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Pharmacological studies in an herbal drug combination of St. John's Wort (*Hypericum perforatum*) and passion flower (*Passiflora incarnata*): *In vitro* and *in vivo* evidence of synergy between Hypericum and Passiflora in antidepressant pharmacological models

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ABSTRACT

Extracts of Hypericum, Passiflora and Valeriana are used for the treatment of mild depression and anxiety.
We were interested whether a combination of Hypericum and Passiflora exerts comparable effects to Hypericum alone. We used two well-established models for investigating extracts for their anti-depressant activity, namely the effects on synaptic uptake of serotonin and the forced-swimming-test. We show here for the first time, that Passiflora significantly enhances the pharmacological potency of Hypericum in both models.
Our data suggest that anti-depressive therapeutic effects of Hypericum are possible with lower doses, when it is combined with Passiflora, than with mono-preparations of Hypericum.

1. Introduction

Extracts of *Hypericum perforatum* (St. John's Wort), *Passiflora incarnata* (passion flower) and *Valeriana officinialis* (valerian) are traditionally used for the treatment of mood and sleep disorders. For these three psychoactive herbal extracts, the extent of the evidence of their pharmacological characteristics varies greatly with most information being available for Hypericum extracts, which is one of the most sold phytomedicines in Europe.

In various models of depression, Hypericum extracts have been shown to act like conventional antidepressant drugs [1–3]. Hypericum exerts a variety of effects on central neurotransmitter systems [4,5]. The neurochemical mechanisms of the central actions of Hypericum are still debated but several components have antidepressant-like and anxiolytic-like effects in animals, or interact with neurotransmitter systems believed to be causally involved in depression, anxiety and in psychiatric illness generally. [6]. Many of the pharmacological activities appear to be attributable to the naphthodianthrone hypericin, the phloroglucinol derivative hyperforin and several flavonoids [6,7].

Passiflora is traditionally used in combination with other herbs as a mild sedative and there are limited published data relating to the pharmacology of the herbal extract alone [8]. After intra-peritoneal administration in mice, a reduction in motor activity, prolongation of sleep and anticonvulsant actions have been reported [9,10]. Sedative effects of passion flower extracts have been demonstrated in rodents [11]. Furthermore, Passiflora reveals anxiolytic effects [12–14]. The Passiflora constituent flavonoid, chrysin, has been shown to act as a partial agonist of benzodiazepine receptors and has

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anxiolytic effects in mice but is not sedative nor a muscle relaxant [15,16].

One study, [17] using extracts of all three medicinal plants (Hypericum, Passiflora and Valerian), revealed that they activate GABA neurotransmission which is a pharmacological target of anxiolytic drugs as well as being a recognized target in the pharmacological treatment of depression. Recently, Elsas et al. demonstrated that elicit GABA currents in hippocampal neurons in vitro [12].

This study is part of a series of studies that aimed to establish the pharmacological characteristics of the proprietary combination of three special extracts of *H. perforatum* (Hypericum), *P. incarnata* (Passiflora) and *V. officinialis* (Valerian) in Neurapas® balance, which is registered in different European countries for the treatment of mild depression, anxiety and sleep disorders. The daily dose of the pharmacologically-active herbal extracts in the maximal recommended daily dose of Neurapas® balance are at the lower end of the recommended dose ranges that are presented in the respective monographs of the Kommission E of the German Drug Authorities [18–20]. The monographs describe the medicinal use of these herbs which differ from each other but together include the treatment of low mood (Hypericum), anxiety (Passiflora) and sleeping disorders (Valerian). These are common co-morbidities that may share a single pathophysiology [21] and this is the therapeutic rationale for the triple combination in Neurapas® balance. A neuropharmacological rationale for the combination of herbal extracts in Neurapas® balance has been proposed [22] and is based on the evidence that the combination is considered to act by activating serotonin and GABA neurotransmission, which are established targets in the pharmacological treatment of depression [23]. A randomized placebo-controlled clinical trial demonstrated that the drug has efficacy in treating mild depression showing a significant decrease in the Hamilton Depression Score of greater than 50% (own, unpublished data).

The aim of this study was to investigate possible synergistic effects of a Passiflora extract if it used in combination with a Hypericum extract using the forced swimming test [24,25] and *in vitro* uptake of neurotransmitters in rat synaptosomes [26], where extracts of Hypericum are reported to be active in both models ([7] own data).

