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The antidepressant activity of *Hypericum perforatum* L. measured by two experimental methods on mice

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The pharmacological approach to the treatment of depression includes a long-term employment of antidepressants, either in the form of monotherapy or as a combination of several antidepressants with various mechanisms of action. *Hypericum perforatum* L. (St. John's wort) is the only natural antidepressant. Several constituents of the extract, such as hypericin and hyperforin, seem to be important for this effect. *H. perforatum* is considered to be an effective alternative to other therapeutic agents in the treatment of mild to moderate depression.

The paper describes the investigation of the antidepressant effect of *H. perforatum* (doses 7, 35 and 70 mg kg^{-1} b. m.) on mice using the forced-swimming and tail-suspension methods. As an indicator of the antidepressant effect, it was shown that the immobility time of animals in the forced-swimming and tail-suspension experiments was shorter, *i.e.* the activity of the animals was higher. With single doses of extract suspension increasing from 7 over 35 to 70 mg kg^{-1} the antidepressant effect increased in proportion by 10.1%, 25.8% and 38.6% in the swimming method, and by 12.7%, 16.5% and 24.5% in the tail-suspension method compared to controls. *H. perforatum* extract displays dose-dependent antidepressant effect at a dose as low as 7 mg kg^{-1}. Both models have proved to be equally valuable for demonstration of substances with a potential antidepressant effect.

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From the phytochemical point of view, *Hypericum perforatum* L. (*Hypericaceae*), commonly known as St. John's wort, is one of the best medicinal plants investigated. *H. perforatum* is used in many countries for the treatment of mild to moderate forms of depression (1, 2). Several clinical studies provide evidence that *H. perforatum* is as effective as conventional pharmacological antidepressants, with the great advantage that it has

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very few undesirable reactions (3, 4). Some recent studies have suggested that *H. perforatum* extract could exert an anxiolytic and antipanic effect as well (5). A series of bioactive compounds have been detected in crude material, namely phenylpropanes, flavonol derivatives, biflavones, proanthocyanidines, xanthones, phloroglucinols, naphthodianthrones, some amino acids and essential oil constituents (6).

Although *H. perforatum* has been subjected to extensive scientific studies in the last decade, there are still many open questions about the pharmacology and the mechanism of its action. In fact, the active constituents with a potential antidepressant effect are not fully known. However, recent reports have shown that hyperforin, hypericin, pseudohypericin as well as some flavonoids contribute to the antidepressant efficacy of *H. perforatum*. Therefore, several compounds from different structural groups and with different mechanisms of action are likely to be responsible for the antidepressant effect. These mechanisms seem to correspond to the pharmacodynamics of conventional antidepressants, the basic action of which is to increase monoamine and serotonine transmission in the central nervous system (7, 8). The antidepressant effect of St. John's wort seems to be a consequence of various effects, including inhibition of the inactivation of neurotransmitters and a change in the expression of monoaminergic receptors and cytokine. *H. perforatum* is only a weak inhibitor of monamine oxidase activity but it also inhibits the synaptosomal uptake of serotonine, dopamine and noradrenaline with approximately equal affinity and has also an affinity for adenosine, GABA and glutamate receptors (7, 9). *In vivo*, St. John's wort extract leads to downregulation of beta-adrenergic receptors and up-regulation of serotonin receptors in the rat frontal cortex and causes changes in neurotransmitter concentrations in the brain areas implicated in depression (7). On the other hand, divergent results were obtained regarding the influence of *H. perforatum* extract on the function of hypothalamus-pituitary-adrenal (HPA) axis whose dysregulation may be involved in the aetiology of major depressive disorder. Thus, data from some studies confirmed the changes of brain cortisol and corticosterone concentrations in the rat brain, while other experiments showed that the plant extract had no effect on the modulation of HPA axis function (10, 11).

The purpose of this experimental work on mice was to test the antidepressant effect of *H. perforatum* plant extract on mice using two experimental methods. In the present study, the amount of the applied plant extract was chosen to be lower than that commonly employed in the literature in order to minimize the side effects and to find out the lowest effective dose of the extract.

EXPERIMENTAL

Animals

The experiments involved male mice, strain BlC_{57}, approximately three months old and weighing about 30 g, under standard laboratory conditions (temperature 23 °C with changes of light and dark every 12 hours and constant access to food and water). The mice were divided into four groups for each testing method, five animals in each group. One group served as control and received 2% aqueous Tween 80 suspension, and the others received various doses of the extract suspension under the same conditions 30 min before antidepressant testing.

Test solutions

The material for the experiment was the extract of *H. perforatum*, which was a gift from Belupo, Croatia. It was a dry extract containing 0.2% hypericine. The characteristics of the extract are: grey-green powder, strong fragrance and bitter taste.

Plant extract was suspended in 2% Tween 80 immediately prior to use and given as a single intraperitoneal injection in a volume of 0.5 mL per 30 g body mass.

