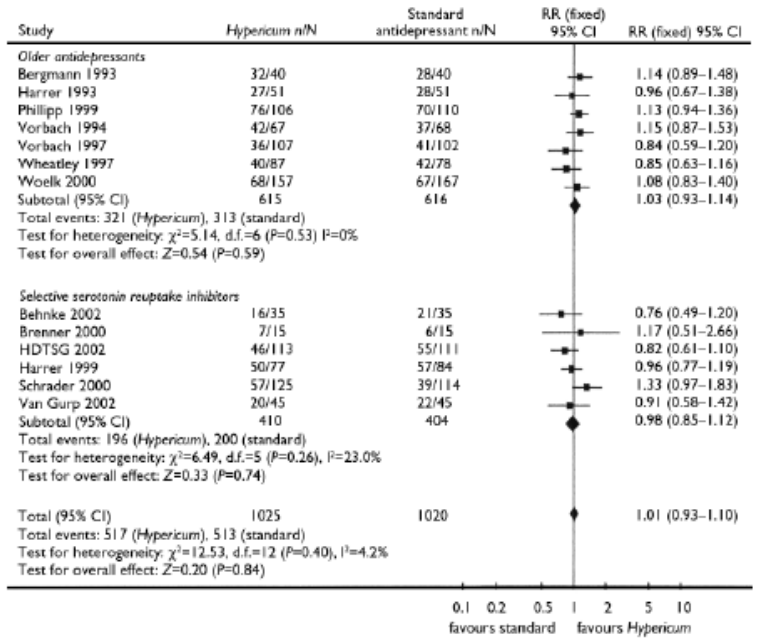


Fig. 5 Response to *Hypericum perforatum* extracts in depression: results from controlled trials stratified by type of comparison drug. Studies identified by first author and year (HDTSG, Hypericum Depression Trial Study Group; *n*, number of responders; *N*, number of patients per group; RR, response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

		solvent	Hypericins	Hyperforin	Flavonoids	dosage	
LI 160	3-6:1	methanol 80% V/V	0.12-0.28	app. 4.5%	pp. 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 relapse +
STW3-VI	3-6:1	ethanol 80% V/V	0.26% (mean)	4-5%	7.17% (mean)	900 mg	6 weeks 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.

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; Calmigen: 20.0 -> 10.0 / 55% Fluoxetine: 20.7 -> 8.7 / 66%	
Comment	Number of patients too small for a comparison of efficacy. 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.

Liquid extract, DER, extraction solvent ethanol 50%

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.

Liquid extract (1:2, ethanol 50%):

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%. Responder: *Hypericum*: 80% 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 times 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 per day
	Multicentre	n=1
	number of patients	80
	Statistics	Per protocol
Outcome	HAMD reduction / % responder: Esbericum: from 15.82 to 6.43 / 84.2% Amitriptyline: from 15.26 to 6.65 / 73.7% 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.

Dry extract (no information on DER and extraction solvent)

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. 29 patients *Hypericum* group, 27 citalopram, 25 placebo. Drop outs: citalopram 4 (side effects), *Hypericum* 1, placebo 2 Effects in all 3 groups were similar. Citalopram and *Hypericum* not superior to placebo.	
Comment	Insufficient characterisation of the herbal preparation.	

<u>Other clinical trials, oral use:</u>

Seasonal affective disorders

Martinez *et al.* (1994) compared light therapy (2h daily) and *Hypericum* (3 times 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 per 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 times 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 times 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 1h. 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 times 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 times 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 times 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 per 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 times 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 □acemose 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 Parsons *et al.* (2009) smokers received 3 times 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 per 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 times 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 times 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 times 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 times 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).

Kacerovská *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 2h 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 times 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 times 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 per 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 times 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/EC 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 '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

An overview of most important safety data/ adverse events from clinical studies is given in Table 15.

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 4 weeks	LI 160 3 times 300 mg	51 *Hypericum* 51 Maprotiline	Moderately severe depressive episodes, according to ICD-10, F 32.1 (HAMD 17-items >- 16)	*Hypericum*: 25% of patients Maprotilin: 35% *Hypericum*: gastrointestinal complaints, dizziness, confusion	Side effects considered in the monograph
Hänsgen *et al.,* 1994	Controlled study 4 weeks	LI 160 3 times 300 mg	33 *Hypericum* 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 4 weeks	LI 160 3 times 300 mg	42 *Hypericum* 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 6 weeks	LI 160 3 times 300 mg	67 *Hypericum* 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-III-R (HAMD >-	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 times 300 mg	50 Placebo	16)		monograph
Wheatley 1997	Controlled study 6 weeks	LI 160 3 times 300 mg	83 *Hypericum* 73 Amitriptyline	Mild to moderate major depression (HAMD-17 score: 17-24; according to DSM-IV)	37 % of the patients Dry mouth (5%) Drowsiness (1%) Sleepiness (2%) Dizziness (1%) Lethargy (1%) Nausea/Vomiting (7%) Headache (7%) Constipation (5%) Pruritus (2%)	Gastrointestinal and nervous symptoms considered in the monograph
Vorbach *et al.,* 1997	Controlled study 6 weeks	LI 160 3 times 600 mg	107 *Hypericum* 102 Imipramine	Severe episode of a major depression according to ICD-10 F 33.2, recurrent, without psychotic symptoms	23% of the patients n=37 Dry mouth (3) Gastric symptoms (5) tiredness/sedation (5) Restlessness (6) Tremor (2)	Gastrointestinal and nervous symptoms considered in the monograph
Czekalla *et al.,*	Randomized, placebo-	LI 160	84 patients in *Hypericum* group;	Patients suffering from depression	Imipramine: significant increase in first degree AV-blocks and	Authors conclude that *Hypericum* extract is