2. Materials and methods

2.1. Herbal extracts

We used special dry extracts of *H. perforatum* (Hypericum), *P. incarnata* (Passiflora) and *V. officinialis* (Valerian) as combined in Neurapas® balance (PASCOE pharmazeutische Präparate GmbH). In addition, one other Hypericum extract with higher hyperforin content (2.7%) was included in the tests. The extracts were prepared by validated methods and the extraction solvents are all ethanol–water based.

The Hypericum extract is a dry extract of the aerial parts of *H. perforatum* with a ratio of the herbal drug to the herbal drug preparation of 4.6–6.5:1. Its hyperforin content is specified with not more than 1.5% (low hyperforin content). The extraction solvent is ethanol 38% (m/m). The hyperforin content of the tested Hypericum extract was 1.1% (low hyperforin extract). The second tested Hypericum extract had a hyperforin content of 2.7% (high hyperforin extract).

The Passiflora extract is a dry extract of the cut and dried aerial parts of *P. incarnata* containing flowers and leaves/or fruits. The extraction was performed in 60% (m/m) ethanol. The ratio of the herbal drug to the herbal drug preparation: 6.25–7.1:1 Extraction solvent: ethanol 60% (m/m).

As Harpagophytum extract, used as a negative control here, is a dried aqueous-ethanol (40:60, vol:vol) extract (batch no. 7873/03) of the secondary storage roots of *Harpagophyti procumbens* (Hp) (PASCOE pharmazeutische Präparate GmbH). The content of the marker substance harpagoside in the native extract was calculated as 2.9% according to HPLC analysis.

The described dry extracts were used in the pharmacological studies and were dissolved in a measured small amount of dimethylsulfoxide (DMSO) and diluted in the application solution immediately prior to testing. All test doses contained the same quantity of DMSO as did all control test solutions. For animal studies, extracts were first dissolved in a small volume of 0.5% polyethylenglycol and added an equivalent amount to the vehicle control solutions.

2.2. Serotonin re-uptake in rat synaptosomal preparations

Rat cerebral cortex was dissected freshly from Sprague–Dawley rats and immediately immersed in 10 volumes of ice-cold 0.32 M sucrose, buffered with 10 mM HEPES, pH 7.4 and homogenised with a Potter–Elvehjem homogenizer. Following centrifugation at 900 ×*g* for 10 min at 4 °C the supernatant was collected and centrifuged at 10,000 ×*g* at 4 °C. The pellet from this centrifugation was then kept in 0.32 M sucrose, buffered with 10 mM HEPES, pH 7.4 on ice prior to testing. For testing, the pellet was re-suspended in Famebo buffer (121 mM NaCl, 1.8 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 11 mM glucose, 0.57 mM ascorbic acid, 0.03 mM EDTA and 50 μM paragyline) with a pH of 7.4.

Assays were carried in 180 μl Famebo buffer and each drug was tested at different final concentrations by addition of 10 μl of the diluted test substance, followed by 50 μl of the synaptosomal pellet suspension in 96-well filtration plates (Millipore Multiscreen) pre-wetted with Famebo buffer. The effect of the selective serotonin-reuptake inhibitor, Fluvoxamine® (10 μM), was tested in order to assess the potency of the test system (positive control). Following 10 min incubation, the tritiated serotonin ligand was added to a final concentration of 2 nM and the incubation proceeded for 20 min. The incubation samples were then filtered with additional Famebo buffer. The synaptosomes with the internalized radiolabel remained on the filters which were then counted in a liquid scintillation counter with 50% efficiency. Mean values and 95% confidence intervals (CI_{95}) were calculated for each concentration of test solution from 8 replicates. The specific uptake is defined astotal uptake minus uptake in the presence of 10 μM fluvoxamine. The EC_{50} values werecalculated using the following formula:

$$\text{specific uptake} = 1 - \left(I_{max} * 10^{\lg[\text{conc}]}\right) / \left(10^{-pEC50} + 10^{\lg[\text{conc}]}\right)$$

[conc] is the concentration of the test substance or dry extract.

3. Animals

All animal experiments were carried out according to the National Institute of Health (NIH) guidelines for the care and use of laboratory animals, and approved by the Institutional Animal Use and care committee at the University of Florida.

