Long Term Administration of Hypericum perforatum Improves Spatial Learning and Memory in the Water Maze

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The aim of the present study is to investigate the effects of long-term Hypericum perforatum treatment on spatial learning and memory in rats. Hypericum preparation (HP) standardized to 0.3% hypericin content was administered orally for 9 weeks in doses of 4.3 and 13 μg/kg corresponding to therapeutic dosages in humans of 0.3 and 0.9 mg of total hypericins daily. A Morris water maze paradigm was used. The mean escape latency over 4 d 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 (15.8 s). 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). After completion of the behavioral experiment, the regional brain concentrations of monoamines and metabolites were estimated in selected brain regions, i.e., prefrontal cortex, hippocampus and hypothalamus. Analysis of variance (ANOVA) demonstrated significant differences in the content of monoamines and metabolites between the treatment groups compared to the Control. The increased 5-hydroxytryptamine (5-HT) levels in the prefrontal cortex correlated positively with the retention of spatial memory. These findings show that the long-term administration of Hypericum perforatum can improve learning and spatial memory with significant changes in the content of monoamines in several brain regions.

Key words Hypericum perforatum; water maze; spatial memory; monoamine; prefrontal cortex; 5-hydroxytryptamine

Depressive illness is universal and, according to WHO estimates, by the beginning of the third millennium major unipolar depression will be one of the most important causes of ill health. A systematic review of controlled, randomized, double blind clinical trials with the phytotherapeutic antidepressant Hypericum perforatum L. revealed that it was significantly superior to a placebo and similarly effective as a low dose of standard antidepressant drugs,1,2 as well as selective serotonin-reuptake inhibitors (SSRIs).3 Recently, the efficacy of Hypericum perforatum in antidepressant medication therapeutically equivalent to imipramine in treating mild to moderate depression both in adults4 and children5 has been reported. It offers encouraging results in the treatment, with response rates of 60–70% estimated by analysis of pooled data. After treating thousands of patients with hypericum products, no major side effects have been reported so far, contrary to conventional antidepressants.6

Until now, Hypericum products have been tested mostly in regard to their antidepressive activity, and presented changes similar to those seen with other antidepressants, potent inhibitors of the uptake of several neurotransmitters. Alterations in behavior were documented in motor activity, tail suspension test, exploration, rat forced swimming test, learned helplessness, escape deficit models and animal models of alcoholism.7–12 Memory enhancing properties in rodents were observed only after acute or short-term Hypericum administration.13,14

Depression is often accompanied by diminished ability to think or concentrate and impairment of memory.15 Commonly used synthetic antidepressants are known to have antimuscarinic side-effects associated with sedation, and delirium symptoms, increasing already impaired cognition processes.

Therefore, in this study, we report the assessment of the effects of long-term Hypericum perforatum administration on spatial learning and memory in the water maze paradigm to test an alternative treatment devoid of side-effects characteristic of tricyclic antidepressants.

MATERIALS AND METHODS

Animals Twenty-seven 6-month-old male Wistar rats (WAG, Charles River), initially weighing 350–360 g, were used in this study. The rats were housed under a 12:12 light:dark schedule (lights off at 20:00 h) with food and tap water continuously available. Herbal tablets made of the dried crude herb of Hypericum perforatum (Hyperherba®, Laboform) standardized to 0.3% hypericin were used in the study. The herb was administered daily for nine weeks by gastrointestinal gavage in a suspension of 2% solution of carboxymethyl cellulose in a volume of 0.1 ml/kg b.w.

Animals were divided into three groups and treated as follows: 1) eight rats received a 2% solution of carboxymethyl cellulose (Control); 2) ten rats received Hypericum perforatum supplement (HP) equivalent to 4.3 μg/kg b.w. of hypericins (HP 4.3), and 3) nine rats received HP equivalent to 13 μg/kg b.w. of hypericins (HP 13). These doses corresponded to the human recommended daily dosage of 0.3 and 0.9 mg of total hypericins.

All animal testing was carried out according to the European Communities Council Directive of 24 November 1986 (86/609/EEC), after approval of the Ethical Committee for Animal Experiments at Medical University of Warsaw.