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Adverse reactions	Comments
1997	controlled, multicentre safety study 6 weeks	*Hypericum* extract (DER 4-7:1, methanol 80% V/V): 1800 mg daily, 6 weeks Imipramine: 150 mg daily, 6 weeks	76 patients in imipramine group Mean age 48-49 years		abnormalities of repolarization. *Hypericum*: significant decrease of such findings	safer with regard to the cardiac function than tricyclic antidepressants
Grube *et al.,* 1997	Non interventional study 5 weeks	LI 160 *Hypericum* extract (DER 4-7:1, methanol 80% V/V): 3 times 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 Single dose	LI 160 *Hypericum* extract (DER 4-7:1, methanol 80% V/V) 900, 1800 or 3600 mg; single administration	13 volunteers	Healthy volunteers	No dose-related trend in light sensitivity. Sensitivity to UV-A increased only at highest dose	Common therapeutic doses do not influence photosensitivity

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Adverse reactions	Comments
		1800 mg daily, 15 days	50 volunteers		Slight increase in sensibility	
Holsboer-Trachsler & Vanoni 1999	Non interventional study 6 weeks	LI 160 *Hypericum* extract (DER 4-7:1, methanol 80% V/V): 3 times daily 300 mg	647 patients	Patients suffering from mild to moderate depressive mood disorders	Adverse reactions in 17% Diarrhoea, Nausea (10%) Photodermatosis (3%) Headache, tiredness (7%)	Gastrointestinal and nervous symptoms considered in the monograph
Brenner *et al.,* 2000	Controlled study 7 weeks	LI 160 3 times 300 mg	15 *Hypericum* 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 12 weeks	LI 160 3 times 300 mg	124 *Hypericum* 124 Placebo	Mild to moderate depression (DSM-IV)	No information	
Shelton *et al.,* 2001	Controlled study 8 weeks	LI 160 3 times 300 mg, if no response 4 times 300 mg	98 *Hypericum* 102 Placebo	Major depression (HAMD: ≥ 20 for more than 2 years, according to DSM-IV: major depression disorder, single episode or recurrent, without psychotic features)	Headache (41%) Abdominal pain (≥ 10%)	Side effects considered in the monograph

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Adverse reactions	Comments
Hypericum depression trial study group 2002	Controlled study 8 weeks	LI 160 3 times 300 mg to 5 times 300 mg	113 *Hypericum* 111 Sertraline	Moderately severe major depressive disorder (according to DSM-IV; HAM-D ≥ 20; GAF ≤ 60)	Diarrhoea (21%) Nausea (19%) Anorgasmia (25%) Forgetfulness (25%) Frequent urination (27%) Sweating (18%) Swelling (19%)	Gastrointestinal and nervous symptoms considered in the monograph
Schempp *et al.,* 2003b	Controlled safety study 7 days	LI 160 Dry extract (methanol 80%, DER 3-6:1) In the single-dose study the volunteers received 6 or 12 coated tablets (5400 or 10800 mg hypericin). In the steady-state study the volunteers (n = 24) received an initial dose of 6 tablets (5400	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. The results do not provide evidence for a phototoxic potential of the *Hypericum*

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Adverse reactions	Comments
		mg hypericin), and subsequently 3 times 1 tablets (2700 mg hypericin) per day for 7 days.				extract
Sarris *et al.,* 2012	Continuation of the study above 26 weeks	LI 160 3 times 300 mg to 5 times 300 mg	35 *Hypericum* 49 Sertraline 40 Placebo	Moderately severe major depressive disorder (according to DSM-IV; HAM-D ≥ 20; GAF ≤ 60)	No information	
Bjerkenstedt *et al.,* 2005	Controlled study 4 weeks	LI 160 3 times 300 mg	54 *Hypericum* 54 Fluoxetine 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 Patients with adverse events: 35.1% Adverse events possibly related to study medication: 24 Body as a whole (13) Gastro-intestinal system disorders (6) Autonomic nervous system disorders (10) Central & peripheral nervous system disorders (10) Skin and appendages disorders	Gastrointestinal and nervous symptoms considered in the monograph

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Adverse reactions	Comments
					(9) Psychiatric disorders (2) Others (5)	
Fava *et al.,* 2005	Controlled study 12 weeks	LI 160 3 times 300 mg	45 *Hypericum* 47 Fluoxetine 43 Placebo	Major depressive disorder (HAMD-17 ≥ 16)	Most common adverse events. Headache (42%) Dry mouth (22%) Nausea (20%) Gastrointestinal upset (20%) Sleepiness (18%)	Gastrointestinal and nervous symptoms considered in the monograph
Mannel *et al.,* 2010	Controlled study 8 weeks	LI 160 3 times 300 mg	100 *Hypericum* 100 Placebo	Depression with atypical features	13 patients No systematic adverse drug reaction	
Lecrubier *et al.,* 2002	Controlled study 6 weeks	WS 5570 *Hypericum* extract (DER 3-7:1, methanol 80% V/V) 3 times 300 mg	186 *Hypericum* 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 Nausea (4.8%) Headache (1.6%) Dizziness (2.2%) Abdominal pain (1.1%) Insomnia (1.6%)	Gastrointestinal and nervous symptoms considered in the monograph
Szegedi *et al.,* 2005	Controlled study 6 weeks	WS 5570 900 mg or 1800 mg daily	125 *Hypericum* 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 WS 5570 900 mg (0.029) WS 5570 1800 mg (0.039)	Gastrointestinal and nervous symptoms considered in the