3.1. Forced swimming test

The forced swimming test (FST) is based on the observation that rats when forced to swim in a restricted space from which they cannot escape will cease apparent attempts to escape and become immobile apart from small movements necessary to keep their heads above the water [24]. This characteristic immobile posture reflects a state of despair in the rat; it is assumed that the animals have "given up hope of escaping". Immobility is reduced by a variety of antidepressant drugs including tricyclics, monamine-oxidase-inhibitors (MAOs) and selective serotonin reuptake inhibitors. There is a significant correlation between clinical potency and potency of antidepressants in the forced swimming test that is unique to this model. In order to establish the specificity of the test data, a parallel testing in the open field model is required to exclude possible effects on mobility in general.

The Hypericum extract (low hyperforin content) was administered alone or in a combination with the Passiflora extract (Hypericum:Passiflora, 2:1 (m:m)). Different groups of rats (n = 10 animals per group) were treated with a single dose of the Hypericum extract or of the combination. Different doses of each were tested and administered by gavage three times (24, 5 and 1 h) prior to testing. The tests were performed as previously described [27]. Two sets of experiments were run each with a vehicle-treated and active control groups.

3.2. Open Field test

Open Field test was performed as previously described [27] in male rats (n = 8 animals per group) following the same pretreatment regime and with the same experimental groups as in the FST. This test is essential to exclude false positive results in the FST by a non-specific motor stimulation.

3.3. Statistical analysis

The forced swim test data was analyzed using Tukey's Multiple Comparison Test.

4. Results and discussion

We investigated the effects of Hypericum extract alone or in combination with Passiflora to characterize possible synergistic effects on well established *in vitro* and i*n vivo* models used to identify anti-depressive effects of pharmacological compounds and herbal extracts.

Depressive disorders are commonly attributed to an imbalance in serotonergic neurotransmission. One approach to bring this imbalance back to balance consists in inhibiting the reuptake of serotonin into the neurons, thus increasing its extracellular levels. Serotonin transporter inhibitors have been shown to improve the clinical picture of depression. Among them are chemical entities like tricyclic antidepressants or herbal extracts as demonstrated here and by many others [28].

In the first of these experiments, the effect of pure tannins on serotonin uptake was tested in order to exclude that their presence have possible non-specific effects on the synaptic uptake of serotonin. This group of substances is commonly present in herbal extracts and is known to cause protein denaturation and may disturb *in vitro* biological experiments. The tannin mixture inhibited serotonin uptake and the IC_{50} value was 39.3 μg/ml as calculated using the maximal inhibitory effect of the mixture, which was only 61.6% of the control value. Thus, tannins were unable to reduce synaptosomal serotonin uptake completely and had effects only at concentrations greater than 50 μg/ml (see Fig. 1), which is far in excess of the levels of tannins present in the herbal extracts tested here. The almost 90% inhibitory effect of the Fluvoxamine® (the positive control) confirmed the potency of the synaptosomal system. An extract of Harpagophytum was used as a negative control to avoid false positive effects in the assay due to the general use of extract preparations dissolved in DMSO. The traditional use of Devils claw extract is not linked to anxiety and depression and is not expected to exert a specific effect on 5-HT re-uptake. As shown in Fig. 1, right column, the extract of Harpagophytum did not affect 5-HT re-uptake. In contrast, Hypericum (500 μg/ml) inhibited serotonin uptake by greater than 80% and so confirmed previous published finding [25]. These results indicate that the effect of Hypericum is a pharmacological effect and not a non-specific effect of any general herbal constituent.

In further experiments using this model, the effects of Hypericum combined with Passiflora on the inhibition of serotonin uptake were studied. Synergistic effects of this combination had been previously observed in rat brain slices (unpublished results). Different batches of the same proprietary extract were tested for their efficacy in the synaptosomal model. These different batches differed in their content of hyperforin, and were tested separately in the presence of increasing concentrations of the Passiflora extract. The results of these experiments are summarized in Table 1 and the dose response curves for Hypericum and Passiflora and the combination of both (with the low hyperforin extract) are shown in Fig. 2. As has been reported by others (for review see: [27–29]), these results demonstrate that the inhibitory potency of Hypericum extracts in this *in vitro* pharmacological model is related to the concentration of hyperforin present in the extract. The presence of the Passiflora extract to the Hypercium extract leads to a multiple increase of the potency of a low-hyperforin-containing Hypericum extract (Fig. 2); the IC_{50} value for inhibition of serotonin uptake was 88.2 μg/ml for the low hyperforin-containing Hypericum extract alone compared to a value of 14.0 μg/ml in the presence of the Passiflora extract (50 μg/ml) (Table 1).