In this paper, all doses are expressed as mg of the extract per 1 kg body mass. The following doses were used: 7, 35 and 70 mg kg^{-1}.

Methods

Forced-swimming test. – Measurement of immobility time was carried out by observing the motoric activity of the mice, which were placed in a pool of water. A glass cylinder, 25 cm in diameter, height 23 cm, was filled with water to a height of 12 cm. The temperature of water was 23 ± 1 ºC. Each mouse was injected once with a respective dose of the extract suspension or 2% Tween 80. Thirty minutes later, the animals were subjected to the test. Measurement was carried out for six minutes; the first two minutes the animal was allowed to adjust to the new conditions; after these two minutes, the immobility time that alternated with conditions of enhanced motor activity was measured. Immobility time was measured with a stopwatch for the next four minutes (12, 13).

Immobility time is the time during which the animal floated on the surface with front paws together and made only those movements which were necessary to keep afloat.

Shorter immobility time is an indicator of the stronger antidepressant effect of the tested substance.

Tail-suspension test. – The tail-suspension test was the second method for assessing the antidepressant effect of the extract. Thirty minutes after the single drug or vehicle injection, mice were subjected to the test.

A cord of about 50 cm in length was stretched between two metal tripods at a height of ca 70 cm, to which the mice were attached by the tail with sticky tape. After the initial period of vigorous motor activity, the mice became still and the immobility time was measured with a stopwatch, for a total duration of 4 minutes (13, 14). Mice were considered immobile when they hung passively and completely motionless.

Statistics

Results are presented as means ± SD and were evaluated using Student's *t*-test for independent variables.

RESULTS AND DISCUSSION

Our pre-clinical experiments demonstrate the antidepressant effect of *H. perforatum* extract using two animal models of depression. In the present study, however, we used several times lower doses of *H. perforatum* extract than doses commonly employed in the

literature. Namely, while the highest dose in our study was 70 mg kg^{-1} b.m., doses of the extract reported in the literature usually range from 250 to 1000 mg kg^{-1}.

The results obtained after a single administration of the extract suspension showed that the immobility time of animals decreased dose-dependently, namely, the animals were more active in both employed models, which means that the antidepressant effect was stronger. For all three doses administered there were differences compared to the control, that is, they led to reduction of immobility time, in the forced-swimming method by 10.1% for 7 mg kg^{-1} to as much as 38.6% for 70 mg kg^{-1}. Similar results of increased antidepressant effect, that is, of immobility time depending on the concentration administered, were obtained with the tail suspension test (Table I).

Table I. Antidepressant effect of Hypericum perforatum *extract*

Dose (mg kg^{-1})	Forced-swimming test		Tail-suspension test	
	Immobility time[a] (s)	Change (%)	Immobility time[a] (s)	Change (%)
0 (control)	158.0 ± 8.2	–	134.5 ± 10.9	–
7	142.0 ± 19.4	–10.1	117.4 ± 17.3	–12.7
35	117.2 ± 16.7[b]	–25.8	112.3 ± 8.3[b]	–16.5
70	97.0 ± 11.2[c]	–38.6	101.6 ± 11.0[c]	–24.5

[a] Mean ± SD, $n = 5$
[b] Statistically significant difference compared to the control group of animals: [b] $p < 0.05$, [c] $p < 0.01$.

The immobility displayed in rodents subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioural despair, which in turn may reflect depressive disorders in humans. In fact, there is a significant correlation between the potency of antidepressants in both forced-swimming and tail-suspension tests and clinical potency of the drugs (13). Comparing the results obtained by the two models employed in this study, which use different stress situations to induce states of terror and despair, it can be observed that the effect of the extract on the reduction of immobility time was expressed more strongly in the forced-swimming model than in the tail-suspension test (Table I). Although the mean value of immobility time for the control group of animals was higher in the forced-swimming than in the tail-suspension test, this difference was not significant. However, when comparing the changes of immobility time in extract-treated animals, expressed as the percent of control values, between two tests, the reduction of immobility time was more marked in the forced-swimming method. This means that the forced-swimming test is more sensitive and better reflects the state of depression.

CONCLUSIONS

The results of this pre-clinical study on mice after an acute administration of various doses of *H. perforatum* plant extract using two antidepressant models show that St. John's wort displays an antidepressant effect in doses several times lower than those used in pre-clinical experiments reported in the literature. The antidepressant effect was dose-dependent. Future experiments are needed to further characterize the mechanism of the antidepressant effect of *H. perforatum*, especially of single compounds of the extract, as the only natural drug with antidepressant action.