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Adverse reactions	Comments
					Upper abdominal pain (9.6%) Diarrhoea (9.6%) Dry mouth (12.8%) Nausea (7.2%) Fatigue (11.2%) Dizziness (7.2%) Headache (10.4%) Sleep disorder (4%) Increased sweating (7.2%) Highest incidence: Gastrointestinal disorders (59 events in 42 patients) Nervous system disorders (35 events in 29 patients) 2 serious adverse events (psychic decompensation attributable to social problems, hypertensive crisis), both not caused by *Hypericum*	monograph
Anghelescu *et al.,* 2006	Controlled study	WS 5570 900 mg or 1800	71 *Hypericum* 62 Paroxetine	Moderate to severe depression according to DSM-IV criteria: 296.22, 296.23, 296.32 and 296.33 (HAMD	26.8% of 71 no "typical adverse events (except: 1 allergic reaction to	

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Adverse reactions	Comments
	6 weeks	mg daily		17-item: ≥ 22)	sunlight → early study termination) 0.006 AE/d	
Kasper *et al.,* 2006	Controlled study 6 weeks	WS 5570 600 mg or 1200 mg	119 *Hypericum* 600 mg 124 *Hypericum* 1200 mg 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%) Serious events 1 (tendon rupture attributable to accidental injury) Ear and labyrinth disorders 3 (2.4%) Gastrointestinal disorders 24 (19.5%) General disorders and administration site conditions 2 (1.6%) Infection and infestations 7 (5.7%) Injury, poisoning and procedural complications 1 (0.8%) Investigations 1 (0.8%) Metabolism and nutrition disorders 1 (0.8%) Musculoskeletal and connective	Gastrointestinal and nervous symptoms considered in the monograph

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Adverse reactions	Comments
					tissue disorder 1 (0.8%) Nervous system disorder 6 (4.9%) Psychiatric disorders 2 (1.6%) Renal and urinary disorders 1 (0.8%) Reproductive system and breast disorders 1 (0.8 %) Respiratory, thoracic and mediastinal disorders 4 (3.3%) Skin and subcutaneous disorders4 (3.3%) Vascular disorders 1 (0.8%)	
Kasper *et al.,* 2008	Continuation of study above 12 months	WS 5570 3 times 300 mg	282 *Hypericum* 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 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	1264 patients *Hypericum*; 126 patients paroxetine; 271	Acute major depression	No significant effects on sedation, no significant anticholinergic reactions, gastrointestinal disturbances,	Percentage of patients with adverse events similar for *Hypericum* or

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Adverse reactions	Comments
		methanol 80% V/V	patients placebo		no sexual dysfunction. Highest number reported for diarrhea and nausea (1.7% of patients).	placebo, but significantly lower compared to paroxetine
Musselmann *et al.,* 2011	Open non-interventional study 8-10 weeks	WS 5570: DER 3-7:1, extraction solvent methanol 80% V/V 1 times 600 mg per day 8-10 weeks	1300 patients	Mild to moderate derpession	Incidence of adverse reactions 0.46%. No serious ADRs. In total 9 different ADRs were reported, each ADR occurred once.	No influence on monograph
Laakmann *et al.,* 1998a, Laakmann *et al.,* 1998b	Controlled study 6 weeks	WS 5572 (hyperforin 5%), WS 5573 (hyperforin 0.5%) 3 times 300 mg	49 *Hypericum* WS 5572 49 *Hypericum* WS 5573 49 Placebo	Mild or moderate depression according to DSM-IV criteria, HAMD ≥ 17	WS 5573 (28.6% of 49 patients) WS 5572 (28.6% of 49 patients) Bronchitis (3/1) Influenza-like symptoms (2/0) Cough (2/0) Infection (1/0)	No influence on monograph
Lemmer *et al.,* 1999	Open non-interventional	WS 5572 (hyperforin	6154	Mild to moderate derpession	0.7% adverse events, only 0.1% were considered causally	No influence on monograph

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Adverse reactions	Comments
	study Ca. 6 weeks	5%), WS 5573 (hyperforin 0.5%) 3 times 300 mg			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 cases of interactions were observed.	
Kalb *et al.,* 2001	Controlled study 6 weeks	WS 5572 3 times 300 mg	37 *Hypericum* 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 Bronchitis Common cold	No influence on monograph
Rychlik *et al.,* 2001	Observational study 7 weeks	WS 5572 3 times 300 mg or 4 times 300 mg	2166	Mild to moderate depression (based on Clinical Global Impression CGI scale)	17 patients n=21 (13 with relation to *Hypericum*) AEs frequency < 1% Skin irritation, pruritus Allergic exanthema Nervousness, restlessness Gastrointestinal disorders (4) Diarrhea Insomnia	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.,* 1998	Controlled study 6 weeks	Ze 117 2 times 250 mg	81 *Hypericum* 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
Schrader *et al.,* 2000	Controlled study 6 weeks	Ze 117 2 times 250 mg	126 *Hypericum* 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%) Abdominal pain (2) Moderate Diarrhoea (1) Moderate Melancholia (1) Moderate Acute deterioration (1) Moderate Dry mouth (1) Mild	Gastrointestinal and nervous symptoms considered in the monograph
Woelk 2000	Controlled study 6 weeks	Ze 117 2 times 250 mg	157 *Hypericum* 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%) Dry mouth (13) Headache (3) Sweating (2) Asthenia (2) Nausea (1)	Gastrointestinal and nervous symptoms considered in the monograph
Brattström *et al.,* 2009	Open trial Up to 1 year	Ze 117 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