Passiflora extract alone exhibited little potency in this model but the data reveal that the Passiflora extract exerts a novel synergistic effect on the inhibitory potency of Hypericum in this pharmacological model. The Passiflora extract exerted a dose-dependent shift of the Hypericum dose–response curve of both low- and high-hyperforin containing Hypericum extracts. The effect of the Passiflora extract was

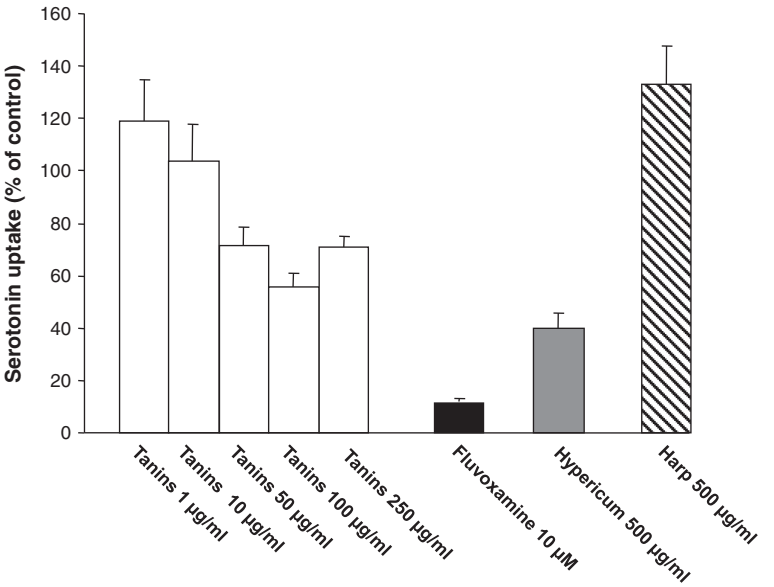

Fig. 1. Effects of tannins on the uptake of radiolabelled serotonin by rat synaptosomes (open bars) compared to single concentrations of Fluvoxamine (positive control, black bar, 10 µM), the hypericum extract (grey bar, 500 µg/ml) and an extract of *Harpagophytum procumbens* (striped bar, 500 µg/ml). The mean uptake of radiolabelled serotonin is expressed as a percentage of the mean amount taken up by untreated synaptosomes.

more pronounced for the low-hyperforin extract with the effect that the difference in potency of the low- and high-hyperforin containing Hypericum extracts was eliminated. These findings are very significant with regard to the safe medicinal use of Hypericum-containing preparations. Besides being considered to be an important active ingredient of Hypericum, hyperforin is responsible for the well-documented and well-publicized problem of drug interactions that is associated with Hypericum [30]. The Hypericum extract in Neurapas® balance contains little hyperforin, a Hypericum constituent which is highly unstable and readily degrades [31]. These studies and our data clearly demonstrated that, although the potency of the inhibitory effect of Hypericum extract on serotonin-uptake increases with increasing hyperforin content, the presence of the Passiflora can compensate for the low hyperforin-content of the Hypericum extract used here. This implies that preparations that contain Passiflora in combination with a low-dose of a low-hyperforin containing Hypericum extract can be as effective in treating mood disorders as mono-preparations of Hypericum with a high hyperforin-content.

To confirm the observed synergy between Passiflora and Hypericum *in vivo* and to prove the hypothesis that a combination Passiflora and Hypericum using low doses of Hypericum is at least as potent as high doses of Hypericum alone, we used a well-established and validated animal model for screening of antidepressant activity of drugs, the rat forced swimming test [24]. A significant correlation exists between clinical efficacy and potency of antidepressants in reducing the induced immobility that is the basis of this pharmacological model. To proof the effects in the synaptosomal assay, we used Hypericum alone and in combination with Passiflora. The extract of Passiflora was not used alone, since it already failed to show significant effects of 5-HT reuptake in the *in vitro* model.