Water Maze Behavioral testing was conducted on five consecutive days during the ninth week of treatment. The experiment took place during the light portion of the cycle between 8:00—15:00 h.

A circular water maze was used as described by Morris, with some modifications.16 The pool was 1.40 m in diameter and 0.50 m high and was filled with 23°C water (0.30 m). The pool was divided into four quadrants and designated Northeast (NE), Northwest (NW), Southeast (SE) and South-
west (SW) arbitrarily. Rats were trained to locate a transparent hidden plexiglas platform (10 cm x 10 cm), 29 cm high and 1 cm below the water surface. The pool was surrounded by several prominent cues to spatial coordinates, including items such as a shelf, several posters, a video camera, illumination lights and the presence of the researcher and one assistant. All rats were given one session of four trials daily for four consecutive days. For each trial, the rat was placed in the water facing the wall of the pool at one of three equally spaced starting points, excluding the quadrant with the platform. The order in which these starting points were used was determined randomly for each trial and changed each day to prevent the use of a simple taxis strategy, but the location of the escape platform was always centered in the SE quadrant. A trial was terminated when the rat reached and entered the platform. If the animal did not find the platform within 60 s it was placed on the platform for 15 s before the next trial was initiated. Rats that failed to find the platform were given a latency score of 60 s. At the end of the day’s session, the rat was wiped in a cloth to dry it, and returned to its home cage. The probe trial was conducted the day after the last training session. There was no platform in place during the probe trial, and none of the animals received any drug treatment prior to being placed in the maze. The rats were allowed to swim for 60 s before the end of the session. Data were recorded by a VHS image analyzing system (Chromotrack, San Diego Instruments). A remote switch was used to start and stop recording. Data from the water maze included latency to find the platform and distance travelled. For the probe trial, the number of visits to where the platform had been and the time spent in the goal quadrant were measured.

A three factor repeated measures analysis of variance (ANOVA) (treatment x day x trial) was used to assess differences during acquisition learning. All posthoc tests were performed using Student’s t-test to identify the origin of any significant differences, when appropriate. In addition, Pearson’s product moment correlation coefficient and the correlation coefficient r were calculated with simple linear regression based on the results of the behavioral testing in the probe trial in the water maze and the level of monoamines and their metabolites in the prefrontal cortex, hippocampus and hypothalamus. All hypothesis testing used a significance level of 0.05.

**Biochemistry** Biochemical measurements were conducted after the end of the behavioral experiment. The animals were killed by decapitation 24 h after last treatment, and brains were rapidly removed. The cerebral cortex, hippocampus and hypothalamus were dissected out on an ice-cold plate according to the method of Glowinski and Iversen. Each tissue sample was rapidly weighed and quickly frozen, then stored in a deep freezer at −80 °C until assayed. Tissues were homogenized in 1000 μl ice-cold 0.1 M HClO4, and centrifuged at 13000 x g for 15 min to precipitate proteins. The supernatant was removed and filtered (0.2 μm pore size; Whatman) and examined for neurotransmitter content. Dopamine (DA; standard substance supplied by RBI), its metabolite, 3,4-dihydroxyphenylethylamine (DOPAC) (RBI); 5-hydroxytryptamine (5-HT) Sigma; 5-hydroxyindolacetic acid (5-HIAA) Sigma; and 3,4-dihydroxyphenylethanolamine (NA) Sigma were measured using high-performance liquid chromatography (HPLC) with electrochemical detection and a glassy carbon electrode. The electrochemical potential was set at 0.8 V with respect to an Ag/Ag Cl reference electrode. The chromatograph system consisted of an autosampler automatic injector (Knauer Basic Marathon), a pump (Mini-Star K-500; Knauer), and an electrochemical detector (L-3500A; Merck). The mobile phase comprised 58 mM sodium phosphate (Sigma), 31 mM citric acid (Sigma), 1 mM octane sulfonic acid (Aldrich), and 27 μM ethylenediaminetetraacetic acid (EDTA, Sigma) in deionized, 18.3 mMΩ polarized water containing 1% acetonitrile (Merck) and 12% methanol (Merck). Separation of monoamines was achieved with a C-18 column (250 mm x 4 mm reverse phase, Nucleosil, 5 μm particle size; (Macherey-Nagel, Germany) and the mobile phase flow rate was maintained at 0.8 ml/min. Samples were quantified by comparison with standard solutions of a known concentration using HPLC software, and area under the peaks was quantified. Data were collected and analyzed by Eurochrom 2000 for Windows (Knauer). Contents of neurotransmitters and metabolites were expressed as ng/g fresh tissue. Comparison between neurotransmitters and metabolites of the groups was accomplished by one-way analysis of variance, followed by Student’s t-test.