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Adverse reactions	Comments
		1 times 500 mg				
Gastpar *et al.,* 2005	Controlled study 6 weeks	STW3 612 mg daily	123 *Hypericum* 118 Sertraline	Moderate depressive disorder (according to ICD-10 criteria: F32.1 or F33.1; HAMD 17-items: 20-24)	9.8% related to study medication Diarrhoea (1) Serious adverse events (3): shoulder blade after falling down the stairs, somatic disorder, cerebral haemorrhage	Gastrointestinal and nervous symptoms considered in the monograph
Uebelhack *et al.,* 2004	Controlled study 6 weeks	STW3-VI 1 times 900 mg	70 *Hypericum* 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 12 weeks	STW3-VI 1 times 900 mg	4188 patients	Moderate depressive disorders ICD-10 F 32.0: 34.8% F 33.0: 16.1% F 32.1: 32.7% F 33.1: 10.9% 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	*Hypericum* extract (DER 5-8:1, ethanol	4337 patients	Depressive outpatients	Adverse reactions in 0.09% (= 4 patients), no further details. No reports for drug interactions.	No influence on monograph

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Adverse reactions	Comments
	12 weeks	50% V/V) 600 mg daily				
Gastpar *et al.,* 2006	Controlled study 6 weeks	Dry extract (DER 3-6:1, ethanol 80%, STW3-VI) 1 times 900 mg	131 *Hypericum* 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% Total AEs. 58 Related: 10 Gastrointestinal disorders (6) Ear and labyrinth disorders (1) Skin and subcutaneous tissue disorders (1)	Gastrointestinal and nervous symptoms considered in the monograph
Schulz *et al.,* 2006a	Open trial Determination of the minimal erythema dose 2 weeks	Dry extract (DER 3-6:1, ethanol 80%, STW3-VI) Dry extract (DER 5-8:1, ethanol 50%, STW3) 1 tablet per day (no further information)	20	healthy volunteers	No significant changes in the minimal erythema dose	No influence on monograph
Kresimon *et al.,* 2012	Non-interventional study	STW3-VI *Hypericum* extract (DER 5-	281 *Hypericum* 128 other SSRIs	Moderate depression	97% of the *Hypericum* patients rated the tolerability with very good or good compared to	No influence on monograph

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Adverse reactions	Comments
	6 months	8:1, ethanol 80% V/V) 1 times 900 mg daily			86.4% in the SSRI group. 2.3% of the adverse events were rated as potentially caused by *Hypericum*	
Philipp *et al.,* 1999	Controlled study 8 weeks	STEI 300 3 times 350 mg	100 *Hypericum* 105 Imipramine 46 Placebo	Moderate depression according to ICD-10 (codes F32. 1 and F33.1) (HAMA score ≥ 18)	0.5 events per patient (22%) most frequently reported adverse event: Nausea	Gastrointestinal and nervous symptoms considered in the monograph
Schempp *et al.,* 2000a	Randomised, prospective safety study 24h	*Hypericum* oil containing 110 µg/ml hypericin 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 Skin types II and III	No change of minimal erythema dose (visual erythema score). 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.,* 2010	Open non-interventional study 12 weeks	Dry extract, DER 3.5-6:1, extraction solvent ethanol 60% (m/m)	1778 patients included, 1541 finished the study	Depressive episodes F32.0-F33.9 in 83.3% of the patients	Incidence of adverse reactions 3.54%. No serious ADRs. 65% of the patients rated the tolerance as 'very good'. Only ADR reported by >1% of	Gastrointestinal and nervous symptoms considered in the monograph

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Adverse reactions	Comments
		Mean daily dose app. 750-822.5 mg 12 weeks			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%	
Köppel *et al.,* 2008	Open trial Determination of the minimal erythema dose 2 weeks	Dry extract (ethanol 60% V/V, DER 3.5-6:1) 180 mg, 2 times daily	20	healthy volunteers	No significant changes in the minimal erythema dose	No influence on monograph
Reuter *et al.,* 2008	Controlled study 24h	Bath oil containing a *Hypericum* extract (sypercritical CO2, 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 Selective serotonin reuptake inhibitors (SSRIs) is between 20% and 50%, including more serious side effects.

5.2. Patient exposure

Patients included in clinical trials according to specified extract:

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 per 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 to take *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 α in a patient with chronic hepatitis C infection. Although the authors admit that interferon α 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.

Yildirim & 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 per 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, CYP2B6, CYP2C9, CYP2C19 and P-glycoprotein. The concomitant use with coumarin-type anticoagulants, cyclosporine, tacrolimus for systemic use, fosamprenavir, indinavir and other protease inhibitors in the treatment of HIV infection, irinotecan, imatinib and other cytostatic agents metabolised by CYP3A4, CYP2B6, CYP2C9, CYP2C19 or transported by P-glycoprotein is therefore contraindicated.

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 several enzymes of the CYP-family. Induction of CYP enzymes and P-glycoprotein is most probably caused by hyperforin via activation of the pregnane X receptor (Mueller *et al.,* 2006, Pal & Mitra 2006, Izzo & Ernst 2009, Izzo 2012). Table 16 summarises the available published data.

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

Reference	**Study Design**	**Test Product(s): herbal preparation, pharmaceutical form;** **Dosage**	**Probe drug**	**Number Subjects**	**Outcome**
Bolley *et al.,* 2002	Case report	3 times 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 2 weeks	600 mg *Hypericum* extract (methanol 80%, DER 3-6:1)	Tacrolimus 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 per day to 8 mg per day was necessary. The pharmacokinetics of mycophenolate mofetil remained unchanged
Hebert *et al.,* 2004	Controlled study 18 days	3 times 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 times 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 times 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 returned to normal immediately
Barone *et al.,* 2000	Case report	1-2 times daily 300 mg (0.3% hypericin, no further information)	Ciclosporin	Patient 54 months after kidney and pancreas	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