Table 1
IC50 values of the effects of Hypericum extract (high or low hyperforin content) alone or in combination with Passiflora extract on the mean uptake of radiolabelled serotonin by rat synaptosomes.

Test extracts	Hyperforin content	Mean IC50 µg/ml
Hypericum	1.1%	88.2
Hypericum	2.7%	13.0
Hypericum + Passiflora 8 µg/ml	1.1%	23.4
Hypericum + Passiflora 8 µg/ml	2.7%	13.9
Hypericum + Passiflora 50 µg/ml	1.1%	14.0
Hypericum + Passiflora 50 µg/ml	2.7%	9.7

The results of the forced swimming test experiments are shown in Fig. 3. The Hypericum extract alone exerted a significant inhibitory effect on the immobility of the rats at the two test concentrations (180 and 360 mg/kg), which were comparable to the effect of the positive control, Imipramine® (30 mg/kg). However, the most potent inhibitory effects in this series of experiments were seen with the combination of Hypericum and Passiflora. The combination at a dose of 135 mg/kg, and which contained just 90 mg/kg of the Hypericum extract (and 45 mg/kg passiflora extract), exerted the largest inhibitory effect that was greater than that of 360 mg/kg Hypericum alone or that of 30 mg/kg Imipramine®. Higher doses of the combination of Hypericum and Passiflora were, however, less effective and exerted no significant effect. Thus, the combination of Hypericum with Passiflora exhibits a U-shaped dose–response curve in this test model. This is a well-known phenomenon for the forced swimming test model and is also observed with chemical antidepressants [32]. All test substances and mixtures were without effects in the open-field mobility test which excludes possible direct effects on motor function (not shown). Swimming and climbing was not determined in this set up [24].

We show here for the first time that Passiflora synergistically enhances the anti-depressive effects of Hypericum on

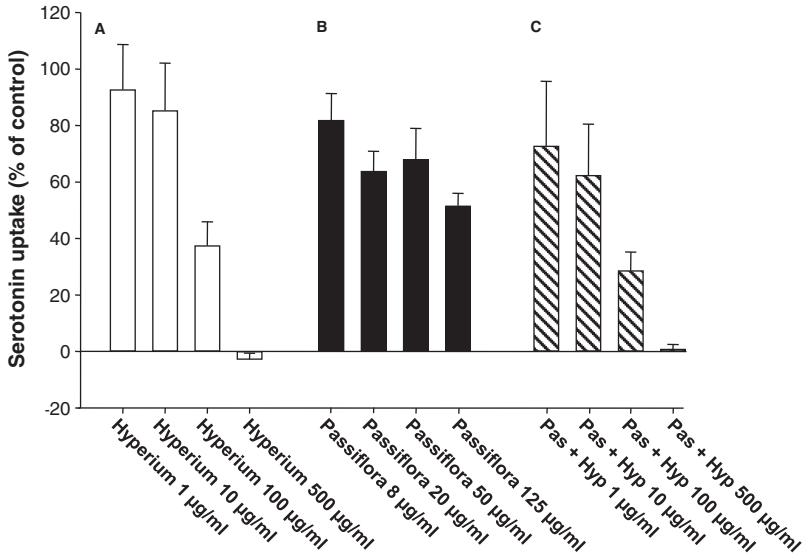

Fig. 2. Effects of Hypericum extract (low hyperforin) (A), Passiflora extract (B), and Hypericum extract (low hyperforin) in the presence of Passiflora extract (50 µg/ml) (C) on the mean uptake of radiolabelled serotonin by rat synaptosomes. The mean uptake of radiolabelled serotonin is expressed as a percentage of the mean amount taken up by untreated synaptosomes.

serotonin up-take *in vitro* and in the forced swim test *in vivo*. Passiflora extracts have been shown to reveal anxiolytic effects in models of anxiety [33–35] possibly mediated by the GABAergic system [36,37] as also shown for the triple combination of Hypericum, Passiflora and valerian in a synergistic manner [17]. So far, no reports are available of the effects of Passiflora on the serotonergic system and as anti-depressive herbal phytomedicine, whereas Hypericum is a well established anti-depressive drug (for review see [38]) in part due to the inhibition of serotonin re-uptake (for review see [29]) as also confirmed by the data shown here.