**RESULTS**

After eight weeks of treatment, at the time of the beginning of the behavioral experiment, the body weight of the rats ranged from 475 to 500 g and did not differ between the Control and experimental groups (F(2,24) = 0.968, p > 0.05).

**Water Maze** Acquisition Trials (Days 1—4): The results of the acquisition in the water maze test (escape latency) are presented in Fig. 1. The effect of treatment was significant in escape latency among groups F(12,24) = 4.32, p < 0.02. Latencies were significantly reduced as a function of training: F(3,72) = 59.493, p < 0.001 for training days and trials F(13,72) = 12.93, p < 0.001. There was significant group x day interaction: F(18,72) = 3.280, p < 0.001, but not for group x trials (p > 0.05). Newman–Keuls analysis of latencies showed the mean escape latency for the HP 13 μg/kg group to be significantly smaller than both the HP 4.3 μg/kg and the vehicle control.

![Fig. 1. Mean Escape Latency (±S.E.M.) during Acquisition of the Spatial Navigation Task for Control and Treated Rats That Received Hypericum perforatum Preparation for 9 Weeks](image-url)
groups ($p<0.05$). The vehicle Control and HP 4.3 $\mu$g/kg groups did not differ (Fig. 1). The result did not show a significant main effect for swim distance ($F_{2,24} = 0.260$, $p = 0.775$). It was significant for the day of training ($F_{15,405} = 17.357$, $p < 0.001$) but not for the trials ($F_{15,405} = 1.776$, $p > 0.05$), group x day ($F_{6,72} = 0.982$, $p > 0.05$) or group x trials ($F_{2,72} = 0.667$, $p > 0.05$) interaction.

The Probe Trial, Day 5: As can be seen from Fig. 2, platform crossings over the previous SE position showed a significant improvement in the HP 13 $\mu$g/kg group ($p < 0.05$, t-test). Hypericum (HP) 13 $\mu$g/kg treated rats swam preferentially in the target quadrant (SE), where the platform was previously placed during training ($p < 0.05$, t-test). The quadrant preference of HP 4.3 $\mu$g/kg treated rats was not significantly different from that of the Control rats, but though the HP 4.3 $\mu$g/kg group did not cause statistically significant changes, there was a trend for improvement in comparison with the Control group.

Biochemistry

The levels of monoamines and their metabolites in the prefrontal cortex, hippocampus and hypothalamus are given in Table 1.

Noradrenaline (NA): ANOVA demonstrated statistically significant differences between the content of NA in the prefrontal cortex ($F_{2,24} = 3.481$, $p < 0.05$). Posthoc comparisons showed a significant increase in the level of NA in the HP 4.3 $\mu$g/kg group compared to the Control group ($p < 0.01$).

Dopamine (DA): The HP 4.3 and HP 13 $\mu$g/kg treatment significantly reduced DA levels in the hippocampus ($F_{2,24} = 6.509$, $p < 0.005$) and hypothalamus ($F_{2,24} = 9.536$, $p < 0.001$). Further analysis showed that the level of DA did not differ between the treatment groups.