Reference	Study Design	Test Product(s): herbal preparation, pharmaceutical form; Dosage	Probe drug	Number Subjects	Outcome
				transplantation	returned to therapeutic levels a chronic rejection developed
Ruschitzka *et al.,* 2000	Case report	3 times 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 times 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 times 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 further information)	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 levels after stopping the intake of the herbal tea
Bauer *et al.,* 2003	Controlled study 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, C_{max} and C_{trough} values decreased by 46%, 42% and 41%, respectively
Mai *et al.,* 2004	Controlled study	3 times 300 mg, methanol 80% V/V, 3-	Ciclosporin	10 renal transplant	The low hyperforin extract did not alter significantly the pharmacokinetics of ciclosporine, while the high hyperforin extract reduced the plasma levels of

Reference	Study Design	Test Product(s): herbal preparation, pharmaceutical form; Dosage	Probe drug	Number Subjects	Outcome
	2 weeks	6:1, 2.3% hyperforin Low hyperforin extract: removal of hyperforin, 0.03% hyperforin		patients	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%
L'homme *et al.,* 2006	Controlled study 2 weeks	Herbal tea, 2 times daily	Nevirapine	36 healthy volunteers	No changes in the half-life of nevirapine
Jackson *et al.,* 2014	Controlled study 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 2 weeks	3 times daily 300 mg; extraction solvent methanol 80%, DER 3-6:1	Ritonavir (CYP3A inhibitor)	12 healthy volunteers	Probe drug midazolam Combined administration of inducer and inhibitor resulted in a predominance of enzyme inhibition: co-administration of *Hypericum* 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

Reference	Study Design	Test Product(s): herbal preparation, pharmaceutical form; Dosage	Probe drug	Number Subjects	Outcome
					decreased to 33% of the values observed during combined administration ($P < 0.001$ for each)
Piscitelli *et al.,* 2000	Controlled study 2 weeks	3 times 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%
Goey *et al.,* 2014	Controlled study 2 weeks	3 times 300 mg, no information regarding type of herbal preparation; 0.36-0.84 mg hypericin and 9-19 mg hyperforin per tablet	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
Frye *et al.,* 2004	Controlled study 2 weeks	3 times 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 2 weeks	3 times daily 300 mg for 2 weeks, no further information	Imatinib	10 healthy volunteers	*Hypericum* reduced in 10 healthy volunteers the AUC, C_{max} and half-life of imatinib
Mathijssen *et al.,* 2002	Controlled study 18 days	900 mg *Hypericum* extract (no further information)	Irinotecan	5 cancer patients	Plasma levels of the active metabolite SN-38 decreased dramatically

Reference	Study Design	Test Product(s): herbal preparation, pharmaceutical form; Dosage	Probe drug	Number Subjects	Outcome
Hall *et al.,* 2003	Controlled study 3 cycles	*Hypericum* extract (food supplement, 900 mg per day)	Ethinylestradiol Norethindrone 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 ± 19.5h to 12.2 ± 7.1h, 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 contraceptive efficacy. The authors interpret the changes in pharmacokinetics of norethindrone and ethinylestradiol as 'modest'
Pfrunder et al., 2003	Controlled study 2 cycles	2 times 300 mg or 3 times 300 mg *Hypericum* extract daily (LI 160: DER 3-6:1, methanol 80% V/V)	Ethinylestradiol Desogestrel	18 women	No change in follicle maturation, serum estradiol or progesterone concentrations were found. Significantly more subjects reported intracyclic bleeding, the AUC and C_{max} of ethinyl estradiol remained unchanged, whereas the AUC and C_{max} 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 Dienogesterol	1 woman	Unwanted pregnancy
Will-Shahab	Controlle	2 times 250 mg, ethanol 50% V/V, DER	Ethinylestradiol	16 healthy female	*Hypericum* intake resulted in a small loss of bioavailability but remained

Reference	Study Design	Test Product(s): herbal preparation, pharmaceutical form; Dosage	Probe drug	Number Subjects	Outcome
et al., 2009	d study 2 weeks	4-7:1, < 1mg hyperforin per daily dose	Desogestrel	volunteers	within the limits for bioequivalence
Fogle *et al.,* 2006	Controlled study 2 cycles	3 times daily 300 mg extract (standardised to 0.3% hypericin and 3.7% hyperforin)	Ethinylestradiol Norethindrone	15 healthy women	No significant changes in the androgene levels and in the level of sex hormone-binding globuline (SHBG) were observed
Murphy *et al.,* 2005	Controlled study 2 cycles	3 times 300 mg, alcoholic extract, 0.3% hypericin, 3.7% hyperforin	Ethinylestradiol 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 2 weeks	3 times 300 mg *Hypericum* (no further information)	Clopidogrel	15 *Hypericum* 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 2 weeks	3 times 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	3 times 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

Reference	Study Design	Test Product(s): herbal preparation, pharmaceutical form; Dosage	Probe drug	Number Subjects	Outcome
	11 days				
Jiang *et al.,* 2004	Controlled study 2 weeks	each tablet equivalent to 1 g flowering herb; dry extract containing 0.825 mg hypericin and 12.5 mg hyperforin per tablet; 3 times daily 1 tablet	Warfarin	12 healthy volunteers	*Hypericum* significantly induced the apparent clearance: the apparent clearance from S-warfarin changed from 198 ml/minutes to 270 ml/min, form R-warfarin it changed from 110 ml/minutes to 142 ml/min
Lei *et al.,* 2010	Controlled study 2 weeks	325 mg, 3 times 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 2 weeks	3 times 300 mg *Hypericum* (0.3% hypericin; no further information)	Quazepam	13 healthy volunteers	*Hypericum* significantly decreased C_{max} and AUC of quazepam. However, the pharmacodynamics profile of quazepam remained unchanged
Johne et al., 2002b	Controlled study 2 weeks	3 times 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