The synergistic effects of Passiflora and Hypericum on serotonin re-uptake might be explained by different binding sites on the transporter molecule affected by the extracts,

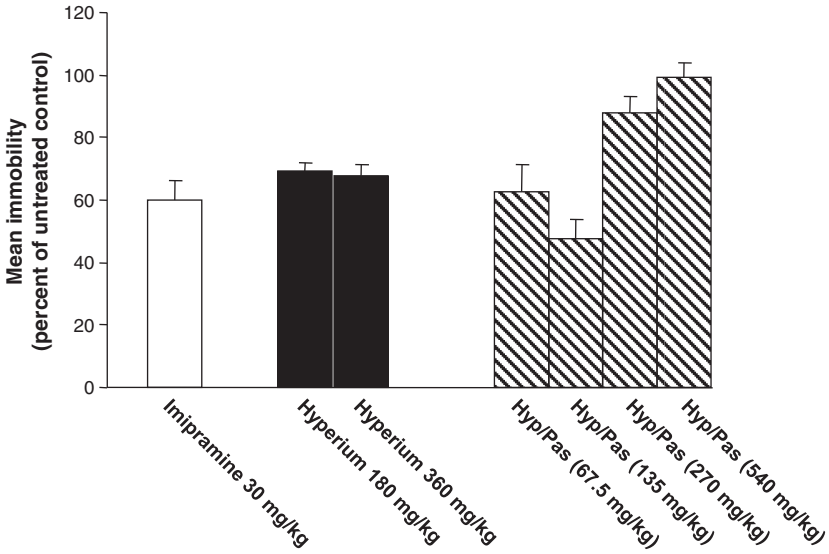

Fig. 3. Effects of Hypericum extract (black bars) and the combination of Hypericum and Passiflora extract (striped bars) on immobility in the forced swimming test. The data were obtained from two identical sequential experimental series (A and B) in each of which a group of un-treated animals and a group of Imipramine® (30 mg/kg)-treated animals (as positive control) (open-bar) were included. In each experiment, the mean immobility was expressed as a percentage of the mean immobility of the respective untreated vehicle control group (240 s in series A and 207 s in series B). In both experimental series, the positive control (Imipramine®) significantly ($p<0.01$) reduced immobility compared to control. Two doses of Hypericum extract (closed bars) each significantly ($p<0.01$) decreased immobility compared to control. Increasing doses of the combination of Hypericum and Passiflora (2:1, m:m) exhibited a U-shaped dose response curve with a significant ($p<0.05$) decrease in immobility compared to control occurring at 135 mg/kg of the combination containing 90 mg/kg Hypericum and 45 mg/kg Passiflora.

which would result in an allosteric modulation of the transporter. A similar effect is known e.g. for the binding of the two antidepressants escitalopram and R-citalopram to the serotonin transporter [39]. Modulations of the serotonin transporter activity might also result from altered phosphorylation states of the transporter molecule due to inhibition of protein kinases or phosphatases by the herbal extracts or altered protein–protein interactions caused by conformational changes after binding [40].

Further studies will reveal the mechanisms by which Passiflora synergistically enhances the effects of Hyperiucm especially on the serotonergic system.

The demonstration of this possible synergy in two separate tests for antidepressant pharmacology, one *in vitro* and the other one *in vivo*, strongly strengthens the significance of the finding which has important implications for the medicinal use of these two herbal drugs. Additionally, the data imply that the specific low-dose combinations of Passiflora and Hypericum are an alternative to high-dose Hypericum mono-preparations.

Taken together, our data prove that the anti-depressive therapeutic effects of Hypericum are possible with lower doses, when it is combined with Passiflora as in Neurapas® balance, than with mono-preparations of Hypericum. This might be due to different mechanisms by which both extracts act on the neurotransmitter system.

5. Conflict of interest statement

Financial support for the analysis was provided by PASCOE pharmazeutische Präparate GmbH, the manufacturer of Neurapas® balance. The sponsor had no influence on the conduct of the analysis. T. Kammler and G. Weiss are employees of PASCOE pharmazeutische Präparate GmbH. B. L. Fiebich and R. Knörle have received funding from PASCOE pharmazeutische Präparate GmbH.

6. Submission declaration

The article has not been published previously and is approved by all authors. If accepted, it will not be published elsewhere including electronically in the same form, in English or in any other language, without the written consent of the copyright-holder.