DOPAC: The HP 4.3 $\mu$g/kg and HP 13 $\mu$g/kg groups had significantly lower DOPAC levels when compared to the control group in the prefrontal cortex ($F_{2,24} = 18.616$, $p < 0.0001$), in the hippocampus ($F_{2,24} = 25.142$, $p < 0.0001$) and in the hypothalamus ($F_{2,24} = 14.424$, $p < 0.0001$). Posthoc analysis showed that the content of DOPAC was significantly lower in the HP 4.3 group vs. the HP 13 group in the cortex ($p < 0.01$), in the hippocampus ($p < 0.001$) and in the hypothalamus ($p < 0.001$).

The DOPAC/DA Ratio: The ANOVA demonstrated statistically significant differences between the groups in the DOPAC/DA ratio in the prefrontal cortex ($F_{2,24} = 15.03$, $p < 0.0001$), in the hippocampus ($F_{2,24} = 14.384$, $p < 0.0001$) and in the hypothalamus ($F_{2,24} = 4.01$, $p < 0.03$). Further analysis by means of Student’s t-test showed that the DOPAC/DA ratio in the cortex was significantly lower in the HP 4.3 group vs. the Control group ($p < 0.0001$) and vs. the HP 13 group ($p < 0.001$). In the hippocampus, it was also significantly lower in the HP 4.3 group vs. the Control group ($p < 0.0001$) and vs. HP 13 group ($p < 0.001$). In the hypothalamus, the DOPAC/DA ratio was decreased in the HP 4.3 group compared to the HP 13 group ($p < 0.01$).

5-HT: One way ANOVA did not reveal statistically significant differences between the groups in 5-HT content in the prefrontal cortex ($F_{2,24} = 2.325$, $p = 0.118$), but differences were observed in the hypothalamus ($F_{2,24} = 17.156$, $p < 0.001$). Further analysis showed a significant increase in 5-HT content in the hippocampus between the HP 4.3 group compared to the Control group ($p < 0.05$), and a lower level of 5-HT in the hypothalamus in HP 4.3 group vs. Control group ($p < 0.001$) and vs. the HP 13 group ($p < 0.01$).

5-HIAA: Overall ANOVA did not show significant differences between groups in 5-HIAA content. Posthoc analysis revealed a significant decrease in the level of 5-HIAA for the HP 13 group vs. the Control group in the cortex ($p < 0.05$), and an increase in the HP 4.3 group vs. the Control group in

![Fig. 2. Spatial Probe Data from the Platform Area Crossings of Control and Hypericum perforatum Treated Rats in the Water Maze Task on Day 5 (Trial 17)](image-url)

The test was run in the same manner as the acquisition trials, except that the target platform was absent and the trial was terminated after 60 s. The measure given is platform crossings: S.E.M.: the number of times the rat passed through a nominal area defining the originally correct platform position. *p<0.05 vs. Control (t-test). Open bar represents Control group (n=8); hatched bar represents HP 4.3 $\mu$g/kg group (n=10), filled bar represents HP 13 $\mu$g/kg group (n=9).

<table>
<thead>
<tr>
<th>Group</th>
<th>Brain region</th>
<th>Monoamine and metabolite levels in ng/g wet tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>Control</td>
<td>Cortex</td>
<td>217.1±15.1</td>
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<tr>
<td></td>
<td>Hippocampus</td>
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<td>HP 4.3</td>
<td>Cortex</td>
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<td>Hippocampus</td>
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<tr>
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<tr>
<td></td>
<td>Hypothalamus</td>
<td>2190.0±116.5</td>
</tr>
</tbody>
</table>

Data are presented as mean±S.E.M. levels (ng/g tissue). *p<0.05, **p<0.01, ***p<0.001 when compared with the corresponding Control in vehicle treated rats; †p<0.05, ‡p<0.01, §§p<0.001 compared to the other treatment group.
the hypothalamus (p<0.05).