Reference	Study Design	Test Product(s): herbal preparation, pharmaceutical form; Dosage	Probe drug	Number Subjects	Outcome
Bonetto *et al.,* 2007 Evans (2008)	Case report Reply to the 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 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
Lantz *et al.,* 1999	Case report	900 mg, no further information	Sertraline Nefazodone	5 depressant patients	The patients developed a serotonin syndrome after combining prescribed antidepressants with *Hypericum*
Waksman *et al.,* 2000	Case report	600 mg per 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 1998	Case	600 mg powder per	Paroxetine	1 depressant	A female patient (50 years old) stopped taking paroxetine 40 mg and started

Reference	Study Design	Test Product(s): herbal preparation, pharmaceutical form; Dosage	Probe drug	Number Subjects	Outcome
	report	day, no further information		patient	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 Up to 47 days	LI 160 (3 times 300 mg per 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 2 weeks	3 times daily 300 mg dry extract (methanol 80%, DER 3-6:1)	Ketamine	12 healthy volunteers	*Hypericum* decreased the AUC by 58% and C_{max} by 66%. The decrease was not associated with significant changes in the analgesic or behavioral effects of ketamine
Galeotti *et al.,* 2014	Controlled study 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 al.,* 2009	Controlled study 2 weeks	500 mg of powdered Hyperici herba with a content of 0.06 mg total hyperforin per	Midazolam	20 healthy volunteers	The pharmacokinetic parameters of the probe drug midazolam did not change significantly

Reference	Study Design	Test Product(s): herbal preparation, pharmaceutical form; Dosage	Probe drug	Number Subjects	Outcome
		capsule			
Imai *et al.,* 2008	Controlled study 2 weeks	3 times 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	Controlled study 2 weeks	3 times 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 C_{max} 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	Controlled study 2 weeks	LI 160 (3 times 300 mg per 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	Controlled study 3 weeks	2 times 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 2h 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.,* 2011	Controlled study 2 weeks	3 times 325 mg (no further information)	Repaglinide	15 healthy volunteers	Volunteers with specific solute carrier organic anion transporter family member 1B1 (SLCO1B1) genotypes *Hypericum* did not influence the pharmacokinetic parameters of a single dose of repaglinide. Also no changes in the pharmacological effects of repaglinide were observed

Reference	Study Design	Test Product(s): herbal preparation, pharmaceutical form; Dosage	Probe drug	Number Subjects	Outcome
Sugimoto *et al.,* 2001	Controlled study 2 weeks	3 times 300 mg *Hypericum* extract (0.3% hypericin, no further information)	Simvastatin 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 4 weeks	3 times 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 4 weeks	3 times 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
Wang *et al.,* 2009	Controlled study 2 weeks	3 times 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	Controlle	3 times 300 mg dry extract, methanol 80%	Verapamil	8 healthy	*Hypericum* significantly decreased the bioavailability of R- and S-verapamil in healthy volunteers. This effect is caused by induction of first-pass CYP3A4

Reference	Study Design	Test Product(s): herbal preparation, pharmaceutical form; Dosage	Probe drug	Number Subjects	Outcome
et al., 2004	d study 2 weeks	V/V, DER 4-7:1		volunteers	metabolism
Schwarz *et al.,* 2007	Controlled study 12 days	3 times 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 10 days	3 times 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 10 days	3 times daily 300 mg extract (methanol 80%, DER 3-6:1)	Ambrisentan	20 healthy volunteers (10 CYP2C19 extensive, 4 poor and 6 ultrarapid metabolisers)	Ambrisentan is metabolized by CYP2C19. Midazolam was used as probe drug in order to quantify changes in CYP3A4 activity. At 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 2 weeks	3 times 300 mg extract (methanol 80%, DER 3-6:1)	Ivabradine	12 healthy volunteers	AUC and C_{max} were significantly decreased

Reference	Study Design	Test Product(s): herbal preparation, pharmaceutical form; Dosage	Probe drug	Number Subjects	Outcome
Xie *et al.,* 2005	Controlled study 10 days	3 times 300 mg, no further information	Fexofenadine 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 2 weeks	3 times 300 mg, 0.3% hypericin, no further information	Fexofenadine	12 healthy volunteers	After the single dose the C_{max} of fexofenadine increased while the oral clearance decreased significantly. Long term administration resulted in a 35% decrease of C_{max} and a 47% increase in oral clearance
Rengelshausen *et al.,* 2005	Controlled study 15 days	LI 160 (3 times 300 mg day day	Voriconazole	16 healthy volunteers	Day 1: Short-term clinically irrelevant increase in voriconazole parameters. This is limited to the absorption phase of voriconazole Day 15: Extensive reduction of voriconazole exposure
Burstein *et al.,* 2000	Controlled study 3 weeks	3 times 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 2 weeks	3 times 300 mg, 0.3% hypericin, no further information	Mephenytoin Caffein	6 extensive metabolisers of CYP2C19 and 6 poor	*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

Reference	Study Design	Test Product(s): herbal preparation, pharmaceutical form; Dosage	Probe drug	Number Subjects	Outcome
				metabolisers	
Van Strater & Bogers 2012	Case report	3 times 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 15 days	3 times 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.,* 2007a	Controlled study 3 weeks	3 times 300 mg standardised to 0.3% hypericin	Ibuprofen	8 healthy volunteers	*Hypericum* had no apparent impact on the pharmacokinetic parameters of ibuprofen. It is concluded that *Hypericum* does not influence CYP2C9
Wang *et al.,* 2004b:	Controlled study 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:	Controlled study 2 weeks	3 times 300 mg, methanol 80% V/V, 3-6:1	Cortisol Dextrometorphan	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