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References

[1] Chatterjee SS, Noldner M, Koch E, Erdelmeier C. Antidepressant activity of *Hypericum perforatum* and hyperforin: the neglected possibility. Pharmacopsychiatry 1998;31(Suppl 1):7–15.
[2] De Vry J, Maurel S, Schreiber R, de Beun R, Jentzsch KR. Comparison of hypericum extracts with imipramine and fluoxetine in animal models of depression and alcoholism. Eur Neuropsychopharmacol 1999;9:461–8.
[3] Gambarana C, Tolu PL, Masi F, Rinaldi M, Giachetti D, Morazzoni P, et al. A study of the antidepressant activity of *Hypericum perforatum* on animal models. Pharmacopsychiatry 2001;34(Suppl 1):S42–4.
[4] Burstein AH, Horton RL, Dunn T, Alfaro RM, Piscitelli SC, Theodore W. Lack of effect of St John's Wort on carbamazepine pharmacokinetics in healthy volunteers. Clin Pharmacol Ther 2000;68:605–12.
[5] Muller WE, Singer A, Wonnemann M. Mechanism of action of St. Johns wort extract. Praxis 2000;89:2111–21 (Bern. 1994).
[6] Caccia S, Gobbi M. St. John's wort components and the brain: uptake, concentrations reached and the mechanisms underlying pharmacological effects. Curr Drug Metab 2009;10:1055–65.
[7] Butterweck V. Mechanism of action of St John's wort in depression: what is known? CNS Drugs 2003;17:539–62.
[8] Dhawan K, Dhawan S, Sharma A. Passiflora: a review update. J Ethnopharmacol 2004;94:1–23.
[9] Oga S, de Freitas PC, Gomes da Silva AC, Hanada S. Pharmacological trials of crude extract of *Passiflora alata*. Planta Med 1984;50:303–6.
[10] Soulimani R, Younos C, Jarmouni S, Bousta D, Misslin R, Mortier F. Behavioural effects of *Passiflora incarnata* L. and its indole alkaloid and flavonoid derivatives and maltol in the mouse. J Ethnopharmacol 1997;57:11–20.
[11] Speroni E, Minghetti A. Neuropharmacological activity of extracts from *Passiflora incarnata*. Planta Med 1988;54:488–91.
[12] Elsas SM, Rossi DJ, Raber J, White G, Seeley CA, Gregory WL, et al. *Passiflora incarnata* L. (Passionflower) extracts elicit GABA currents in hippocampal neurons in vitro, and show anxiogenic and anticonvulsant effects in vivo, varying with extraction method. Phytomedicine 2010;17(12):940–9.
[13] Ernst E. Herbal remedies for anxiety—a systematic review of controlled clinical trials. Phytomedicine 2006;13:205–8.
[14] Akhondzadeh S, Naghavi HR, Vazirian M, Shayeganpour A, Rashidi H, Khani M. Passionflower in the treatment of generalized anxiety: a pilot double-blind randomized controlled trial with oxazepam. J Clin Pharm Ther 2001;26:363–7.
[15] Brown E, Hurd NS, McCall S, Ceremuga TE. Evaluation of the anxiolytic effects of chrysin, a *Passiflora incarnata* extract, in the laboratory rat. AANA J 2007;75:333–7.
[16] Wolfman C, Viola H, Paladini A, Dajas F, Medina JH. Possible anxiolytic effects of chrysin, a central benzodiazepine receptor ligand isolated from *Passiflora coerulea*. Pharmacol Biochem Behav 1994;47:1–4.
[17] Gramowski A, Jugelt K, Stuwe S, Schulze R, McGregor GP, Wartenberg-Demand A, et al. Functional screening of traditional antidepressants with primary cortical neuronal networks grown on multielectrode neurochips. Eur J Neurosci 2006;24:455–65.
[18] Kommission E. Monographie Hyperici herba (Johanniskraut). Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM); 1989.
[19] Kommission E. Monographie *Passiflorae herba* (Passionsblumenkraut). Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM); 1990.
[20] Kommission E. Monographie *Valerianae radix* (Baldrianwurzel). Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM); 1990.
[21] Nutt DJ, Stein DJ. Understanding the neurobiology of comorbidity in anxiety disorders. CNS Spectr 2006;11:13–20.
[22] McGregor GP. Kombination von Johanniskraut-, Baldrian- und Passionsblumen-Extrakten in einem pflanzlichen Arzneimittel. Arztezeitschrift Naturheilverfahren 2002;43:348–53.
[23] Pacher P, Kecskemeti V. Trends in the development of new antidepressants. Is there a light at the end of the tunnel? Curr Med Chem 2004;11:925–43.
[24] Porsolt RD, Anton G, Blavet N, Jalfre M. Behavioural despair in rats: a new model sensitive to antidepressant treatments. Eur J Pharmacol 1978;47:379–91.
[25] Singer A, Wonnemann M, Muller WE. Hyperforin, a major antidepressant constituent of St. John's Wort, inhibits serotonin uptake by elevating free intracellular Na + 1. J Pharmacol Exp Ther 1999;290:1363–8.
[26] Lieb K, Fiebich BL, Herpfer I, Mantovani M, Loffler M, Feuerstein TJ. No modulatory effect of neurokinin-1 receptor antagonists on serotonin uptake in human and rat brain synaptosomes. Eur Neuropsychopharmacol 2005;15:641–6.
[27] Butterweck V, Christoffel V, Nahrstedt A, Petereit F, Spengler B, Winterhoff H. Step by step removal of hyperforin and hypericin: activity profile of different Hypericum preparations in behavioral models. Life Sci 2003;73:627–39.
[28] Iversen L. Neurotransmitter transporters and their impact on the development of psychopharmacology. Br J Pharmacol 2006;147 (Suppl 1):S82–8.
[29] Muller WE. Current St John's wort research from mode of action to clinical efficacy. Pharmacol Res 2003;47:101–9.
[30] Madabushi R, Frank B, Drewelow B, Derendorf H, Butterweck V. Hyperforin in St. John's wort drug interactions. Eur J Clin Pharmacol 2006;62:225–33.
[31] Orth HC, Rentel C, Schmidt PC. Isolation, purity analysis and stability of hyperforin as a standard material from *Hypericum perforatum* L. J Pharm Pharmacol 1999;51:193–200.