The 5-HIAA/5-HT Ratio: The ANOVA demonstrated statistically significant differences between groups in the 5-HIAA/5-HT ratio in the prefrontal cortex (F_{2,24}=5.788, p<0.01), in the hippocampus (F_{2,26}=3.364, p<0.05) and in the hypothalamus (F_{2,24}=12.165, p<0.001). Analysis of differences between groups showed that the 5-HIAA/5-HT ratio in the cortex is significantly lower in the HP 13 group vs. the Control group (p<0.01) and vs. the HP 4.3 group (p<0.01). In the hippocampus, the 5-HIAA/5-HT ratio was also significantly higher in the HP 13 group vs. the Control group (p<0.05) and vs. the HP 4.3 group (p<0.05). In contrast in the hypothalamus the 5-HIAA/5-HT ratio was significantly increased both in the HP 4.3 group vs. the Control group (p<0.001) and vs. the HP 13 group (p<0.05).

Correlation between Monoamine Levels and Spatial Memory: A measure of spatial learning accuracy was used to determine whether the monoamine levels were associated with cognitive ability in the Hypericum perforatum treated rats. The number of annulus crossings over the previous SE position of the platform during the probe trial was correlated with the levels of monoamines in the prefrontal cortex, hippocampus and hypothalamus.

The accuracy of spatial memory was not reliably correlated with any monoamine or metabolite level in the hippocampus (p>0.05).

Prefrontal cortex 5-HT levels correlated positively with the mean annulus crossing of the HP 13 group during the probe trial: r=0.676; F(1,7)=5.72; p<0.05; (Fig. 3).

There was no correlation in the prefrontal cortex between the two measures for the other groups or for the other monoamines and metabolites tested (p>0.05).

In none of the experimental groups was there a correlation between behavioral performance and monoamine level detected in the hypothalamus.

DISCUSSION

The findings presented indicate that in Hypericum-treated rats, the HP 13 μg/kg dose elicited improvement of learning performance and consolidation of spatial memory. The maze test of spatial memory can be affected by a motor influence. In our experiment, swim speed was not altered by Hypericum treatment. The absence of effects of Hypericum perforatum on the motor task reduces the probability of gross changes in motor function that could affect water maze performance.

Hypericum perforatum was shown to induce the enhancement of retrieval memory of a conditioned avoidance response and a passive avoidance-conditioning paradigm in mice and in rats. Similarly, in several models of cognitive dysfunction, Indian Hypericum perforatum L. presented nootropic activity which was qualitatively comparable with that induced by piracetam. Our results also agree with studies showing significant enhancement of the cognitive performance in depressive patients treated for 4 weeks with Hypericum extract. In a controlled, randomized double blind clinical trial, Hypericum extract improved cognitive functions better than maprotiline. Electroencephalographic estimation of the influence of St. John’s Wort in humans revealed different activity in terms of improving mental performance when compared with tricyclic antidepressants.

It is generally accepted that individuals with depression have mental symptoms that reflect changes in brain amine neurotransmitters, but the specific impairment that is critical for depression is unclear. Most of the effective antidepressive drugs increase the availability of monoamines, a phenomenon considered to decrease depression. Chronic administration of most antidepressants increases the monoaminergic transmission in the synapses (NA, 5-HT), due to different mechanisms.

Most of the biochemical and behavioral models of antidepressant activity of Hypericum perforatum are performed in acute experiments, with pre-treatment time lasting hours, and usually no longer than 14 d. In the present experiment, Hypericum was administered daily for 9 weeks. It is considered that the treatment in rat, lasting nine weeks, may be equivalent to treatment for 5—6 years in humans and may therefore be classified as “long term”. It is expected that such long treatment may induce chronic changes in the brain mechanisms leading to distinct consequences in monoaminergic neurochemistry, resulting in the occurrence of central adaptive mechanisms.

A variety of neurochemical and pharmacological effects have been reported recently for Hypericum perforatum. Attempts to clarify the mechanisms involved did not yet clarify the issue of its active antidepressant constituents. The pharmacological quality of Hypericum herbal supplements is standardized to the putative antidepressants: hypericin and/or hyperforin content. However, many other constituents or groups of components, including naphthodiantrones, acylphloroglucinols, flavonol glycosides, biflavones, tannins and proanthocyanidines may contribute to Hypericum’s pharmacological effects. In the crude dry herb of Hypericum perforatum used in this study, we can attempt to identify the other active constituents which are not present in the extract: different mono and sesquiterpenes, fatty acids and the higher molecular-weight aliphatic primary alcohols.