Reference	Study Design	Test Product(s): herbal preparation, pharmaceutical form; Dosage	Probe drug	Number Subjects	Outcome
Bauer *et al.,* 2002	Controlled study 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	Controlled study 4 weeks	3 times 300 mg standardised to 0.3% hypericin	Prednisone Prednisolone	8 healthy volunteers	Although the test substances are metabolised by CAP3A4 no influence on the pharmacokinetic parameters was observed
Lundahl *et al.,* 2009	Controlled study 2 weeks	2 times 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 C_{max}, 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 10 days	900 mg extract (methanol 80%, DER 3-6:1)	Digoxin	13 healthy volunteers *Hypericum* 12 Placebo	*Hypericum* treatment resulted in a decrease of digoxin AUC by 25%. After multiple dosing a reduction in trough concentration (33%) and C_{max} (26%) was observed, the effects were time-dependent

Reference	Study Design	Test Product(s): herbal preparation, pharmaceutical form; Dosage	Probe drug	Number Subjects	Outcome
Dürr *et al.,* 2000	Controlled study 2 weeks	3 times 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 *al.,* 2004a	Controlled study 2 weeks	Dry extract (methanol 80%, 3-6:1, LI 160): 28.9 mg hyperforin / day Hyperforin-containing powder: 21.1 mg hyperforin per day *Hypericum* oil: 3 times 2 capsules containing 200 mg oil extract each. 0.13 mg hyperforin per day. Tea (prepared from 1.75 g): 2 times 1 cup. 0.04 mg hyperforin per day. Hyperforin-reduced powder: 2 g per day: 0.3 mg hyperforin per	Digoxin	96 healthy volunteers	Co-medication with 2 g powder without hyperforin, tea, juice, oil extract, hyperforin-free extract (Ze 117), 1g or 0.5 g hyperforin-containing powder: No significant interaction Co-medication with LI 160: reduction of AUC -24.8%, C_{max} -37%, Ctrough -19% Co-medication with 4 g hyperforin-containing powder: Similar interaction compared to LI 106

Reference	Study Design	Test Product(s): herbal preparation, pharmaceutical form; Dosage	Probe drug	Number Subjects	Outcome
		day Fresh plant juice: 2 times 10 ml. 3.56 mg hyperforin per day Dry extract (ethanol 50%, 4-7:1, Ze 117): 0.38 mg hyperforin / day			
Nebel *et al.,* 1999	Case report	300 mg per day, no further information	Theophylline		The patient was established to 2 times 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 per day). After stopping *Hypericum* the plasma concentration of theophylline increased about 100% after 7 days
Morimoto *et al.* (2004)	Controlled study 2 weeks	3 times 300 mg standardised to 0.3% hypericin	Theophylline	12 healthy volunteers	The treatment of *Hypericum* produced no significant difference compared to the control group *Assessor's comment:* *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	3 times 300 mg per day, 900 µg hypericin per capsule, no further	Tolbutamide Caffeine	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

Reference	Study Design	Test Product(s): herbal preparation, pharmaceutical form; Dosage	Probe drug	Number Subjects	Outcome
	2 weeks	details	Dextrometorphan Midazolam		observed
Gurley *et al.,* 2005	Controlled study 28 days	3 times daily 300 mg (no details regarding herbal preparation, 0.3% hypericin; app. 5.5 mg hyperforin per day	Midazolam Caffeine	12 elderly volunteers	*Hypericum* induced the activity of CYP3A4 and CYP2E1
Dresser *et al.,* 2003	Controlled study 12 days	3 times 300 mg (methanol 80% V/V, 3-6:1)	Midazolam Fexofenadine 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 18 days	3 times 300 mg (methanol 80% V/V, 3-6:1)	Cimetidine Carbamazepin 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 cimetidine and carbamazepine

Reference	Study Design	Test Product(s): herbal preparation, pharmaceutical form; Dosage	Probe drug	Number Subjects	Outcome
Arold *et al.,* 2005	Controlled study 11 days	240 mg extract (ethanol 60% V/V, 3.5-6:1) containing 3.5 mg hyperforin	Alparzolam (CYP3A4) Caffeine (CYP1A2) Tolbutamide (CYP2C9) 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 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 16 days	3 times daily 600 mg *Hypericum* (0.15% hypericin, no further information)	-	15 healthy volunteers	P-glycoprotein expression increased 4.2 fold from baseline
Zahner et al., 2019	Controlled study 16 days	1 times daily 500 mg dry extract ZE 117 (extraction solvent ethanol 50% m/m, DER 4-7:1), hyperforin content 0.96 mg per	Caffeine (CYP1A2), bupropion (CYP2B6), flurbiprofen (CYP2C9),	20 healthy volunteers	Probe cocktail at days 1, 8 and 17, administration of *Hypericum* extract daily from day 8 to day 17 The authors conclude that no significant changes occur, all probe drugs remained in the predefined bioequivalence range of 80-125% However, an inhibition of CYP2D6 can be seen from the data given in the

Reference	Study Design	Test Product(s): herbal preparation, pharmaceutical form; Dosage	Probe drug	Number Subjects	Outcome
		tablet	omeprazole (CYP2C19), dextromethorphan (CYP2D6), midazolam (CYP3A4), fexofenadine (P-gp).		publication. Although the authors conclude that this inhibition is considered not significant a risk of interactions with drugs metabolised by this enzyme cannot be excluded