[32] Butterweck V, Jurgenliemk G, Nahrstedt A, Winterhoff H. Flavonoids from *Hypericum perforatum* show antidepressant activity in the forced swimming test. Planta Med 2000;66:3–6.
[33] Grundmann O, Wahling C, Staiger C, Butterweck V. Anxiolytic effects of a passion flower (*Passiflora incarnata* L.) extract in the elevated plus maze in mice. Pharmazie 2009;64:63–4.
[34] Barbosa PR, Valvassori SS, Bordignon Jr CL, Kappel VD, Martins MR, Gavioli EC, et al. The aqueous extracts of *Passiflora alata* and *Passiflora edulis* reduce anxiety-related behaviors without affecting memory process in rats. J Med Food 2008;11:282–8.
[35] de Castro PC, Hoshino A, da Silva JC, Mendes FR. Possible anxiolytic effect of two extracts of *Passiflora quadrangularis* L. in experimental models. Phytother Res 2007;21:481–4.
[36] Grundmann O, Wang J, McGregor GP, Butterweck V. Anxiolytic activity of a phytochemically characterized *Passiflora incarnata* extract is mediated via the GABAergic system. Planta Med 2008;74:1769–73.
[37] Lolli LF, Sato CM, Romanini CV, Villas-Boas LB, Santos CA, de Oliveira RM. Possible involvement of GABA A-benzodiazepine receptor in the anxiolytic-like effect induced by *Passiflora actinia* extracts in mice. J Ethnopharmacol 2007;111:308–14.
[38] Linde K, Berner MM, Kriston L. St John's wort for major depression, Cochrane. Database Syst Rev 2008:CD000448.
[39] Zhong H, Hansen KB, Boyle NJ, Han K, Muske G, Huang X, et al. An allosteric binding site at the human serotonin transporter mediates the inhibition of escitalopram by R-citalopram: kinetic binding studies with the ALI/VFL-SI/TT mutant. Neurosci Lett 2009;462:207–12.
[40] Jayanthi LD, Ramamoorthy S. Regulation of monoamine transporters: influence of psychostimulants and therapeutic antidepressants. AAPS J 2005;7:E728–38.