REFERENCES

1. S. S. Chatterjee, K. Bhattacharya, M. Wonnemann, A. Singer and W. E. Müller, Hyperforin as a possible antidepressant component of Hypericum extracts, *Life Sci.* **65** (1998) 2395–2405
2. H. P. Rang, M. M. Dale and J. M. Ritter, *Pharmacology*, 5th ed., Churchill Livingstone, London 2003, pp. 492–499.
3. R. Brenner, V. Azbel, S. Madhusoodanan and M. Pawlowska, Comparison of an extract of Hypericum (LI 160) and sertraline in the tretment of depression: a double-blind, randomized pilot study, *Clin. Ther.* **22** (2000) 411–419.
4. J. De Vry, S. Maurel, R. Schreiber, R. de Beun and K. R. Jentzsch, Comparison of hypericum extracts with imipramine and fluoxetine in animal models of depression and alcoholism, *Eur. Neuropsychopharmacol.* **9** (1999) 461–468.
5. V. Beijamini and R. Andreatini, Effects of Hypericum perforatum and paroxetine on rat performance in elevated T-maze, *Pharmacol. Res.* **48** (2003) 199–207.
6. V. Butterweck, V. Christoffel, A. Nahrstedt, F. Petereit, B. Spengler and H. Winterhoff, Step by step removal of hyperforin and hypericin: activity profile of different *Hypericum* preparations in behavioral models, *Life Sci.* **73** (2003) 627–639.
7. V. Butterweck, Mechanism of action of St John's wort in depression: what is known?, *CNS Drugs* **17** (2003) 539–562.
8. G. Calapai, A. Crupi, F. Firenzuoli, G. Inferrera, F. Squadrito, A. Parisi, G. Sarro and A. Caputi, Serotonin, norepinephrine and dopamine involvement in the antidepressant action of Hypericum perforatum, *Pharmacopsychiatry* **34** (2001) 45–49.
9. M. Rolli, C. Schäfer and W.E. Müller, Effect of Hypericum extract (LI160) on neurotransmitter receptor binding and synaptosamal reuptake system, *Pharmacopsychiatry* **28** (1995) 207–210.
10. M. Franklin, A. Reed and H. Murck, Sub-chronic treatment with an extract of Hypericum perforatum (St John's wort) significantly reduces cortisol and corticosterone in the rat brain, *Eur. Neuropsychopharmacol.* **14** (2004) 7–10.
11. P. Frost, S. Bornstein, M. Ehrhart-Bornstein, F. O'Kirwan, C. Huston, D. Heber, V. Go, J. Licinio and M. L. Wong, The prototypic antidepressant drug, imipramine but not Hypericum perforatum (St. John's Wort) reduces HPA-axis function in the rat, *Horm. Metab. Res.* **35** (2003) 602–606.
12. R. D. Porsolt, A. Bertin and M. Jalfre, Behaviour despair models in mice: a primary screening test for antidepressants, *Arch. Int. Pharmacodyn.* **229** (1977) 327–336.
13. R. D. Porsolt, Animal models of depression: utility for transgenic research, *Rev. Neurosci.* **11** (2000) 53–58.
14. J. M. Vangeois, G. Passera, F. Zuccaro and J. Costentin, Individual differences in response to imipramine in the tail mouse suspension test, *Psychopharmacology* **134** (1997) 387–391.

S A Ž E T A K

Antidepresivni učinak *Hypericum perforatum* L. mjeren pomoću dvije eksperimentalne metode na miševima

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Farmakološki pristup liječenju depresije uključuje dugotrajnu primjenu antidepresiva, pri čemu se oni mogu primjenjivati kao monoterapija ili pak kao kombinacija više lijekova s različitim mehanizmima djelovanja. *Hypericum perforatum L.* (gospina trava) je jedini prirodni antidepresiv koji je djelotvoran u liječenju depresivnih poremećaja blagog i srednje jakog intenziteta. Za antidepresivno djelovanje gospine trave čini se da su najzaslužniji hipericin i hiperforin.

U ovom radu ispitivan je antidepresivni učinak niskih doza ekstrakta *H. perforatum* i to: 7, 35 i 70 mg kg^{-1} na miševima pomoću metode »prinudnog plivanja« i »vješanja za rep«. Kao pokazatelj antidepresivnog djelovanja mjereno je vrijeme mirovanja životinja – što je ono bilo kraće, aktivnost životinja je bila veća, pa je antidepresivni učinak bio jači. Povećavanjem doze sa 7 mg kg^{-1} na 35 odnosno 70 mg kg^{-1}, antidepresivni učinak ekstrakta *H. perforatum* bio je veći u odnosu na kontrolnu skupinu životinja i to za 10,1%, 25,8% i 38,6% u metodi plivanja, odnosno za 12,7%, 16,5% i 24,5% u metodi vješanja za rep. Rezultati ovog rada ukazuju na antidepresivno djelovanje ekstrakta gospine trave već nakon jednokratne primjene niskih doza u oba korištena eksperimentalna modela.

Ključne riječi: *Hypericum perforatum* L. *(Hypericaceae)*, antidepresivni učinak, životinjski modeli depresije

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