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, Borrelli & 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 (e.g. centrally authorised medicinal products, generics with harmonsid SmPC) 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	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	SmPC: concomitant use of strong inducers of CYP3A4 may cause a lower starting dose	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 (CYP3A4, CYP2C19)
Clopidogrel	SmPC; induction of CYP2C19 may increase plasma levels of active metabolites; *Hypericum* not mentioned in SmPC	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 (CYP3A4)
Fentanyl	SmPC; induction of CYP3A4 may reduce plasma levels	Relevant
Fexofenadine	Clinical trial; not in SmPC	Relevant
Finasteride	Clinical trial; induction of CYP3A4 may reduce plasma levels	Relevant
Fluoxetine	Case report, SmPC; mild forms of pharmacodynamics interactions may be possible (serotonin 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;	Considered not relevant
Midazolam	Clinical trials; induction of CYP3A4 may reduce plasma levels	Relevant
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
Oxcarbazepine	*Hypericum* not explicitely mentioned in SmPC,	Not 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 SmPC	Indirectly considered as a warning regarding to elective surgery is taken up into the monograph
Propranolol	No published information regarding pharmacokinetic or pharmacodynamics interactions; *Hypericum* not mentioned in SmPC	Relevant
Quazepam	Clinical trial; obsolete	Considered not relevant
Quetiapine	*Hypericum* not explicitely mentioned in SmPC, but induction of CYP3A4 may reduce plasma	Relevant

	levels	
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
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 pharmacodynamic interactions	Relevant
Trazodon	Possible pharmacodynamics interactions	Relevant
Triazolam	*Hypericum* not explicitely mentioned in SmPC, but induction of CYP3A4 may reduce plasma	Relevant

	levels	
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 serotonin syndrome, possible pharmacodynamics interaction	Relevant
Zolpidem	Clinical trial; induction of CYP3A4 may reduce plasma levels	Relevant

It can be concluded that there is scientific evidence that hyperforin induces the the activity of CYP3A4, CYP2B6, CYP2C9, CYP2C19 and P-glycoprotein. Follwing the conclusions also from the EMA's Pharmacovigilance Risk Assessment Committee (PRAC) (PSUSA/00001701/201801) the evidence regarding induction of CYP1A2, CYP2D6 and CYP2E1 is currently lacking. Potential interactions should be closely monitored.

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 herbal preparations of *Hypericum* containing more than 1 mg hyperforin per daily dose:

Concommitant use contraindicated:

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, CYP2B6, 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, warfarin

- Anticonvulsants: Mephenytoin, , phenobarbital, phenytoin

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

- Antidiabetics: Gliclazide

- Antifungals: Itraconazole, voriconazole

- Antihistamines for systemic use: Fexofenadine

- Antipsychotics: Clozapine, quetiapine, risperidone, ziprasidone

- Benzodiazepines: Alprazolam, diazepam, midazolam

- Beta blockers: Carvedilol, propranolol

- 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

- Platelet aggregation inhibitors: Clopidogrel

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

- Statins: Atorvastatin, simvastatin

<u>Herbal preparations containing less than 1 mg hyperforin per daily dose:</u>

There is evidence (Zahner *et al.,* 2019) that *Hypericum* preparations containing less than 1 mg hyperforin per daily dose of 500 mg dry extract do not induce enzyme activity of CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP3A4 and P-glycoprotein. The study design is considered appropriate for interactions with CYP3A4. As no information is given on the time for maximum induction of the other enzymes the relevance of the findings for these enzymes remain unclear. In this study an inhibition of CYP2D6 has been reported. The possible omission of contraindications and warnings in the product information for this particular herbal preparation

should be assessed within procedures for marketing authorisation for a concrete product case by case.

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

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

For the monograph section 4.5 the following more general wording is proposed:

Pharmacokinetic interactions:

Daily dose of hyperforin ≤ 1 mg and duration of use <2 weeks (= TU):

As the daily intake of hyperforin is less than 1 mg and of a duration of use not longer than 2 weeks no clinically relevant interactions are reported for concomitantly administered drugs which are metabolised via CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP3A4 and P-glycoprotein. Pharmacokinetic interactions with drugs which are metabolised via other CYP-enzymes have not been investigated.

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

Daily dose of hyperforin > 1 mg (both WEU and TU):

Hyperici herba preparations induce the activity of CYP3A4, CYP2B6, CYP2C9, CYP2C19 and P-glycoprotein. Concomitant use with coumarin-type anticoagulants, cyclosporine, everolimus, sirolimus, tacrolimus for systemic use, fosamprenavir, indinavir and other protease inhibitors, nucleoside reverse transcriptase inhibitors, irinotecan, imatinib and other cytostatic agents metabolised by CYP3A4, CYP2B6, CYP2C9, CYP2C19 or transported by P-glycoprotein 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, CYP2B6, CYP2C9, CYP2C19 or P-glycoprotein (e.g., amitriptyline, fexofenadine, alprazolam, diazepam, midazolam, 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 preparations may contribute to serotonergic effects when combined with antidepressants such as serotonin reuptake inhibitors (e.g. sertraline, paroxetine) or buspirone.

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

In addition, the following information is included in the monograph section 5.2: "Hyperforin induces the activity of the metabolic enzymes CYP3A4, CYP2B6, CYP2C9, CYP2C19 and P-glycoprotein dose-dependently via activation of the PXR system. Therefore, the elimination of other drug substances may be accelerated, resulting in decreased plasma concentrations".

5.5.5. Fertility, pregnancy and lactation

Klier *et al.* (2002) report from a mother with post-natal depression. She took 3 times 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 18h 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 per 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 per 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: Ze 117, 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 times 300 mg LI 160 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 are missing in order to clarify this aspect.

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 times 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, CYP2B6, 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 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* preparations.

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

Adequate interaction studies with extracts with low hyperforin content are available. Exemptions in the wording of contraindications, special warnings and in the interactions section of the SmPC should be assessed within a concrete procedure.

No influence on the mental performance at least in case of low-hyperforin extracts was observed. *Hypericum* preparations 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'. Also the supportive treatment of mild gastrointestinal discomfort and of nervous restlessness are considered as traditional indications for oral use. The cutaneous application of oil preparations is also plausible based on long-standing medicinal use and experience.

Benefit – Risk – Assessment

Well-established use:

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 2006b). 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.

Traditional use:

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