In this study, catecholamine levels and metabolism were measured in specific brain regions involved in memory processing. Many studies suggest that the hippocampal formation is critically involved in spatial learning and memory.
The prefrontal cortex in primates is involved in behavioral flexibility and navigational strategies.  

Animal behavioral studies implicated catecholamines in learning, reinforcement, memory and attention. The cognitive deficits in depression and dementia in humans have been also associated with evidence of disturbance in central catecholaminergic activity. Central noradrenergic pathways have been associated with arousal, attention and learning. Dopaminergic neuron activity has been identified with locomotor, orienting and strategies of exploratory behavior. The involvement of 5-HT in a variety of functions probably reflects the fact that 5-HT neurons are broadly represented in the central nervous system (CNS).  

Nevertheless, the relevance of the neurochemical effects of Hypericum perforatum to its influence on learning and memory is not yet clear. Many studies have examined the effects of acute and long-term antidepressive treatment on monoamine metabolism, showing variable and inconclusive findings, depending on the particular treatment.  

The administration of HP 4.3 μg/kg decreased the DA concentration in the hippocampus, leading to a decreased the DOPAC/DA ratio in the prefrontal cortex and in the hippocampus. In the hypothalamus, dopamine turnover was also diminished after treatment with HP 4.3. The reduction of DA turnover indicates the inhibition of dopamine reuptake into presynaptic neurons. In contrast, noradrenaline (NA) content in the prefrontal cortex was significantly increased.  

Changes in 5-HT turnover in the hippocampus were more evident after the administration of higher dose of Hypericum (15 μg/kg) (increased content of 5-HT and reduced metabolism evaluated as the 5-HIAA/5-HT ratio in the prefrontal cortex). In the hypothalamus, Hypericum treatment significantly decreased 5-HT concentration and increased turnover after the administration of both doses of HP. Our findings indicated that serotonin levels were increased following repeated long-term Hypericum treatment, which is consistent with that published by Yu (after 14 d treatment).  

The observed lack of dose-response on monoamine metabolism in our experiment may reflect the dose-response curve of the Hypericum extract as an antidepressant agent which is inverted U-shaped.  

Recently the influence of chronic treatment using hypericin and Hypericum extract on monoamine levels in rat hypothalamus and hippocampus were compared with imipramine, but the dose-response effect was not shown. However, like in most animal studies, the dose of hypericum supplements was 15—45 the average clinical dose. Therefore, there is no relevance of these pharmacological effects to the doses used in the long-term treatment in our study, which closely corresponds to the therapeutic doses used in humans.  

Taken together, the present study has shown that the increased 5-HT levels in the prefrontal cortex correlated positively with the retention of spatial memory. We interpret the correlation between the prefrontal cortex 5-HT concentration and memory as a reflection of the role of 5-HT in behavioral performance. The reported relations in a single neurotransmitter system may be important for understanding and explaining the behavioral influence of long-term administration of Hypericum perforatum.  

Hypericum extract is known to inhibit the sodium-dependent uptake of catecholamines and amino acids into synaptic terminals. It was found that low doses of hyperforin caused a significant elevation in striatal acetylcholine (ACh) release. These observations suggest that part of the antiangenic properties of Hypericum may be based on ACh-dependent learning and memory.

On the other hand, procognitive activity may also indicate the recently reported potent antioxidant effect of Hypericum on free radical production. Deterioration related to free radical damage is implicated in several neurodegenerative disorders, including Alzheimer’s disease, which are accompanied by states of depression.  

Moreover, we should add that the higher aliphatic primary alcohols present in the crude dry plant used in our study, but not in the extract, are thought to decrease cholesterol synthesis by modifying the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (HMG CoA), comparable to a low dose of statin treatment. Recently, such effect is considered of promising value in the management of atherosclerosis and developing dementia.  

In conclusion, treatment with the crude medicinal herb Hypericum perforatum improves not only mood but also learning and memory processes which have deteriorated in depression, and may provide a new and holistic therapeutic approach.

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